Global Assessment of Biological Engineering & Manufacturing

WTEC Report

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INTERNATIONAL ASSESSMENT OF RESEARCH IN BIOLOGICAL ENGINEERING & MANUFACTURING

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WTEC Panel Report on

INTERNATIONAL ASSESSMENT OF RESEARCH IN BIOLOGICAL ENGINEERING & MANUFACTURING

Final Report on Europe and Asia

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R.D. Shelton

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WTEC MISSION

WTEC provides assessments of international research and development in selected technologies under awards from the National Science Foundation (NSF), the Office of Naval Research (ONR), and the National Institute of Standards and Technology (NIST). Formerly part of Loyola University Maryland, WTEC is now a separate nonprofit research institute. Sponsors interested in international technology assessments or related studies can provide support through NSF or directly through separate grants or GSA task orders to WTEC.

WTEC's mission is to inform U.S. scientists, engineers, and policymakers of global trends in science and technology. WTEC assessments cover basic research, advanced development, and applications. Panels of typically six technical experts conduct WTEC assessments. Panelists are leading authorities in their field, technically active, and knowledgeable about U.S. and foreign research programs. As part of the assessment process, panels visit and carry out extensive discussions with foreign scientists and engineers in their labs.

The WTEC staff helps select topics, recruits expert panelists, arranges study visits to foreign laboratories, organizes workshop presentations, and finally, edits and publishes the final reports. R.D. Shelton is the WTEC point of contact: telephone (717) 299-7130 or email shelton@wtec.org.

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EXECUTIVE SUMMARY

The Emerging Fields of Regenerative Medicine and Cell Therapy

The regenerative medicine and cell therapy community has made great steps forward in bringing new methods of medical intervention to fighting progressive disease and to repairing or restoring lost tissue and organ function. Fundamental insights and conceptualized therapies raise the hope that tissue damage such as spinal cord transection (Tabakow et al. 2013) and loss of tissue function in diabetes mellitus (Pagliuca et al. 2014) can be overcome through cell therapy. The promise of a future in which we are able to wield regenerative medicine therapy to minimize tissue damage and prevent age-associated degradation of tissue function is compelling and suggests a way to control the expanding costs of healthcare for aging populations. However, the barriers to widespread implementation of regenerative medicine are legion. We are just beginning to recognize the importance of biological, chemical, physical, and electrical interfaces across tissue complexity in normal and regenerative human physiology. Furthermore, the path from insight and conceptualized therapy to clinical practice cannot be achieved without process engineering and manufacturing.

This study focused on identifying engineering and life science principles that lead translation from conceptualized intervention in complex disease states to manufacture of safe and effective products in cell therapy and regenerative medicine. It quickly became apparent that integration of lessons from different disciplines is essential to rapid progress in technology transfer from the laboratory to the medical theater. Technology transfer becomes successful by building on the basic sciences and moving forward to clinical practice; process engineering is a central thread in this process, moving to manufacture of cell therapies and regenerative medicine. The most successful initiatives observed in this study are those where engineering principles are engaged early in conceptualizing medical intervention in disease and where natural barriers between disciplines are replaced with shared insight to the most rapid paths to medical practice. The need for more insight, more information, and more effective integration between engineering and other disciplines in regenerative medicine is of paramount importance to realizing the potential of this field of medicine. Collaboration and integration across disciplines is essential to successful translation from basic research to clinical and commercial implementation. This study documents some extraordinary examples of this in laboratories and manufacturing facilities across the world. The authors strongly recommend careful review of the individual site reports in Appendices B and C and perusal of their references.

To reliably manufacture safe and efficacious cell therapies (autologous or allogeneic), process engineers must know and understand the critical quality attributes (CQAs) of cell therapy and regenerative medicine. What are the biological, chemical, physical, and cellular characteristics that determine physiologic function and performance (the CQAs from basic biochemistry to manufactured tissue function)? How do process variables (e.g., kinetic and transport limitations in scale-up) influence these CQAs during changes in scale to the manufactured product? The leading institutions in regenerative medicine and cell therapy are involving engineering logic earlier and earlier in the basic processes leading to conceptualization of therapy and translation to real products and important outcomes. The scientific and technical communities of the United States must encourage earlier integration of engineering logic to speed the development of this field of medicine and to realize the economic impact of radically effective therapies for disease intervention.

Although we have some of the tools for analysis of tissue interfaces in normal and regenerative human physiology and some of the methodologies for tracing communication and networking

across cellular and tissue boundaries, we do not yet understand enough of the languages and linkages of whole tissue function. What constitutes successful intervention of regenerative medicine? How is it achieved? How is it maintained? What is its longevity? What are the dynamics of regenerative medicine integration in resolving tissue repair and regaining tissue function?

Chondrocytes derived from induced pluripotent stem cells can repair damaged knee cartilage, regaining some of the lost function of the knee (Makris et al. 2015), but does the benefit result from engraftment of the implanted tissue, or is the function of the implant to create microenvironments that favor autologous repair? Do limbal epithelial stem cells implanted into the limbal pocket of a human eye alter the transient amplifying cells leading to terminally differentiated cells of the human cornea; do they differentiate *in vivo* to become transient amplifying cells; or do they simply aid in providing the right microenvironment for corneal repair (Levis, Daniels, and Ahmad 2013)? Regenerative medicine is intrinsically multidisciplinary, ranging from electron transfer to routine manufacture of multilayer polymorphic functional tissue and delivery of therapies to patients. Across the leading teams in this study, engineering logic is helping to provide the answers to questions such as those raised above.

Everywhere the WTEC panel went, panelists observed teams of people who have brought the basic sciences of tissue function through to process engineering and on to manufacturing. World-class teams in cell therapy and regenerative medicine advance through integration of multidisciplinary and cross-disciplinary systems. Interdisciplinary learning was evident at all sites, but transdisciplinary maturity is still elusive (Stember 1991), where engineering is a core discipline in discovery and development as well as in scale-change and manufacturing. Engineering in the United States needs a catalyst that will broaden its core role in regenerative medicine.

National organizations (e.g., regulatory authorities, government economic initiatives, insightful legislative bodies) and technical associations (e.g., economic development councils and scientific associations) that help combine expertise in process engineering and manufacturing with the underlying sciences and with the economics of outcome analysis, add impetus to the fields of regenerative medicine and cell therapy in Europe and Asia. Examples of their impact were evident at the Cell Therapy Catapult Ltd., UK; the Scottish Enterprise Edinburgh BioQuarter; the 21st Century Frontier R&D Program, Ministry of Science and Technology, Korea; across all of the sites the panel visited in Japan, where changes in legislation are changing the landscape in regenerative medicine regulation (ACCESSWIRE 2014); in China's investment of US$5 billion in translational and regenerative medicine and its awaited accelerated regulatory framework for cell therapy; and in the international Alliance for Regenerative Medicine (http://alliancerm.org/). The National Academies study on stem cells provides an analysis of the changing regulatory landscapes (Berger, Beachy, and Olson 2014).

The chapters in the present final report ask, and in some cases answer, the questions of how can engineering accelerate understanding in the safe and effective development of cellular therapy and individualized medicine; how can nanotechnology and genetic engineering impact the outcomes of medical intervention and regenerative medicine; and how can biology and engineering interact to discover new approaches to the treatment of disease and to the manufacture of therapies and interventions that have both the complexity of functional tissue systems and the reliability required to ensure safety and efficacy. Through all of the panel's observations, it found that including engineering at the earliest stages of development establishes the most rapid path to product realization. Engineering insights, some elementary (e.g., determining how to remove all animal-derived components from media), and some much more complex (e.g., understanding and modeling subtle cellular control parameters to establish and maintain states of differentiation) are enabling regenerative medicine in Europe and Asia.

The science of regenerative medicine in Europe and Asia is also changing the way that scientists worldwide think about the traditional roles of engineering and manufacture. It is clear that the development of cellular materials and systems must begin in the clinic where acquisition initiates.

The range of thinking for manufacture of cell therapy and regenerative medicine products needs to expand from cell acquisition through manipulation, scale-up, formulation, and even to outcomes follow-up.

The path from concept to commercial cell therapy is long and arduous, requiring revenue streams that can support the people, equipment, and clinical trials necessary to launch product-driven revenues. MEDINET (Japan) has integrated long-range strategic development with its tactical capabilities for contract manufacture to finance growth in cell therapy. MEDIPOST (South Korea) has successfully leveraged its operational infrastructure for its private cord blood banking business to support its long-range vision of regeneration of tissue function through endogenous regeneration via paracrine (trophic factor-mediated) mechanisms (Jang et al. 2014; Jeong et al. 2013). PharmaCell (The Netherlands) is the commercial manufacturer of two of the three cell-based products approved by the European Medicines Agency and marketed in Europe; further, it utilizes its substantial manufacturing capability to support preclinical development and clinical manufacture of other cell therapy products. Still, the revenue streams necessary to bring the potential of regenerative medicine to those who need it most are not yet fully developed, nor are they adequate to the task.

Stephen W. Drew

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WTEC International Assessment of Research in Biological Engineering & Manufacturing

CHAPTER 1

INTRODUCTION

Stephen W. Drew

BACKGROUND

Tremendous progress has been made in recent years in developing new treatments for human diseases based on fundamental discoveries in tissue engineering, regenerative medicine, stem cells, cell-based therapies, personalized medicine, and many other fields. To make these treatments available at affordable prices, the resulting products have to be manufactured in quantity to high standards of efficacy and safety, with cost being a critical constraint. This translation phase of the innovation cycle is challenging since it requires much more capital than research, has to meet strict regulations, and has to be delivered to patients through practitioners who may be resistant to new therapies. It is particularly hard to do in the United States where the infamous "Valley of Death" limits funding for the transition between Government funding of fundamental research and commercial funding of applied research and manufacturing. Researchers in many countries are trying to bridge this gap, and much progress is being made.

It is particularly interesting to learn from the alternate universes abroad that have different environments for this translation process. In some countries governments do more to help bridge the gaps to final production. There are alternate mechanisms for cooperation among the many players: government, universities, profit-seeking startup and established companies, clinical physicians, and the patients themselves. Countries that have long provided healthcare for everyone through vast national systems obviously have very different environments for translation than the United States. Thus there is much to be learned abroad.

National Science Foundation (NSF) program Directors Dr. Ted Conway and Dr. Athanassios Sambanis and others (the Biomedical Engineering and Engineering Healthcare cluster) initiated the study International Assessment of Research in Biological Engineering and Manufacturing in the NSF Chemical, Bioengineering, Environmental, and Transport Systems (CBET) division during the first half of 2013. Among other purposes, those programs fund fundamental research in technologies that can be applied to improving healthcare:

> The mission of the Biomedical Engineering and Engineering Healthcare cluster is to provide opportunities to develop novel ideas into projects that integrate engineering and life science principles in solving biomedical problems that serve humanity. The cluster focuses on high impact transforming technologies for deriving information from cells, tissues, organs, and organ systems, extraction of useful information from complex biomedical signals, new approaches to the design of structures and materials for eventual medical use, biophotonics, and new methods of controlling living systems. This cluster is also directed toward the characterization, restoration, and/or substitution of normal functions in humans (NSF 2013).

The study uses an existing cooperative agreement with WTEC that provides a structure for such international studies. WTEC asked Dr. Stephen Drew to chair the study, and an additional five experts were recruited (Table 1.1). A kickoff meeting was held on November 6, 2013, where Dr. Drew finalized the technical scope of study. The study methodology was to send a delegation of U.S. experts to some of the top labs in Europe and Asia. The study was also informed by a workshop held in August 2013 that surveyed U.S. activities in the field (Kaplan 2013). This introductory chapter primarily provides a summary of the process of the study.

Table 1.1. Panelists

Panelist	Affiliation
Stephen Drew, Ph.D.	Drew Solutions, LLC (*Chair*)
Gang Bao, Ph.D.	Georgia Institute of Technology (Georgia Tech)
Christopher Bettinger, Ph.D.	Carnegie Mellon University
Kam Leong, Ph.D.	Duke University (now at Columbia University)
Madhusudan Peshwa, Ph.D.	MaxCyte, Inc.
Kaiming Ye, Ph.D.	Binghamton University, State University of New York (SUNY)

WTEC BACKGROUND

The study was organized on behalf of the NSF sponsors by WTEC, which is a nonprofit research institute. With core funding and management from the NSF Directorate for Engineering, WTEC has conducted over 70 international technology assessments. Additional support for WTEC studies has come from the National Institutes of Health (NIH), Department of Energy (DOE), National Institute of Standards and Technology (NIST), U.S. Environmental Protection Agency (EPA), National Aeronautics and Space Agency (NASA), and several agencies of the Department of Defense (DOD), whose funds are made available via NSF. WTEC peer review panels have assessed international R&D in numerous technologies, including flexible electronics, nanocatalysis, simulation-based engineering and science, and systems engineering for renewable energy. This study specifically builds on previous WTEC international studies of R&D on tissue engineering, rapid vaccine manufacturing, stem cell engineering, and mobility technologies for people with disabilities. Final reports are posted at http://www.wtec.org/.

SCOPE OF THE STUDY

The study's target field involves translation of scientific discoveries to clinical applications through manufacturing of resulting products. Some of the areas of interest include:

- Imaging/sensing
- Functional nanoparticles
- Biomimetics in healthcare delivery
- Cell-based delivery and therapy
- Personalized medicine manufacturing and regulation
- Genome editing, e.g., stem cell differentiation

To further sharpen these focus areas, a list of questions was developed to guide discussions with researchers. It is provided in Appendix E.

METHODOLOGY OF THE STUDY

The panel of U.S. experts listed in Table 1.1 conducted the study. The team includes an expert in regenerative medicine commercialization who was able to join the panelists after launch of the study: Dr. Madhusudan Peshwa. Patricia Foland, Hassan Ali, and Clay Stewart from WTEC accompanied the expert panelists. The sites to be visited were identified by an iterative process. The particular sites visited were limited by geography, availability of hosts, logistics, and funding. Omission of a site does not imply that it was less important than those visited. The final list of sites for Europe is shown in Table 1.2 and Figure 1.1. To allow the study to engage in in-depth discussions with a larger group of hosts during the limited timeframe of the study, the panel was divided into Group A, which visisted England, Scotland, the Netherlands, western Germany, Sweden, and Switzerland, and Group B, which visited the UK, Portugal, central and eastern Germany, and Italy. Group A generally included Drew (head), Bettinger, Ye, and Ali. Group B generally included Leong (head), Applegate, Bao, Peshwa, and Foland. However, individuals switched between the groups to visit sites related to their areas of technical focus. Dr. Grant Lewison of Evalumetrics in London arranged the itinerary. The site visits took place during March 3–10, 2014. Appendix B includes a site report from each European site visited.

Table 1.2. Sites Visited in Europe

Location	Site
Group A	
London, England	Imperial College London (ICL)
London	University College London (UCL)
London	Cell Therapy Catapult, Ltd (CTC)
London	London Regenerative Medicine Network (LRMN) networking event
Edinburgh, Scotland	Several centers at one site: Roslyn Cells, Scottish Centre for Regenerative Medicine, BioQuarter, Systemic, et al.
Utrecht, the Netherlands	PharmaCell B.V.
Idar-Oberstein, Germany	EUFETS, GmbH
Linköping, Sweden	University of Linköping
Lausanne, Switzerland	École Polytechnique Fédérale de Lausanne (EPFL)
Basel, Switzerland	ETH-Basel
Royston, England	TAP Biosystems
Loughborough, England	University of Loughborough
Group B	
Würzburg, Germany	University of Würzburg
Stuttgart, Germany	Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB)
Lisbon, Portugal	Technologic Institute of Experimental Biology/Institute of Chemical and Biological Technology (IBET/ITQB), Instituto Superior Técnico, Cell2B
Lisbon, Portugal	INFARMED
Berlin, Germany	Berlin-Brandenburg Center for Regenerative Therapies (BCRT)
Leipzig, Germany	Fraunhofer Institute for Cell Therapy and Immunology (IZI)
Milan, Italy	University of Milan
Milan, Italy	MolMed S.p.A.
Milan, Italy	The San Raffaele Telethon Institute for Gene Therapy (TIGET)
Leeds, England	University of Leeds

Figure 1.1. Sites visited in Europe.

The Asian phase of the WTEC study visits also was divided into two groups, which traveled during different weeks. The sites visited by both are shown in Table 1.3 and Figure 1.2. Group A met with leaders in Korea and Japan during May 24–31, 2014. The group consisted of Drew, Leong, Bao, Peshwa, and Sambanis, with Ali, who made the travel arrangements for WTEC. Group B visited China during July of 2014. This group included Ye, Bettinger, and Conway, with Stewart providing WTEC support. Bao was able to attend for a day or two. These visits were also arranged by Ali. Appendix C includes a site report from each Asian site visited.

After the second study tour in Asia, the results were presented in a full-day public workshop in Arlington, Virginia, on November 5, 2014. The workshop was webcast and placed in an archive available at http://wtec.org. The webcast can also be accessed at: http://www.tvworldwide.com.

Table 1.3. Sites Visited in Asia

Location	Site
Group A	
Beijing, China	Bureau of International Cooperation at NSFC
Beijing, China	Peking University, College of Life Science
Beijing, China	Tsinghua University
Guangzhou, China	Sun Yat-sen University (SYSU)
Guangzhou, China	Guangzhou Institutes of Biomedicine and Health (GIBH), CAS
Shanghai, China	Shanghai Jiaotong University
Soochow, China	Soochow University

Location	Site
Group B	
Seoul, S. Korea	MEDIPOST
Seoul. S. Korea	Korea Institute of Science and Technology (KIST), South Korea
Seoul, S. Korea	Sungkyunkwan University School of Medicine, South Korea
Daegu, S. Korea	Kyungpook National University School of Medicine (virtual visit)
Tokyo, Japan	MEDINET
Tokyo, Japan	CellSeed, Inc.
Kyoto, Japan	Center for Induced Pluripotent Stem Cell Research & Application (CiRA), Kyoto Univ.
Aichi, Japan	Japan Tissue Engineering Co. Ltd. (J-TEC)
Otsu Shiga, Japan	Takara Biosystems, Inc.
Tokyo, Japan	Nanocarrier Co. Ltd.

Figure 1.2. Sites visited in Asia.

OVERVIEW OF THE REPORT

Madhusudan Peshwa in Chapter 2 addresses personalized medicine manufacturing and regulation, introducing the common threads of cell therapy and regenerative medicine as they flow through each of the subsequent chapters. Kam Leong in Chapter 3 illuminates the role of functional nanoparticles in regenerative medicine, including delivery of medical intervention in disease and the analytics that allow monitoring and control. Kaiming Ye in Chapter 4 develops concepts in analytics, including imaging and sensing in regenerative medicine. Christopher Bettinger traces biomimetic applications in medicine and cell therapy in Chapter 5. Gang Bao presents concepts in genome editing in Chapter 6.

Appendix A contains short biographical sketches of the panelists, and Appendix B and C have the detailed trip reports from the sites studied in Europe and Asia respectively. A glossary of technical terms is given in Appendix D, and Appendix E contains the questions submitted to hosts.

This study covered seven broad topics: (1) cell-based therapy and delivery, (2) manufacturing of personalized medicine, (3) functional imaging and sensing in regenerative medicine, (4) functional nanoparticles, (5) biomimetics in regenerative medicine, (6) genome editing in medical intervention of disease states, and across all of these areas, (7) the regulatory affairs that guide translation of new concepts into commercial and clinical utilization. These seven areas share two common characteristics:

- *Regenerative medicine*: the use of tissues to rebuild, repair, and extend the functionality of diseased or injured tissues to bring them back to normal function and form
- *The underlying area of cell therapy*; the use of cells, cell components, and cell systems, to generate new tissue and regenerate tissue function

In many ways, the areas of regenerative medicine and cell therapy define the boundary of this study. This study focuses on the convergence of biological, chemical, physical, and temporal interfaces with respect to cellular and tissue activity and function; and on the translational engineering and manufacturing that will bring new products and therapies into medical practice. An example of this convergence is in the functioning of three-dimensional tissue. It is in the chemical, biological, physical, and electrical communication, over time, within and between cells and tissues, that function arises. An example of exemplary biological engineering in this arena might be creation of a functioning biomimetic analog of a biological, three-dimensional, complex tissue.

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CHAPTER 2

PERSONALIZED MEDICINE MANUFACTURING AND REGULATION

Madhusudan Peshwa

INTRODUCTION

This chapter presents a chemical engineer's perspective on preclinical translation and clinical development of cell therapy and regenerative medicine products in the sites I visited in Europe and Asia. Particularly, I will focus on how the companies the WTEC panel visited have developed tangible solutions within the constraints of a particular situation and identified practical, implementable outcomes. The chapter begins with a brief overview of the history and current status of the field, followed by discussions of the activities at seven sites in Europe and Asia. The chapter concludes with recommendations based on what cell therapy and regenerative medicine mean to the National Science Foundation (NSF) and our national economy.

CELL THERAPY AND REGENERATIVE MEDICINE: WHAT IT IS, WHY IT IS NEEDED, AND WHERE IT IS HEADED

Cell therapy and regenerative medicine can be traced back almost 200 years to the work of Dr. James Blundell, an obstetrician in the UK who performed the first human blood transfusion to treat women suffering from hemorrhage after childbirth (Rowlinson n.d.). This was an engineering solution for providing a biological response that has since been industrialized, commercialized, and scaled to the point where 14 million transfusions of blood products are performed annually around the world. More recently, in 1957, Donald Thomas first demonstrated the principle of the intravenous infusion of bone marrow (Thomas et al. 1957)—an early example of regenerative medicine that has since led, in December 2012, to the one-millionth stem cell transplantation being performed (Worldwide Network for Blood and Marrow Transplantation 2013). Today, this therapy is used in 130 countries. As with blood transfusions, the scaling and commercialization of stem cell therapy has been driven by engineering innovation.

From an engineering perspective, I believe that the United States should focus on developing robust, reliable, and scalable manufacturing practices for the cost-effective delivery of safe and efficacious cell therapies and regenerative medicine products. Chris Mason of University College London refers to regenerative medicine and cell therapy as the "fourth pillar" of managed health care, along with small-molecule drugs, biological drugs, and medical devices (Mason et al. 2011). We are in the early stages of validating this fourth pillar, which has the potential to replace or regenerate biological activity and is focused on curing the underlying causes of disease, not simply on providing a means for controlling the symptoms of disease. The question we face is, how can we develop potentially curative therapies that are one-time interventions and that do not have a chronic taxation impact on the healthcare system globally?

The commercialization of cell therapies and regenerative medicine is still in its earliest stages. Estimates place the total sale of regenerative medicine products in 2014 at around US$2 billion globally. To put that into perspective, during the same time frame the sale of antibody therapies, which have a 20-year head start on regenerative medicine, accounted for US$55 billion in annual

sales. The pharmaceutical industry has been gradually realizing that it needs to accommodate the manufacturing and delivery of these novel products, although at this stage it still trying to identify and acquire the required core competencies to accomplish this goal. In the meantime, the primary driver of development has been our increasing understanding of biological processes. As an example of the growth in this area, the number of papers published about stem cells has been steadily increasing as more types of pluripotent stem cells are being discovered and the ability to use stem cells and somatic cells for disease modeling, drug screening, toxicity testing, and cell therapy increases.

Cell therapy has also had an impact on our understanding of immunological diseases. We have learned how to build traditional biologics and vaccines, and we understand better how the immune system takes up antigens and generates immune responses, but we have found that there is a gap between what we can do with a single immunogen and our ability to, for example, provide an immunogen for an entire cancer cell. To that end, researchers have been looking at how to use the uptake of sentinel cells to introduce antigens into the immune system and modulate them *ex vivo* to give them a biological function that they either don't have *in vivo* or are unable to perform *in vivo*. They are also investigating the development of therapeutics as active cellular vaccines and ways to use them to activate T-cells outside of the body and then reintroduce the T-cells as means of fighting cancer or immune diseases. The first metabolically active cell therapy product, Provenge® (sipuleucel-T) by Dendreon Corporation, was approved by the U.S. Food and Drug Administration (FDA) in 2010. Ultimately, the field is headed toward a transformation in which we are able to use cells rather than pills to provide treatment generically and uniformly across the board.

Taking advantage of the discovery that antibodies have both an antigen recognition arm and an immune system signaling arm, researchers have developed what is called the chimeric antigen receptor (CAR) T-cell that hooks both arms together to create an artificial molecule that, when inserted into T-cells or K-cells, combines the power of antibody technology with the natural mechanisms of stimulated immune responses to host pathogens. In 2013, *Science* named cancer immunotherapy using CAR T-cells as the top scientific breakthrough of the year (Couzin-Frankel 2013). What must happen next is for us to determine how to translate this breakthrough into a meaningful impact on healthcare and the economy.

Of the more than 700 companies worldwide working on cell therapies and regenerative medicine, over half—386 firms, or 56 percent—are located in the United States, due primarily to the existing economic infrastructure here. However, other countries are quickly catching up to the United States; 54 industry-sponsored cell therapy trials (sponsored by 43 companies) are in Phase III, with over 200 trials (sponsored by 114 companies) in Phase II, and the remaining 63 trials (sponsored by 49 companies) in Phase I (Alliance for Regenerative Medicine 2013).

THE ROLE OF ENGINEERS IN DEVELOPMENT, MANUFACTURE, AND DELIVERY OF CELL THERAPY AND REGENERATIVE MEDICINE IN REGULATED ENVIRONMENTS

The successful engineering of cell-therapy and regenerative-medicine products and solutions requires researchers to address a host of manufacturing, commercialization, and regulatory issues. The manufacturing issues include:

- Lack of complete understanding of the biological manifestations of cellular systems
- Manufacturing process controls and robustness
- Product characterization and stability
- Delivery (implementation and administration) of therapy

The biggest problem facing this field today is moving from cell source to final product. Multiple technological solutions exist for cell isolation, as well as for the fill and finish activities. However, many aspects of biological function—cell expansion and differentiation—remain unknown. The

development of "enhanced" potency cellular therapies requires determining how to develop products that, early in development, can demonstrate the level of biological activity that will lead to objective clinical benefit. Characterization is the key; the process or product can only be as good as the yardstick that is used to measure it. The challenge for engineers, then, is to determine how to make products with the desired biological characteristics consistently, at a scale that is relevant for cost-effective clinical and commercial delivery.

To accomplish this, engineers must be able to control the quality of the source, whether it is autologous or allogeneic. Cell collection, characterization and stability, and testing must all be controlled. Robust engineering solutions must be found to reduce the impact of variability of source material and to process the components of the product or process, including being able to identify whether a specific source is better suited and whether preconditioning is required. Then, manipulation requirements must be addressed. For example, is cell isolation/purification needed? Is cell differentiation needed? And what part of the manufacturing process imparts potency? Because a cell receives signals from many different sources—from the extracellular matrix, from soluble factors, and from cell–cell interactions—a means may be found by which one could directly intervene in the molecular pathways inside the cell to control molecule flux. From an engineering perspective, it is important not to think about the cell in isolation, but rather to consider where the cell will function, the signals it will receive, and the ways in which it will respond to these signals.

All of these factors need to be considered in the design of a cell therapy product or process. As engineers, our focus should be on how to control the biology not just in the manufacturing process, but also in the intended use in a patient. We should be considering the functional relevance of the traditional minimalistic approach to characterization focusing on variability, quality and release requirements, and the formulation and shelf life of the final product. The focus should be on approaches to controlling biology that lend themselves to robust, specific, and high-fidelity controls, in order to obtain scalable, cost-effective, current good manufacturing practice (cGMP).

Nor does the process end with the successful manufacture and distribution of the product. Delivery is an integral part of the manufacturing process. We need to think of manufacturing as starting in the clinic, not in the manufacturing facility. Our conventional thinking about what constitutes manufacturing needs to expand to include the patient. Dosage, treatment schedule, and administration modality all need to be considered as part of the manufacturing process, as does the clinical scale-up that comes with implementation. In order to ensure that these drugs respond to the *in vivo* environment, we must ensure robustness, reproducibility, and biological activity.

Regulatory Issues

From a regulatory perspective, the 1938 Food, Drug, and Cosmetic Act and associated current FDA provisions of Title 21 of the Federal Code of Regulations (FDA 2014) classify cell therapy and regenerative medicine products into three broad categories:

- Human cells, tissues, and cellular and tissue products (also called 361 products or HCT/Ps), which are not more than minimally manipulated and are intended for homologous use
- Biological products (also called 351 products), which encompass most current cell and gene therapy products, which are more than minimally manipulated and not intended for homologous use, and which also include Investigational New Drugs (INDs) required for clinical trials and Biologics License Applications (BLAs) for which approval is required for marketing
- Devices, which provide two approaches to regulatory oversight for marketing: the 510(k) clearance, which provides substantial equivalence to predicate marketed devices, and for medical devices, the Investigational Device Exemption (IDE) required for clinical trials and Premarket Approval (PMA) required for marketing

The goals of this regulatory approach are to ensure that products are safe and effective and that manufacturing processes are adequately controlled. From an engineering perspective, these broad goals appear straightforward, but there are many different ways and means of accomplishing them.

To quote former Secretary of Defense Donald Rumsfeld (2002), “There are known unknowns. That is to say there are things that we now know we don't know. But there are also unknown unknowns. There are things we don't know we don't know.” It is the unknown unknowns that engineers must identify and address in order to achieve successful scale-up and manufacture of cell therapy and regenerative medicine products.

CELL THERAPY AND REGENERATIVE MEDICINE EFFORTS IN EUROPE AND ASIA

The Japanese government has recognized regenerative medicine as a priority for medical research and product development to provide healthcare to an aging population and to stimulate economic growth. The government has passed legislation to provide an expedited approval pathway to market specifically for regenerative medicine products, and it is successfully recruiting U.S. companies to do business there.

In 2012, China announced plans to invest US$5 billion into stem cell research, application-oriented research, translational medicine, and regenerative medical treatments. An announcement of a new regulatory framework for cell therapy is anticipated in 2015.

In South Korea, the Ministry of Science and Technology has designated stem cell research and regenerative medicine as a 21st Century Frontier R&D Program. A total allocation of US$90 million has been set aside for promoting stem cell research and for reforming that country’s regulatory framework to facilitate the acceleration of clinical studies and commercial licensing.

The Technology Strategy Board of the United Kingdom has established regenerative medicine as one of the emerging industries that will revolutionize healthcare. The government has allocated £50 million to establish basic and translational research, and £55 million to establish a cGMP manufacturing facility under the aegis of the Cell Therapy Catapult, a centre of innovation excellence for the UK cell therapy industry. In parallel, the Government of Scotland, through Scottish Enterprise, is providing significant financial support to establish an ecosystem to support regenerative medicine from discovery research through to market.

Observations of several specific companies in Europe and Asia follow.

Cell Therapy Catapult Ltd., UK

The vision of Cell Therapy Catapult (CTC) is for the UK to become a global leader in the development, delivery, and commercialization of cell therapy, where businesses can start, grow, and confidently develop cell therapies, delivering them to patients rapidly, efficiently, and effectively. CTC defines cell therapies as any treatment for a medical condition that employs at its core one or more types of viable human cells. This encompasses both use of the patient’s own cells (autologous) and donor derived (allogeneic) cells, and associated interventions required thereof in related areas; for instance, manipulated cells used in gene therapy, devices used to process human cells for therapy, and tissue/biomedical-engineered replacement organs are all within the scope of the CTC’s definition of cell therapy. The mission of CTC is to grow the industry in the UK to substantial and sustainable levels by:

- Taking products into clinical trial, de-risking them for further investment
- Providing clinical expertise and access to National Health Services (NHS) clinical partners
- Providing technical expertise and infrastructure to ensure products can be made in compliance with cGMP standards and delivered cost-effectively
- Providing regulatory expertise to ensure that products get to the clinic safely in the shortest time
- Providing opportunities for collaboration, nationally and internationally
- Providing access to business expertise, grants, and investment finance so that commercially viable products are progressed and investable propositions generated

CTC is governed by a Commercial Board and a Management Team consisting of key opinion leaders with 300+ years of collective experience across pharmacology, biotechnology, small- and medium-sized enterprises, the National Health Service, contract research organizations (CROs), academia, healthcare, not-for-profits, charities, and consultancy.

CTC has created an operating plan based on its stated goal to facilitate development and de-risking of advanced therapeutic (cell therapy) products from preclinical through end-of-Phase II trials to ensure successful paths to commercialization. CTC's approach to executing its operating plan is guided by the creation an integrated product development plan for each candidate product encompassing several considerations—basic science, clinical and regulatory, product development and manufacturing, and business and reimbursement—based on a target therapeutic product profile (TPP) for the future commercial product. To provide for such an integrated approach to product development, CTC has organized itself (Figure 2.1) into four business units, each of which is focused on these four functional challenges to product development.

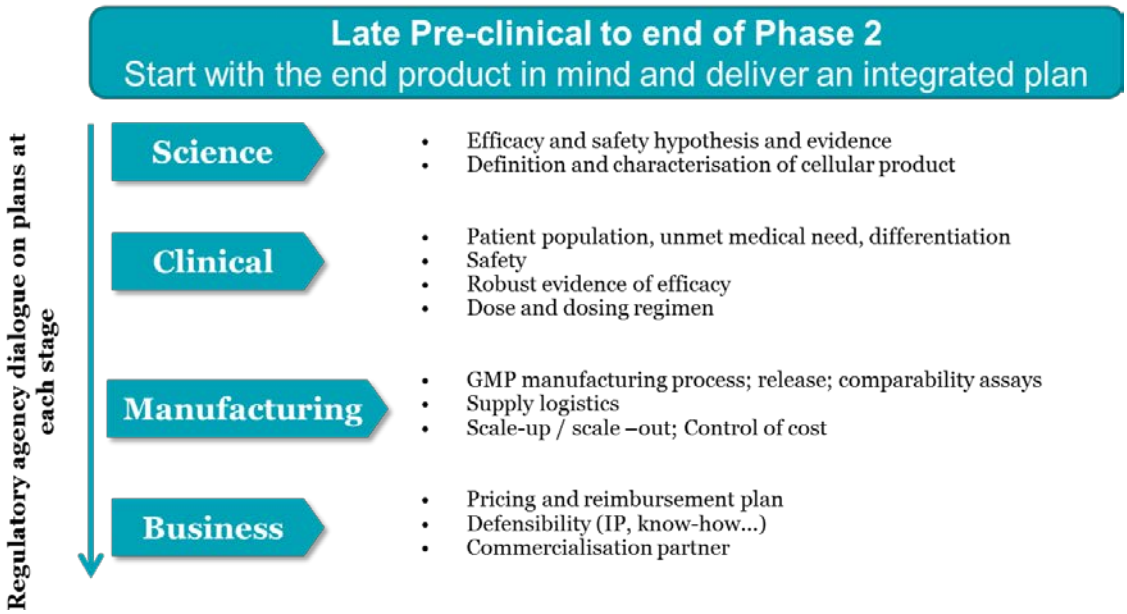

Figure 2.1. Organization of CTC along lines of four functional business units to drive integrated product development for advanced cell therapy products (courtesy of Cell Therapy Catapult).

CTC is flexible in considering/discussing other approaches to working together. As part of its continuing efforts at facilitating development of cell therapy manufacturing innovation in the UK, in a recent announcement, CTC and University of Leeds agreed to work together on acellular scaffolds for the delivery of cellular therapies. The University's Regener8 (the N8 Centre for Translational Regenerative Medicine) and Medical Technologies Innovation and Knowledge Centre have extensive expertise in regenerative devices, scaffolds, and biomaterials, which are pivotal to the development of new cell therapies. The two centers and CTC will collaborate to identify projects, including those from the university's research base and project teams supported by Regener8 funding, which they can develop further together.

The WTEC panel benefitted from having a first-hand opportunity to visit with the Cell Therapy Catapult to observe "what it takes" to build an ecosystem and infrastructure to promote development of innovative manufacturing approaches to facilitate de-risking and drive commercial development of novel biological therapeutics. The level of commitment from the UK Government and the UK Technology Strategy Board in having developed a strategy and funding the creation/operation of CTC is exemplary. Furthermore, the execution ability of CTC to identify key gaps/challenges and align its internal organizational structure to address these challenges, thus permitting an integrated commercial TPP-driven developmental program, is noteworthy. Specific

to driving innovation in manufacturing, CTC's identification of unique/customized nature of cell therapy products, and taking a modular platform-driven approach to product/process development—not just in terms of technology platforms employed for rapid, automated, closed-system bioprocessing and associated analytical capabilities for control of process and product quality, but extending into design of laboratory facilities and operations to mimic current standards in cGMP manufacturing to ensure ease of modular technology transfer—is commendable.

I suggest that the NSF consider opportunities to promote similar strategies for driving innovation in cell therapy manufacturing, and to seek potential collaborative opportunities to work with the Cell Therapy Catapult in fostering international collaborations for precompetitive development of manufacturing and analytical platforms that will benefit the entire industry.

EUFETS GmbH, Germany

Based on extensive expertise in molecular biology, virology, and cell biology as well as an understanding of the regulatory prerequisites, the major goal of EUFETS is the support of clients developing innovative gene and cell therapies through development, testing, and manufacturing services. EUFETS offers its academic, biotechnology, and pharmaceutical customers a complete service spectrum from gene through clinical trial medication to in-market supply of novel advanced therapy medical products (ATMP), providing every step from gene expression to cGMP manufacturing and integrated project management support.

EUFETS has been certified and licensed as a cGMP manufacturer of cell therapy products since 1999. It employs more than 50 experienced scientists, technicians, and project managers (a total of 67 FTEs) and occupies 600 m^2 of R&D, quality control (QC) and certified Good Laboratory Practice (GLP) laboratories and a 500 m^2 cGMP facility comprised of six clean room suites of class A/B (class 100) and four suites of class C (class 10,000) for multipurpose, multiproduct concurrent manufacturing. All suites are equipped with state-of-the-art apparatus and are classified as biosafety level (BSL) 2 or 3**, which also allows the handling of infectious material such as HIV-positive cells. To date, EUFETS has produced more than 1,000 cGMP product lots and has been involved in numerous studies employing cell therapy and *ex vivo* gene-modified cell therapy in Europe.

EUFETS provides its customers with fully integrated product/process and analytical development and program management services extending from preclinical through translation into cGMP clinical manufacturing and marketed products. It also provides process development and cGMP manufacturing services for synthesis of messenger RNA therapeutics and vaccines to its parent company (BioNTech AG). EUFETS currently processes/manufactures multiple complex ATMP products with appropriate segregation, chain of identity, and chain of custody, allowing for concurrent manufacture of multiple product types and for multiple customers within its facility.

The WTEC panel met with Dr. Klaus Kühlcke (CEO) and Dr. Reinout Hesselink (Business Development Manager). Drs. Kühlcke and Hesselink provided an overview of EUFETS activities and led the WTEC panel on a tour of the development labs and cGMP facility.

EUFETS services encompass the entire spectrum from preclinical through completion of late-stage clinical trials and translation to market, and encompass multiple activities at various stages of product development:

- Process development
  - Selection of raw material
  - Transduction optimization
  - Optimization of culture conditions
  - Assay development & qualification
  - Process adaptation & validation
  - Preparation of authority communication

- Preclinical/Qualification for IND (Investigational New Drug, with the U.S. FDA) and CTA (Clinical Trial Authorization in Europe) filings
  - Production of preclinical material
  - Development of functional *in vitro* assays
  - Preclinical *in vitro* testing (according to GLP standards)
- Early clinical development (phase I/II)
  - Application for manufacturing license
  - Cell banking and characterization
  - Production of clinical grade product
  - QC analytics of product
  - Molecular patient monitoring
- Late clinical development (phase II/III)
  - Production of clinical grade product
  - Cell banking and characterization
  - QC analytics of product
  - Molecular patient monitoring

The development laboratory is well equipped with 10 modularly designed cell culture workspaces that comprise biological safety cabinet, CO_2 incubator, centrifuge, and microscope. In addition, the laboratories are equipped with three WAVE bioreactor platforms, two large-capacity roller bottle incubators, an automated single-use disposable-based cell processing system, four flow cytometers, a BioProfiler, and an Äkta Explorer 100 chromatography skid.

EUFETS is continuing to invest in acquiring equipment/technologies that permit automated, closed-system, single-use disposable-based platforms that can be applied across multiple different types of cell therapy products. Such modularity, flexibility, and adaptability of platform technologies that meet the manufacturing requirements for cell therapy ATMP products, independent of the autologous or allogeneic nature of product and independent of whether the product is an immune cell, stem cell, or somatic cell therapy, will permit developers to build core competencies in use and control of unit operations that are employed across multiple ATMP product types.

Analytical capabilities encompass multiple testing services, including:

- Basic analyticals (turbidity, visible particles, sub-visibles, pH)
- Biosafety (toxicity, clonogenicity)
- Bio-analytics (capillary electrophoresis, qPCR)
- Cell biology (mechanism of action studies, development of potency assays)
- Immunochemistry (FACS, ELISA, cytokine release)
- Molecular biology (gel analysis, RT-PCR)
- Retrovirology (replication competent retrovirus and/or replication competent lentivirus [RCR/RCL], infectious particles)
- Microbiology (sterility, mycoplasma, endotoxin)

As for other developers of innovative biotherapy products in Europe, one of EUFETS's largest challenges is lack of adequately trained entry-level personnel who understand the requirements of and have a working knowledge of cell therapy manufacturing and its GMP regulations. Most entry-level workers lack the practical knowledge/experience required for development and manufacture of cell therapy products. Thus, EUFETS has had to develop its own internal program to train and qualify development, manufacturing, and quality control personnel. Once trained, these individuals are in high demand, and retention of its skilled workforce is another challenge. This gap of skilled personnel is primarily at the entry level for operators and not at M.S./Ph.D. levels—given the

relatively large proportion of Associate/Bachelor-level personnel required for every M.S./Ph.D.-level staff member.

The WTEC panel's hosts at EUFETS shared their perspective that early indication of definitive biological/clinical efficacy with some of these novel advanced gene-modified cell therapy products is the primary driver of current interest and investment from traditional large pharmaceutical companies in biomanufacture of ATMP products. Long-term and sustained involvement of large pharmaceutical companies is a must for continued innovation of biomanufacturing for ATMP products. However, such sustainability will depend on two critical outcomes in the near-term (within the next 5 years) timeframe, namely: (1) that efficacy outcomes remain robust on treatment of larger numbers of patients, and (2) whether large pharmaceutical companies can make the "business case" work for delivery of novel ATMP products under socialized and/or managed healthcare systems where, in addition to efficacy, the cost-effectiveness of comparative therapies must continue to be unequivocally demonstrated.

It was of great value that the WTEC panel was able to see firsthand at EUFETS how to build technologies, analytical capabilities, and operational infrastructure to drive development of commercializable, innovative manufacturing approaches for novel biological therapeutics. The ability to manufacture multiple complex products concurrently in a small facility with limited staff is truly exemplary. Furthermore, the execution ability of EUFETS to identify key gaps/challenges with product characterization and analytical methodologies and to integrate these into process/product characterization for development of automated, closed system, single-use disposable-based manufacturing processes is commendable. EUFETS's insights into requirements for training a skilled entry-level workforce by faculty who have practical skills/experiences of developing and manufacturing cell therapy products is a clear recommendation to consider in design of appropriate Associate/Bachelor degree programs for NSF-funded training grants. NSF should consider opportunities to collaborate with EUFETS (and similar organizations) to establish an in-plant training program as part of the curriculum for biomedical engineering students to foster training and development of a future skilled workforce for the cell therapy industry.

PharmaCell B.V., The Netherlands

PharmaCell has the prestige and responsibility of being the commercial manufacturing partner for two of the three European Medicines Agency (EMA)-approved cell-based products commercially marketed in Europe. PharmaCell operates two cGMP manufacturing facilities, located in Maastricht and Chemelot, respectively. The specific characteristics for each of these two facilities are detailed in Figure 2.2.

The Maastricht facility is 1,400 m^2 and is built and operated in a manner that offers flexibility in handling multiple products from different clients. It has three class-B rooms with laminar air flow cabinets that are class A-compliant to the European requirements for open handling during manufacturing. The manufacturing area also has one class C non-cellular room, two class D cell therapy closed processing rooms, one class D equipment preparation room, and one class D incubator room. A separate wing of the facility is specifically designed for handling virus-positive material, where the class B room has negative pressure and has a separate flow of materials and personnel compared to the rest of the facility. The facility has equipment monitoring with automated alarm reporting, HVAC system, and uninterrupted power supply connection for all critical material in the clean rooms and labs. PharmaCell also has cryogenic storage capacity, warehouses, QC labs, and process development labs that are fully controlled under a cGMP-compliant quality system. The QC labs are separated for cell-related testing and microbiological testing. The QC labs and process development labs contain equipment such as LAFC, incubators, FACS CANTO 6 colors, fluorescence luminescence, UV-visible light spectrometry, and endotoxin devices.

Figure 2.2. Characteristics of Maastricht and Chemelot cGMP manufacturing facilities (courtesy of PharmaCell).

The ability to handle multiple products within the Maastricht facility is guaranteed by change-over procedures designed to offer the flexibility to use the class B rooms for multiple products per day. Incubation of cells is done in a D room (with project dedicated incubators) to keep the B rooms assigned for manufacturing purposes. Additionally, the facility is operated around the clock to allow flexibility of use.

The Chemelot facility is 2,400 m^2 with 750 m^2 of clean room space consisting of two independent class B suites, 325 m^2 of QC space, warehouse, lockers, and offices. There is 500 m^2 of expansion space in an adjoining shell area within the facility. This facility is operated as a single-product facility (PharmaCell 2014).

The WTEC panel's visit to PharmaCell's Chemelot facility, which is currently used for commercial manufacture of TiGenix's EMA-approved autologous cartilage product, provided understanding of what is "state of the art" today in commercial manufacture of ATMP products in Europe. The visit also enabled panel members to appreciate the challenges of control of raw materials, chain of

identity, and chain of custody for manufacture of autologous patient-specific cell therapy products. The requirement of flexibility in manufacturing operations and having round-the-clock readiness to process patient samples, coupled with the challenges of logistics and need to ensure minimal batch failures requires multiple systems of checks and balances. The development of novel and sensitive analytical methodologies will certainly provide higher degrees of assurances in pushing quality further upstream into the manufacturing process for ATMPs.

MEDINET Co., Ltd., Japan

MEDINET has continued to grow its Immuno-Cell Therapy (ICT) Total Support Service business and has cumulatively manufactured more than 137,000 ICT product infusions for treatment of more than 17,000 cancer patients in Japan through 8 contracted medical institutions comprising four Seta Clinic Centers, four hospitals (Tokyo, Kanazawa, Osaka, and Fukuoka), and 61 allied medical institutions (that receive contracted cell processing services through one of the 8 contracted medical institutions). MEDINET generates revenues through receipt of royalties for manufacturing services provided to the contracted and allied medical institutions. MEDINETIt has 158 employees (as of September 30, 2013) with a paid-in capital of JPY6.4 billion (approximately US$64 million) (as of December 31, 2013).

Medical doctors at the Seta Clinic have developed a decision tree matrix (Figure 2.3) for selection of a specified ICT product for each patient based on specific patient tumor characteristics.

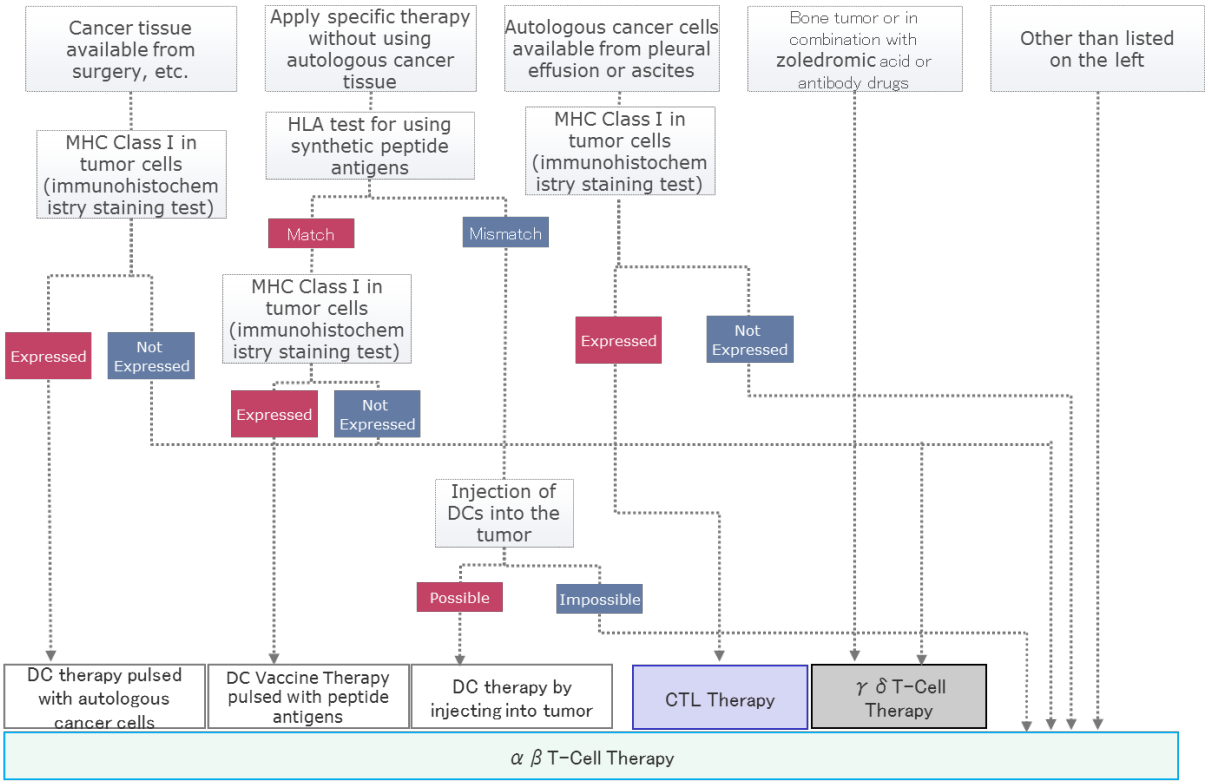

Figure 2.3. Decision tree matrix for choice of immune cell therapy for a given patient (courtesy of MEDINET).

For example, in instances of higher levels of HLA expression on patient tumors, an antigen-specific therapy may be better suited compared to lower levels of HLA expression, which may favor a passive immunotherapy approach (Figure 2.4). MEDINET's product portfolio thus provides for a continuum of treatment options for cancer patients through all stages of disease evolution and progression—providing for comprehensive, patient-focused delivery of immunotherapies.

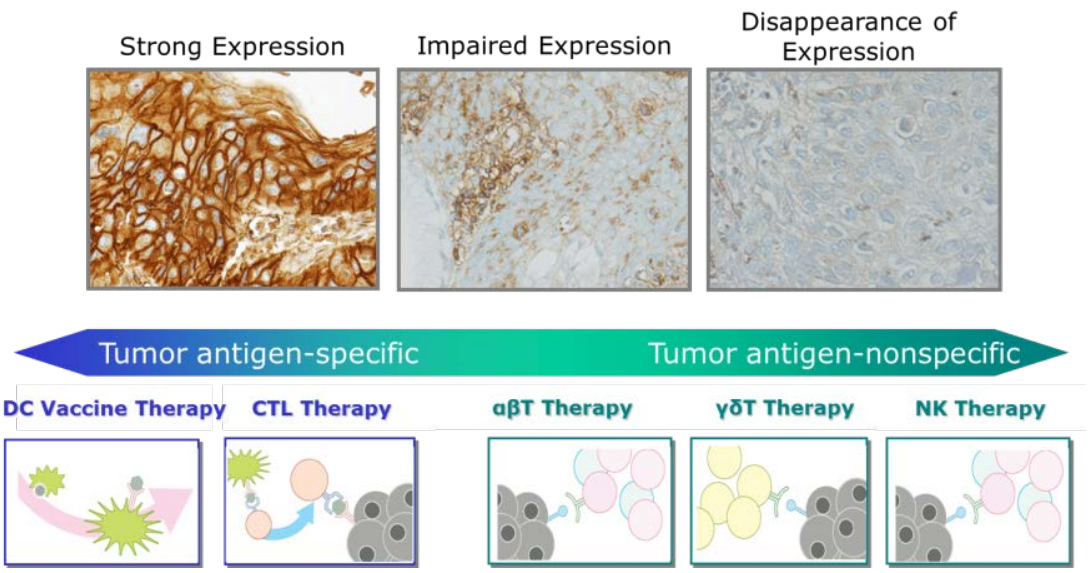

Figure 2.4. HLA expression on patient tumor cells as criterion for selection of immune cell therapy (courtesy of MEDINET).

MEDINET Medical Institute is a self-sufficient facility with laboratory spaces dedicated for standard molecular biology techniques, controlled access cell culture suites, analytical laboratories for flow cytometry and immune functional assays, and its own rodent facility for conduct of *in vivo* preclinical studies. MEDINET has built its cGMP cell processing centers (CPCs) close to Seta Clinic Group's Medical Centers or its other contracted medical institute locations. For example, at the Shin-Yokohama facility, the CPC is located less than half-a-minute walk from the Seta Clinic patient reception area. The proximity of the manufacturing site to the clinical site permits ease of logistics for high-throughput commercial manufacturing of autologous products and enables decentralized, on-site manufacturing for patient treatment.

In summary, the MEDINET approach to design of novel ICT products and manufacturing processes thereof appears to be strongly driven by:

- Understanding of the biological mechanism of action of the ICT product and specifically augmenting product potency for enhancement of immunological efficacy (Noguchi et al. 2014, Hosoi et al. 2014)
- Using small-molecule drugs to modulate biological activity and function of cells, permitting development of cost-effective manufacturing processes (Kondo et al. 2008, Sato et al. 2009)
- Developing a portfolio of products that are a unique fit with the stage of progression of disease to permit comprehensive clinical options for management of oncology patients

Such integration of basic science and immunology, coupled with development of cost-effective processes for manufacture of patient-specific (autologous) immune cell therapy products in the context of understanding of clinical disease progression to provide patients with a suite of product options, is a unique approach to development and delivery of cancer care.

Under its current business operations structure, MEDINET has continued to grow its Immuno-Cell Therapy Total Support Service business. Figure 2.5 depicts the cumulative growth in number of products administered by MEDINET.

ICT products delivered under Japan's Medical Practitioners' Act are not reimbursed by national insurance. Patients pay for these treatments out of pocket. One course of treatment consists of six cycles of ICT product administration and is priced at approximately JPY2 million (approximately US$20,000) in payments made to the clinic. The clinic pays a portion of that fee to MEDINET as reimbursement for manufacturing services.

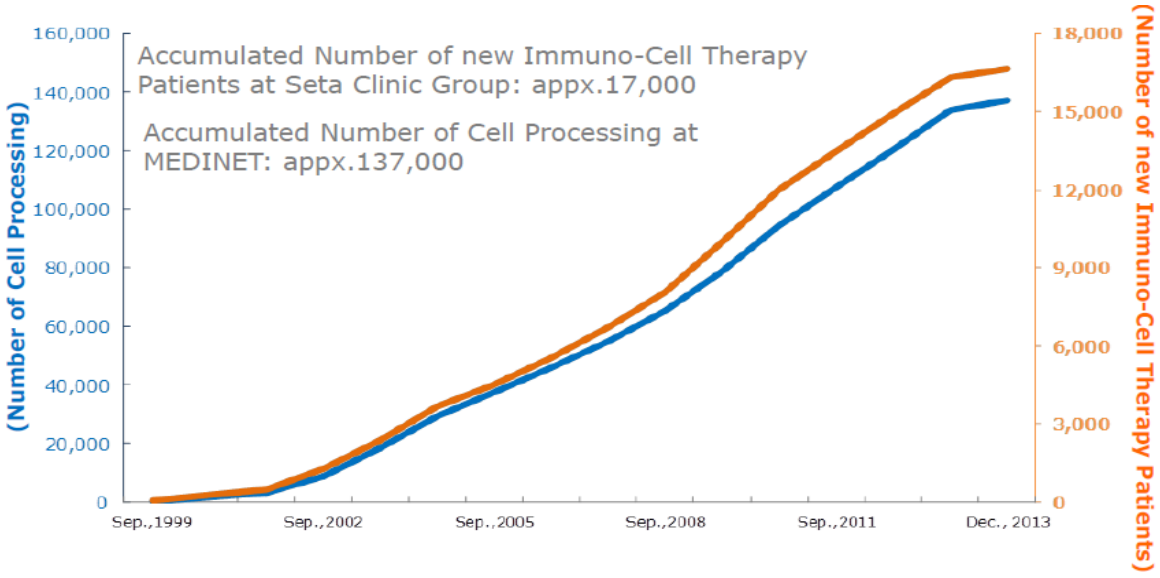

Figure 2.5. Accumulated number of cell processes manufactured by MEDINET under the Medical Practitioners Act, as of December 31, 2013 (courtesy of MEDINET).

Regulatory Framework

A new regulatory framework for regenerative medicine products in Japan has been enacted by the Japanese parliament as the Regenerative Medicine Promotion Law. This new law was scheduled to take effect in November 2014 and contains two major provisions (as depicted in Figure 2.6):

- The Act on the Safety of Regenerative Medicine (Safety Act)
- The Act on Pharmaceutical and Medical Devices (PMD Act)

The Safety Act includes Risk Classification; Rules for Hospitals and Clinics; and Rules for Contract Cell Processors in the manufacture and delivery of regenerative medicine products (including ICT Products).

The PMD Act, in effect, creates an adaptive licensing approval process for regenerative medicine/cell therapy products in Japan (Figure 2.7). The PMD Act is aimed at accelerating the development of regenerative medicine products by allowing for conditional marketing approval following demonstration of safety in human clinical trials with adequate numbers of patients, with a 7-year period to submit comprehensive safety and efficacy data on the product for consideration of full marketing approval. During the conditional approval period there is no reimbursement by the government's national insurance plan; patients will have to pay out-of-pocket or get coverage through private insurance providers.

MEDINET Business Model

Given the significant changes in the regulatory environment in Japan, MEDINET continues to rapidly evolve its business model and is embarking on multiple other business initiatives to build on its leadership position in cell therapy product development and manufacturing experience. To accomplish this, it is creating a new Contract Manufacturing Organization (CMO) Business Unit and a new Cell Medicinal Products (CMP) Business Unit.

CMO Business. In Dec 2013, MEDINET decided to build new, stand-alone GMP Facility in Tokyo with easy access to Haneda Airport for the express purpose of providing regenerative medicine and cell therapy CMO services to academic investigators, research institutes, medical institutes, and commercial entities. This new facility has a total floor area of 2,990 m^2 (about seven times the size of its Shin-Yokohama CPC), is estimated to cost (CAPEX) JPY1.5 billion (approximately US$15 million), and was to be validated and operational by the end of 2014.

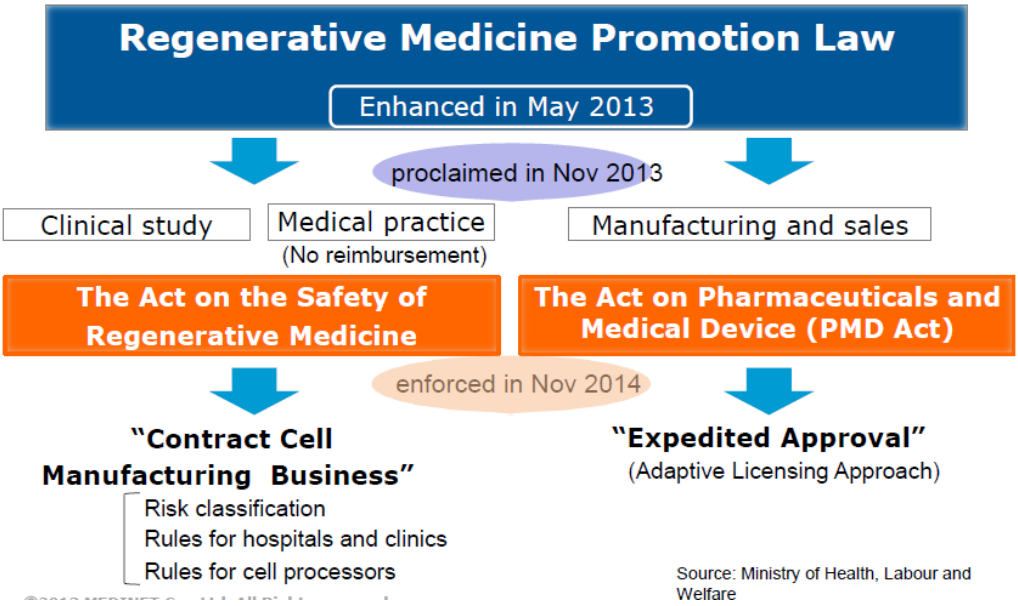

Figure 2.6. New Regenerative Medicine Promotion Law enacted in Japan Japan (from Japan's Ministry of Health, Labour and Welfare, courtesy of MEDINET).

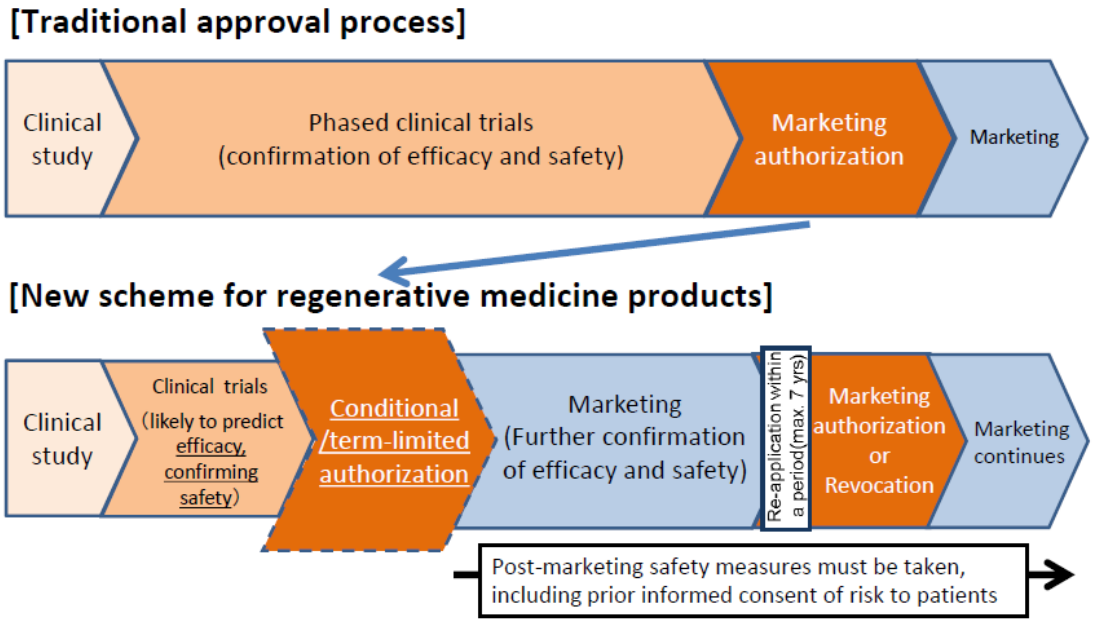

Figure 2.7. Expedited Approval System under PMD Act for regenerative medicine products in Japan (from Japan's Ministry of Health, Labour and Welfare, courtesy of MEDINET).

CMP Business. MEDINET has refocused its priorities to obtain regulatory approval under the new PMD Act for selected ICT products developed by the MEDINET Medical Institute. MEDINET is compiling patient safety data from ICT products with the objective of seeking conditional approval as a Cell Medicinal Product under the new PMD Act, specifically for:

- αβ T-cells from clinical studies in pancreatic cancer at Nagoya University and Seta Clinic
- Dendritic cell vaccine electroloaded with tumor lysate for clinical studies at the University of Tokyo in renal cell carcinoma and in esophageal cancer
- γδ T-cells from clinical studies at Tokyo University in multiple separate cancer indications

To support these business endeavors, MEDINET has invested JPY100 million (approximately US$1 million) in creating a wholly owned subsidiary (MEDcell Co., Ltd.) that is co-located at the MEDINET Medical Institute site for the purpose of development and approval of ICT products as cell medicinal products.

In addition, MEDINET has partnered with PURPOSE Co., Ltd., and Mutoh Co., Ltd., to create a joint venture (PURPOSE Biomedical Co., Ltd.) with the objective of in-licensing, development, regulatory approval, and marketing of an autologous engineered neo-cartilage product (NeoCart) which is currently in phase III clinical trials in the United States and is being developed by Histogenics (Waltham, Massachusetts). PURPOSE Co. Ltd. has licensed rights from Histogenics for NeoCart for the Japanese market. Mutoh has a commercial sales, marketing, and distribution channel for medical devices in Japan. MEDINET will provide cell processing services for manufacture of NeoCart products in Japan through its CMO business.

More recently, MEDINET also invested £0.63 million (approximately US$1.1 million) for a 50% stake in another joint venture (TC BioPharm, Ltd.) in the United Kingdom for the purpose of clinical development of γδ T-cell therapy in Europe. Clinical data generated in Europe is potentially useful in regulatory filings for conditional approval in Japan under the PMD Act.

The key findings of the WTEC panel based on the visit to MEDINET Co., Ltd., are that the cell-therapy and regenerative medicine field needs to:

- Focus and fund preclinical research efforts on “attribute sciences” to understand how to design/engineer novel therapeutic products based on assessment of their biological activity/function per hypothesized mechanism of action.
- Support translational product development and manufacturing efforts with focus on establishing cost-effective processes for cell expansion and sensitive analytical methodologies for assessments of product characteristics that correlate with biological function and hypothesized mechanisms of action.
- Develop an understanding of approaches to training and competency assessment of scientific, engineering, manufacturing, and quality personnel to enable industrialized operations for routine cost-effective production (10,000+ units annually) of individualized (autologous) product lots.
- Develop appreciation for how process economics and scale considerations determine priority of investment in manufacturing process automation. (Automation may not be a critical path item for translational/clinical or even commercial development.)

MEDIPOST Co., Ltd., S. Korea

In January 2012, MEDIPOST received commercial license from the Korean Ministry of Food and Drug Safety (MFDS), previously known as the Korea Food and Drug Administration (KFDA), for marketing of its CARTISTEM® product in South Korea. CARTISTEM was approved for treatment of repetitive and/or traumatic cartilage degeneration, including osteoarthritis for adult patients without any (minimum or maximum) age limit. The CARTISTEM product was codeveloped in collaboration with Prof. Chul-Won Ha, M.D., Ph.D., Professor of Orthopedic Surgery and Director of Stem Cell and Regenerative Medicine Center, Samsung Medical Center, Sungkyunkwan University School of Medicine in Seoul, South Korea (Yang et al. 2004; Oh et al. 2008; Lee et al. 2014). MEDIPOST America, Inc., is currently conducting a two-center Phase I/IIa clinical trial for CARTISTEM in the United States under an IND application filed with the U.S. FDA. Sourcing of cord blood and cGMP clinical manufacture of products for the U.S. clinical trials is supported through CMOs in the United States.

In addition to the CARTISTEM product, human umbilical cord blood-derived mesenchymal stem cell (hUCB-MSC) drug products are currently in clinical development at MEDIPOST for other indications (Figure 2.8; Jin et al. 2013; Jang 2014). The PNEUMOSTEM® product is currently in phase II trials in Korea for treatment of bronchopulmonary dysplasia in premature infants and has

been provided with "orphan" drug designation[1] in the United States (Chang et al. 2014). The NEUROSTEM® product is starting Phase IIa studies using Ommaya reservoir for intraventricular delivery as treatment of Alzheimer's disease, based on demonstration of safety in a Phase I study following stereotactic delivery to the subventricular zone (Kim et al. 2010 2011, 2012; Jeong et al. 2013).

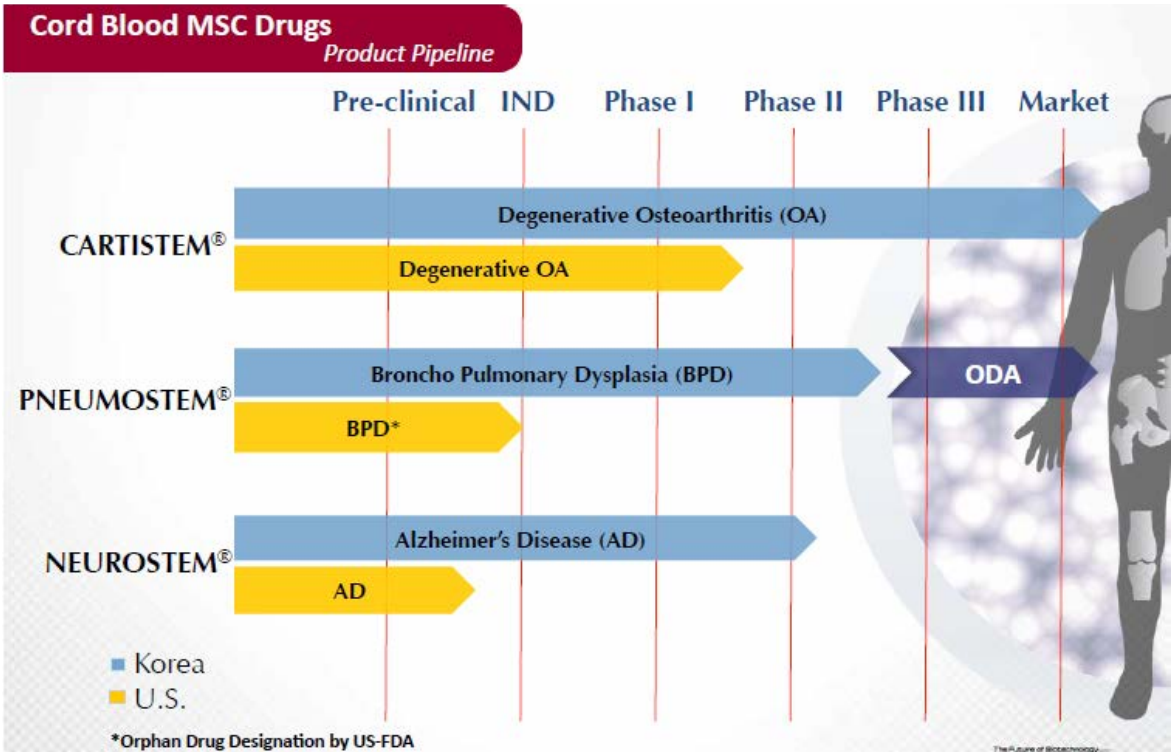

Figure 2.8. MEDIPOST's hUCB-MSC cell drug product pipeline (courtesy of MEDIPOST).

MEDIPOST has worldwide coverage for its core intellectual property in use of allogeneic hUCB-MSC as cell drugs and has been able to obtain approximately US$26 million in nondilutive grant funding in South Korea to advance development of its hUCB-MSC platform (Figure 2.9).

At the company's Guro-gu facility, donated cord blood (CB) samples are received and subjected to incoming inspection. Following release from incoming inspection testing, CB samples are processed to isolate and expand plastic-adherent MSCs to manufacture bulk drug substance, which is harvested and banked (cryopreserved). In-process assessment of bulk drug substance consists of cell surface phenotypic markers and safety testing. At this stage cells must be human leukocyte antigen (HLA) class II negative (no detectable expression of HLA-DR, DQ, or DP by flow cytometry).

Cryopreserved bulk drug substance has a 3-year shelf life. On a periodic basis (currently, weekly), based on receipt of prescription orders from treatment clinics and hospitals, a selected number of bulk drug substance vials are thawed and cultured for an additional 5 days to manufacture and release drug product (7.5×10^6 cells per vial in 1.5 mL aqueous solution) that is shipped at ambient temperature (8–20 °C), with a 48-hour shelf life, for clinical/commercial administration. Final product is tested for viability (cell83 enumeration and Trypan blue exclusion), purity and identity (FACS), potency (multiple assays), and safety (sterility, endotoxin, and mycoplasma).

[1] The U.S. Orphan Drug Act (ODA) "…provides for granting special status to a drug or biological product ('drug') to treat a rare disease or condition…" (see http://fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/default.htm).

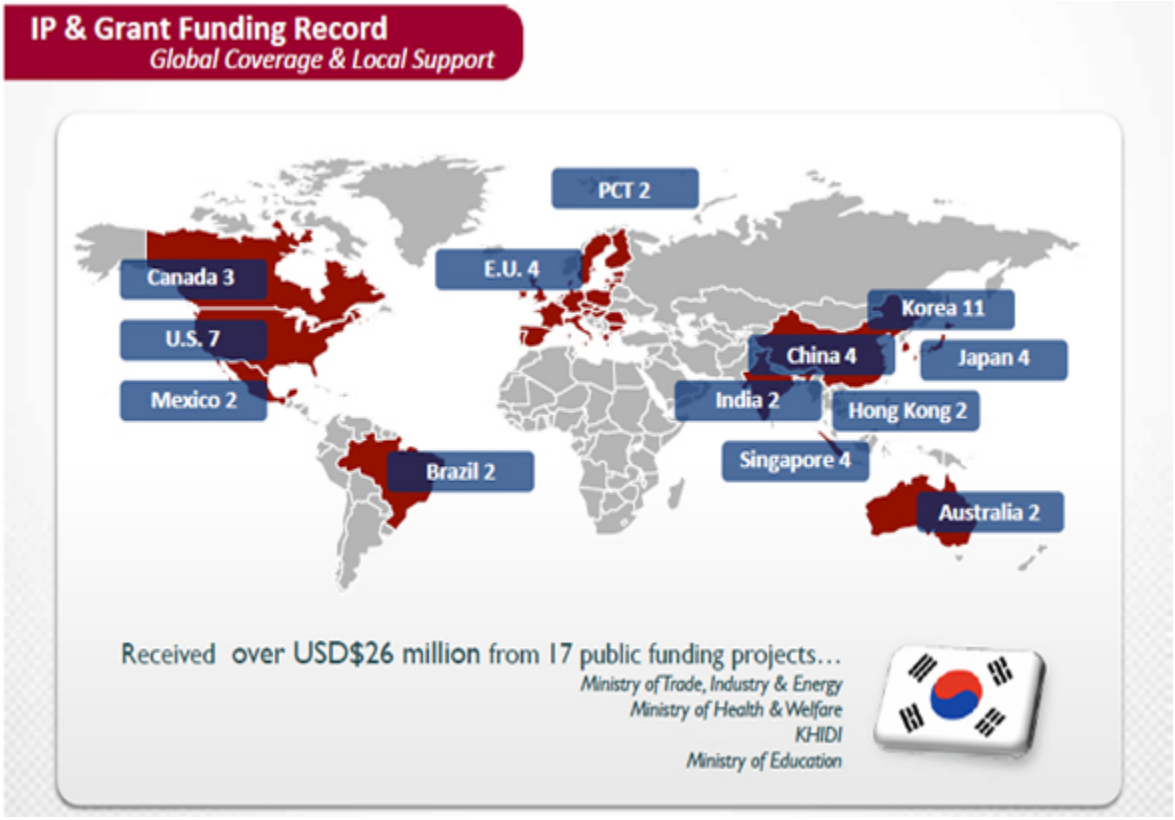

Figure 2.9. MEDIPOST's global intellectual property coverage (courtesy of MEDIPOST).

The hypothesized mechanism of action of hUCB-MSC products is through stimulation of endogenous regeneration via paracrine (trophic-factor-mediated) mechanisms (Jang 2014; Chang et al. 2014; Kim et al. 2010, 2011, 2012; Jeong 2013). Hence, potency testing consists of assessment of differentiation, anti-inflammatory, anti-apoptotic, and mitogenic bioactivities. In preclinical studies, it was determined that there were no safety or toxicology issues associated with transplantation of CARTISTEM product in animals (i.e., <1% of administered cells detectable in any major organ system in the body). Also, the transplanted cells survived only transiently (for approximately 8 to 12 weeks) at site of administration and were replaced by endogenous cartilage that uniformly expressed Safranin-O and Type II collagen (representative of articular hyaline cartilage). These observations confirm a trophic-factor-mediated mode of action that is different than in the case of transplant of *ex vivo* culture-expanded autologous chondrocytes (cartilage cells obtained from biopsy of non-load-bearing areas of the knee)—similar to the product marketed by Aastrom Biosciences (CARTICEL® developed by Genzyme) in the United States or the Sewon Cellontech (Chondron) product in South Korea.

At the clinical site the cells are mixed with lyophilized viscous formulation of 4% hyaluronic acid (excipient) and applied to prepared (shallow drilled) knee defects (one vial of cells is used per 3 cm^2 of defect; multiple [up to three] vials can be used for larger-sized defects) using arthroscopic delivery without any membrane covering. Patients are required to wear a brace overnight and are then equipped with non-weight-bearing support for two weeks.

One dose of CARTISTEM is priced at approximately US$5000 (inclusive of VAT). There are additional hospital costs and surgeon's costs that need to be considered as part of the treatment. On average, total cost of CARTISTEM is US$10,000–12,000 per patient. Treatment is currently not reimbursed by national insurance, and therefore patients pay either out-of-pocket or may have reimbursement through premium private insurance providers. By contrast, microfracture (the current standard of care) has a total cost to patient of approximately US$300–400 per knee, and is

reimbursed by national insurance at US$200 per knee. In South Korea it typically can take 3–5 years post-approval for reimbursement by national insurance. However, if a product is covered by national insurance, it ensures that the pricing is fixed. Therefore, therapeutic developers in South Korea have to consider whether they want to pursue national insurance at a fixed price or continue to market the product without national insurance coverage but have flexibility in independently determining pricing.

In January 2013, MEDIPOST licensed its CARTISTEM product to Cell Therapies Pty., Ltd., (Melbourne, Australia) for commercial development and marketing in Australia and New Zealand (MEDIPOST 2013a). At the time of the WTEC study, Cell Therapies was in the process of submitting a Biologics License Application, based on Korean clinical data, to Australia's Therapeutics Goods Administration for commercial marketing approval in Australia and New Zealand. MEDIPOST plans to commercially manufacture the final drug product at its GMP facilities in Seoul and will ship under controlled temperature to the Australia/New Zealand market.

MEDIPOST has also signed a licensing agreement for clinical development and commercial marketing of CARTISTEM in India, with Alkem Laboratories, Inc., in Mumbai, India (MEDIPOST 2013b). MEDIPOST is currently seeking strategic development partners and licensing partners in multiple geographies. These business development activities are being conducted through MEDIPOST America Inc.

MEDIPOST has been able to execute its transition into a leading cell therapy product company largely supported by and building on its operational infrastructure established for support and growth of its primary, revenue-generating, private cord blood banking business. This transition has also been largely funded by public sector grants supporting systematic scientific investigations and translational collaborations with the founder's lab at Samsung Medical Center and Sungkyunkwan University, around the use of an allogeneic hUCB-MSC platform as the modulator/catalyst of endogenous tissue repair and regeneration.

MEDIPOST has been successful in partnering with a large Korean pharmaceutical company for commercial marketing and distribution of the CARTISTEM product, thus saving costs and leveraging the pharmaceutical partner's sales and marketing infrastructure. It has also been successful in out-licensing CARTISTEM for regulatory approval and commercial marketing in Australia/New Zealand, while leveraging clinical data from Korean clinical trials and market. Specific to North America and Europe, MEDIPOST has decided to invest in human proof-of-concept (POC) studies to permit generation of clinical data in-country with the goal of out-licensing to strategic partners for phase III clinical development and commercial marketing in these territories.

MEDIPOST has, however, struggled with how to accelerate adoption of its commercially approved CARTISTEM product in the Korean marketplace. Although, in the approximately two years since approval, MEDIPOST has treated more than 1,200 patients at more than 160 centers; the revenue ramp at US$5000 per patient indicates that commercial product revenues from sales of CARTISTEM are in the range of US$2–6 million on an annual basis (roughly <10% of total revenues for the private cord blood banking business). There may be multiple factors that impact revenues from sales of CARTISTEM, including completion of the 600-patient post-marketing study commitment; lack of national insurance coverage; lack of publication of human clinical data in scientific and medical literature; possible concerns among prescribing physicians of improvements in objective clinical benefit to patients (versus microfracture—the current standard of care, which is reimbursed and costs approximately <5% of the price of CARTISTEM treatment); lack of long-term safety data on use of allogeneic cell therapy products in non-life-threatening indications; and possible lack of acceptance or level of comfort among physicians recommending treatment for their patients of a novel of cell therapy product. In conversations with the scientific co-founder of MEDIPOST, Prof. Chul-Won Ha, Dr. Ha indicated that as a treating physician he himself would feel more confident in prescribing CARTISTEM to his patients if there was a good 10-year record of safety data in humans with the use of the CARTISTEM product.

At the present time, given that the hUCB-MSC is an allogeneic platform wherein a single cord blood donation can generate sufficient CARTISTEM product for treating hundreds of patients, and given the market trends and MEDIPOST's share of the cord blood banking market in South Korea, it does not appear that the availability of donor cord blood will be rate-limiting for MEDIPOST to scale up commercial production over a multiple-log-order increase in demand. Whether, in addition to the domestic market, medical tourism will lead to any significant increase in commercial demand remains to be seen. The current manufacturing process is largely manual and controlled by standard operating proceedures. There does not appear to be sufficient incentive at the current commercial demand levels (or even if the demand increases by a log-order magnitude) to warrant investments in automating the manufacturing process. When in the future automation-driven reduction in cost of goods sold (COGS) will be needed is undetermined.

The key findings of the WTEC panel from this site visit are as follows:

- Companies like MEDIPOST should consider focusing on and funding preclinical research efforts on "attribute sciences" to understand how to design/engineer novel therapeutic products based on assessment of their biological activity/function per hypothesized mechanism of action.
- There must be support for translational collaborations between researchers, engineers, and clinicians that have a clear definition of a therapeutic product profile for an eventual commercial product.
- Efforts in the United States should focus on continuing to educate all stakeholders on the scientific principles, manufacturing platforms, safety, and clinical utility aspects associated with the programs that NSF supports in the area of cellular medicines.

Japan Tissue Engineering Co., Ltd. (J-TEC), Japan

The Japan Tissue Engineering Co., Ltd. (J-TEC) has continued to invest in core competencies in its personnel and facilities to become the leading tissue-engineered medicinal product (TEMP) developer and manufacturer in Japan. All of J-TEC's products are currently autologous products. J-TEC has built infrastructure to support commercial manufacture of patient-specific products with complete control over logistics and supply chain infrastructure for control over chain of identity and chain of custody for TEMP Products.

The J-TEC facility was completed in October 2004 and consists of a site area of 5,045 m^2 comprising the Corporate Headquarters and GMP Facilities, which have both clean room and containment suites. Currently, nine clean room suites are dedicated to manufacture of its products. Another floor in the building is being built out to accommodate additional GMP suites.

J-TEC is the leading regenerative medicine company, with three TEMPs: (1) J-TEC autologous cultured epidermis (JACE) for treatment of burns, (2) J-TEC autologous cultured cartilage (JACC) for treatment of knee defects, and (3) autologous cultured corneal epithelium for treatment of eye disease. TEMPs are subject to Japan's Pharmaceutical and Medical Devices Act, which requires approval of the Welfare, Health and Labor Ministry in order to sell these products. The process of manufacturing typically entails the following three steps:

1. The doctor (medical facility) takes a sample of healthy tissue from the patient.
2. J-TEC cultures cells from the treated tissue, carries out a preshipment inspection, packages the product, and sends it to the medical institution.
3. A tissue transplant operation is carried out by a doctor (medical facility) for the same patient.

There is significant patient-specific variability in the manufacture of JACE products. J-TEC has established a two-stage manufacturing process to control this variability: (1) a primary culture stage, in which the patient biopsy is cultured to an intermediate expanded cell product and cryopreserved and has most variability, and (2) a post-amplification process in which, once the patient treatment date is finalized, the intermediate expanded cell product is subsequently expanded

to final product dose. The second stage is significantly robust and permits delivery of final product in compliance with the clinical schedule. Given the current two-stage process, variability in clinical/market demands, and the use of mouse feeder cells in the expansion process, automation of the manufacturing process for JACE is challenging and not being pursued at present.

J-TEC has recently completed a 7-year, post-market surveillance study and submitted the data for expansion of indication to deep second- and third-degree burns, which require coverage of >30% of body surface area.

The JACC product was approved in July 2012 as a three-dimensional, tissue-engineered cartilage in collagen gel. This product was developed in collaboration with Prof. Mitsuo Ochi (Department of Orthopedic Surgery, Hiroshima University). Autologous chondrocytes biopsied from a non-load-bearing area are cultured in 3D collagen matrix, implanted in area of knee defect, covered with periosteum flap, and sutured in place. Standard of care in Japan for treatment of such knee defects is mosaicplasty (transplant of osteochondral plug). Clinical efficacy of the JACC product has been assessed by MRI-based studies. Reimbursement for JACC was approved in April 2013 (approximately 9 months after product approval) and pays JPY2 million (~US$21,300) per knee.

The cultured corneal epithelium product, in clinical development, is being developed for the Japanese market in collaboration with Prof. Teruo Okano (Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University and CellSeed, Inc.).

Unlike conventional drug treatment or organ transplants, regenerative medicine draws out the regenerative capabilities of our own bodies' tissues, focusing on the utilization of living cells to recover the lost functions of tissue or organs of the body. The main business goal at J-TEC is to use autologous culturing techniques to develop tissue-engineered medical products and then to manufacture and sell these same tissue-engineered products to medical institutions for the purpose of medical treatment. J-TEC's tissue-engineered medical products are meant to be used in "autologous transplant" cases, where living cells are taken from the actual patient, cultured, and then transplanted back to that same patient.

The new Regenerative Medicine Law in Japan (effective November 2014) has created an expedited regulatory framework for development of cell therapy/tissue-engineered medical products. This permits conditional approval over a seven-year time frame with demonstration of safety and biological activity.

Takara Bio, Inc., Japan

At the time of the WTEC visit, Takara Bio had recently built and was in the process of commissioning a new 6,500 m^2 cGMP facility, which the WTEC panel toured, for vector and cell manufacturing that was projected to become operational in the second half of 2014. This facility will support the following activities:

- Plasmid DNA vector manufacturing
- Viral vector manufacturing. primarily gamma-retrovirus and (some) lentivirus
- RetroNectin® manufacturing
- Cell product and gene-modified cell product manufacturing
- Cell banking and cell storage
- Biosafety assays

Takara Bio's core competencies and cGMP facilities facilitate translation of its proprietary gene-modified cell therapy products. The facility has three floors and is designed as a one way flow-through system for materials and personnel with clean and dirty corridors to ensure separate flow streams for clean and waste products. The first floor comprises five suites for cell banking, six suites for *E. coli* fermentation, cell storage area, and QC labs dedicated to sterility and mycoplasma testing. The second floor comprises viral vector production suites (with exit air being HEPA-

filtered) for manufacture of HVJ (hemagglutinating virus of Japan), adeno-associated virus, adenovirus, herpes simplex virus, lentivirus, and gammaretrovirus; protein and vector purification suites; automated and aseptic filling suites; and a lyophilization suite. The third floor comprises three suites for cell processing that can handle both unmodified and gene-modified cell therapy product manufacturing and QC laboratories for conduct of FACS, qPCR, general biological assays, and testing for cells and viruses. The third floor suites are accessed through a gowning room on the second floor; which also provides access to future expansion suites.

Takara Bio is the leading commercial company in Asia producing gene-modified cell therapy and gene therapy, with multiple programs in clinical development and core competency and facility infrastructure to drive translational development of gene-modified cell therapy products. The WTEC panel's visit to the new cGMP manufacturing facility clarified the effort needed for development and manufacture of all critical materials, viral vectors, and analytical methodologies in development of gene-modified cell therapy products. The WTEC panel also learned of innovative, manufacturing-driven opportunities for successful business execution in building a long-term stable foundation for development novel gene-modified cell therapy products.

CONCLUSIONS AND FEEDBACK FOR NSF

To successfully develop a manufacturing capacity for cell therapy and regenerative medicine products as discussed in this chapter, the field needs several things. First, focused investments must be provided in basic and translational research, wherein programs are multidisciplinary and collaborative, have clear paths to product development, and technologies are available for the robust and controlled means of enabling biological activity. Such programs should be designed to solve unique engineering challenges related to manufacturing or analytical characterization of cell therapy and regenerative medicine products. These investments should be shared among stakeholder agencies to ensure a translational and collaborative focus.

I also suggest establishment of a Center of Excellence (CoE) for Biomedical Engineering for Cell Therapy and Regenerative Medicine under a leadership that provides translational and industrial experience and governance by an external translational advisory board. The CoE would establish standards for manufacturing platforms and analytical methodologies that are relevant for developing cell therapy and regenerative medicine products. It would coordinate and harmonize efforts with international agencies, precompetitive public–private consortia, and regulatory agencies, and operate as a "working accelerator" to facilitate the provision of external investment and strategic guidance on early risk reduction and on the development of therapeutic product profiles for selected programs. It would also provide on-the-job training for faculty and students who are interested in pursuing translational and industrial research.

The field needs to change its mindset regarding the skills that are required to enable the development of an effective workforce for cell therapy and regenerative medicine products. Training programs for biomedical and engineering students need to be revamped to ensure that they are effective at meeting translational and industrial needs, and student internships should be made integral to training and graduation requirements. Faculty should likewise be offered opportunities and incentives to undertake sabbaticals in translational programs outside their normal university research and teaching environments. Lastly, networking and mentorship programs for faculty and students, along the lines of those maintained by business schools, should be encouraged.

Finally, we need to work in partnership with other stakeholders in the Federal Government and with other groups such as professional societies, patient groups, legislators and policymakers, healthcare providers and payers, the investment community, and the general public to educate, communicate, and collaborate in fostering the development of an ecosystem that promotes innovation and translational development in the cell therapy and regenerative medicine field.

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CHAPTER 3

MANUFACTURING OF DELIVERY SYSTEMS FOR REGENERATIVE MEDICINE

Kam Leong

INTRODUCTION

Regenerative medicine and cell-based therapies have the potential to revolutionize healthcare by changing treatment strategies from being reactive to preventative and restorative (Heslop et al. 2015). Although various forms of stem cells (embryonic, progenitor, induced, or transdifferentiated) often play a central role in regenerative medicine, biochemical cues in the form of drugs, proteins, or nucleic acid can provide a supportive or even decisive role in determining the fate of the stem cells and the eventual outcome of the tissue regeneration (Lorden, Levinson, and Leong 2015). These soluble therapeutics alone can also in some cases achieve a regenerative outcome by acting on the resident cells at the tissue site. For example, heparan sulfate is a form of regenerative therapeutic that can be administered to recruit endogenous growth factors at the site of injury to initiate repair due to the specific interactions of heparan sulfate with many growth factors (Rai, Nurcombe, and Cool 2011; Saez et al. 2014).

Many therapeutics relevant to regenerative medicine are delicate growth factors and nucleic acids, often with short half-lives that require intracellular delivery. Effective drug delivery systems are needed to realize their potential. Fortunately, needs for other therapies have already stimulated the development of drug delivery technologies for decades. The sophistication of drug delivery has progressed from macroscopic (1960-80), to microscopic (1980–1990), and recently (1990–present), to nanoscale delivery systems (Zhang, Chan, and Leong 2013).

CONSIDERATIONS FOR DELIVERY OF THERAPEUTICS

Recombinant DNA technology has enabled protein-based molecules, such as growth factors, zinc finger protein transcription factors, zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and monoclonal antibodies, to be developed and used as drugs (Ferrer-Miralles et al. 2009). Protein-based therapeutics promote tissue regeneration because they can mimic, activate, or inhibit endogenous pathways, helping the body to heal. Bioengineers can mimic biochemical cues in nature by developing recombinant proteins and engineering their delivery. However, the use of protein-based drugs for regenerative therapeutics is limited by their propensity for instability *in vitro* and *in vivo*, presenting a challenge for handling, and implying a need for repeated doses over time and the possibility for unwanted side effects. Both micro- and nanoparticles can be applied to deliver these biologics because they can be functional in the extracellular space.

For nucleic acid therapeutics, nanoscale delivery systems would be needed, because they must act at the molecular level intracellularly (Williford et al. 2014). Nucleic acid therapeutics in naked form are highly inefficient because they will not passively cross the plasma membrane of cells due to their size and negative charge. Several forms of nucleic acids have been applied for regenerative medicine. The three most prominent are plasmid complementary DNA (cDNA), small RNA, and

aptamers. Complementary DNA encoding for a therapeutic gene is delivered directly to cells *in vivo* or *in vitro* so that they will express that gene or protein of interest. Small RNAs are used to regulate gene expression *in vivo* by controlling protein transcription and translation at the messenger RNA (mRNA) level; this process is known as RNA interference (RNAi). Aptamers are similar to monoclonal antibodies (mAbs) in that they bind and inhibit a specific biological target, such as an enzyme or receptor, but they are generated by chemical methods. Table 3.1 summarizes the advantages and disadvantages of various forms of therapeutics important for regenerative medicine.

Table 3.1. Summary of soluble factors from small molecule drugs to nucleic acids, including their most common uses in regenerative medicine, and general advantages and disadvantages of the systems
(Lorden, Levinson, and Leong 2015)

Class of Drug	Soluble Factor	Most Common Regenerative Application	Pro's	Con's
SMDs	Small Molecule Drugs	Local delivery for nervous system regeneration	Infrastructure and popular acceptance exists for manufacture and use	Costly to identify develop novel formulations
Protein Drugs	Growth factor	Tissue Regeneration and angiogenesis	Naturally regulate cell function. Recombinant manufacturing	Can be toxic in excess, short half-life, subject to enzymatic cleavage
	Zinc Finger Proteins	Genome editing	Activate endogenous genes expressing all splice variants, or repress endogenous genes	High cost and difficulty of generation
	TALENs	Genome editing: repress endogenous genes	Simplistic design methods, low cost of production	Development of these as therapeutics is in its infancy
	Moncolonal Antibody	Inactivate pathways associated with disease states	Long half-life compared to other protein-based therapeutics, high specificity	Large size inhibits trafficing within tissues; immunogenecity
Nucleic Acids	cDNA/pDNA	Introduce genes into cells	Well-developed technique for *in vitro* manipulation of cells to express a protein or gene of interest	Delivery method and risk of insertional mutageneis
	RNAi	Inhibit endogenous RNA to regulate gene expression	Regulate endogenous gene expression *in vivo*	Rapidly cleared *in vivo*, non-tissue-specific, negatively charged
	Aptamer	Inhibit action of target	Essentially non-immunogenic, and chemical generation results in little batch-to-batch variation	Rapidly degraded *in vivo*, & costly to generate

These therapeutics may need to be delivered in different ways, from systemic administration to intracellular delivery. Systemic delivery is appropriate for systemic diseases but is rarely the most attractive or effective option for regenerative medicine applications because soluble factors degrade rapidly without an efficient carrier. For localized tissue regeneration, the ideal therapeutic would have a controlled, local delivery to limit toxicity and minimize the amount of drug needed to achieve a therapeutic effect. There are several approaches for controlled local delivery (Figure 3.1); suspension in an implantable or injectable scaffold and immobilization on or inside of biomaterial constructs are the most common examples. For example, the drug can be chemically linked to the network of a hydrogel or covalently immobilized to the surface of a scaffold, such as an

electrospun fibrous matrix (Piantino et al. 2006; Censi et al. 2012). Scaffolding systems such as these provide the option of controlled release by varying porosity and degradation rate.

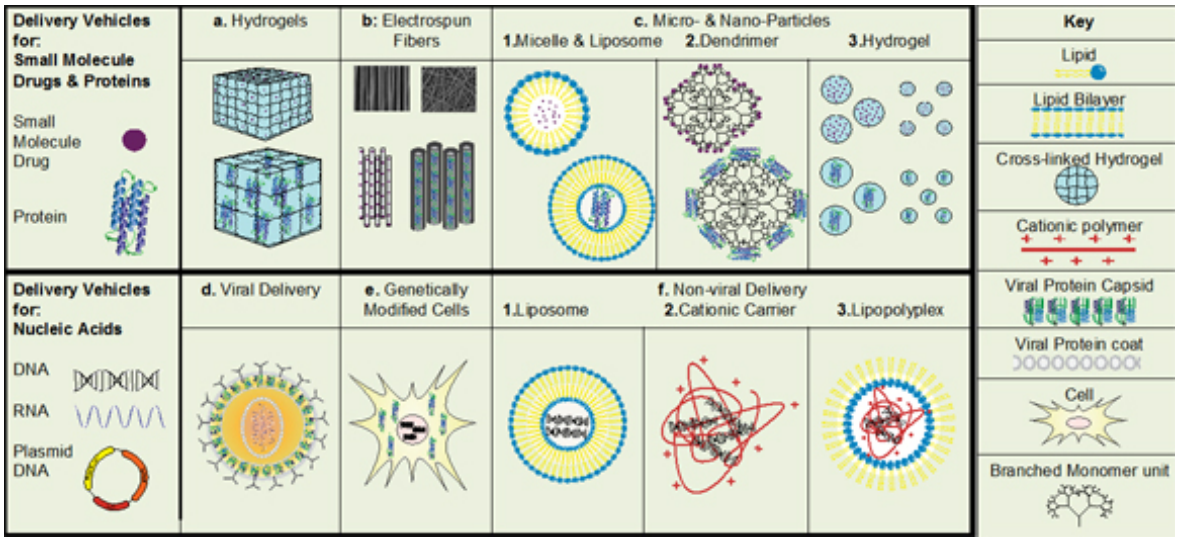

Figure 3.1. Delivery vehicles for small-molecule drugs (SMDs), proteins, and genes. Carriers for soluble factors can be macroscopic, such as hydrogels (A) and electrospun fibers (B), or microscopic, such as micelles and liposomes (C.1), dendrimers (C.2), or particulate hydrogel systems (C.3). Carriers for nucleic acids such as DNA, RNA and plasmid DNA have unique design requirements in that they must be able to carry their negatively charged cargo across the negatively charged cellular membrane. Viral carriers (D) can be used to introduce DNA into cells, most commonly done *in vitro* to generate genetically modified cells (E). Nonviral delivery methods such as liposomes (F.1), cationic polymers (F.2) and lipopolyplexes (F.3) can also be used to generate genetically modified cells or deliver nucleic acid cargo *in vivo* (Lorden, Levinson, and Leong 2015).

Particulate delivery systems such as micro- and nanoparticles have emerged as one of the popular delivery vehicles because they are injectable and their size can be tailored to deliver the cargo of interest either extra- or intracellularly. Viral vectors can be used to deliver and promote the expression of DNA based therapeutics *in vitro* by the generation of genetically modified cells or, less frequently, *in vivo*. Genetically modified cells can be used as carriers, typically following transduction or transfection with a gene encoding the protein of interest. These carrier systems are often combined to create an optimal release profile in the tissue of interest.

Delivery of nucleic acid therapeutics by viral vector has been the predominant method because of its effectiveness. Excellent science is being done on the viral approach to make the viral vector safer, as evidenced by the commercialization of a gene product in Europe in 2012 (Flemming 2012). However, concerns about the immunogenecity and long-term safety of viral vectors remain, and increasingly, effort has been shifting to nonviral delivery via DNA nanoparticles or DNA nanocomplexes.

MANUFACTURING TECHNIQUES

Innovations in materials chemistry have initially fueled the development of drug delivery systems, creating carriers that are biodegradable, less toxic, targeted, and stimulus-responsive. Nanotechnology has joined forces with materials chemistry in the past decade. The realization that the sizes and shapes of nanoparticles can help navigate biological carriers has stimulated the application of nanofabrication technologies, both top-down and bottom-up, to develop more effective particulate drug delivery systems. For instance, the size of nanoparticles determines their biodistribution. Whereas particles smaller than 20 nm will be cleared from a person's circulation via the reticuloendothelial system within a few hours when injected intravenously, larger ones will

be trapped in the liver and the spleen within minutes (Yu and Zheng 2015). Size and shape also determine the endocytic pathways by which nanoparticles enter cells, which has important implications for the intracellular delivery efficiency of the nanoparticles (Canton and Battaglia 2012). Fabrication techniques such as nanoprecipitation, emulsion-based phase inversion, microfluidics-based self-assembly, layer-by-layer synthesis, and nanoimprinting have been used to generate particulate drug delivery systems to deliver a wide range of therapeutics for regenerative medicine (Zhang, Chan, and Leong 2013). These nanomanufacturing techniques will play a prominent role in the future to fully realize the potential that a particulate drug delivery system with controlled size and shape can offer in improving therapeutic outcomes.

Top-Down Nanoparticle Fabrication

While the influence of particle size on drug delivery efficiency has been well established, it is not until recently that the role of particle shape on drug delivery has been revealed. For instance, rod-like structures demonstrate the highest cellular uptake, followed by spheres, cylinders, and cubes (Champion, Katare, and Mitragotri 2007; Gratton et al. 2008). Conventional nanoparticle synthesis typically relies on bottom-up approaches; however, the capacity to achieve large size differences and shape variation is greatly limited by the nature of the self-assembly process. Top-down fabrication methods can overcome this drawback.

Particle Replication in Non-wetting Template (PRINT)

The PRINT method, first introduced in 2005, is a top-down technique to fabricate monodisperse particles with precise particle geometry (Rolland et al. 2005). A non-wetting perfluoropolyether (PFPE) elastomeric mold containing wells or cavities of predefined shape and size is used to fabricate the particles. Polymer solution containing the cargo is confined in the cavities by pressure applied between the mold and the PFPE surface, followed by crosslinking or solvent evaporation. The low surface energy of PFPE prevents the overflow of polymer solution to the non-cavities region, leading to well-isolated nanoparticle formation. With this method, particles from 80 nm to 20 μm have been fabricated with a variety of polymers, producing structures such as discs, cubes, rods, and cones.

Step-Flash Imprint Lithography (S-FIL)

S-FIL is a commercially available nanoimprint technique that uses a quartz template with predesigned patterns for particle synthesis. Polymer solution containing cross-linkable monomer is added to the cavities of template and polymerized via UV light. A PVA layer is deposited beneath the polymer layer and on top of a silica wafer for the release of imprinted particles. S-FIL relies on oxygen plasma treatment to release the nanoparticles from the PVA layer (Glanchai et al. 2008). On the one hand, it does not involve any mechanical stretch and maximally preserves the structure of the nanoparticles. On the other hand, oxygen plasma generates a large quantity of reactive oxygen species and free radicals, which could damage biological materials such as DNA and protein, and it induces polymer degradation. Furthermore, this method is restricted to photo-crosslinkable polymers.

Bottom-Up Nanoparticle Fabrication

Conventional nanoparticle fabrication techniques are prone to polydispersity and batch-to-batch variations. For instance, the final size of nanoparticles generated by emulsion-based techniques is directly determined by the size of the emulsion droplets, which itself could be heterogeneous due to bulk mixing. While heterogeneity remains an insurmountable obstacle in bulk preparation of drug delivery systems, microfluidics, the manipulation of fluid in nano/picoliter-scale channels, presents interesting opportunities to improve the fabrication and manufacturing of particulate drug delivery systems. The general benefits of conducting reaction in microfluidics include rapid mixing of reagents, an homogeneous reaction environment, flexibility for multistep reaction design, enhanced processing accuracy and efficiency, better heat transfer due to high surface-to-volume ratio,

miniaturization, and cost savings from reduced consumption of reagents (DeMello 2006). This approach is particularly attractive for fabrication of nucleic acid nanoparticles.

While the PRINT technique is capable of fabricating a variety of controlled release nanopartcles, it is not easily adaptable for making nucleic acid nanoparticles. DNA, mRNA, miRNA, or siRNA nanoparticles are formed by complexation between polycations and the negatively charged nucleic acids. It is a highly energetic reaction; the nanoparticles are formed in milliseconds via bulk mixing. A top-down fabrication technique that requires filling of the mold with the reagents is not suitable for dealing with such fast kinetics.

One of the companies that the WTEC panel visited in Japan, NanoCarrier Co., Inc., is a world leader in polymer-based nanocarrier systems that can be used to deliver different cargoes both extra- and intracellularly. NanoCarrier currently has several delivery system designs in various stages of development. One design encapsulates hydrophobic drugs in a hydrophobic core with a hydrophilic corona; another method provides a more sustained release through conjugation with a di-block copolymer comprising a hydrophobic segment and a hydrophilic segment. NanoCarrier has also developed a system that uses ligands for targeted delivery, while an even more advanced version of this approach uses antibodies as the ligand.

Using such a di-block copolymer design, the company is able to produce small nanoparticles loaded with chemotherapeutics that can reach the tumor tissue by intravenous injection via the enhanced permeation-retention (EPR) effect. The EPR effect is a consequence of leaky vasculature coupled with impaired lymphatic clearance at the tumor site. Nanoparticles small enough can cross the leaky blood vessels and then stay at the tumor site, the combination of which leads to a favorable therapeutic outcome due to accumulation at the tumor tissue that releases the drug in a local and sustained manner. These nanomedicines are at an advanced stage of clinical trial and product development. However, Nanocarrier is not currently looking beyond conventional manufacturing techniques. The types of delivery technologies being developed by NanoCarrier typically have a wide size distribution, and if they were used to deliver nucleic acid cargo, the size distribution problem would be even greater and the characteristics of the nanoparticles would be even more non-uniform.

Assembly of nanocomplexes by charge neutralization is done by bulk mixing in the vast majority of laboratories. While preparation in bulk formats by pipetting, shaking, or oscillatory mixing in a 1-mL Eppendorf tube is convenient, these methods are poorly suited to reproducibly generate uniform particles given the kinetically determined nature of the formation process. Irreproducibility is typical; slight perturbations of bulk mixing protocols often yield particles of varied properties. The poor quality of these polyplexes exacerbates the challenge of establishing precise structure–function relationships and precludes mechanistic understandings of the delivery barriers, because subpopulations of particles may be responsible for observed phenomena. Inability to manufacture nonviral delivery systems in a reproducible and scalable manner may hinder their clinical translation in the future.

To bypass the drawbacks of bulk mixing, an alternative to fabricating DNA nanocomplexes is to *not* mix the polycations and nucleic acids and simply allow them to self-assemble under equilibrium. This process is kinetically determined by the encounters between oppositely charged polyelectrolytes, which may take a long time, so one option is to miniaturize the volume. This has been done in microfluidics-generated droplets. Self-assembly of polycation-DNA nanocomplexes occurs rapidly in a microfluidic droplet that is in the order of 100 picoliters, which is a reduction of seven orders of magnitude over the bulk mixing process described above in a 1-mL Eppendorf tube. This approach yields DNA or mRNA nanocomplexes that are smaller and have tighter size distribution, lower surface charge, improved colloidal stability, and significantly improved transfection efficiency (Ho et al. 2011; Grigsby et al. 2013).

Yet another approach to nonviral DNA nanoparticle production is the formation of DNA nanocomplexes by 3D hydrodynamic focusing in a continuous flow. This technique, developed by

Tony Huang at the Pennsylvania State University, uses centrifugal force to squeeze the third dimension of the interface. This approach reduces the sample volume and minimizes flocculation after nanoparticle formation. The technique, also known as microfluidic drifting, provides for the retention of DNA bioactivity with notably improved transfection efficiency (Lu et al. 2014).

Complex Multifunctional Nanoparticles

The field of nanomedicine is moving increasingly toward complex multifunctional nanoparticles using recombinant polymer rational design and combinatorial synthesis of carriers. The advantages of these nanoparticles include improved stealth characteristics; the ability to provide multistaged targeting of vasculature, tissue, and cell; greater responsiveness to internal and external triggers such as pH, enzymes, heat, and magnetism; the ability to provide multimodal imaging; and the co-delivery of both the drug(s) and an imaging agent or a drug and a gene (e.g., Figure 3.2). This combination therapy is having a significant impact on cancer therapy and promises significantly enhanced therapeutic efficacy. For example, if one were to deliver Paclitaxel and a Polo-like kinase 1 (PLK1) siRNA to the same cancer cell, the PLK1 would sensitize the drug to the chemotherapeutic compound. As a result, the dose of Paclitaxel could be reduced 100 to 1,000 times while still achieving the same cell-kill effect (Sun et al. 2011).

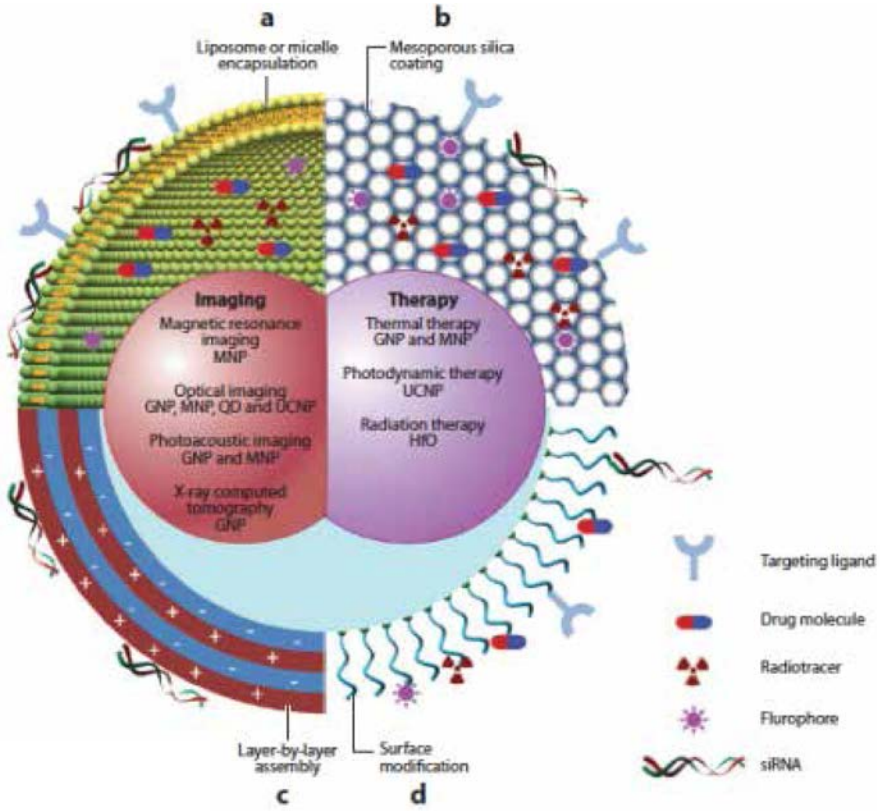

Figure 3.2. Complex multifunctional nanoparticle systems combine therapeutics and imaging and/or other functions (Bao et al. 2013).

As multifunctional nanoparticles become increasingly sophisticated, they require more advanced manufacturing techniques to produce them. Capillary microfluidics has been used to couple nanoparticle synthesis with online monitoring to sort particle size and composition. It may be possible to couple this approach with a flow system to first form the DNA nanoparticles, followed by surface decoration of the nanoparticles with ligand conjugation in a subsequent microfluidic system.

CONCLUSIONS

Manufacturing will play an important role in nanoparticle commercialization. The performance of nanotherapeutics is extremely sensitive to their physical characteristics. Size can have a tremendous influence on biodistribution, on whether therapeutics can get into cells, or even on specifying which endocytic pathway the nanoparticle will enter. Nanotherapeutics are also highly sensitive to surface charge. As a result, the current processes of making nanotherapeutics are uncontrolled and suboptimal.

Advanced manufacturing technologies can make a difference not only for scaling up but also for influencing product performance. As has been noted in this chapter, the field has so far focused on chemical innovations (polymer chemistry, carriers, bioreactive carrier design, etc.) but very little attention has been paid to processing. Yet processing is tremendously important to realizing the full potential of these chemical innovations. As nanotherapeutics require more sophistication in the future, fabrication and manufacturing applications will be even more important. At some point, when nonviral gene therapy overtakes viral gene therapy as the predominant mode of delivery, manufacturing will be a critical barrier both to therapeutic outcomes and to commercialization.

To ensure that these manufacturing innovations are in place when we are ready for them, we must begin training of personnel right now, for example, to teach automation technologies and manufacturing concepts, systems engineering and techno-economic analysis, micro- and nanofabrication technologies, interfacing between physics and biology for biomaterial design, theory and computation for modeling, and statistics and machine learning. As is pointed out elsewhere in this report, the bioengineering field needs a new model for partnering between industry and academia to facilitate such training. At the professional level, academic faculty and industrial scientists must spend time in each other's domains to learn what is being done there that can be taken away and applied in their respective areas of endeavor. At the student level, an effort must be made to change the perception that manufacturing is not "cool." The National Science Foundation has done a great job in attracting students to science, technology, engineering, and mathematics (STEM) fields; perhaps it could develop some similar programs for biomanufacturing.

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CHAPTER 4

AUTOMATED BIOMANUFACTURING AND THREE-DIMENSIONAL TISSUE AND ORGAN FABRICATION

Kaiming Ye

INTRODUCTION

The creation of highly organized multicellular constructs, tissues, and organoids will revolutionize regenerative medicine. The production of these high-order tissues and organs will enable the generation of personalized tissues and organs from human pluripotent stem cells for patient-tailored transplantation. They can also be used as disease models for pathophysiological study and drug screening. With the advent of stem cell biology, tissue engineering, and the discovery of the ability to reprogram patient-specific cells into human induced pluripotent stem cells (iPSCs), the production of personalized tissues—unthinkable just a few decades ago—is now within reach.

Recent studies, however, have been more focused on creating multicellular constructs at a laboratory scale. The conventional wisdom for producing tissue products of interest is to differentiate and maturate stem cells stepwise toward desired tissues and organs. These approaches are less controllable and hard to scale up or out. These limitations have made it virtually impossible to manufacture tissues and organs at an industrial scale, which is critical for the success of regenerative medicine and tissue engineering. Industrial-scale manufacturing of tissues and organs in a controllable fashion requires completely new theories and new technologies, which in turn requires the breaking of new ground.

To help realize this vision, the WTEC panel conducted a global assessment of advanced biomanufacturing by visiting and speaking with leading scientists in the field particularly in Europe and Asia. The purposes of this assessment were to identify the challenges that scientists and engineers face in developing advanced biomanufacturing technologies, as well as the opportunities for international collaboration, education, and training. It has become clear that biomanufacturing is an emerging field that requires much additional effort to create the new technologies and tools that enable tissue and organ production to eventually reach an industrial scale.

One of the most critical findings to come out of this study is the recognition that greater efforts to coordinate the study of biomanufacturing are needed. The concept of automated biomanufacturing and three-dimensional (3D) tissue and organ fabrication has become a key focal point for advanced biomanufacturing. During the study, we observed several examples of national-level coordination in advanced biomanufacturing in both Europe and Asia.

CREATION OF PROFESSIONAL ECOSYSTEMS

One of the most significant observations made by the WTEC panel during its visits to leading biomedical institutions in Europe was the remarkable efforts being made to build ecosystems that bring clinicians, life scientists, and biomedical engineers together in highly integrated environments. An excellent example of this approach is the Edinburgh BioQuarter Science Triangle in the UK (Figure 4.1).

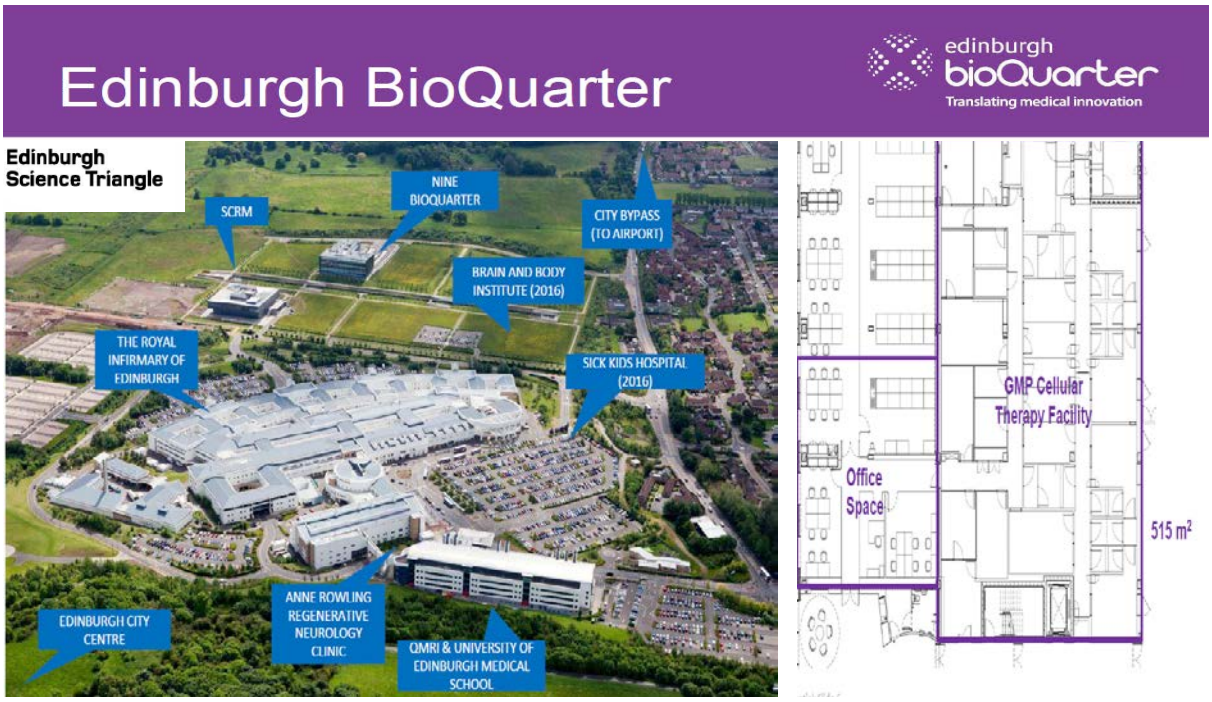


Figure 4.1. Edinburgh BioQuarter—an academic medical center that integrates teaching hospitals, biomedical research facilities, biomedical product manufacturing facilities, and commercial business centers (courtesy of Edinburgh BioQuarter).

The Edinburgh BioQuarter is an academic medical center that integrates teaching hospitals, biomedical research facilities, biomedical product manufacturing facilities, and commercial business centers. The teaching hospitals include the Royal Infirmary of Edinburgh, the Royal Hospital for Sick Children, Western General Hospital, and Easter Bush Veterinary Center. University and research institutes in the BioQuarter include the University of Edinburgh Center Area, King's Building, Medical School, the Queen's Medical Research Institute, the Scottish Center for Regenerative Medicine, the Royal School of Veterinary Medicine, the Roslin Institute, Heriot-Watt University, Napier University, Queen Margaret University, Moredun Research Institute, Scottish Agriculture College, and Heriot-Watt School of Textiles and Design. The science parks and incubators include Edinburgh BioQuarter Life Science Park, Edinburgh Technopole, Pentlands Science Park, Roslin BioCentre, Hariot-Watt University Research Park, Alba Campus, Alba Innovation Center, Institute and Systems Level Integration, Innotek Centre, Elvingston Science Centre, Edinburgh Technology Transfer Centre BioSpace, Scottish Microelectronics Centre, and PROSPEKT Center. More than 1,300 researchers, including 120 academic clinicians, work in the BioQuarter, which has 960 inpatient beds, an 85,000 sq. ft. bioincubator area, a Medicines and Healthcare Products Regulatory Agency (MHRA)-accredited phase I clinical trial suite, a clinical research imaging center providing PET, computer tomography (CT), and MRI services, a good manufacturing process (GMP) cell therapy manufacturing facility, and National Health Service (NHS) clinical service laboratories.

The WTEC panel discovered that the on-site commercialization teams become engaged in clinical product development at a very early stage. These teams work closely with Scottish Enterprise to stimulate, initiate, and work side-by-side with biomedical researchers and clinicians to develop biomedical technologies and clinical products. They offer support and guidance for concept development, R&D and investment funding, regulatory matters, marketing, and product development. They also offer "one-stop-shop" assistance to BioQuarter researchers from the concept development stage through to the creation of a company.

The outcomes have been significant: in the last four years, ten spin-off companies have been created, resulting in the creation of more than 100 new jobs, and BioQuarter plans to spin off another 18 new companies over the next seven years. It has also raised £50 million of venture capital located in Edinburgh in 2015.

Another example of a successful BioQuarter venture is Roslin Cell's iPSC Service Core Facility. Roslin Cell focuses on the development of cell therapies and pluripotent stem cells for biomedical research, drug discovery, and therapy. The company was spun off from Roslin Institute in 2006 and was the first group in Europe to derive a clinical-grade human embryonic stem cell line. It offers a full portfolio of iPSC development services from human tissue procurement to the derivation and delivery of high-quality, fully characterized iPSCs. It helps custom users develop tailored iPSC differentiation protocols and drug screening strategies. It also helps custom users develop iPSC-based pharmacology models for toxicity testing and physiopathological studies. It serves as a European bank for iPSCs as a means of accelerating the translation of iPSC technologies to therapies from the laboratory to clinical development.

Life Sciences Scotland is another example of the integration of stem cell and regenerative medicine research into clinical product manufacturing development. Life Sciences Scotland has developed two cell therapies—a pancreatic islet program and an Epstein-Barr virus (EBV) cytotoxic T lymphocyte (CTL) bank—into clinical practice. The CTL bank has been developed to treat post-transplant lymphoproliferative disease, while the pancreatic islet program has been developed to coordinate Scottish liver/islet transplantation. Life Sciences Scotland plans to develop four more regulatory-approved stem cell therapies: for stroke, critical limb ischemia, corneal epithelial treatment, and chronic liver failure.

The Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB) in Stuttgart, Germany, offers R&D solutions for medicine, pharmaceuticals, chemistry, the environment, and energy. The institute consists of several divisions including molecular biotechnology, physical process technology, environmental biotechnology, bioprocess engineering, and tissue engineering. By bringing materials scientists, life scientists, process engineers, and business entrepreneurs together in an integrative environment, the institute creates a path for scientists, engineers, and investors to work together from the technology development phase through to eventual product commercialization. For instance, the institute's biomedical engineers have successfully developed an inkjet printer for the production of artificial tissues using gelatin derived from collagen (Figure 4.2). The gelatin has been chemically modified so that it remains fluid during the printing process. When exposed to UV light, the printed gelatin cross-links and cures to form hydrogels. By controlling the chemical modifications of the gelatin, the engineers have been able to regulate the mechanical properties and swelling characteristics of the gelatin to mimic natural tissues ranging from solid cartilage to soft adipose tissues.

The institute's engineers are also currently developing a technology that will offer a platform for generating fine blood vessels from synthetic materials. The ability to print blood vessels is of critical importance for eventually being able to print biologically functional tissues and organs. The rapid development of this printing technology at the institute is an excellent example of the ways tissue engineers, life scientists, and biomaterials scientists are able to work together to accelerate process development and the translation of new technology into products.

TRANSLATING 3D PRINTING TECHNOLOGIES INTO MEDICAL PRODUCTS

The WTEC panel was impressed by the high degree of development that automated and on-demand biomanufacturing has attained through the use of 3D bioprinting. Until recently, the laboratory development of tissues and artificial implants had been very labor intensive and highly dependent upon experienced operators and lab technicians, which made commercial scale-up of these processes unthinkable. Today, in contrast, the widespread availability of 3D tissue and organ printing and biorobotic manufacturing of tissues and organs has placed this outcome within reach.

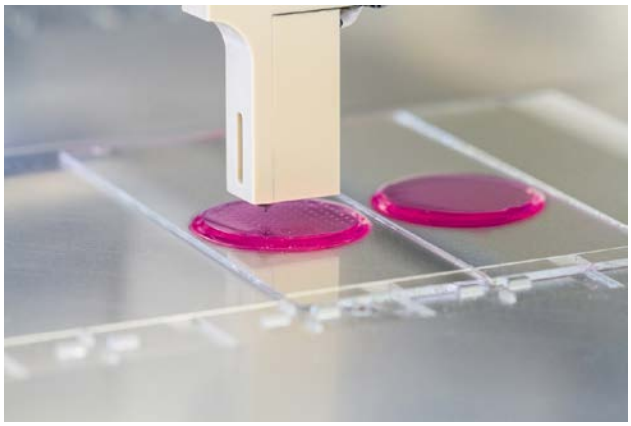


Figure 4.2. An inkjet printer developed by Fraunhofer IGB scientists for printing cell suspensions onto shimmering pink hydrogel pads, which prevent desiccation (courtesy of and © Fraunhofer IGB).

The panel visited the Institute for Bone and Joint in Shanghai, China. Founded in 1986, the institute consists of the Shanghai Key Laboratory of Orthopedic Implants, the Laboratory of Orthopedic Cell and Molecular Biology, and the Engineering Research Center of Digital Medicine and Clinical Translation. It is a joint venture between the Shanghai Ninth People's Hospital and Shanghai Jiao Tong University School of Medicine, and it was one of the pioneer institutions focused on the biomechanics of musculoskeletal systems. The laboratory is well known internationally for its research in orthopedic implants, training, and translational medicine. Its research infrastructure ranges from fundamental stem-cell biology to 3D bioprinting and orthopedic implant banks. The panel was particularly impressed by the laboratory's individualized implants, such as 3D printed joints, which were developed through collaborative research among industries, universities, and hospitals. This industry- and hospital-driven research model will be a good example of promoting the types of advanced biomanufacturing that are the focus of this study.

The Shanghai Key Laboratory of Orthopaedic Implants supports a number of multidisciplinary teams consisting of orthopedic surgeons, life science investigators, and engineers. The lab has been actively carrying out clinical-oriented medical researches on orthopedic translation, and has accomplished great things in the optimization, design, and application of artificial joints; stem-based therapy for bone repair and regeneration; the development and evaluation of functional bone substitutes; and the understanding and prevention of periprosthetic osteolysis, osteoporotic fractures, and fracture healing (Figure 4.3). The lab also focuses on translating lab discoveries into clinical products by developing individualized artificial joints and bone allografts using 3D printing technologies. The China Food and Drug Administration (CFDA) has approved these implants for industrialization and clinical applications. The lab provides technical services and support for other research institutes and enterprises in Shanghai through collaborative research, training, and joint R&D.

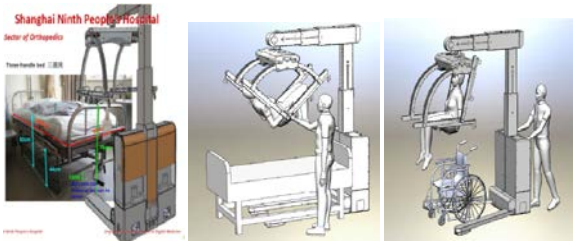
The key lab, which is led by Professor Dai, has been awarded more than twenty prizes, including the Second Prize of National Invention, the Second and Third Prize of National Scientific and Technological Progress Award, and other science and technology progress awards. Three of these achievements have resulted in the obtainment of medical equipment registration certificates and have been successfully translated to clinics. The lab's medical 3D printing technology, orthopedic tissue bank, and failure medical implant capability are aiding the development of a new generation of individual implants to meet clinical demands. RoboNurse, an intelligent patient lifting and handling device developed by a team led by Professor Dongyun Gu in collaboration with the Toronto Rehabilitation Institute, is a noteworthy approach to the dual problems of moving patients safely and reducing occupational nursing injuries. In the new field of mobile digital medicine, the

center has successfully developed a computer-aided fracture diagnosis and clinical treatment decision support system on both PC and mobile terminal platforms.

This system has been widely applied and promoted by nearly 400 hospitals in 29 provinces and regions in China. In the field of minimal invasive technology, the team led by Professor Chengtao Wang and Professor Yun Luo has developed a number of scientific research breakthroughs that have been translated successfully into clinics as a result of cooperation among industry, academia, and research (Figure 4.3).

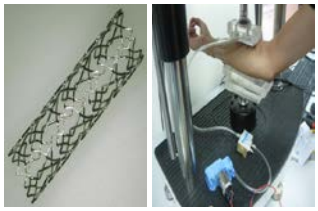
3D printed implants

Orthopedic tissue banks



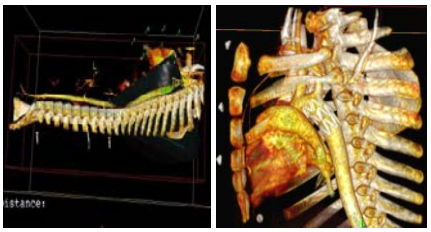
China-Canada science & technology cooperation project: RoboNurse

Computer-aided fracture diagnosis systems used at hospitals

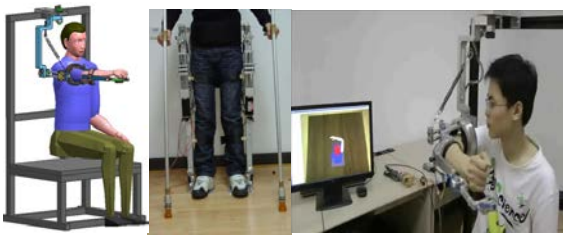


Stents with high radial strength and HMI unit device

Maxillofacial surgical navigation system and a digital operating theater



A cardiac intervention surgical navigation system



Exoskeleton robot and lower limb rehabilitation robot

Figure 4.3. Advanced biomanufacturing research activities at the Shanghai Key Laboratory of Orthopaedic Implants, Shanghai, China (courtesy of Shanghai Jiao Tong University).

From 2006 to 2010, the Shanghai Key Laboratory of Orthopedic Implants received 73 grants totaling CNY38.5 million[2] (including four National 863 Program, two National 973 Program, and seventeen National Natural Science Foundation awards). The lab also won the second prize of the Shanghai Science and Technology Progress Award, and the third prize of the Shanghai Medical

[2] CNY is the ISO code for Chinese yuan, China's currency, also known as *renminbi*

Advancement Award. The lab has applied for and received 22 national patents, has published over 250 papers in international and domestic journals, and has received a number of awards for training talented young clinical researchers. These training programs include the Program for New Century Excellent Talents in University; the New Century Hundred, Thousand, Ten Thousand Talent Project; the Shanghai Cultivation Program for Academic Leaders; the Shanghai Science and Technology Committee Rising-Star Program and Rising-Star Tracking Program; the Shanghai Pujiang Talent Program; the Shanghai Dawn Program and Dawn Tracking Program; and the China Scholarship Council Program for Constructing High-level Universities, among other honors.

The Engineering Research Center for Digital Medicine and Clinical Transplantations currently consists of seven principal investigators: Professors Kerong Dai, Dongyun Gu, Chengtao Wang, Jinwu Wang, Le Xie, Lixu Gu, and Yun Luo. Over the last five years, the center has received 90 research grants totaling over CNY58 million, including five projects supported by the National 863 Program, two projects supported by the National Key Technology Support Program, seven sub-projects supported by the National 973 Program, and 30 projects supported by the National Natural Science Foundation of China, among other programs. It has published more than 200 research papers and received 48 patents.

The panel was also impressed by the center's activities in training talented young clinical scientists by acquiring funding from many levels, from the central government to local cities. One of the critical challenges facing biomanufacturing organizations around the world is the lack of adequate mechanisms for attracting and training clinical scientists to industrial- and hospital-driven research; the training model developed by the center offers a good solution to this challenge.

ROBOTIC MANUFACTURING OF CELL AND TISSUE PRODUCTS: SCALE-UP VS. SCALE-OUT

Robotic and automated biofabrication are key technologies for realizing advanced biomanufacturing of cell and tissue products. Unlike conventional biomanufacturing, in which fermentation is a cornerstone of the entire process, the manufacture of tissue products relies upon the culturing, differentiating, maturing, and assembling of cells into tissue structures. These requirements pose new challenges for engineering and process development, primarily that on-demand production of personalized tissue products for transplantation or organ regeneration requires "scale-out" rather than scale-up.

It has long been known that individual patients respond to medical treatments differently as a result of genetic differences. Thus, not only medical treatments, but also diagnoses, need to be tailored to individual patients. This requirement reduces the need to produce bioproducts on a large scale. Rather, they can be produced or manufactured on a small scale, but tailored to individual patients. This in turn necessitates the development of standardized and automated production methods that allow the quality of the bioproducts to be monitored and controlled. Attempts have been made to develop such production technologies.

One good example that the panel observed is the concept of "cell factories" developed by Teruo Okano, the director of biomedical engineering and science at Tokyo Women's Medical University in Japan. Dr. Okano has developed a truly automated and standardized cell production technology based on a temperature-responsive polymer that his group developed. Dr. Okano discovered that a coating of poly(N-isopropylacrylamide) (PIPAAm) on the surface of a cell culture dish allows cells to attach and proliferate. The hydrophobicity of the polymer layer changes from hydrophobic at or above 37 °C to hydrophilic at or below 32 °C, which leads to cell detachment from the surface (Matsuda et al. 2007). This behavior allows for the collection of cell sheets without the need to use proteolytic enzymes such as trypsin or dispase. This technology allows contiguous cell sheets to be harvested while preserving cell–cell connections and the extracellular matrix, which is hard to achieve using conventional cell cultures in which proteolytic enzymes such as trypsin or dispase are used to detach cell sheets from cell culture dishes.

Dr. Okano's cell-sheet technology has been used to generate corneal epithelium sheets for corneal surface reconstructions (Figure 4.4). In this process, corneal epithelium stem cells that reside in the limbus are isolated from limbal tissue biopsy, enabling the production of personalized corneal tissues for transplantation. Multilayered corneal epithelial cell sheets can be collected simply by reducing the temperature from 37 °C to 20 °C. The harvested corneal epithelium sheets are then transplanted on the corneal surface to reconstruct the deteriorated corneal tissues. Dr. Okano's team has shown that oral mucosal epithelium cell sheets can also be used replace corneal epithelium cell sheets for reconstructing deteriorated corneal tissues.

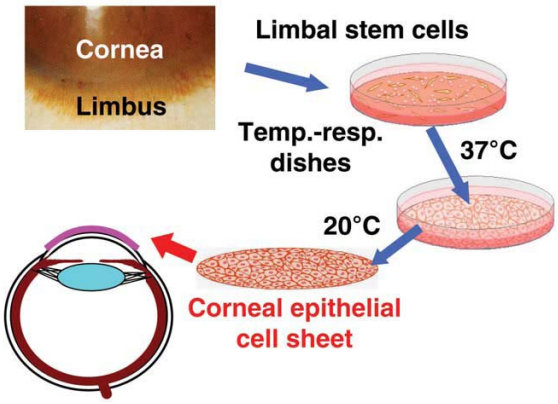


Figure 4.4. Corneal epithelial cell sheet transplantation (from Yamato and Okano 2004).

Limbal stem cells are isolated from a small limbal tissue biopsy and cultured on temperature-responsive culture dishes at 37 °C. Transplantable corneal epithelial cell sheets are harvested by reducing the temperature to 20 °C and grafted onto a damaged cornea.

To accomplish automated production of these cell sheets, Dr. Okano's group developed and built a 280 m^2 automated tissue factory equipped with a standardized docking interface (Figure 4.5) inside a GMP facility at the Cell Processing Center. While this tissue factory is still a very preliminary design, the concept of developing automated tissue production lines is significant. More work is necessary to improve tissue production. For instance, the incorporation of noninvasive detection technologies will allow for the monitoring and controlling of tissue production.

Figure 4.5. Cell sheet factory-automated cell culture system (courtesy of Prof. Teruo Okano).

LESSONS FROM THE STUDY

The panel recognized that advanced biomanufacturing is an emerging field that requires advances in scientific and technological development as well as in training. Following the study, the panel has the following recommendations for the United States:

- Create a scientific ecosystem that integrates engineering and business principles into the life sciences and clinical sciences in order to develop successful models of advanced biomanufacturing.
- Develop new technologies and engineering principles to automate, modularize, standardize, and industrialize bioproduction.
- Initiate and develop educational and training programs in advanced biomanufacturing.
- Offer training opportunities to create the future workforce for the field, for example an M.S. degree in advanced biomanufacturing.

A VISION FOR THE FUTURE

Regenerative medicine offers the possibility that someday doctors may be able to replace damaged or diseased organs and tissues with new ones grown from a patient's own cells. Although regenerative medicine is still a relatively young field, the advent of improved stem-cell therapies, advanced scaffolds, and now 3D printing have greatly accelerated progress to the point where, as Wendell Lim of the University of California, San Francisco, has predicted that bioengineering could soon become the "third pillar" of medicine, along with pharmaceuticals and biologics (Fischbach, Bluestone, and Lim 2013).

To succeed, however, such revolutionary therapies first require the establishment of a dedicated engineering discipline and an industry dedicated to advanced biomanufacturing. The National Science Foundation is actively supporting the development of the former, sponsoring workshops to bring advanced biomanufacturing researchers together to define the field, establish its parameters, and seek consensus for proposed methodologies.

Issues of scaling, the development of related technologies for imaging and monitoring, and the need for specialized academic degrees must also be addressed; in the meantime, bioengineering may need to focus on shorter-term goals such as drug screening as alternatives to *in vivo* trials. Then, as familiarity and experience increase, decisions about these key areas will be easier to make.

(The ideas discussed by the author in this section are derived from his and others' work as reported in Thomas K. Grose's article "Human Spare Parts," in the February 2015 issue of *PRISM*, the monthly publication of the American Society for Engineering Education.)

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CHAPTER 5

BIOMIMETICS IN BIOMANUFACTURING: MOLECULAR, CELLULAR, AND TISSUE-SCALE BIOMIMICRY

Christopher J. Bettinger

INTRODUCTION

Definition and Scope

Biomimetics is broadly defined as the imitation of processes, materials, and structures found in nature in engineered systems for use in solving problems of humanity. This classic definition encompasses a wide scope of active research and a broad range of disciplines including microbiology, chemistry, materials science, mechanical engineering, physics, computation, and many other fields of study. The application of biomimetics to biomanufacturing further increases the scope of biomimicry. This chapter will focus on the use of biomimetics and biomimicry in the design of novel materials, structures, processes, and fabrication techniques that are related to the field of biomanufacturing of regenerative medicine products. The envisioned therapeutic products include, but are not limited to, production of high-value compounds such as protein therapeutics and vaccines, novel biomaterials, cell-based products, biosensors, and medical devices. Particular focus will be granted to the production of new biomimetic materials, structures, and devices for applications in controlled release, generative medicine, organ replacement, and rehabilitation technologies such as brain–machine interfaces. The treatment of these topics in the survey of worldwide research activities is not intended to be exhaustive or comprehensive. Rather, these examples will highlight some generalizable observations in the application of biomimetic principles to biomanufacturing. The topics of this chapter are categorized by the characteristic length scale of the specific system. The topics will be organized and segmented into the following length scales: molecular, micro- and cellular, and tissue/organ-scale (Figure 5.1). This chapter focuses on therapeutic products at the following length scales: molecular (nanometer-scale); cellular (micron-scale); and tissue/organ (millimeter-scale).

SURVEY OF WORLDWIDE RESEARCH ACTIVITIES

Biomimetics in Molecular-Scale Manufacturing

Biological processes are often leveraged for the production of a wide range of high-value compounds such as fine chemical building blocks, protein-based therapeutics, antibodies, and vaccines. A classic example of bioprocess engineering is the use of bioreactors and downstream purification methods in combination with genetic engineering methods to produce proteins on an industrial scale (5 g/L). Parallel strategies are also being pursued in microorganisms for the production of commodities such as biofuels. Despite the variation in cost pressures and economic drivers, both arenas focus on use biomimetic strategies to improve production efficiency by exploring non-native biosynthetic pathways and reaction conditions.

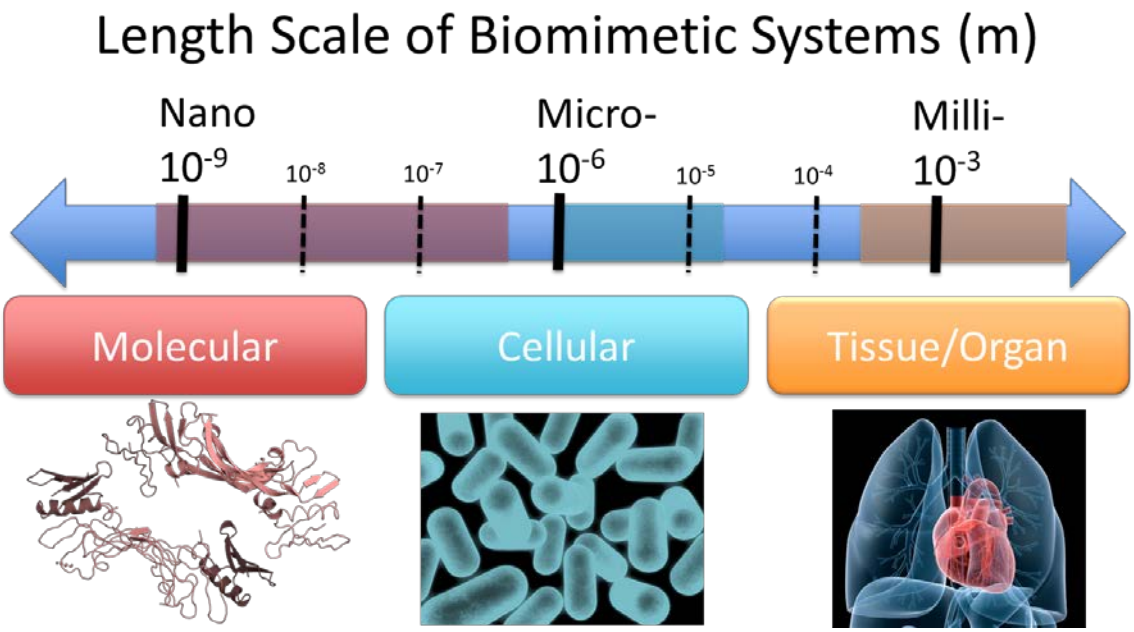

Figure 5.1. Biomimicry can be applied to many biological length scales when creating therapeutic products (courtesy of C. Bettinger).

Genomic and Metabolic Engineering in Biochemical Production

Biochemical engineering is the application of engineering principles and technology to alter and improve natural biosynthetic processes. Biochemical engineering is an established discipline that rose to prominence with the advent of genetic engineering and the maturation of chemical engineering principles and sophisticated instrumentation. There are many organizations in academia and industry throughout the world that conduct research programs in this area, including U.S. industry stalwarts Genentech and Biogen. These organizations pioneered biochemical engineering with the help of academic partners such as University of California-San Francisco and the Massachusetts Institute of Technology (MIT). The Biotechnology Process Engineering Center (BPEC) at MIT is an academic leader in this discipline and has populated both academia and industry with many of its former trainees. Biochemical engineering is a fairly broad classification for biomanufacturing that provides a key intellectual foundation for many subsequent research directions. Microbial fermentation is a decades-old technology that is still widely used today for the large-scale production of compounds such as antibodies, protein-based therapeutics, and vaccines.

Much of the R&D in process optimization from the standpoint of hardware and instrumentation has shifted to the private sector. However, there are many exciting research opportunities related to the genetic and metabolic engineering of microbes to synthesize products of interest at increased efficiencies. One such global academic leader in biochemical engineering is the Technology Division within the Instituto de Tecnologia Química e Biológia (ITQB), located in Lisbon, Portugal. This organization is led by Prof. Paula Marques Alves and consists of approximately ten laboratories that are focused on applying principles of engineering science to chemical and biochemical systems. Prof. Manuel J.T. Carrondo directs the Animal Cell Technology Unit, which engages in research that integrates a variety of engineering tools and concepts for improved production of novel biopharmaceuticals, including protein-based therapies, monoclonal antibodies, vaccines, and reagents for gene delivery. This research group is ultimately interested in tools to understand the genotypic and phenotypic profiles associated with cells that produce biochemical products in an efficient manner. This strategy designs models to describe cell behavior by incorporating data from genomics, transcriptomics, proteomics, and metabolomics. These comprehensive models are then combined with cellular data to predict metabolic fates and ultimately estimate the amount of protein that is expected to be produced from a given process.

Predictive models can also provide a basis for cellular and metabolic engineering of both microbial and animal cultures. One such example of a valuable model is the stochastic simulation of protein expression in transfected mammalian cells (Roldão et al. 2008). A predictive model was developed to predict infection events by genetically engineered recombinant baculovirus (rBAC) and downstream protein production within insect cells. This particular model incorporates stochastic infection events and mass action kinetics in multistep reaction pathways that lead to protein production. The final result is a predictive model that describes protein production as a function of both multiplicity of infection (MOI) and the size of the gene (thousand base pairs, or kbp). Future research directions in this area focus on cost-effective acquisition of cellular data to provide as much information as possible into predictive models.

Another interesting evolution in biomimetic manufacturing will be a paradigm shift in which the cell genotype/phenotype defines the product rather than the process (Figure 5.2). This idea means that the cell genotype/phenotype can be engineered to create a specific product as opposed to previous frameworks in which the cell was considered a "black box" element of the process to be controlled, if possible. This fundamental shift has been made possible by recent advances in scalable cost-effective biochemical assays, real time genetic analysis, novel biosensors, microfluidics, and the abundance of sophisticated computational methods.

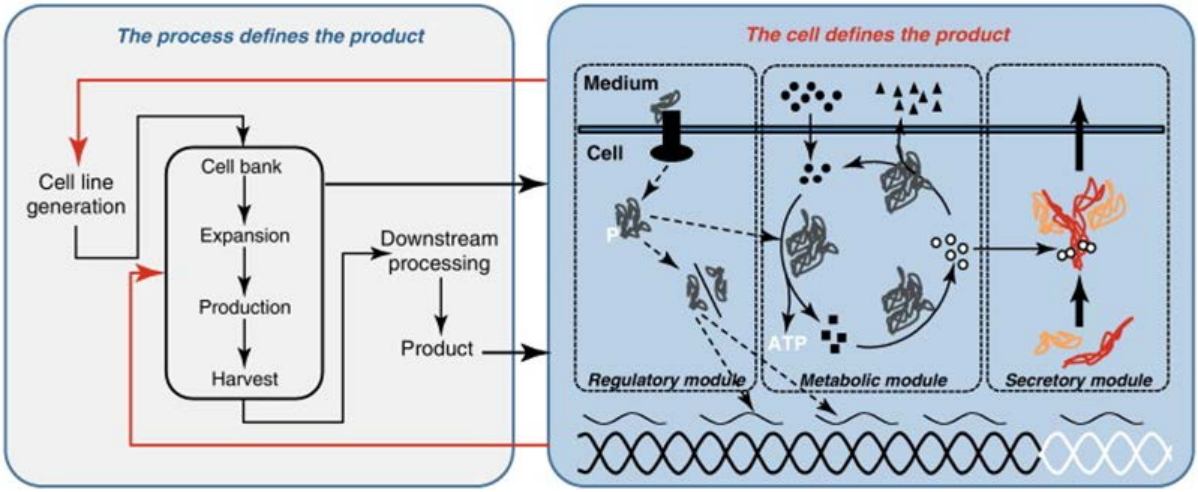

Figure 5.2. Defining the product in molecular-scale biomimetic biomanufacturing (Carinhas et al. 2012).

In the early days of recombinant biologics production, sophisticated analytical tools for product characterization were in their infancy and were of limited utility in process control for biochemical engineering. This previous paradigm defined the product by controlling the process sequence. The advent of sophisticated analytical tools permits the use of well-defined cell populations in biochemical production (cell-defined product design).

Biomimetic Cell-Free Systems for Fine Chemical Production

The advent of both systems biology and synthetic biology offers additional paradigm shifts that can alter the landscape for biomimetic manufacturing of molecular products. There are many internationally recognized research groups working on the emerging area of synthetic biology in the context of biochemical process engineering. One such group is led by Prof. Sven Panke of the Department of Biosystems Science and Engineering (D-BSSE) at the ETH campus in Basel, Switzerland. Panke and others are interested in designing robust enzyme-based biochemical pathways that can be modeled and monitored in real time. One of the key innovations in this approach is the use of cell-free extracts for metabolite production. One representative example of this work is a project in the Panke research group at D-BSSE that aims to increase the production of dihydroxyacetone phosphate (DHAP) using cell-free extract isolated from *E. coli* (Bujara et al. 2011). DHAP is a fine chemical product that serves as a building block for many high-value small

molecules. The group of Prof. Panke discovered a method to design and engineer enzymatic networks involved in glycolysis to produce DHAP with a 2.5-fold increase over previously reported strategies. This achievement is notable because of the ability to both monitor the temporal evolution of the metabolites in real time and predict these trends using modeling and simulation. The end result is a tractable cell-free system that can produce DHAP with increased efficiencies using a biomimetic glycolytic pathway. Cell-free systems have been studied extensively by many groups in the United States and abroad.

For example, the laboratory of Prof. James Schwartz at Stanford University and others have pioneered many advances in cell-free systems. Cell-free approaches are advantageous because they can be more cost-effective and facile compared to microbial fermentation of animal cell culture. From a biomimetic standpoint, cell-free methods offer a more tractable blueprint for engineering complex biochemical pathways that rely on sequential enzymatic activity. Cell-free extracts may therefore address one of the key emerging challenges in biomanufacturing products containing mammalian cells—seemingly unbounded complexity.

Despite worldwide efforts to apply good laboratory practice and good manufacturing practice (GLP and GMP) frameworks to cell-based products, intrinsic limitations in the noise, complexity, and variability of cell-based systems leads to significant challenges in manufacturing. There is an urgent, unmet need to improve our collective understanding of relevant signaling pathways to bias cell fate towards different genotypes/phenotypes more reliably. It may be possible to leverage advances in cell-free systems as representative models for relevant signaling pathways in mammalian cells. Deconstructing complex cellular systems may identify robust pathways that determine cell fate in a reliable manner. Simplified modular networks that can be perturbed and monitored *in vitro* in real time may provide insight into network dynamics. This framework could then predict the impact of small molecules on downstream signaling cascades that can alter cell fate, for example.

Molecular Biomimicry in Polymeric Biomaterials: Representative Activities

Biomimetics can also be leveraged to produce acellular materials and devices for use in regenerative medicine and diagnostics. Biohybrid polymers combine the inherent advantages of both synthetic and natural polymers. Synthetic polymers afford advantages in terms of scalable materials synthesis and well-defined compositions. Natural biopolymers may provide some unique capabilities such as unique physicochemical properties or functionalities such as self-assembly.

One representative example of biohybrid materials synthesis from the laboratory of Prof. Molly Stevens at the London Center for Nanotechnology and Imperial College London is a polymer composed of a poly(γ-glutamic acid) backbone with pendant groups composed of beta-sheet motifs (Mart et al. 2006). This polymer self-assembles into hydrogel networks in which the composition of the beta-sheet motifs determines the mechanical properties. Hydrogel networks can serve as scaffolds that support the seeding and proliferation of many cell types for potential applications in tissue engineering.

One of the challenges in biomimetic approaches for tissue engineering is the complexity of milieu of soluble factors that vary with both space and time. Spatiotemporal complexity can be addressed, in part, by exploring new kinds of multiphase release profiles and micropatterning molecules within networks using photolithography and other soft matter fabrication techniques. One well-characterized example of this approach is the use of unreactive caged molecules that can be rendered bioactive when exposed to light. Another strategy is to use molecules in which the reactivity requires cellular processes as a prerequisite. Hyaluronic acid hydrogels are used in many applications for regenerative medicine, including cartilage tissue engineering. One class of cross-linked hyaluronic acid hydrogels contain tissue growth factor-beta (TGF-beta) that is incorporated into a small latent complex (Place et al. 2012). This is achieved by covalent conjugation of a latency associated peptide with TGF-beta. Basal function of seeded chondrocytes leads to the

activation of TGF-beta, which induces chondrogenesis. This example of biomimetic materials design on the molecular level can recapitulate select aspects of tissue regeneration.

Biomimetic molecular systems are also widely being used as nanoparticle systems for a wide range of potential applications in regenerative medicine, including drug delivery and *in vitro* diagnostics. For example, pH-sensitive particles are used for selective delivery of small molecule agents to cells after phagocytosis (Chen et al. 2010). Prof. Zhiyuan Zhong (College of Chemistry, Chemical Engineering and Materials Science, Soochow University) directs a laboratory focused on the design of stimuli-responsive biodegradable nanosystems for targeted drug delivery. Prof. Zhong and colleagues have made several key advances in this regard. Many polymer synthesis strategies use artificial building blocks that contain biomimetic properties. One example of this overarching strategy is to incorporate redox-sensitive disulfide bonds into the polymer as an actuation component (Zheng et al. 2011; Wei et al. 2012). Lipoic acid is a naturally small molecule compound that exhibits a disulfide bond. Lipoic acid can be conjugated to primary amines in branched polyethyleneimine (PEI) networks to create redox-sensitive polymer reagents for DNA delivery. Low molecular PEI networks conjugated with lipoic acid can deliver DNA to cells with comparable efficiency to high molecular PEI, but with reduced toxicity.

There are many prominent research groups working in the area of drug delivery that are incorporating molecular-scale biomimetics into their designs. For example, (MEA)-grafted poly(L-aspartic acid) has recently been used as a pH-sensitive cross-linked in PEG-based nanoparticles for programmable intracellular drug delivery (Dai et al. 2011, Wang et al. 2012). This materials design strategy delays the onset of burst release and maximizes the delivery of small molecule payloads into cells. Other biomimetic strategies use folate receptors for targeting particles (Lee, Na, and Bae 2003; Yoo and Park 2004). These overarching design strategies can be used as particle formulations to control cell fate in a variety of contexts.

Scalable Synthesis of Natural Pigments

Another class of biologically derived natural products that may draw commercial interest is melanin pigments. Melanins are a natural biomaterial with a wide range of biomedical applications. The manufacturing of melanin using scalable biomimetic approaches would be advantageous. Melanins are a diverse class of pigments that are found in fungi, plants, and animals. Melanins represent an interesting class of biopolymers because they are composed of amino acids but exhibit mechanical properties that are comparable to biomineralized compounds (Meredith et al. 2006). Melanin pigments include eumelanins (black) and pheomelanins (red) (Eisenmann and Casadevall 2012). Eumelanins (hereby referred to as simply melanins) are hyperbranched oligomers of aromatic substituents with extended heterogeneous structures (Meredith et al. 2006; Duff, Roberts, and Foster 1988). Planar protomolecules stack into structures to form densely packed disordered sheets (Simon, Hong, and Peles 2008; Yu et al. 2014). Melanins are totally amorphous. This property confers unique physical characteristics including high broadband optical absorption, efficient photon-phonon conversion, and hybrid electronic/protonic conductivity (McGinness, Corry, and Proctor 1974; Mostert et al. 2012; D'Ischia et al. 2009; Tran, Powell, and Meredith 2006; Mostert et al. 2012). These material properties can be leveraged in the following applications: water purification (Gao et al. 2013), electrochemical storage, biomedical devices, bioimaging (Delogu et al. 2012), light harvesting, and functional coatings (Jiang et al. 2011; Ejima et al. 2013). Many of the chemical functionalities in melanin pigments can also be used in bioinspired surgical adhesives (Lee et al. 2010; Sáez, Escuder, and Miravet 2010).

The chemistry and microstructure of melanins depends on the molecular precursors, the synthesis scheme, and the local microenvironment of melanin assembly (Simon, Hong, and Peles 2008; Blois 1978; Morris-Jones et al. 2005). The structure and composition of natural melanins found in living organisms is much different than that of synthetic melanins that are assembled *in vitro* (Zecca et al. 2008; Seraglia et al. 1993). The chemical and structural variations impact the function of the material. Natural melanins are composed of homogeneous nanoparticles with porphyrin structures.

Synthetic melanins consist of heterogeneous microparticles without mesoscale features (Nicolaus, Piattelli, and Fattorusso 1964; Ito 1986; Zajac et al. 1994). Synthetic melanins are composed of heterogeneous aggregates with a large size distribution. Furthermore, synthetic melanins exhibit topological disparities compared to natural melanins. Understanding the process by which living organisms produce melanin could lead to new *in vitro* synthetic schemes to create melanins and other natural biopolymers in a highly scalable manner. Primary knowledge in this arena could transform melanin from a highly specialized pigment into a commodity chemical with impactful technological applications. Generating porphyrin-based structures in a reliable manner could lead to the scalable synthesis of other molecules with unique catalytic activity.

There are many groups within the United States, Europe, and Asia that actively research many topics related to biomimetic synthesis of melanin pigments. Select groups are highlighted here. The laboratory of Prof. Vincent Ball of the Université de Strasbourg and Unité Mixte de Recherche, Strasbourg, France, actively study many aspects of biomimetic *in vitro* melanogenesis. Prof. Jin-Kyu Lee also studies many aspects of melanin nanomaterials within the research group at the Department of Chemistry within the Bio-MAX Institute of Seoul National University. The efforts of these groups and many others throughout the world seek to understand how melanin pigments can be manufactured in a scalable manner using biomimetic processes. Primary knowledge from this transformative research can potentially translate melanin into a therapeutic biomaterial for applications in tissue engineering, drug delivery, imaging, and beyond.

Microscale Biomimetic Strategies for Reliable Cell-Based Manufacturing

Tools and technologies for *in vitro* sensing and stimulation of cultured cells represent an important strategy for devising reliable manufacturing strategies for cell-based therapies. *In situ* genotyping and real time measurements of soluble factor is challenging because of the latency between sampling and analysis. Functional assays and physiological metrics are viable alternatives to traditional biochemical analysis. Functional assays are advantageous because they can provide real-time data with facile parallelization and integration with large-scale cell production facilities. Select examples of worldwide research activities in this arena are summarized here.

In Vitro Microfabricated Electrochemical Sensor Arrays

Electrophysiology of excitable tissues such as neurons, cardiac cells, and beta-islet cells can be characterized using microelectronic devices that are optimized for this specific biosensing application. For example, substrates with silicon-based sensors operating in aqueous environments can resolve ionic currents on the order of ~1 nA or smaller. Planar biosensors integrated into circuits can detect ionic currents that originate from single unit action potentials in depolarized neurons that are cultured on the top surface of these devices (Franks et al. 2005). Microfabricated biosensors can be multiplexed into two-dimensional sensor arrays for real-time mapping of action potential propagation down axons of isolated neurons (Figure 5.3). The active sensing component is a conductive microstructure composed of bioinert metals coated with a material to reduce the interfacial impedance. Materials such as conducting polymers or platinum black promote efficient charge transfer between ionic current in cells and the electronic current that is processed in back-end hardware. Hybrid electronic-ionic conductors can serve as biomimetic interface materials that bridge abiotic and biotic matter. Multiplexed microfabricated biosensor arrays can resolve the spatiotemporal coordinates of a propagating action potential along the axon of a single neuron and the dynamics of synaptic junctions (Hierlemann et al. 2011). Electrode arrays can also be used for spatiotemporal control of neuronal stimulation. Intriguing *in vitro* experiments can be performed in which depolarization events can be mapped and measured as a function of the stimulation location.

There are many challenges that must be addressed with this technology. First, there are potential complications associated with multiplexing hundreds of sensors within a confined working volume. Furthermore, reliable microelectronic devices must operate reliably in environments with elevated temperatures, increased hydration, and potentially corrosive electrolytes.

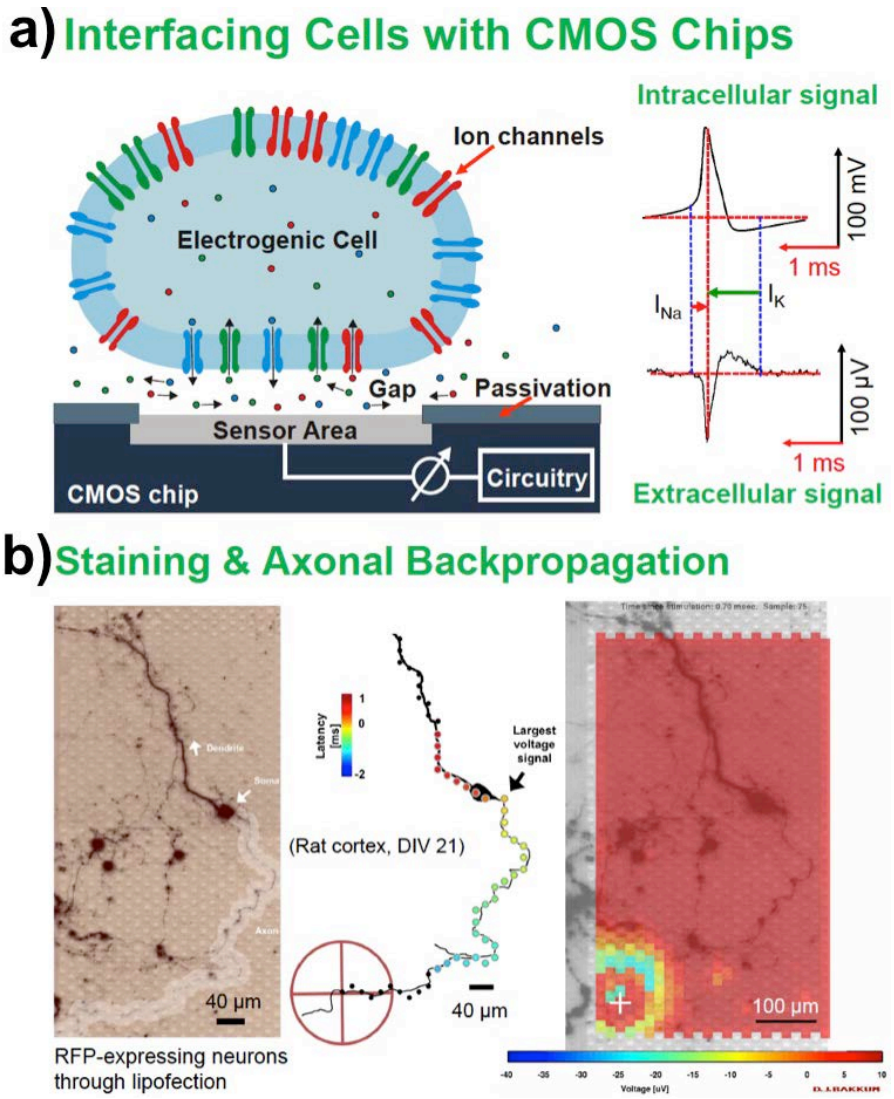

Figure 5.3. Using microfabricated sensor arrays to measure field potentials of neurons (adapted from Hierlemann et al. 2011).

Multiplexed microfabricated sensors can measure extracellular ionic currents in neurons with subcellular spatiotemporal precision. These devices have the potential to track the depolarization of axons in excited neurons. Biomimetic sensor arrays could be used as tools to measure *in situ* physiology of excitable cells and serve as a functional assay for tissue structures.

Second, neuron signals produce ionic signals in the form of field potentials on the order of 10-100 µV. These signals are potentially challenging to resolve and require backend hardware to process the physiological signals into meaningful data. Taken together, microfabricated electronic devices permit *in situ* functional characterization of neuronal networks in real time. This capability could be leveraged as a rapid and distributed sensing platform during the cultivation of tissue constructs. Sensor networks may be used with other excitable types of excitable tissue, including cardiac cells, muscle, cells, and even beta-islet cells derived from the pancreas. Non-neuronal phenotypes produce signals that arise from field potentials that can exceed 100 µV, which may be easier to resolve compared to field potentials that arise from neurons.

In vitro sensor arrays can be used in a wide range of applications in regenerative medicine and biomanufacturing. Perhaps the most likely application of multiplexed sensor arrays is to assess organotypic functionality via functional assays. Genetic analysis and biochemical assays can be

costly, time-consuming, and stochastic when assessing the function of cells, tissues, and organ constructs *in vitro*. Electronic sensing arrays that can quantify cellular function rapidly offer the promise of expedited screening with more relevant outcomes.

For example, measuring synchronized depolarization of cardiac tissue using 2D sensor arrays could provide a functional biomarker for the presence of tight junctions in cardiomyocytes. Furthermore, measuring the frequency and amplitude of depolarization events in beta-islet cells may provide insight into glucose sensitivity. Both of these scenarios permit phenotypic characterization without the need for expensive reagents to measure soluble factors or destructive characterization techniques. Of course, one challenge in this approach is the continuous validation in which physiological outcomes can be consistently mapped to genotypic and phenotypic benchmarks. Regular genotyping and analysis of markers would be important components of GMP for the robust manufacturing of tissues and organs. The most likely application of these kinds of sensor arrays would be as a tool to assess the organotypic response of a construct to a specific chemical, electrical, or mechanical microenvironment. For example, sensors that can measure cardiac function may be used to assess *in vitro* cytotoxicity profiles of prospective drugs. The key advantage of this approach is that electrical biosensors can perform sensing operations in a rapid, distributed, precisely quantitative manner. Quantifying protein-based markers or secreted soluble factors requires some combination of optical microscopy, flow cytometry, ELISA, or other boutique-based technique purification methods prior to obtaining a reliable assessment of the marker of interest. In contrast to direct electrical characterization of cell function, many of the aforementioned analysis techniques are difficult to perform in parallel.

Organic Ionics for In Vitro and In Vivo Biosensors

Bridging the biotic–abiotic interface in an important challenge in assessing the functionality of tissue constructs. Microfabricated electronics and living cells exhibit several fundamental incompatibilities in terms of both physical properties and communication medium. Perhaps the most obvious challenge is exchanging signals based on electron flow in synthetic devices with signals based on ion flow in cells. Faradaic reactions at the interface generate an impedance element that reduces the sensitivity of *in vitro* sensors. Recent advances in conducting polymers can exchange ionic signals with electronic signals and can reduce the overall impedance of these devices. Conducting polymers are useful for other types of biomimetic communication. They can be fabricated into ionic devices that can control the flow of charged solutes in aqueous environments.

The Organic Bioelectronics Laboratory at Linköping University, Sweden, led by Prof. Magnus Berggren, is a pioneer in this concept. This group has developed a suite of devices in which conducting polymers with complementary charges can be assembled into structures that are analogous to silicon-based logic elements (Figure 5.4; Tybrandt, Forchheimer, and Berggren 2012). In one representative example, oxidized poly(3,4-ethylenedioxythiophene) (PEDOT) is over-oxidized using poly(styrene sulfonate) (PSS) to form PEDOT:PSS blends with a net negative charge. The immobilized anionic network can therefore transport mobile cations in a field-dependent manner. Complementary cationic networks are composed of poly(vinylbenzylchloride) that have been cross-linked and quarternized (q-PVBC) to form quarternary amines, which are positively charged in aqueous environments at physiological conditions. Cross-linked q-PVBC is therefore an immobilized cationic network that can transport mobile anions. PEDOT:PSS and q-PVBC are therefore complementary materials that can be fabricated into unique microstructures. A three-terminal device can be constructed in which a reservoir of anionic fluorescent solute is set as the emitter. A positive bias is applied between the base and emitter (V_{EB}) to generate a flux of this solute. Conversely, a bias of $V_{EB} < 1V$ restricts solute flow. The operation principles of this device have been leveraged as an *in vivo* controlled release system. PEDOT reservoirs can be doped with PSS and processed into microstructures that serve as systems to deliver charged small molecule neurotransmitters via electrophoretic transport. Neurotransmitters used in this study include glutamate, aspartic acid, and γ-amino butyric acid (Figure 5.5; Simon et al. 2009).

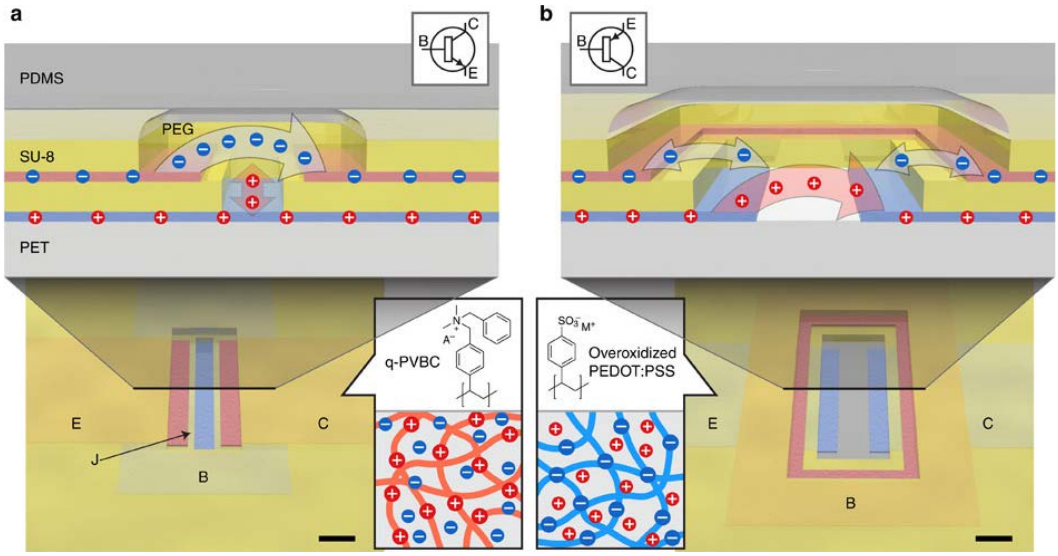

Figure 5.4. Design of organic ionic transistors (from Tybrandt, Forchheimer, and Berggren 2012).

Cationic and anionic polymeric networks can modulate the flow of anionic and cationic small molecules, respectively. The flow of both cations and anions from the emitter to the collector is modulated by the injection of oppositely charged ions from the base into the channel. This principle can bias ionic flow use microfabricated structures that resemble logic elements in traditional microelectronics. (*a*) q-PVBC and (*b*) PEDOT:PSS polymers serve as the emitter and collector in these microstructures.

The general device structure is composed of the following components: (1) electrolyte source, (2) electrolyte target, (3) anode, and (4) cathode. These components are fabricated into a variety of geometries and packaged using several widely accepted encapsulation strategies. However, the mechanism for delivery of neurotransmitters through the device is conserved. These devices can produce zero-order release kinetics of small molecule neurotransmitters, the rate of which depends upon the magnitude of the applied voltage along with the mass and net charge of the molecule. The *in vitro* delivery of bioactive small molecule neurotransmitters can be verified by intracellular calcium recordings. In addition to serving as an *in vitro* model, this technology can be potentially utilized as an implantable therapeutic device. *In vivo* delivery of glutamate, a small molecular neurotransmitter, was verified by measuring the auditory brainstem response in the cochlea. Device-based therapies that use electroactive controlled release are particularly attractive because the rate of therapeutic release can be precisely controlled through electrochemical methods. These materials may also be compatible with previously established remote-controlled release implants (Santini, Cima, and Langer 1999). Implants with glutamate reservoirs deliver this payload to the round window membrane of a guinea pig animal subject. Organic ionics represents an exciting new direction in interfacing excitable tissue with synthetic devices.

Advances in conducting polymers and microfabrication permit robust signal transduction mechanisms between electrons, ions, and soluble factors. Closed-loop devices could measure an external voltage from an excitable cell and convert this into an electronic signal that can be processed and analyzed. This signal could further be transduced into spatiotemporal controlled delivery of small molecules for local control of cell fate. There are many potential barriers to the adoption of such as complex system in biomanufacturing of cell-based therapies. First, although microfabrication and multiplexing are straightforward in principle, the parallelization of these devices for large-scale cell culture is challenging. Furthermore, economic pressures may prohibit the use of sophisticated microfabricated devices as tools for quality control during the production of cell-based products. Rather, one may anticipate that these devices would provide utility as a discovery tool to understand how the precise spatiotemporal control of small molecular concentrations can influence cell fate.

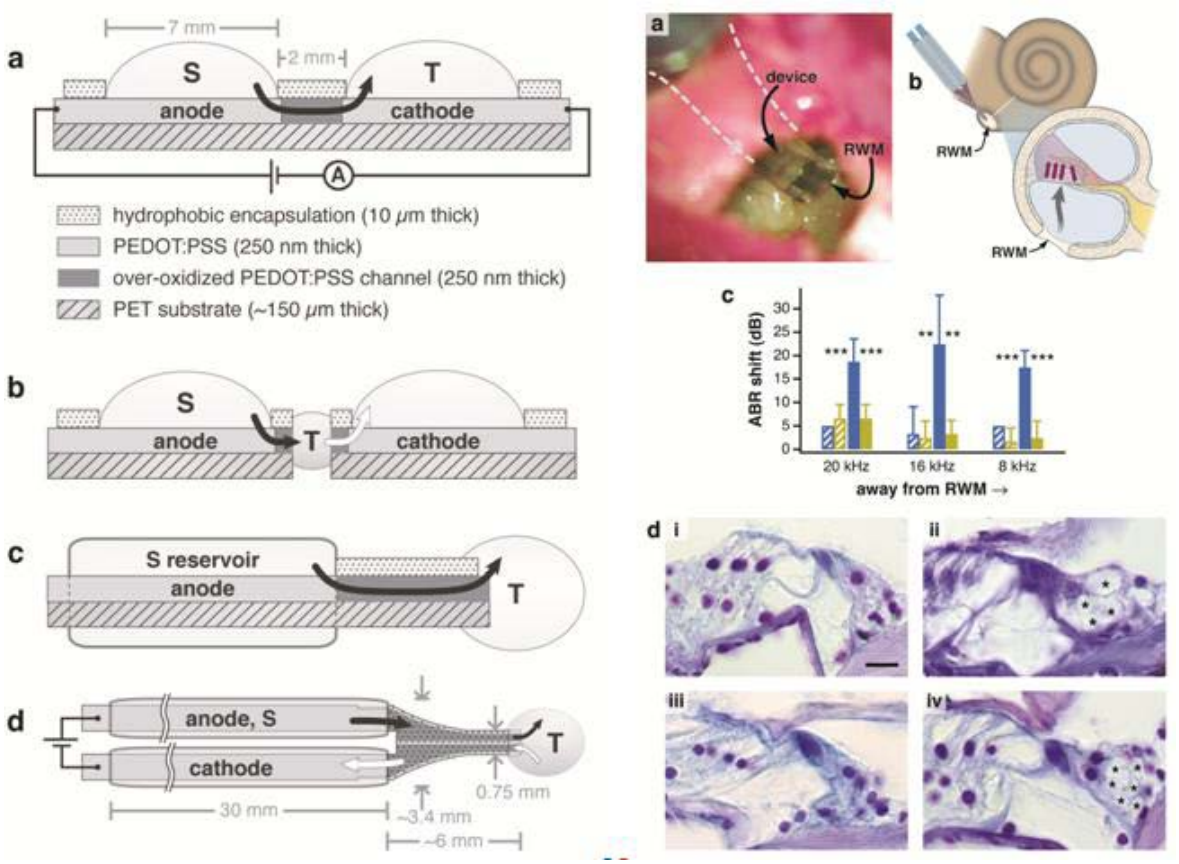

Figure 5.5. Microfabricated organic electronic device for delivery of neurotransmitters to modulate mammalian sensory function *in vivo* (from Simon et al. 2009).

Side view of the planar device used in small molecule neurotransmitter transport studies (*left, a*). The black arrow indicates the flow of neurotransmitters from the source, S, through the anode and over-oxidized channels, and finally out into the target electrolyte, T. Side view showing various configurations in which the white arrow indicates the flow of cations from T to the cathodic electrolyte (*left, b*). Side view of the encapsulated device shown with the arrow indicating ion flow (*left, c*). Top view of the encapsulated device, showing both electrolyte chambers and the target, T (*left, d*). The electrolyte reservoir tubes are 2 mm in outer diameter.

In vivo validation of the device (*right*). Briefly, the device is placed on the round window membrane of a guinea pig. The electronically activated release of neurotransmitters produces a detectable shift in the auditory brainstem response (*right, c)*. Local release of neurotransmitters produces an excitotic-induced damage to auditory dendrites (*d, right)*, indicated by stars (*d ii, iv*) compared to controls (*d i, iii*).

Biomimetic Electronic Materials for Rehabilitation

Principles of Brain–Machine Interfaces

Biomimetic structures to bridge the abiotic–biotic interface can be leveraged in other technologies to augment and restore organ function that is lost due to disease or injury. Engineering devices with increased biocompatibility could empower numerous clinically relevant diagnostic and rehabilitation technologies including brain–machine interfaces (BMI) (Schwartz et al. 2006), retinal prostheses (Chader, Weiland, and Humayun 2009), peripheral nerve regeneration, and real-time cardiac monitoring (Viventi et al. 2010). Other potential applications include improved electrode materials for vagus nerve stimulation and systems to measure electrophysiological performance of *in vitro* tissue models. Biomimetic *in vivo* device interfaces serve as a key

foundation for electrical communication by providing a high-fidelity channel for electronic communication between synthetic devices and excitable tissue. Stable electronically active biotic–abiotic interfaces are essential components of implantable devices that are intended to sense, monitor, and stimulate tissues for applications in regenerative medicine.

Implantable biosensors are an emerging technology in which electrical signals generated by excitable tissue are recorded and analyzed to map electrophysiology to organ function. One clinical application of this strategy is brain–machine interfaces (BMI) in which cortical electrodes record neuronal activity in the motor cortex. Patterns of neural activity are then mapped and used to predict patient intent, which is then translated into specific robotic operations. BMI may link external devices with the peripheral nervous system as well. Peripheral neural interfaces (PNI) represent a minimally invasive strategy to connect external electronic devices with the nervous system. PNI have applications in fine motor control of lower arm prostheses. PNI also have the potential to discover and administer transformative therapies based on targeted electrical stimulation of the peripheral nervous system. This concept, termed "bioelectroceuticals," posits that neurological and inflammatory pathologies can be ameliorated through electrical excitation. Bioelectroceuticals and other exciting possibilities have been tempered by technical challenges associated with designing PNI and interfacing them with tissue to achieve clinically relevant outcomes.

There is much worldwide activity in designing new materials and devices to bridge the abiotic–biotic interface in BMI and PNI. The current state of the art includes a variety of geometries including epineural and intrafascicular electrode arrays. Despite the varying degrees of success of these strategies, the reliability and long-term performance of these devices is unpredictable. There are two primary failure modes: electronics failure or deterioration in device performance from histological responses. The latter usually results from the use of materials that are optimized for fabrication and intrinsic electronic performance without in-depth consideration of tissue–materials interactions. The design of many synthetic devices is predicated on microfabrication strategies that were pioneered in the 1990s. Silicon-based devices are rigid, brittle, and planar. Silicon and other noble metals used in the microfabrication industry exhibit three properties that render them ill-suited for intimate communication with neurons. First, silicon and noble metals are electron/hole conductors, whereas neurons and other excitable tissues communicate using ions. Second, silicon has a Young's modulus of E_{Si} = 70 GPa, which is 7 orders of magnitude stiffer than brain matter, a linear elastic hydrogel with a storage modulus of G' ~1 kPa (Figure 5.6). Taken together, silicon and other inorganic materials are convenient for BMI/PNI device fabrication, but the reliability could be improved by engineering the impact of tissue–biomaterials interactions.

Flexible Electronics as Biomimetic Materials for Brain–Machine Interfaces

Flexible electronics represents a potential tool to overcome the challenges associated with incompatibilities between synthetic materials and natural tissues. The laboratory for neuroprosthetics at École Polytechnique Fédérale de Lausanne (EPFL) is a world leader in advancing the field of biosensors for monitoring neurological function. This center has made significant contributions in this field by combining expertise in clinical neuroscience, cognitive behavior, and device fabrication. Specifically, the laboratory of Prof. Stephanie Lacour has made several important advances in flexible electronics for the integration of electronic devices with tissue. Prof. Lacour and her team address these challenges at multiple levels of integration. New polymers are continuously being synthesized and evaluated for use as substrate and encapsulation materials in flexible electronic devices. These classes of materials include flexible plastics, elastomers, and gels. New electronically active materials include thin films, nanowire networks, and liquid metals. These materials are being processed using various strategies, including microelectromechanical (MEMS)-based fabrication techniques, low-temperature processing, and dry patterning methods. Finally, the electromechanical properties of these devices are being evaluated both *in vitro* (integration with primary neuronal culture) and *in vivo*.

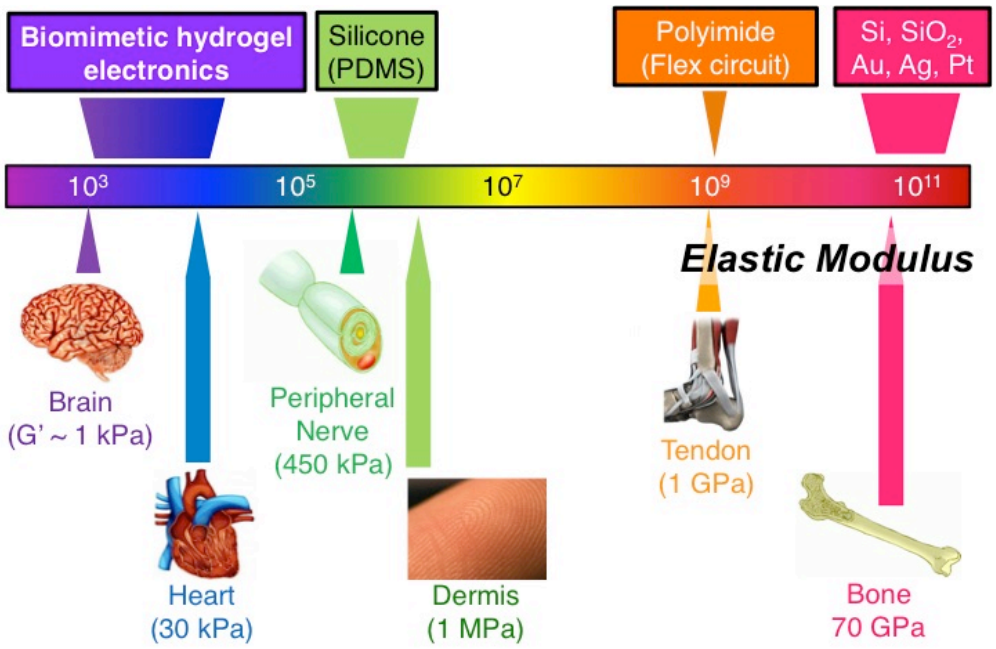

Figure 5.6. The range of mechanical properties between tissues and electronic materials (courtesy of C. Bettinger).

The range of mechanical properties (compressive/tensile Young's modulus; storage modulus; given in Pa) for excitable tissue does not align well with existing materials that are often employed in semiconductor manufacturing. Therefore, a new class of ultracompliant electronically functional biomimetic materials is required to interface soft tissues with synthetic devices. These materials may include conducting hydrogels and electronics.

Prof. Lacour has engineered devices with potential applications as *in vivo* sensors to measure and modulate organ function. These structures serve as a critical enabling technology for other therapeutic and rehabilitation strategies such as electroceuticals. These devices could be implanted in a simple procedure, permit high-density information transfer, and maintain functionality for many years by mitigating the mechanical mismatch at the tissue–device interface. The tissue–electrode interface plays a critical role in the stability of many devices designed to modulate the neuromuscular system. There have been substantial advances in electrode materials, including the use of biocompatible low impedance materials (iridium oxide (IrO_2); carbon nanomaterials (e.g., carbon nanotubes, grapheme, and carbon black); and conducting polymers (e.g., polypyrrole, PEDOT). Chronic electrode stability remains elusive due to issues such as corrosion and isolation from excessive fibrous capsule formation. For example, invasive biosensors incorporated into implantable devices produce local inflammatory responses, which reduce signal-to-noise ratios and increase charge injection requirements. There is renewed interest in engineering tissue–materials interactions in the context of improving the biotic–abiotic, tissue–device interface. Strategies include reducing the onset of inflammation via controlled release of therapeutics, electrode surface modification using bioactive molecules, and reduced device dimensions as previously described. These approaches, which are partially effective, are not able to fully address the fundamental issues of the monocyte/macrophage-mediated foreign body response. This realization has spawned the next-generation of biomimetic electrode materials that can obviate failure modes associated with these inevitable interactions.

Polymer and Hydrogel Electronics

Materials systems with both ultralow mechanical stiffness and low electrical impedance are elusive, yet critical to engineering chronically stable biotic–abiotic interfaces for tissue-level device integration. Currently available flexible conductors for use in biotic–abiotic interfaces use

ubiquitous engineering elastomers as bulk materials and then devise methods to embed conductive structures. This overarching strategy produces elastomeric devices (E ~ 0.1–1 MPa). However, the elastic modulus of tissue in the brain and the retina, two key targets of interest for interface technology, can be at least three orders of magnitude smaller than elastomeric conductors. The resulting modulus mismatch contributes significantly to the failure of chronic BMI. Low modulus hydrogels play a key role in currently available biotic–abiotic interfaces by serving as coatings, immunoisoloation barriers, and reservoirs for drug delivery. Conducting hydrogels offer a potential material framework to match the mechanical properties of devices with soft tissues. The laboratory of Prof. Gordon Wallace at the University of Wollongong in Australia has pioneered many advanced materials for these applications. His group works extensively at the interface of organic conductors and nanomaterials for improving the functionality of polymeric systems. Hydrogel matrices based on aliphatic backbones can be functionalized with conducting polymers via the *in situ* synthesis of conducting polymers such as PEDOT and polypyrrole (PPy). Other leading researchers in this area include Prof. Marc in het Panhuis, also of the University of Wollongong. Prof. Panhuis has pioneered many advances in biomimetic ultra-compliant conducting hydrogel-based materials for a variety of biomedical applications (Higgins et al. 2011).

Biomimetic hydrogel materials for biointerfaces have also been developed by the laboratory of Prof. Matsuhiko Nishizawa at the Tohoku University Department of Bioengineering and Robotics in Sendai, Japan. In one demonstration, two-dimensional microfabricated PEDOT structures are integrated into agarose matrices (Sekine et al. 2010). First, PEDOT is polymerized into hydrated agarose networks. Next the composite structure is removed through electrochemical-actuation-assisted composite delamination. The electrodes exhibit a resistivity of 11 k-Ohm-square-1, which is comparable to previously reported values in PEDOT films. Furthermore, printed electrodes can stimulate the contraction of hydrogel networks seeded with C2C12 myotubes. Electrical stimulation produces simultaneous contraction of myotubes.

There are many promising biomimetic materials design strategies that can improve the fidelity of *in vivo* biotic–abiotic interfaces. Tissue–device interfaces based on well-defined nanomaterials can improve sensing and modulation of excitable tissue at many length scales, including the cellular length scale.

Biomimetic Strategies for Organ-Scale Therapies

There are many exciting new directions that leverage biomimetic strategies for tissue and organ scale replacement. This section is not intended to be a comprehensive summary of worldwide activities. Rather, select examples for host laboratories that were visited by the panel will be highlighted. These examples are chosen because they demonstrate overarching principles that can be used in many different strategies for tissue regeneration and restoration.

Acellular Organ Replacement Strategies

The emergence of three-dimensional printing (3DP) has catalyzed a new era in the design of patient-specific implants. Orthopedic devices such as artificial hips and knees have been manufactured using a limited number of geometries. This strategy is suitable for many standardized procedures such as total artificial hip and total artificial knee replacements. However, there are many examples of morbidity and trauma where standard implant geometries are poorly suited. Rather, patient-specific geometries are more appropriate for orthopedic implants in these situations. 3DP is a manufacturing strategy that is ideal for implants with arbitrarily complex geometries. 3DP of orthopedic devices requires the integration of imaging, segmentation, and materials fabrication (Figure 5.7). The process flow begins with imaging the defect area and rendering of an implant geometry that can best restore function. The device geometry is sectioned into 2D slices that provide a template for layer-by-layer fabrication of the device. This general strategy is widely employed in 3DP of many polymeric materials. However, 3DP of orthopedic implants require unique materials processing for implant materials such as titanium.

The research group led by Professors Kerong Dai and Liao Wang in the Department of Orthopedics at Shanghai Jiao Tong University in Shanghai, China, uses an additive manufacturing technique for processing of metals based on sintering of metal powers. Electron beam welding (EBW) selectively melts metal powders into layers with thicknesses of 70–250 µm. EBW has been used for various implant alloys including Co-Cr and Ti-6Al-4V (Harrysson et al. 2008). The general process begins by acquiring CT data from the patient. A 3D rendering of the bone structure at the defect site is generated using imaging software. This image is then used to create a hypothetical implant with a pre-specified geometry. The 3D implant geometry is rendered and segmented into a series of 2D slices that produce the final structure when assembled. This workflow is essentially constant for the production of any 3D object composed of nonviable material using additive manufacturing.

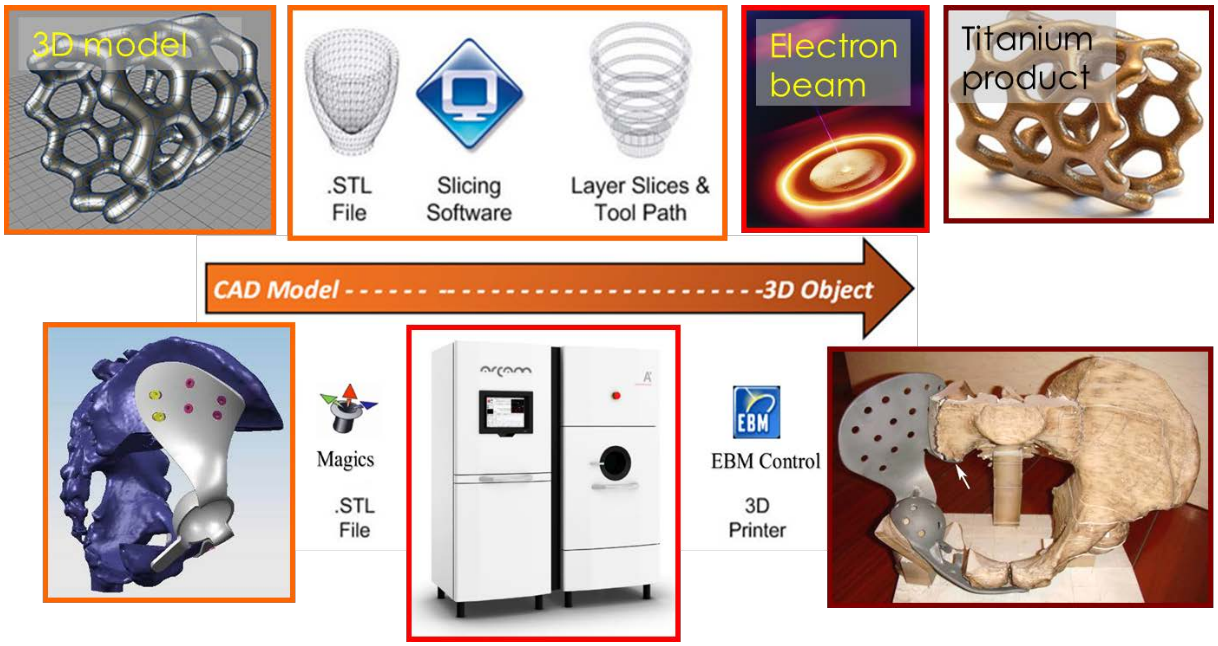

Figure 5.7. 3D printing of patient-specific implants (courtesy of Liao Wang; adapted from the slide presentation to the WTEC panel).

Patient-specific orthopedic implants are fabricated from additive manufacturing of biocompatible metals. Electron beam welding is a strategy to fabricate custom implant geometries with robust mechanical properties. This process involves the following sequence: imaging the defect site, rendering the desired implant geometry, segmentation into 2D slices, and fabrication using EBW.

Additive Biomanufacturing of Cell-Based Therapies

There are dozens if not hundreds of laboratories and start-up companies that are interested in leveraging 3DP and microfabrication for the production of tissue constructs for a wide range of applications. This section will briefly highlight some overarching principles of these activities. More in-depth technical reviews have been previously published elsewhere (Norotte et al. 2009, Murphy and Atala 2014).

Stochastic Tissue Assembly. Stochastic tissue assembly refers to the spontaneous assembly of tissue structures from cellular building blocks that are assembled without prescribed placement of cells during manufacturing. The composition of cellular precursor solutions ultimately determines the makeup of the final tissue structure. However, the spatial distribution of specific cells is a function of the emergent behavior of the system. One classic example of *in vitro* stochastic tissue assembly is the preparation of cell clusters using the hanging drop cell culture method. In this approach, a suspension of cells is cultured in a droplet ~10 µL in volume in an inverted format. Homogeneous or heterogeneous cell suspensions spontaneously form 3D organoids. 3D cell culture produces

organotypic function that cannot be easily produced in 2D culture systems. This approach has been scaled into large-format cell culture strategies due in part to the efforts of many companies including InSphero, a company spin off from the D-BSSE in Basel, Switzerland. InSphero is interested in the use of scalable microfluidics and microfabrication techniques for *in vitro* tissue assembly. The central line of products focuses on the fabricating microtissues in a scalable 96-well-plate format. This fabrication strategy has been applied to a range of phenotypes including hepatocytes, pancreatic microislet, and carcinomas. The key advantages of using the cell assembly technology pioneered by InSphero and others are extended timelines for viability (oftentimes > 4 weeks) and organotypic cytoarchitecture. Supporting analytical hardware and assays enhances the value of the cell culture array technology. For example, InSphero features a high-throughput imaging system that permits rapid label-free, reagent-free, bright-field microscopy of 3D cell culture constructs. InSphero has also integrated assays to quantify the amount of ATP with constructs via luminescence. This capability allows for rapid quantification of cell metabolism. High-throughput optical hardware and assays permit quantification of cell behavior for many applications including cytotoxicity screening and drug efficacy studies.

Decellularized tissue represents another strategy for stochastic tissue assembly. In this approach, an intact organ of interest is incubated in a series of detergents and other preservatives to remove all of the cells. What remains is the extracellular matrix of the organ. The organized extracellular matrix can then be perfused with healthy cells that self-assemble into a functioning organ. Strategies for tissue engineering using decellularized matrices have been applied to a wide range of both vascularized and avascular organ systems including skin, cardiac tissue, and functional arteries (Hoshiba et al. 2010). Much of the active research in decellularized tissue scaffolds is focused on optimizing cell removal procedures and identifying the appropriate cocktails of cell phenotypes during reperfusion.

Deterministic Tissue Assembly. The fabrication strategy utilized by InSphero and others is an example of stochastic assembly of cell constructs. One alternative to stochastic cellular assembly is deterministic cellular assembly. This technique is more closely aligned with additive manufacturing of materials. The key distinction in deterministic cell-based constructs is the use of precursor materials that contain viable cells. 3D printing of cells reduces the rate of fabrication, but increases the precision. Therefore, the most likely use cases for products created by cell printing are orthogonal to stochastic cell assembly.

Research in 3D cell printing is performed in dozens of prominent laboratories throughout the world. Prof. Pranav Soman of Syracuse University is pioneering novel methods for optical-based fabrication techniques of cell-seeded constructs (Figure 5.8). One leader in this field is the Tsinghua University Biomanufacturing Research Center in Beijing, China, led by Prof. Wei Sun. This research center is focused on many aspects of deterministic fabrication of 3D tissue constructs. The primary theme that unites most of the research in this area is biomanufacturing. Researchers within Tsinghua who are active in this area define biomanufacturing as follows:

> Biomanufacturing is an emerging interdisciplinary paradigm in which living cells, biologics, proteins, and biomaterials are used as basic building blocks for fabrication of *in vitro* biological structures and cellular systems with application to biology, tissue engineering, disease pathogeneses study, drug test and discovery, and cell/tissue /organ-on-a-chip devices.

The research directions currently explored by the Tsinghua Biomanufacturing Research Center perhaps best capture the philosophy of this nascent field.

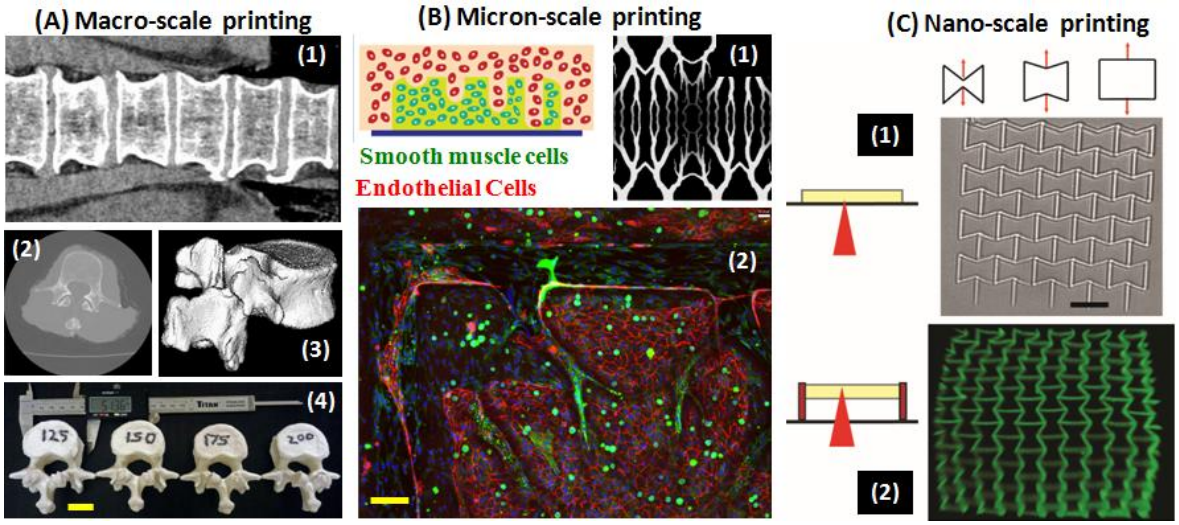

Figure 5.8. Biomanufacturing at the macro-, micron-, and nanoscales.

(*A*) Macroscale printing of human lumbar vertebra (*1*: CT image, *2*: Cross-section of image, *3*: Reconstruction of geometry using set of binary images, and *4*: Printed replica-models in PLA biomaterial using fused deposition modelling). (*B*) Micron-scale printing of multiple cells within gelatin-biomaterial (*1*: Digital mask of vasculature-like structure and *2*: Co-encapsulation of green smooth muscle and red endothelial cells within vasculature geometry, using digital projection stereolithography). (*C*) Sub-micron/nano-scale printing of negative Poisson's ratio structures using the femtosecond laser direct-writing process: Re-entrant honeycomb geometry structure using polyethylene glycol (PEGDA) hydrogel (*1*) on glass support and (*2*) suspended between side supports. Scale bar: *A*: 10 mm, *B*: 0.1 mm, *C*: 0.01 mm. (A 1,2, B 1,2, and C 1,2 courtesy of Pranav Soman, Syracuse University; A 3,4 from Ogden et al. 2015).

Ongoing research activities include, but are not limited to, the following:

- Bioprinting of heterogeneous tissue constructs and *in vitro* cellular function study
- 3D printing of cells and proteins for cell micro-environment reconstruction with application to regulation of stem cell and induced pluripotent stem cell function
- Encoded biological model: design, 3D printing, and *in vitro* reconstruction of biological function
- 3D printing of *in vitro* tumor models, and tumorigenesis characterization
- Precisely controlled cell assembly for construction of three-dimensional *in vitro* biological models
- Investigating the mechanisms of three-dimensional structural formation of assembly of tissue/organ constructs with biomaterial scaffolds
- Computer-aided tissue engineering and design
- Design and manufacture of personalized tissue scaffolds and implants
- Printing *in vitro* cell models for 3D biology, pathology, and pharmacology study
- Integration of bio-, micro- and nanofabrication technology
- Novel 3D cell printing process and equipment development
- 3D cell printing for cell/tissue/micro-organ-on-a-chip and advanced medical diagnostic devices

Many of these multidisciplinary research activities focus on engineering novel tools, techniques, and methods to achieve the following: improved integration of cell-based constructs; advanced materials processing capabilities; and design, invention, and fabrication of new instrumentation for 3DP. There is also active research in these arenas from dozens of groups through the Europe, Asia, and the United States.

One important focus of the group led by Prof. Sun at Tsinghua University is the creation of heterogeneous cell-seeded constructs in terms of both extracellular matrix composition and cell phenotype. This group has invented several novel instrumentation elements and processing techniques to overcome the intrinsic limitations of 3D cell printing. For example, rapid cell printing is required to address the forthcoming economic challenges of production scale-up. Reducing the nozzle sizes may also be desirable to increase the spatial resolution during deposition. Increasing the flow rate and reducing the nozzle diameter can increase the hydrodynamic forces on the deposited cells, which can ultimately reduce viability. Prof. Sun and coworkers have addressed this challenge by inventing a novel technique for generating cell droplets called alternating viscous and inertial force (AVIF). The principle of AVIF involves movement of a printer head that contains an aqueous ink reservoir with a cell suspension. The hydrodynamic forces of the cells during deposition could be modeled as a function of the movement of the printer head. A quadratic waveform achieves the maximum drive efficiency and reduces the damage of hydrodynamic forces to cells leading to the overall increase in viability of printed cell populations.

Other active research areas focus on the printing of multiple cell types and biomaterials compositions. This challenge is being addressed by designing new types of hardware and responsive hydrogel materials (Snyder et al. 2014). Multiplexed printer heads allow co-deposition of multiple materials and/or cells within close proximity to one another. This challenge can be addressed through hardware miniaturization and application of microfluidics technology. The co-deposition of materials is challenging because of the need for coordinated deposition and cross-linking within short time scales. Thermally responsive polymers are suitable materials for these strategies. The general approach is to deposit water-soluble biopolymers that exhibit an upper critical solution temperature (UCST) that is close to physiological temperatures ($T_{gel} = 32 \pm 5$ °C). The reservoir of biopolymer solution can be printed at temperatures of $T_{print} = T_{gel} - 5$ °C into a matrix environment that is maintained at $T_{matrix} = T_{gel} + 5$ °C to initiate gelation. This process is suitable for any polymer that exhibits a UCST near physiological temperature such as collagen (gelatin), matrigel, and poly(N-isopropylacrylamide) (PNIPAAm). This process, termed LDM (low-temperature deposition manufacturing) by Prof. Sun and coworkers, has been optimized for 3DP of porous scaffolds. Other groups have worked on 3DP of extracellular matrix proteins that have been isolated from decellularized organs (Pati et al. 2014).

Emerging Directions in Deterministic Cell Assembly. There are many important unanswered questions in the application of 3DP as a biomanufacturing strategy to produce tissue constructs:

- What is the minimum feature resolution that is desired or required for *in vitro* organotypic models versus therapeutic tissue constructs?
- How do the properties of viable cells complicate biomanufacturing strategies? For example, can mechanical deformation, polarization, or migration of cells be predicted or directed prior to construct assembly?
- What tradeoffs between precision and manufacturing speed are appropriate for cell-based constructs?

Many of these questions are sure to be answered in the coming years as research activity in biomanufacturing continues to grow.

CURRENT CHALLENGES IN BIOMIMETIC MANUFACTURING

Addressing Complexity in Biomimetic Manufacturing

One critical challenge that faces biomanufacturing is harmonizing the inherent complexity and noise of biological systems with precision and reliability that is requisite for manufacturing products in a reliable manner. Addressing this challenge requires a proper definition of the frame of reference and the desired direction of technological progress. There are many potential philosophies to address this challenge.

Deconstructing Biological Complexity

One approach begins by identifying a relevant biological process that may be of interest for manufacturing a specific biologically derived product of interest. The process can be segmented into discrete processes that utilize specific bioactive components, starting materials, and physicochemical environments to yield the product of interest. The cell-free synthesis of DHAP is an example that embodies this philosophy. In this case, a complex multistep metabolic pathway can be engineered in an *in vivo* environment to yield a product of interest. This process can then be recapitulated in a cell-free environment that is cost-effective, scalable, and able to be monitored in real time. The design strategy begins with a biological system that is reengineered to create a synthetic construct. Another prominent example of this approach is decellularized tissue constructs. This tissue fabrication strategy starts with a complex composition of extracellular matrix proteins that are organized into constructs with spatially dependent microstructures and mechanical properties.

De Novo Assembly of Complex Systems

An alternative approach begins with identifying a complex biological structure, materials, process, or product that may provide some therapeutic benefit, for example. This strategy starts by identifying the complex target for biomimicry, followed by the integration of modular components in a serial manner. The eventual goal is to increase the complexity until the necessary level of complexity is reached, but no more. This concept is best applied to materials-based products in which bioactive components can be added in a modular manner with relatively high precision. Consider the rational design of synthetic microenvironments for controlling *in vitro* tissue morphogenesis. Two-dimensional cell culture platforms can use well-defined extracellular matrix compositions. These can be arranged into arrays and screened to identify cocktails that produce organotypic structures of interest. *De novo* assembly can also be applied in three-dimensional tissue culture platforms. Synthetic hydrogels can be modified with adhesive ligands of varying composition and spatial density. Engineering synthetic hydrogels in a modular manner can identify synergistic effects that may be otherwise obscured with more complex biomaterials. Biomaterial building blocks may be used for engineering cartilage or skin. Similar approaches may be used in the design of particles for immunomodulation, imaging, and theragnostics.

Process Integration

While deconstructing the complexity of biological systems is an important starting point for biomimetic manufacturing, integrating discrete components is equally important. There is a broad diversity of challenges to integrate biomimetic strategies into biomanufacturing. The challenges associated with systems integration are largely governed by the specific product of interest. Other kinds of biomanufacturing strategies that are unilateral in terms of length scale and functionality can be leveraged to fabricate multiple diverse products. For example, molecular-level approaches can be leveraged to produce a variety of proteins for use as structural materials, functional materials, or therapeutics. Other examples at the cellular and organ levels have been described previously. These technologies can be used to fabricate specific articles. However, the challenge in systems integration is uniting these articles together in a coherent manner in order to produce a functioning system with higher-order complexity. Systems integration will ideally start with the final product, identify the relevant technology or technologies, and then integrate them into a system that can ultimately be used to fabricate the original product in mind. Among the many challenges in this process are the following:

- Identifying cross-over points where the output of one discrete technology or process can serve as the input of another process.
- Collaboratig with stakeholders and end-users in defining appropriate metrics across molecular, cellular, and organ length scales.

Process integration, defined as the ability to connect discrete unit operations of a broader process in a tractable manner, is a key element of applying biomimetic principles to biomanufacturing.

Strategies for process integration can be inspired from those developed in other more traditional engineering disciplines. For example, inspiration for engineering complex reaction pathways in living cells can potentially be adopted from established principles in reactor design. One example of this strategy is the application of mass action kinetic modeling to understand multistep enzyme reaction networks. Similarly, systems-level design of tissue constructs may be informed by design principles within the microelectronics and aerospace industries. Examples of systems-level design include multiscale fabrication strategies for the integration of materials and cells with electronic devices including biosensors, electronic elements, and logic elements.

Gaps in Fundamental Knowledge

Another key challenge in advancing biomimetic manufacturing is related to the gaps in fundamental knowledge. These knowledge gaps arise because of the intrinsic complexity of biological systems. A comprehensive understanding of cell biology is essential for advancing many products to be created through biomanufacturing processes. However, there is very limited understanding of cell–cell interactions, inter- and intracellular communications, and paracrine/autocrine signaling. A well-defined quantitative predictive framework is essential when applying principles of engineering and manufacturing to biological systems. Furthermore, primary knowledge in this domain is necessary to permit precise control over cellular processes and confer the ability to manipulate these interactions in new and exciting ways. Many fundamental questions must be answered to address these knowledge gaps, including the following examples:

- How do cells respond to chemical, mechanical, and electrical cues? How are these physiological signals monitored, processed, and synthesized into decisions regarding cell fate? Furthermore, how are spatiotemporal variations in these cues convolved with spatially dependent dynamic cell processes? How do these cues impact autocrine and paracrine signals in the context of feedback and feed forward mechanisms? How does the complexity and noise of these signals scale with the size and heterogeneity of cell populations?
- What are the fundamental limits of knowledge about the state of a cell given currently available techniques in imaging, biochemical assays, electrophysiology, biosensing, gene sequencing, and data analysis? How might these limits evolve as the techniques are improved?
- Monitoring and managing heterogeneities both within and across cell populations is a key technical hurdle in improving the consistency and reproducibility of biomanufacturing techniques. What are the fundamental limits of genotypic and phenotypic heterogeneity in cell populations? How does the heterogeneity evolve, for better or worse, from the point of the cell source to the final product? How can cellular heterogeneity be managed in biomanufacturing processes where the cell is part of the process? How may these constraints compare and contrast to intrinsic limitations in heterogeneity of acellular products? The relevant parameters to control each of these processes will be quite different.
- What is the most cost-effective and reliable manner to direct the fate of cells during *in vitro* culture? How might these strategies compare and contrast between cell phenotype and unique culture conditions such as embryoid bodies?
- Are there any opportunities for biomanufacturing paradigms that use unique cell phenotypes (animal, plants, insects, microorganisms)? How can knowledge in one kingdom translate into scientific insights and biomimetic strategies for another? Extracting opportunities for biomanufacturing of chemical and biological products from plant, insect, and bacterial cells could be very important. Similarly, use of cells across these species for the development of cellular machines could be very useful.
- How can emergent behavior of single- and multicellular networks be predicted and potentially controlled? Is it possible to start with a relatively simple metastable cell population and bias it into formation of a much larger multicellular network with increased complexity and function? What are the fundamental limits in expanding complexity in systems with emergent behavior?

Technology Gaps in Biomanufacturing

Many of the knowledge gaps in biomanufacturing arise from the massive intrinsic complexity of biological systems. This is the key differentiator between biomanufacturing and other types of more mature manufacturing industries such as chemical production, assembly of transportation systems, and microelectronics fabrication. In addition to gaps in primary knowledge, there are also gaps in key enabling technologies. Novel technology platforms that can address these gaps in capabilities could accelerate progress in applying biomimetic strategies to biomanufacturing:

- Spatiotemporal control of cell genotypes and phenotype along with methods to bias cells fate to specific fates in a reliable manner would be a critical toolbox in engineering cell-based products and therapies. Small molecule reagent kits for reliable reprogramming would be invaluable for many biomanufacturing processes. Many private companies located throughout the world are addressing this need. One such reagent company located in the United States is Stemgent, Inc., which is based in Cambridge, Massachusetts. For example, Stemgent aims to develop kits composed of small molecules and reagents for genetic manipulation as a toolbox for reliable expansion and reprogramming of stem cells. If successful, technologies developed in the private sector could have a large impact on the application of GMP to large-scale stem cell culture for therapeutic applications.
- Biomimetic cell culture systems will need to be optimized for specific applications such as drug screening or therapeutic tissue fabrication. The approaches might be modular and could be application-specific versus core biomanufacturing modules that are applicable across many potential applications. This technology will ultimately rely on the development of new materials and vectors for genetic manipulation, small molecular signaling agents, and spatiotemporal control of the presentation of these reagents to cells cultured *in vitro*.
- The issue of process control and managing batch-to-batch variation are more important and more challenging for cellular and acellular products of biological origin compared to other types of traditional manufactured products. Similarly, the issues of cell sourcing, product stability, preservation, storage, application-specific biocompatibility, and immunogenicity are important topics that must be considered on a product-by-product basis. There must be coordinated technology platforms to address these potential roadblocks in biomanufacturing.
- The concept of predictive adaptive biomanufacturing by controlling emergent behavior is very alluring. In this scenario, a combination of the starting materials and process will govern the makeup of the final product by leveraging emergent behavior. The product may also continue remodeling in response to changing conditions. Technology platforms that can help understand the dynamics of emergent behavior are essential. Additional strategies to control and direct emergent behavior would also be of interest to the biomimetic biomanufacturing community.
- Biofabrication approaches cannot be considered high-throughput yet. Cell printing and placement, laser-based polymerization, etc., could be integrated with high-speed roll-to-roll printing and other emerging biofabrication approaches to realize new capabilities. This is also related to the balance between high-throughput and low-throughput processes for the appropriate applications.
- Technologies for characterizing and measuring various physical and chemical properties for cell–cell communications and cell-matrix interactions need to be developed. Data acquisition techniques such as high-resolution fluorescent imaging, novel chemical probes, noninvasive sensing, and nondestructive interrogation of cell-seeded constructs are potential cornerstone technologies. These applied systems will ultimately rely on novel imaging agents, spectroscopy/microscopy tools, and biosensor development. Data acquisition systems can be merged with advances in computational methods to gain insight into the spatiotemporal evolution of cell behavior in four dimensions.
- Vascularization of therapeutic tissue constructs is an important aspect of engineering cell-based therapies for organ replacement. There are many emerging strategies for creating vascularized networks, including controlled release of growth factors from scaffolds, bioprinting of vascular structures, and decellularization of vascularized organs. Some combination of these strategies

will ultimately be used in the creation of vascularized cell-based therapies for use in organ replacement therapies.

Nontechnical Challenges

There are numerous nontechnical gaps that need to be addressed through collaborative partnerships across government, academia, and industrial representatives. A variety of nontechnical and regulatory issues and barriers need to be addressed for increasing the impact and pervasiveness of the regulatory barriers. These include the following topics:

- Establishing standards for characterizing cell phenotype and genotype. It is important for manufacturers to agree on standards for specific types of cells. For example, what mosaic of genetic information and protein expression should be assigned to specific cell types? Is it possible to set a range of values that are acceptable and is it feasible to measure these parameters in cell populations that are processed on a production scale? Additionally, it is possible to identify standardize methods for cell culture, transfection, small molecule delivery, and assays?
- It may also be important to establish a common technical language when working in the interdisciplinary field of biomanufacturing. A standardized lexicon can facilitate communication between scientists and engineers that may come from technical backgrounds.
- There are potential ethical issues when dealing with cell sourcing, genetic manipulation of cells, availability of therapeutic organ replacement, and emergent behavior in cell-based systems. These issues must be addressed in concert with the other technical challenges, regulatory hurdles, and economic constraints in regenerative medicine therapies.

A broader goal of biomimetic manufacturing is to establish a roadmap for reproducing this commercial success from products ranging from biologics to cell-based therapies and tissue-engineered organs. It should be noted that there is more uncertainty in cell-based therapies because of the relative newness of these kinds of products. This can lead to risk in regulatory requirements, patient adoption, ethical considerations, and reimbursement approval.

Intrinsic Economic Challenges

Biomimetic manufacturing, to some extent, has already been utilized in the context of traditional biochemical processes. Enzyme catalysts, fermentation, and protein production in mammalian cell bioreactors are representative examples of previously successful biomimetic manufacturing strategies. These processes capture value by accessing natural biological processes that contain a broad spectrum of complexity.

In general, there is a strong positive correlation between the relative tolerance for complexity and imprecision with the intrinsic value of the biomimetic product. This correlation will likely serve as a guiding principle when inventing new biomimetic processes. The economic drivers are an important consideration when choosing products and designing processes. There needs to be alignment between the high risk and production costs of biological processes with the fabrication of high-value products. Monoclonal antibody production represents an ideal registry of these two factors.

One looming issue is the limited resources available for research and development in the private sector. What will be the relative value of committing precious R&D resources to increasing the productivity of biochemical processes in cells compared to the discovery of novel therapeutic products? The relative cost pressures associated with genetic and metabolic engineering of mature cell systems thus require low-cost instrumentation and assays. It is therefore imperative to gain as much knowledge as possible regarding cell function (metabolism, regulation of gene expression, protein processing, protein secretion, and host-virus interactions, etc.) in cost-effective manner. This knowledge will aid in frugal innovation for processes where significant cost pressures exist.

ASSESSMENTS AND CONCLUSIONS

Addressing Regulatory Challenges, Economics of Scale, and Reimbursement

Biomanufacturing follows the essential philosophy of product design and manufacturing process, except that the process is intrinsically more complicated. From a regulatory perspective, it is almost always good to engage discussion with regulatory authorities and understand requirements depending on the area where the product will be used. Some regulatory requirements should be even implemented into the product design and manufacturing process. If the product development involves biological components or studies in animals or human beings, ethics issues should be considered as well.

To drive down the cost of biomanufacturing, scalability and manufacturability should be incorporated as part of product design and manufacturing process design. Other traditional manufacturing considerations, such as quality control, quality assurance, and supply chain validation, apply to biomanufacturing as well, although they could cost more depending on the complexity of the system. As part of the manufacturing process, integration between biological modules and nonbiological modules could incur higher cost due to compatibility, sterilization, and special packaging condition requirements. When delivering the final product to the end user, addressing how to provide the product with longer shelf life and how to make shipping and storage more cost effective would add more value to the product.

Advanced Modeling and Simulation of Biological Systems

Modeling and simulation is a key aspect of biomimetic manufacturing. In the context of systems integration, computational models can be used to highlight some key aspects of biomanufacturing. Specifically, the following provocative questions would be of interest to the biomanufacturing community.

- *Noise and Error in Biological Systems*. How much noise is too much noise? How can these definitions be addressed and modified for specific applications in systems at different levels, including molecular, cellular, and organ-scale devices?
- *Signal Transduction*. How can noise propagation and information transfer be characterized in systems with varied complexity? How can figures of merit be translated to and from different aspects of the various biomanufacturing processes?
- *Fault Tolerance and Failure Modes*. How can fault tolerance be modeled in biological systems? What role can failure mode analysis play? How can these processes be modeled?
- *Abstracting Standards in Molecules, Cells, and Organs*. Can we use modeling to clearly define engineering parameters in cells? For example, in polymeric systems, complex solutions can be abstracted into practical engineering parameters such as molecular weight, viscosity, etc. Can a similar tractable framework be developed for cells and organs? Where and how can computational modeling be used to expedite this process?

Education and Training Programs in Biomimetic Manufacturing

It is important to design new educational programs that are optimized to train the next generation of scientists and engineers to advance biomimicry in biomanufacturing. Challenges in biomimetic manufacturing are highly interdisciplinary and require unique degree programs. It is possible to build off of established educational models that have been successful in training students in other interdisciplinary programs such as computational biology or chemical biology. For example, a general framework for advanced degree programs may be to recruit students with disparate undergraduate backgrounds within cohorts. The students then take complementary courses with each other to bridge intellectual gaps and enhance interdisciplinary communication.

Another model may be to design advanced degree programs that are dedicated to biomimetic manufacturing. Students may have backgrounds in traditional engineering (e.g., chemical,

biochemical, industrial, biomedical, or mechanical engineering). These students may take additional courses that are structured into a biology core and/or a processing core. The biology core may include courses such as zoology, structural biology, anatomy, and molecular biology. A processing core may include courses in control theory, noise and chaos, data management, and predictive algorithms, for example. Yet another possible model may be building off of existing programs in areas of convergence that are somewhat related to biomanufacturing. For example, it may be possible for engineering schools that specialize in robotics and automation to confer minors in biomanufacturing. These students would take core courses in robotics, such as sensors, software, automation, computer vision, etc., and then supplement this training with additional courses in basic biology, biology laboratory, and bioimaging. The broader pedagogical goal is to familiarize roboticists and automation engineers with the specific constraints associated with biomanufacturing.

There are many potential programmatic and philosophical hurdles to implementing formal training programs in biomanufacturing. For example, many students associate manufacturing with undesirable "blue collar" jobs; this social stigma may attenuate their enthusiasm. Another consideration is the influence of market pull from more mature industries. Many of the challenges in biomanufacturing are not specific to the fabrication of biomimetic products. Students may be recruited from biomanufacturing programs to more lucrative positions in industries such as automotive, energy, or consumer electronics.

The WTEC panel noted that integration of research institutes with formal educational programs was prominent within several organizations in Europe and Asia. Two examples are Instituto de Tecnologia Química e Biológica (ITQB) in Portugal and Fraunhofer Institute for Manufacturing Engineering and Automation in Germany. The ITQB is a technology research institute associated with the Universidade Nova de Lisboa and located in Oeiras, near Lisbon, Portugal. The ITQB is primarily a research institute that also provides training for post-graduates. The ITQB also interacts very closely with the Instituto de Biologia Experimental e Tecnológica (IBET), a private, nonprofit research institute. Students enrolled in ITQB can perform their dissertation research at facilities within IBET. IBET also participates in a number of translational research projects.

There are many advantages to this kind of training environment. First, the seamless integration of formal training programs, academic research, and translational development provide students with the full spectrum of activities that will be used in biomanufacturing research once they graduate. Second, pooling intellectual capital, financial resources, and capital equipment can increase the impact of the research. Third, the pipeline of students can be supplemented with other traditional educational institutions that are located in the vicinity. For example, the Instituto Superior Técnico (IST) is a technical institution that is focused on training students in traditional engineering disciplines. One of the campuses of IST is located in Taguspark, a suburb of Lisbon, Portugal. The Instituto de Biotecnologia e Bioengenharia (IBB) is primarily focused on biomedical engineering training, and research projects including stem cell engineering, biomaterials, nanotechnology, and regenerative medicine. This interinstitutional integrated research and teaching environment within metropolitan Lisbon serves as a model for universities and research institutes to support biomanufacturing initiatives in a comprehensive manner.

The Fraunhofer-Gesellschaft is a series of research institutes in Germany with a broad range of research and educational capabilities. The Fraunhofer Institute for Manufacturing Engineering and Automation (IPA) is located in Stuttgart, Germany. This institute has historical roots in automation and robotics research to support the local automotive industry. More recently, the expertise in robotics, machine vision, automation, and instrumentation has been applied to biomanufacturing research. The Fraunhofer IPA works on a broad range of projects in this area, which are funded by a combination of federal and private sources. This research environment provides an opportunity for trainees to work on projects that are of immediate interest to the government, local industry, or both. Like IQTB and IBET, the Fraunhofer IPA provides a training environment that emphasizes

translation and commercialization. This intellectual environment serves as an invaluable educational resource for training the next generation of biomanufacturing engineers.

CONCLUDING REMARKS

Biomimetics represents a viable overarching strategy to advance biomanufacturing as an emerging industry. As with any technology, the risk of product development scales with the complexity of the system. This paradigm explains why advances in microbial fermentation and synthetic biomaterials development can outpace more complex products such as cell-based therapies. The broad range in the maturity of many biomanufacturing technologies is shown in Table 5.1. This table classifies the technology readiness levels of many of the topics discussed in this chapter.

Table 5.1. Technology Readiness Levels (TRLs) of Selected Examples Discussed in this Chapter

TRL	Molecular	Micro-/Cellular	Tissue/Organ
9	Microbial fermentation of protein-based therapeutics, antibodies, and vaccines.		
8			Patient-specific orthopedic implants created by 3D printing.
7			
6	Cell-free biosynthesis of high-value compounds.		
5			
4	Genetic engineering of enzymatic pathways for production of high-value compounds.		Fabrication of *in vitro* "organoids" for drug screening and toxicology studies.
3		Flexible electronics for brain–machine interfaces and *in vivo* biosensors.	
2	Biomimetic polymers for controlled release.	Multiplexed *in vitro* sensor arrays for measurement *in situ* of electrophysiology.	Bioprinting of vascularized constructs containing multiple cell types.
1	Hybrid scaffold materials for tissue engineering. Scalable biosynthesis of natural pigments.	Organic ionics for controlled release and *in vivo* biosensing. Ultracompliant hydrogel electronic materials for *in vivo* biosensing.	Bioprinting of cell-based products for regenerative therapeutics.

The principles of biomimicry, when applied appropriately, can serve as a blueprint to advance many biomedical technologies and therapeutic products. The key is to leverage aspects of biomimicry at the appropriate level of granularity to solve specific problems rather than blindly mimicking biological systems at arbitrary levels of complexity. The inherent complexity of many biological systems leads to an obvious question: What level of biomimicry is ideal when designing a biomanufacturing process or biologically derived product? The answer is dependent upon many factors. One guiding principle is to use select aspects of biomimicry as a means for design inspiration, but not necessarily as an end goal. Engineering is ultimately interested in solving the problems of humanity.

Consider the example of heavier-than-air flight that uses a bald eagle (*Haliaeetus leucocephalus*) as a target for biomimicry (Table 5.2). The problem to be solved is the need to design a system that is capable of heavier-than-air flight as a mode of transportation. Biomimicry is therefore only useful

as a design tool if it assists in solving the aforementioned problem. While strict arbitrary biomimicry will not lead to a feasible solution to this problem, targeted biomimicry can provide insight into the design process. There are many aspects of the structure of a bald eagle that may provide utility in designing an airplane such as the presence of wings and the general aerodynamic structure. The ultimate solution (an A380 jet plane) may look and function very differently compared to the biomimetic target (a bald eagle), but this solution is considered a success in the sense that the problem has been solved.

Table 5.2. Contexualized Biomimicry: Comparison of the Physical Properties of an A380 Jet and a Bald Eagle (*Haliaeetus leucocephalus*)

	Bald Eagle	Jet (A380)
Length (m)	1	73
Wingspan (m)	2	80
Weight (kg)	5	650,000
Propulsion	Flapping wings	Jet engine
Speed (km/hr)	70	980
Range (km)	12	15,000
Altitude (m)	3000	10,000
Fuel source	Sugars	Alkanes

A similar framework must be applied when using biomimicry as inspiration for design in biomanufacturing. Biomimetics will work best when: (1) the problem is well defined, (2) the complexity of biological systems can be deconstructed into tractable components with deterministic behavior, and (3) aspects of biological structure and function provide novel insight into solving a relevant problem. These principles can serve as general guidelines when implementing biomimicry in biomanufacturing strategies.

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CHAPTER 6

GENOME EDITING

Gang Bao

BACKGROUND

The ability to decipher the information stored in genomes and precisely modify them will revolutionize many areas in life, including healthcare, agriculture, the environment, and energy. Over the last few decades a tremendous amount of genomic information has accumulated, thanks to the Human Genome Project. To utilize the growing availability of genome-wide data and increasingly powerful bioinformatics, it is necessary to develop equally powerful molecular tools to rapidly and precisely manipulate genomic content. With the recent development of engineered programmable nucleases such as clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins, transcription activator-like (Tal) effector nucleases (TALENs), and zinc-finger nucleases (ZFNs) (Figure 6.1; Porteus and Baltimore 2003; Reyon et al. 2012; Sander et al. 2011; Cong et al. 2013; Mali et al. 2013), we now have extremely efficient molecular scissors that can cut genomic DNA in cells at preselected locations and introduce mutagenic errors via the non homologous end joining (NHEJ) DNA repair pathway, thus rendering genetic programs nonfunctional. Alternatively, if the nuclease-induced DNA cleavage (DNA double strand breaks or nicks) triggers endogenous homology-directed repair (HDR) with the supplied DNA donor template, precise DNA insertion can be realized (Figure 6.2). These abilities have led to the emerging field of genome editing, a new field in engineering and life sciences focusing on precisely modifying genomes using engineered nucleases.

Genome editing provides a rapid high-throughput method for testing genetic loss-of-function by introducing random mutations and targeted DNA deletions that disrupt gene functions and allow multiplexed gain-of-function by inserting new genetic material or correcting genetic defects. Precise genome editing through targeted gene knockout, gene correction, and gene addition have a wide range of applications in basic biological studies, biotechnology development, and medical research. Specific applications include genetic modification of bacteria to generate cost-effective biofuels, plants, and animals for improved crops with high herbicide and virus resistance, for better food and clothing, and for production of new pharmaceutical reagents. Genome editing can be used to genetically engineer animals and cells for modeling human diseases in drug screening and disease studies, or for basic biological studies of gene function and regulation. Compared with gene therapy, genome editing provides a more precise and powerful strategy for treating human diseases, including infectious diseases (e.g., HIV) by using nuclease-induced site-specific DNA mutations and deletions, and single-gene disorders (e.g., sickle cell disease) using nuclease-enabled gene correction.

The development and application of genome editing are closely related to other areas covered by this study of International R&D in Biological Engineering and Manufacturing, including cell-based therapy and delivery, personalized medicine, functional imaging and sensing, functional nanoparticles, biomimetics, and regulatory affairs. For example, currently the most active medical application of genome editing is cell-based therapy, including the use of ZFNs to treat HIV (Tebas et al. 2014) and X-linked severe combined immunodeficiency (SCID-X1; Genovese et al. 2014).

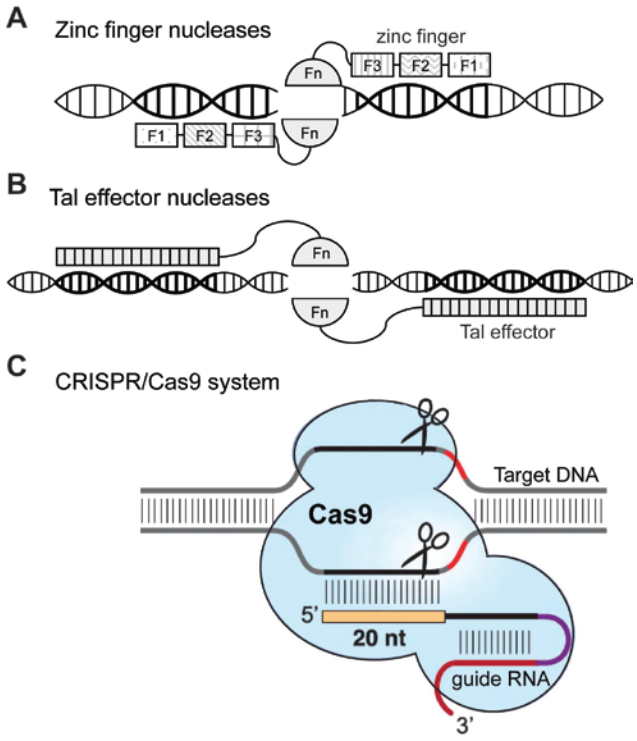

Figure 6.1. Engineered nucleases, including (*A*) zinc-finger nuclease, (*B*) transcription activator-like effector nucleases, (*C*) clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (adapted from Tong et al. 2013).

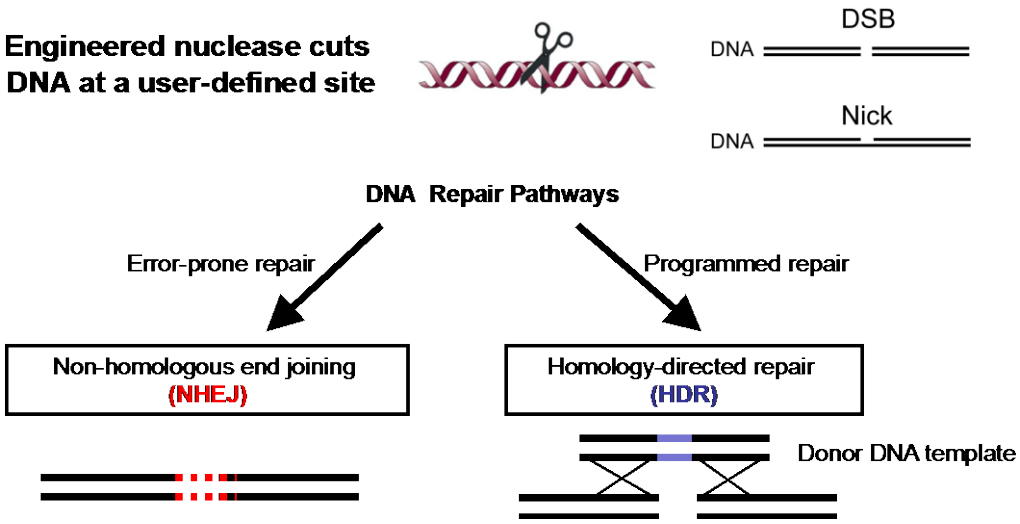

Figure 6.2. The molecular basis of genome editing (courtesy of G. Bao).

Engineered nucleases cut genomic DNA in a living cell at a predefined site, inducing a DNA double strand breaks (DSBs) or nicks, which activate a DNA damage repair pathway, non homologous end joining or homology-directed repair. NHEJ is error-prone and induces mutagenic errors in the genome, while HDR can be used for DNA insertion.

A critical issue in *in vivo* applications of genome editing is delivery, which can be performed using viral- and nanoparticle-based approaches. Functional nanoparticles have the advantage of specific targeting of tissue or organ, and the ability to combine delivery with imaging, allowing tracking and evaluation of site-specific delivery. Functional imaging, including fluorescence-based cellular

imaging and animal imaging using PET, MRI, ultrasound, or CT, offers the ability to study the molecular mechanisms involved in genome editing, and to determine the nuclease/donor delivery efficiency and gene modification efficacy in animals in deep tissue. Since many single-gene disorders are caused by gene defects that vary from patient to patient, their treatment has to be based on the individual patient and thus in the realm of personalized medicine. Genome editing could also be used to create cell lines and animal models according to the unique genetic detect(s) of an individual, facilitating the study of the disease and the development of drug molecules and/or treatment strategies. The construction of ZFNs and TALENs is biomimetic, since these are engineered proteins; the biomimetics could guide the design of next-generation nuclease with better efficiency and specificity.

Finally, there are significant challenges in regulatory and ethics issues in genome editing, and there is an urgent need to develop guidelines for the translation of genome editing into commercial and clinical utilization. For example, what is the safety standard when using genome editing to treat patients? If we could modify human genome at the earliest stages of development, what are the potential consequences? Clearly, modifying one's genome at has significant ethical, social, and legal implications, and altering the natural biological processes of gene modification may cause unwanted results. Therefore, significant efforts need to be devoted to addressing the regulatory and ethics issues in genome editing.

Although genome editing has a huge potential, it also has significant biological and technological challenges, including efficient *in vitro* and *in vivo* delivery of nucleases and donor templates, reducing or eliminating nuclease off-target effects, increasing the rates of homology-directed repair, isolation and expansion of gene-modified cells, having sufficient *in vivo* engraftment, and avoiding possible immunogenicity. Many of these challenges are related to biomanufacturing, as discussed in more detail below.

MAJOR CHALLENGES IN DESIGN AND BIOMANUFACTURING

Nuclease-based genome editing holds great potential for many applications, including disease modeling, molecular pathway dissection, synthetic biology and therapeutics. However, cells overwhelmingly favor error-prone repair pathways, non-homologous end joining and micro-homology mediated end joining. Further, cellular HDR machinery is only active in dividing cells, precluding its use in post-mitotic tissues such as the heart and brain. The engineered nucleases often generate off-target cleavage, causing mutations, insertions, deletions, inversions, and translocations, which may result in diseases including cancer. These limitations constitute the bottleneck for rapid disease modeling based on genome-wide association studies, transgenic animal generation, and gene therapeutics. Therefore, it is necessary to increase the cleavage efficiency of engineered nucleases, maximize the HDR/NHEJ ratio in mitotic and postmitotic cells by having optimized nuclease/donor designs and doses, and minimize genomic risk by reducing or eliminating off-target effects. In certain therapeutic applications, it is necessary to select and enrich gene-corrected cells in order to achieve the desired clinical outcome.

To have widespread applications of genome editing, advanced biomanufacturing and efficient nuclease and donor delivery are essential. To reduce the cost in commercial and clinical applications of genome editing, it is necessary to produce mRNAs encoding ZFNs, TALENs or Cas9 proteins, as well as CRISPR guide RNAs (gRNAs) with large quantity and high quality control (QC). It may also be necessary to chemically modify nuclease mRNAs and gRNAs with high QC.

For cell-based therapies using genome editing, the product is gene-modified cells, and manufacturing them requires efficient nuclease and donor delivery. Although many *in vitro* delivery methods have been developed, including transfection, microinjection, nucleofection, and the use of viral-vectors, delivering nucleases and donor templates into primary cells (especially stem cells) with high efficiency and throughput, controlled amount, and high uniformity and cell

viability remains a significant challenge. It is necessary to optimize the delivery protocols for each cell type involved, and based on delivering DNA (plasmid), RNA (mRNA and gRNA), or proteins. For *in situ* genome editing applications, delivery is even a bigger challenge. Although viral-based delivery, including vectors using adenovirus, lentivirus, and adeno-associated virus (AAV) have been used successfully in delivering plasmids encoding nucleases and gRNAs, they cannot be used to deliver mRNAs, gRNAs, or proteins. Further, for viral-based delivery, safety remains a concern in clinical applications. As an alternative, nanoparticle-based delivery strategies are being developed, which have the potential advantages of specific tissue/organ targeting, versatility (in delivering DNA, RNA and protein), and low toxicity. In both cases, manufacturing the viral vectors and nanoparticles is a challenge, especially when a large quantity is required.

In using genome editing for cell-based therapies of human diseases, a GMP facility is often required to collect cells from a patient (e.g., from bone marrow), perform nuclease or nuclease/donor delivery, select and enrich gene-modified cells, and deliver them back to the patient. It is also necessary to perform quality analysis and quality control steps in the GMP facility. Therefore, developing closed, fully automated systems with multiple components for clinical applications of genome editing is desirable.

Another important challenge for genome editing is to develop unified procedures and safety standards, with "benchmark" assays and common protocols. Due to very limited nuclease off-target analysis and cytotoxicity measures, there is a lack of well-defined parameters for determining safety. Detailed unbiased genome-wide analysis of nuclease off-target cleavage in the relevant cell types and tissues from animal models of the disease is needed to have a better understanding of the effect of off-target cleavage, and the long-term effects of nuclease toxicity, especially the potential tumorigenic effect should be carefully studied. Due to the complexity in determining the safety of genome editing, a close international collaboration would be particularly beneficial.

With all these challenges in mind, the WTEC panelists visited many sites in Europe and Asia, had extensive discussions with colleagues in genome editing and cell-based therapies, and gained a better understanding of the international landscape of biomanufacturing related to genome editing. In what follows, a brief summary is given of selected sites we visited on genome editing.

GENOME EDITING ACTIVITIES AT EUROPEAN AND ASIAN SITES

As shown in detail in Chapter One, for the WTEC International Study of R&D in Biological Engineering and Manufacturing, sponsored by NSF, the WTEC panel visited a total of 21 sites in Europe and 17 sites in Asia. Since genome editing is an emerging field, only a few sites visited have a high level of genome editing activity. However, as shown in Table 6.1, many sites that are focused on regenerative medicine and cell-based therapies have research and commercialization efforts related to the biomanufacturing for and translation of genome editing. Described below are the discussions at selected sites that are most active in genome engineering.

Table 6.1. Sites Visited and their Relationships to Genome Editing

Location	Site	Genome Editing Relevance
EUROPE		
London, UK	Imperial College	Low
London, UK	University College London	Low
London, UK	Cell Therapy Catapult, Ltd	Moderate
Leeds, UK	University of Leeds	Low
Leeds, UK	NanoManufacturing Institute	Moderate

Location	Site	Genome Editing Relevance
Edinburgh, UK	Roslyn Cells, Scottish Centre for Regenerative Medicine, BioQuarter, Systemic, et al.	Moderate
Loughborough, UK	University of Loughborough	Moderate
Royston, UK	TAP Biosystems	Low
Utrecht, the Netherlands	PharmaCell	Low
Idar-Oberstein, Germany	EUFETS, GmbH	Low
Würzburg, Germany	University of Würzburg	Moderate
Stuttgart, Germany	Fraunhofer Institute for Interfacial Engineering and Biotechnology	Moderate
Berlin, Germany	BCRT	Moderate
Leipzig, Germany	Fraunhofer Institute for Cell Therapy and Immunology	Moderate
Linköping, Sweden	University of Linköping	Low
Lausanne, Switzerland	Ecole Polytechnique Fédéral de Lausanne (EPFL)	Low
Basel, Switzerland	Eidgenossische Technische Hochschule(ETH) -Basel	Low
Lisbon, Portugal	Institute for Biological Experimental Technologies (IBET), Cell2B	Low
Lisbon, Portugal	INFARMED	Low
Milan, Italy	MolMed	High
Milan, Italy	TIGET	High
ASIA		
Beijing, China	Natural Science Foundation of China	N/A
Beijing, China	Peking University	High
Beijing, China	Tsinghua University	Low
Guangzhou, China	Sun Yat-sen University	Moderate
Guangzhou, China	Guangzhou Institutes of Biomedicine and Health	Moderate
Suzhou, China	Soochow University	Moderate
Suzhou, China	BioBay	Low
Seoul, South Korea	MEDIPOST	Moderate
Seoul, South Korea	Korea Institute of Science and Technology (KIST)	Moderate
Seoul, South Korea	Sungkyunkwan University School of Medicine	Low
Seoul, South Korea	Kyungpook National University School of Medicine	Moderate
Tokyo, Japan	CellSeed, Inc., at Tokyo Women's University	High
Tokyo, Japan	MEDINET Medical Co.	Moderate
Tokyo, Japan	NanoCarrier	High
Gamagori, Japan	Japan Tissue Engineering Company	Moderate
Kyoto, Japan	Center for Induced Pluripotent Stem Cell Research & Application (CiRA), Kyoto University	Moderate
Kyoto, Japan	Takara Biosystems, Japan	Low

MolMed and TIGET, Milan, Italy

The WTEC panel members visited MolMed S.p.A. in Milan, Italy, on March 6, 2014. MolMed is a leader in cell and gene therapy in Europe, which focuses on research, development, and clinical validation of innovative therapies to treat cancer and genetic orphan diseases. MolMed is located in the San Raffaele Biomedical Science Park, which houses the San Raffaele Research Hospital and San Raffaele Scientific Institute, the largest and most important private research center in Italy. Dr. Claudio Borgignon, Chairman and CEO of MolMed, gave a comprehensive presentation of the R&D activities in MolMed.

The panel members also visited the San Raffaele Telethon Institute for Gene Therapy (TIGET), and talked with Dr. Ferrari Giuliana. TIGET has been collaborating with MolMed and developed the retroviral vector for gene delivery, as well as the first *ex vivo* gene therapies based on HIV vectors. Prof. Luigi Naldini at TIGET is an internationally renowned leader in genome editing and cell-based therapies.

MolMed's R&D efforts have been focused on two innovative technologies: recombinant proteins and cell and gene therapy. Over the last few years, MolMed has been conducting in-house GMP-based manufacturing of cell and gene therapy products, identifying oncology indications that require new therapy options, and improving clinical and pharmaceutical approaches, independently or with partners. In particular, MolMed developed TK cell therapy, a cell-based therapy in Phase III clinical trial, enabling bone marrow transplants from partially compatible donors, in absence of post-transplant immune suppression, for treating high-risk acute leukemia.

The technology developed by MolMed for *ex vivo* genetically engineered TK-T cells has proven to be technically feasible without safety problems. Based on this technology, MolMed has now developed a complete technological platform for *ex vivo* gene therapies including retroviral vector (RVV) for treating TK and adenosine deaminase-severe combined immunodeficiency disease (ADA-SCID), and lentiviral vector (LVV) for treating metachromatic leukodystrophy (MLD), Wiskott-Aldrich syndrome (WAS), β Thal, MPS I, globoid cell leukodystrophy (GLD; Krabbe's disease), and chronic granulomatous disease (CGD). The success of MLD and WAS gene therapies, which were developed through a partnership between MolMed and TIGET, was reported in 2013 in *Science* (Biffi et al. 2013; Aiuti et al. 2013). More recently, Prof. Luigi Naldini's group reported their genome editing work using ZFNs for treating SCID-X1 (Genovese et al. 2014). They found that gene-edited HSCs sustained normal haematopoiesis and gave rise to functional lymphoid cells that possess a selective growth advantage over those carrying disruptive IL2RG mutations. These results open up new avenues for treating SCID-X1 and other diseases.

It is clear that MolMed and TIGET are at the forefront of genome engineering and biomanufacturing. In addition to cutting-edge research in genome editing and cell-based therapies, MolMed has an attractive vision for next-generation biomanufacturing for cell-based therapies that will fully utilize automated production. This vision has the following key elements: (1) flexibility, i.e., to have process and devices easy to adapt to different cell processing applications; (2) scalability, to allow for increasing the production scale to target large indications; (3) feasibility, to have scientific, technological and regulatory experience and know-how. MolMed also has a significant need for well-trained engineers in biomanufacturing. Dr. Claudio Borgignon mentioned that it is necessary to broadly train students in biomanufacturing so that they have a broad range of knowledge, including robotics, automation process, sensor and remote control, cell testing, QC, GMP, regulatory issues, and standards.

Peking University, School of Life Sciences, Beijing, China

The WTEC panel members visited the School of Life Sciences, Peking University (PKU) on July 20, 2014. The School of Life Sciences currently has >60 independent investigators with a broad skill set in developmental biology, molecular biology, plant biology, protein engineering, bioinformatics, and genetics, and an enrollment of >500 undergraduates and >400 graduate

students. The School of Life Sciences is also home to several centers of excellence, including the State Key Laboratory of Protein and Plant Gene Research, the State Key Laboratory of Biological Membranes and Membrane Biotechnology, and the State Key Laboratory of Cell Proliferation and Differentiation.

Professors Bo Zhang and Wensheng Wei gave excellent presentations on the genome editing in their labs. Dr. Zhang's current research focuses on the use of genetics and genome editing tools to model human diseases using zebrafish. These tools involve retroviral vectors for random insertional mutagenesis, and the use of engineered nucleases such as zinc finger nucleases (Xiao et al. 2013), TALENs (Huang et al. 2011), and Crisper/Cas9 systems (Xiao et al. 2014). Dr. Zhang has also made seminal discoveries regarding the role of Kctd10 in cardiac morphogenesis (Tong et al. 2014). The methods and tools developed in Dr. Zhang's lab for genome editing have been widely used in the field.

Dr. Wei's lab is focused on studies in molecular biology and genetics, aiming to address grand challenges in infectious diseases using cutting-edge technologies such as genome editing. Dr. Wei has made significant contributions to genome editing, as demonstrated by the development of a high-throughput screening method for identifying targets for CRISPR/Cas9 (Zhou et al. 2014). Dr. Wei also performed a comprehensive decoding of TAL effectors for DNA recognition, has an active program in identifying receptor-ligand function in the context of C. *difficile* infection, and has shown that genetic manipulation of HeLa cells can reduce the binding of Toxin B to membrane receptors, and this may serve as a potential therapeutic strategy.

Although the WTEC panel members were only able to visit two genome editing groups in China, it is clear that there are many research laboratories and companies in China that have been actively working on genome editing related technologies. For example, a group at the Yunnan Key Laboratory of Primate Biomedical Research in Kunming, China, led by Dr. Weizhi Ji, has successfully demonstrated that CRISPR/Cas9 can be used to create monkey models of diseases (Niu et al. 2014). There are also significant commercialization efforts in China, mostly to provide CRISPR/Cas9-based genome editing services, including gene knockouts and gene activations.

CellSeed and Tokyo Women's Medical University

On May 28, 2014, the WTEC panel members visited the Institute of Advanced Biomedical Engineering and Science at Tokyo Women's Medical University (TWMU), led by Professor Teruo Okano, and CellSeed, Inc., a start-up company based on the technologies developed by Dr. Okano. He and his colleagues have succeeded in harvesting cultured cells as viable and confluent cell layers by modifying the temperature-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm), layered onto the surface of ordinary polystyrene tissue culture dishes. Based on this temperature-responsive surface, these groups have established a new concept of "cell sheet engineering" that introduces an alternate path for tissue and organ regeneration using only manipulated cell sheets. Dr. Okano is a founder and director of the board of CellSeed, which licenses technologies and patents from TWMU. CellSeed is a biotechnology innovator dedicated to providing innovative solutions for tissue engineering through the development of novel cell harvest methods and 3D living tissue replacement products for "cell-sheet therapy" and regenerative medicine.

Dr. Okano has made an extraordinary effort in developing T-Factory, an automation system for cell sheet biomanufacturing. As shown in Figure 6.3, the T-Factory consists of 7 different modules, including cell expansion culture module, CO_2 incubator module, cell sheet layering module, cell seeding/medium changing module, sample loading module, cell isolation/primary culture module, and transfer module. Each module can be decontaminated independently, and this system is capable of implementing a consistent process with high-mix low-volume, and equipped with a standardized docking interface. The T-Factory was established through a collaboration between academia (TWMU) and industry (e.g., Hitachi and Toyota), with significant funding support from the Japanese government.

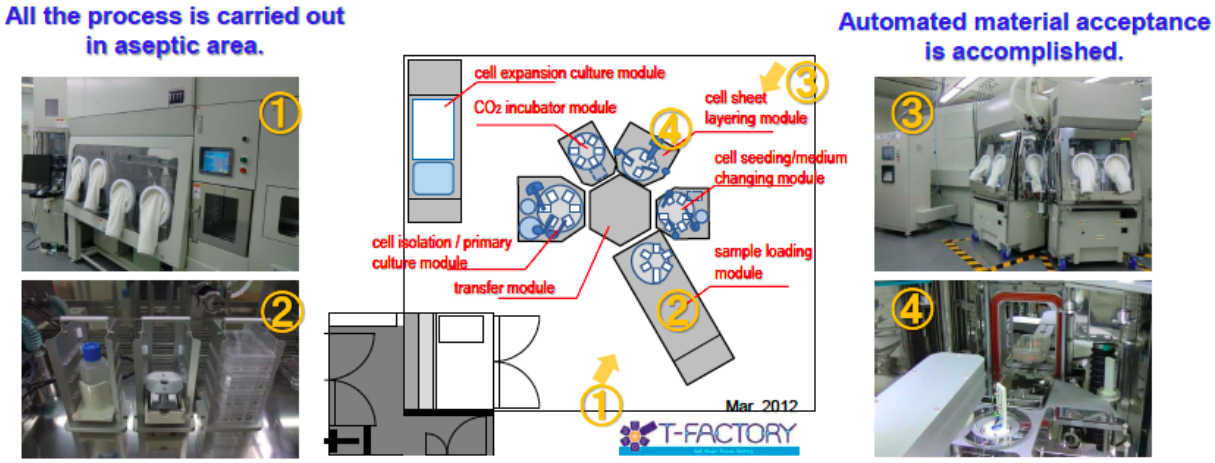

Figure 6.3. T-Factory, an automation system for cell-sheet biomanufacturing (courtesy of Tokyo Women's Medical University).

This system consists of 7 modules, including cell expansion culture module, CO_2 incubator module, cell sheet layering module, cell seeding/medium changing module, sample loading module, cell isolation/primary culture module, and transfer module.

The WTEC panel was very impressed by Professor Okano's ability to integrate disciplines across the entire spectrum, from metabolic research to materials and cell engineering, to clinical application, to development of effective autologous cell systems for regenerative cell therapy. His approach to translating scientific discoveries and engineering innovation into commercial and clinical practice is among the very best that we observed in our study.

ASSESSMENTS AND CONCLUSIONS

Genome editing is an emerging biotechnology that has a huge potential in addressing needs in healthcare, food, energy, and the environment. The WTEC panel's European and Asian studies revealed that, overall, the United States has been taking a lead in genome editing, especially in developing the core technologies and acquiring intellectual properties. There has been a broad range of research, technology development, and commercialization activities in academia and industry in the United States, with increasing emphasis placed on application. However, some groups in Europe and Asia are at the cutting edge of genome editing, such as the translation of genome editing in treating single-gene disorders (Genovese et al. 2014) development of certain nuclease design tools (Xiao et al. 2013, 2014) creating large animal models of human disease (Niu et al. 2014) studies of functional genome (Zhou et al. 2014), and establishing an integrated biomanufacturing system. It is likely that in some application areas, such as genome-editing-based treatment of certain human diseases and the development of fully automated systems for cell-based therapies, Europe and Asia may quickly establish and/or expand their leadership positions.

Although the existing engineered nucleases such as ZFNs, TALENs, and CRISPR/Cas systems have revolutionized precision genome engineering, there are still significant challenges in achieving both high efficiency and specificity, and in making them a robust tool for all laboratories, just like restriction enzymes are today. Therefore, the next-generation genome engineering toolbox should comprise programmable, efficient, and safe molecular tools for direct site-specific DNA modification and reconstruction. These tools should allow for *in vivo* insertion of genetic sequences for gain-of-function, precise mutation and deletion for disease modeling, therapeutic gene correction for disease treatment, and capability to target the entire genome in all cell types with high efficiency and specificity. It is also desirable to further develop engineered nucleases and associated methods into a robust, routine, and pervasive tool for precisely and dynamically modifying, activating, and repressing the genomes. Critical to the successful development and

application of genome editing across the world are: (1) a fundamental understanding of the mechanisms of the DNA damage repair pathways, genome organization, and DNA/RNA, DNA/protein interactions involved; (2) efficient *in vitro* and *in vivo* delivery, (3) creation of robust design, validation, and optimization tools for engineered nucleases, and (4) efficient and low-cost biomanufacturing of genome editing reagents. Addressing these long-term challenges requires a concerted effort by a multidisciplinary team of investigators and a significant investment by the government and private sectors.

Biomanufacturing is a major challenge in realizing the huge potential of genome editing for a broad range of applications. For example, it is essential to have the capability to routinely produce a large amount (> 1 kilogram per batch) of nuclease proteins, mRNAs encoding nuclease proteins, and CRISPR guide RNAs with high quality and low cost. It may also be necessary to chemically modify mRNAs and gRNAs with high quality and low cost. Achieving these requires the establishment of efficient manufacturing processes and facilities. In order to deliver engineered nucleases and donor templates *in vivo*, it is essential to manufacture different viral vectors or nanoparticles as carriers. Although viral vectors such as lentiviral vector have been widely used for *in vivo* delivery of nucleases, packaging Cas9 and effectors into adeno-associated virus remains a challenge. Viral vector-based delivery also has the drawback of being incapable of delivering mRNAs or proteins. To address these issues and avoid potential integration of viral genome into that of host cells, nanoparticle-based delivery has been developed, as described in details in Chapter Three. Clearly, producing a large amount (> 1 kilogram per batch) of functionalized nanoparticles with high quality and low cost is a significant challenge. Given the tremendous needs in genome editing applications, a significant investment in establishing large-scale nanoparticle manufacturing is required.

Ideally, genome editing applications such as disease treatment should be performed using closed, fully automated systems with multiple components, including cell isolation, culture and monitoring, nuclease and donor delivery, selection and expansion of gene-modified cells, and quality control units. The design, integration, optimization and manufacturing of such closed systems require a significant investment by and close collaboration from multiple government agencies and different industries, including biotech, automotive, electronics, and advanced materials. Here again, the T-Factory developed at Tokyo Women's Medical University provides an excellent example.

In conclusion, genome editing, the ability to precisely modify genomes, will revolutionize many areas in our society, including healthcare, agriculture, energy, and the environment. However, there are major challenges in realizing the huge potential of genome editing, including tools development and optimization, large-scale manufacturing of high-quality reagents, and the establishment of closed, fully automated systems. A significant investment from and close collaboration by governments and private sectors across the world are necessary.

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APPENDIX A. DELEGATION BIOGRAPHIES

Stephen W. Drew (Chair), Drew Solutions, LLC

Dr. Drew is a former Distinguished Senior Scientist at Merck & Co., Inc., where his responsibilities encompassed the development of new process technologies for biologics and pharmaceutical manufacturing and technology transfer. At Merck he was also vice president (VP) of Vaccine Science and Technology, VP of Vaccine Operations, and VP of Technical Operations & Engineering. Since retirement from Merck, he has founded two new companies (Drew Solutions, LLC, a direct consulting firm, and Science Partners, LLC, an advocacy company for medicines and technologies) that support the biotechnology and pharmaceutical industries. He joined Merck in 1981 to create the Department of Biochemical Engineering. At Merck, he contributed to the process development and manufacture of several conventional and recombinant microbial products ranging from antibiotics to vaccines. Dr. Drew has expertise in the following areas: manufacturing processes for human and animal vaccines; recombinant biologics; chemical, biological, and engineering technology for bulk manufacture of pharmaceuticals and biologics; and fermentation, cell culture, isolation, and purification processes for sterile products. His specialties are vaccine development and manufacturing and biochemical engineering.

Gang Bao, Georgia Institute of Technology

Professor Bao holds the Robert A. Milton Chair in Biomedical Engineering and is a College of Engineering Distinguished Professor. He received his Ph.D. from Lehigh University and his M.S. and B.S. from Shandong University. Dr. Bao's research areas include biomolecular engineering, molecular imaging, molecular biomechanics and bionanotechnology, and detection of cancer and viral infection. With Lily Yang of Emory University, Bao is developing a new method for detecting pancreatic cancer. The team, together with students, designed a biosensor called a molecular beacon, which uses a single strand of DNA and a fluorescent dye to seek out cancer cells.

Chris Bettinger, Carnegie Mellon University

Professor Bettinger and the Laboratory for Therapeutic Biodegradable Microsystems are broadly interested in the development of biomaterials-based MEMS for use in a wide range of biomedical applications, including regenerative medicine, neural interfaces, and drug delivery. They use

interdisciplinary strategies to develop next-generation medical implants that combine extraordinary properties of biodegradability and biocompatibility with unique mechanical properties and electronic functionality. Specific thrusts include BioMEMS for tissue regeneration, biodegradable electronic devices, biomimetic tissue–device interfaces, nonconventional microfabrication of biomaterials, rational biomaterials synthesis, and quantitative elucidation of biodegradation phenomena.

Kam Leong, Columbia University

Professor Leong's research interest is on biomaterials design, particularly on synthesis of nanoparticles for DNA-based therapeutics and nanostructured biomaterials for regenerative medicine. At Columbia, Dr. Leong and his team are working on nonviral delivery approaches to cellular reprogramming to facilitate clinical translation for treatments for neurodegenerative disorders such as Alzheimer's and Parkinson's disease. They are investigating ways to optimize the biochemical and physical cues dictating direct cellular reprogramming by leveraging biomaterials and biomedical engineering techniques and innovations.

Madhusudan V. Peshwa, Maxcyte, Inc.

Madhusudan Peshwa, Ph.D., is Vice President, Research and Development, at MaxCyte, Inc. Most recently, he was Executive Vice President for Research and Development at NewNeural LLC, a start-up stem cell therapy company. Earlier he served as Vice President of Manufacturing and as Vice President of Process Sciences at Dendreon Corporation, where he was responsible for development, characterization and manufacture of an autologous dendritic cell vaccine product from concept to late Phase III pivotal studies. His expertise is in the areas of design, characterization, scale-up, and implementation of processes, and cGMP systems in the development of engineered cell and tissue products and for biopharmaceuticals' production. Dr. Peshwa obtained his Ph.D. in Chemical Engineering from the University of Minnesota and his B.Tech. in Chemical Engineering from the Indian Institute of Technology, Kanpur, India. He is a co-author on over 35 scientific publications and is a co-inventor on five, issued or under review, patent applications.

Kaiming Ye, Binghamton University, State University of New York (SUNY)

Dr. Kaiming Ye is Professor and Chair of the Department of Biomedical Engineering at Binghamton University, State University of New York (SUNY). He is fellow of American Institute of Medical and Biological Engineering and senior member of IEEE. His scholarly contributions to the field include the development of the concept of advanced biomanufacturing and his leadership role in promoting and growing the field. He is well known for his work in 3D bioprinting and development of 3D differentiation systems for pancreatic organoid development from human pluripotent stem cells. He has invented fluorescent nanosensors for continuous glucose monitoring and yeast-based new influenza vaccines. He has contributed significantly to national policymaking in science and engineering. During his tenure at the National Science Foundation, he directed a biomedical engineering program and was responsible for identifying new direction of biomedical engineering program and coordinated biomedical-related funding programs with other divisions and directorates within NSF. He was member of a number of interagency working groups, including the Interagency Workgroup for Neuroscience under the Office of Science and Technology Policy (OSTP) and the National Council of Science and Technology (NCST), Interagency Modeling and Analysis Workgroup, and the Multiagency Tissue Engineering and Regenerative Medicine Workgroup. In addition, he was involved in NSF CIF21 IGRET program, cyber-enabled science and engineering program, NIH/NSF joint program on interface between physics and life science, and NIH/NCI-NSF Physicals and Engineering Sciences in Oncology (PSEO) funding program.

APPENDIX B. SITE VISIT REPORTS – EUROPE

Site visit reports are arranged in alphabetical order by organization name.

Berlin-Brandenburg Centre for Regenerative Therapies (BCRT)

Site Address: Föhrer Strasse 15
13353 Berlin, Germany

Date Visited: March 5, 2014

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OVERVIEW

The Berlin-Brandenburg Centre for Regenerative Therapies (BCRT) focuses on translating cell-based, factor release, and biomaterials technologies to impact regeneration medicine. While

research is the focus, it also has strategies in place to support the translation research with education programs and interactions with industry and regulatory agencies. It is co-founded and partnered by the Charité University Medicine Hospital and the Center for Materials and the Helmholtz-Zentrum Geesthacht, Institute of Biomaterial Science of the Helmholtz Association. BCRT has strong international ties and a high visibility in the field of regenerative medicine.

FUNCTIONAL FOCUS

The overall goal of BCRT is to develop translational regeneration therapies, from basic science to technology development and to clinical trials. The general strategy of BCRT is to bridge the activities of a typical research unit with eventual commercialization. The Center develops technologies on molecular analysis, cell source derivation, biomaterials, and *in vivo* tissue regeneration to be applied to the immune system, cardiovascular system, and musculoskeletal system. The Center aspires to drive the research and development with unmet medical needs.

RESEARCH & DEVELOPMENT ACTIVITIES

The Center has an emphasis on stimulating endogenous regeneration, particularly on understanding and exploiting the inflammatory process in promoting regeneration. It focuses on the following directions:

- Understanding and modifying inflammation in regenerative processes
- Induction of targeted tissue formation and angiogenesis
- Mimicry of functional cell environments by polymer-based biomaterials, natural materials, and biochemical cues
- Use of physico-mechanical cues for stimulating regeneration
- Control of the fate of stem/progenitor cells (stem cell engineering)
- Development and validation of biomarkers towards personalized therapies

TRANSLATION

The Center is in an advanced stage of developing IP-protected $T_{mem/eff-}$ and T_{reg} cell therapy that is co-developed with t-cell Europe, a spin-off of BCRT/Charité (Abou-el Enein et al. 2013). Promising clinical data are also generated by using anti-CD20 mAb for inflammatory cardiomyopathy and targeting activating alloreactive T_{eff} cells after transplantation. It is also running in co-development industry-sponsored clinical trials on PLX therapy for critical limb ischemia (CLI) and muscle injury protection. Several biomarkers for developing personalized approaches could be commercialized via diagnostic companies. BCRT has an active program on developing cell-based therapy for heart muscle regeneration. It originates from the discovery of cardiac-derived stromal cells with regenerative potential (Haag et al. 2010). Termed CardAP cells, these cells have shown anti-fibrotic characteristics in decreasing the AngII-induced accumulation of collagen I and III in the myocardium (van Linthout et al. 2012, Haag et al. 2013). The technology is being translated via CELLserve, a spin-off from BCRT/Charité. The Center believes it has a cost-effective GMP manufacturing process, presumably due to the fact that the cells are not stem cells in nature and that neither biomaterials nor growth factors are required for application. In other applications, combinations of cells, biomaterials, and/or factors are facilitated.

SOURCES OF SUPPORT

Financial support to the projects comes from, in order of decreasing percentage, government grants, industry-sponsored projects, Charité/HZG, and Helmholtz Association via HZG.

ASSESSMENT

Many academic regenerative medicine units in the world often lack the perspectives of translation and business development. BCRT excels by adopting a comprehensive strategy to link research with education and industrial interactions. Its partnership with the Charité hospital and the Helmholtz Association adds significant strength to the Center. The collaboration between the medical researchers and the biomaterials scientists is commendable. The Center has basic science expertise, technology development capability, clinician scientists, in-house GMP facilities, and business development proficiency. It is an excellent model for translational regenerative medicine.

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OVERVIEW

The Cell Therapy Catapult (CTC) was established as one of eight Catapults in 2012 by the UK Technology Strategy Board (TSB) upon recommendations of the Herman Housel Report to the UK Government, and with input from the Medical Research Council (MRC), to drive innovation and manufacturing competence in building sustainable industry and health care in UK. CTC was financed with a core grant of £70 million over a 5-year period with the goal of promoting development and manufacturing of advanced cellular therapeutic medicinal products in UK. CTC commenced operations in April 2013, is currently hiring up to ~75 staff and will take possession into its new 1,200 m^2 translational development and office facilities in March 2014.

FUNCTIONAL FOCUS

The WTEC panel met with the heads of two functions: Clinical (Dr. Natalie Mount) and Manufacturing (Dr. Stephen Ward) and a representative of the Business function (Mr. Simon Ellison).

- Mr. Ellison provided an overview on CTC's organization and business operations encompassing opportunity search and evaluation, alliance management, IP and contract services, market access & marketing, and reimbursement capabilities.
- Dr. Ward provided an overview of process development, analytical development, and manufacturing and supply chain considerations.
- Dr. Mount provided an overview of non-clinical safety, regulatory, quality, clinical development, and clinical operations capabilities.

RESEARCH & DEVELOPMENT ACTIVITIES

R&D activities encompass providing scientific review expertise on cell therapy products and undertaking preclinical, translational, and analytical development to support clinical and regulatory strategy for development and testing of cell therapy products in human clinical trials. CTC's facilities are well-equipped, have modular laboratories, plus space for meetings and networking. The facility design is intended to promote collaboration and innovation. Laboratories are designed to mimic cGMP manufacturing suites, to facilitate pilot/clinical scale process development in laboratory environment that mimics facility layout and operations of clinical/commercial scale cGMP manufacturing. Tables B.1–B.3 provide a detailed list of equipment and capabilities to support development of cell therapies.

TRANSLATION

CTC has capabilities to undertake academic investigator initiated projects, provide translational services to SMEs, and engages in strategic programs with large pharmaceutical/biotechnology companies both within the UK and globally (Figure B.1).

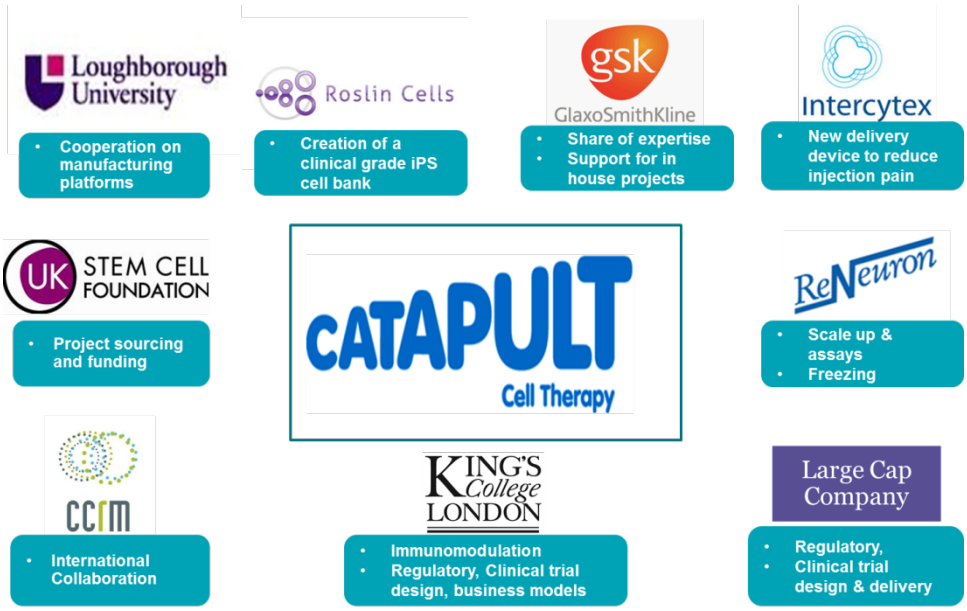

Figure B.1. Publicly disclosed projects and collaborations at CTC (courtesy of Cell Therapy Catapult).

Table B.1. CTC Equipment for Process Development (£1.7 million), Analytical Development (£1.25 million), and Nonclinical Manufacturing (£1.0 million) (courtesy of Cell Therapy Catapult)

	Equipment	Process	Application
Current Capability	• Manual Filling station • Quantum XCELLigence • Tube welder and sealer • ViCell	• Fully integrated vial fill, seal and capping • Hollow fibre bioreactor • Aseptic connection formation between tubing • Trypan blue exclusion	• Small scale closed vial production • Closed system cell expansion • Formation of 'closed' processing between unit operations • Automated cell viability analyser
Guys Facility	• ~ 50 L disposable bag STR • DynaMag • Asymptote (CRF) • Automated filling station • Wave Bioreactor • Sepax • Continuous cell harvest • CliniMACs	• Single use reactor with online monitoring and control • Immunomagnetic separation of cells • Stirling engine freezes vials/bags • Automated vial fill, seal and capping • Wave technology within disposable bag • Automated density gradient separation • Tangential flow filtration/continuous centrifugation • Immunomagnetic separation of cells	• Scalable cell expansion • Selective cell purification • N2 free controlled cryo preservation • High throughput automated closed vial production • Scalable cell expansion • 'Closed' cell separation • High throughput liq/solid separation • Selective cell purification

	Equipment	Analytical Technique	Application
Current Capability	• MacsQuant • M1000 plate reader • LightCycler • QiaCube • Bioanalyser • Nanodrop	• Fluorescent flow cytometry • Fluorescent/Absorbance Spectrophotometry • Real time PCR • Automated DNA/RNA extraction • Microfluidic electrophoresis • UV-Vis spectrophotometry	• Cell marker characterisation • Colourometric/ fluorescent assays • Gene expression analysis • Gene expression analysis • DNA/RNA quality measurements • DNA/RNA concentration
Guys Facility	• In-Cell 6000 • Biomark HD • BD Virse • BioReader • Quant Studio • EpMotion • Eclipse Ti-E	• Laser confocal high content screening • Digital PCR • Flow Cytometer • Elispot imager • Real time PCR • Liquid Handling Robot • Motorised fluorescent Microscope	• High throughput confocal microscopy • Absolute gene expression quantification • Cell marker characterisation • Cytokine analysis • Gene expression analysis • Automated assay preparation • Automated cell imaging

	Equipment	Analytical Technique	Application
Current Capability	• Tube welder and sealer	• Aseptic connection formation between tubing	• Formation of 'closed' processing between unit operations
Guys Facility	• CliniMACs • ViCell • Wave Bioreactor • Sepax • Isolator • Automated filling station	• Immunomagnetic separation of cells • Trypan blue exclusion • Wave technology within disposable bag • Automated density gradient separation • GMP Grade A environment • Automated vial fill, seal and capping	• Immunomagnetic separation of cells • Automated cell viability analyser • Scalable cell expansion • 'Closed' cell separation • Production of clinical grade material • Automated vial fill, seal and capping

Table B.2. CTC Capabilities for Process Development, Analytical Development, and Manufacturing Supply Chain (courtesy of Cell Therapy Catapult)

Process Development	
Resourcing	• 4 FTE expanding to 7 FTE by end of Oct, target 15 FTE by Jan 2014, potential for additional 3-6 secondees • £1.7M of Capital Equipment
Team Experience	• Over 35 yrs • Pharma, Biotech, SMEs, Academia
Capabilities	• QbD, experimental design, TPP, risk analysis, device design control, bioreactor design, automation and software design, CoG reduction • iPS culture, directed differentiation, decellularisation, encapsulation, large-scale cell culture, cell banking, 3D scaffold production, suspension culture • Advising on Clinical production, cGMP manufacturing • Process development for autologous immune therapies, closed processing, large scale adherent and suspension cultures, as well as novel process development for 2D and 3D therapies.
Catapult Projects Executed	• Suitability projects for an autologous 3D scaffold • An iPS-derived source of a blood component • 3+ autologous oncology therapy companies • Plus assessments for 2+ academic Treg organisations. • Major technical programme over 3 years for a Somatic Cell Product for Ph 3 readiness • Capacity modelling for UK manufacturing. • Equipment Technology evaluation to ensure at the leading edge

Analytical Development	
Resourcing	• 5 FTEs expanding to 7 FTEs by end of 2013 • £1.25M of Capital Equipment
Team Experience	• Over 60 yrs total research experience • SME's, Biotech, Large Pharma, Contract R&D, Academia
Capabilities	• Cell therapy product characterisation (identity, purity, impurities, stability, biological function) • Potency assay development and optimisation • Assay design and validation for product release • Analytical method development to support QbD
Catapult Projects Executed	• Optimisation of surrogate potency assay for application in Ph 2 clinical trials • Drug product characterisation using molecular expression profiling • Development of identity tests based on cell surface marker expression • Characterisation of growth media to support manufacture process development. • Equipment Technology evaluation to ensure at the leading edge

Manufacturing & Supply Chain	
Resourcing	• 1 FTE, expanding to a team of nine including head of manufacturing • £1M of capital equipment within a 'GMP proving lab' including a custom designed isolator, and automated cryovial filling and sealing line, wave reactor cell expansion capacity and selective purification potential
Team Experience	• Over 15 yrs • NHS (Blood & Transplant Service)
Capabilities	• Clinical trial supply chain management for Ph. 2 trials, with plans for supporting larger pivotal studies • Validating end to end supply chains (e.g. due diligence testing, risk assessments etc.) for supply routes • Manage relationships with logistics suppliers for 3rd parties • Outsource brokering of manufacturing requirements for 3rd parties • Tech transfer and manufacture of clinical supplies with CMOs • Proving of novel processes under GMP conditions • Process comparability • Stability studies
Catapult Projects Executed	• Comprehensive evaluation of multiple UK-EU logistics routes for time-critical products • Logistics management tendering for a Phase 3 multi-centre European trial on behalf of 3rd party • Part of Consortium awarded £7.7M under UK Government's Advanced Manufacturing Supply Chain Initiative (AMSCI), with Oxford BioMedica, the Heart of England NHS Foundation, Cranfield University and Biotec Services International Ltd • Signposting of 3rd parties to UK GMP manufacturing facilities

Table B.3. CTC Nonclinical Safety, Regulatory (and Quality), Clinical Development, and Clinical Operations Capabilities (courtesy of Cell Therapy Catapult)

Non- Clinical Safety	
Resourcing	• 1 FTE with plans to expand to 2-3 FTEs
Team Experience	• Over 20 yrs • Pharma (Stem Cell Safety), Biotech (Biologics Non clinical Development & Immunology)
Capabilities	• Non clinical Safety: Advise on safety study design and placement. Particular expertise in therapies derived from pluripotent stem cells • Biomarker & Clinical assay Development, including patient biomarker & safety monitoring assays • In house Immunology expertise
Catapult Projects Executed	• Non clinical safety scientific due diligence of opportunities • Pre-clinical safety design, regulatory approval, placement and conduct • Development of patient assays for clinical trials

Regulatory (and Quality)	
Resourcing	• 3 FTE's with plans to expand to around 5 FTEs
Team Experience	• Over 35 yrs. Regulatory submissions > 20 trials & > 7 products covering Adult Stem cells, Somatic Cells, Pluripotent derived therapies, Immune Therapies in EU and US • Pharma, Biotech, NHS, Academia (specialising in cell therapy as well as traditional and blood)
Capabilities	• Expertise in regulation of cell therapies (ATMPs and minimally manipulated): Scientific advice and trial authorisations in UK & EU wide • Clear lines of communication with UK, EMA regulators and policy makers
Catapult Projects Executed	• ATMP EMA classification • Gap analysis, preparing and reviewing submissions for immune therapies, gene modified therapies and adult stem cell therapy clinical trials • Gap analysis, preparing for scientific advice and reviewing clinical trial submissions for immune therapies, gene modified therapies and adult stem cell therapy and tissue engineering products • Working with MHRA, EMA and other industry bodies to improve regulatory environment for cell therapies

Clinical Development	
Resourcing	• 1 FTE, with plans to expand to at least 2 FTEs
Team Experience	• Over 20 yrs • Pharma, Biotech (covering traditional, biologics and cell therapies)
Capabilities	• Clinical development planning and target product profiles including commercial aspects • Clinical trial design including regulatory acceptability • Clinical Safety monitoring and working with DSMBs
Catapult Projects Executed	• Development plans and trial design for immune therapies, gene modified cell therapy and regenerative cell products

Clinical Operations	
Resourcing	• 4 FTE's with plans to expand to at least 5 FTEs
Team Experience	• Over 20 yrs • Pharma, Biotech, NHS, CRO (traditional, biologics and cell therapies)
Capabilities	• Clinical trial feasibility, site selection, set-up, monitoring, recruitment and reporting, across therapeutic areas and including orphan and rare diseases • Cell Therapy clinical trial infrastructure and KOL / site network especially in the UK • Specialist in cell therapy trial operational logistics
Catapult Projects Executed	• Clinical feasibility, site selection, costing, KOL interactions, ethics interactions and R&D approvals for cell therapy product(s) • Clinical protocol, crf and database set-up • Quality system to enable ability to act as clinical trial sponsor

CTC engages with its partners in primarily three different types of relationships:

- Service Provision - wherein CTC provides its services in return for a fee
- Licensing - wherein CTC either (i) in-licenses a product/therapy from Industry and undertakes further development with the Industry having an option to re-acquire the therapy at some future milestone event, or (ii) out-licenses a product/therapy developed in collaboration with an Academic partner to Industry following de-risking the product/therapy at any stage in the preclinical through completion of phase II human trail(s).
- Collaboration - wherein CTC and the Collaborator work together to generate mutual benefits from a development project; such as for example: the Collaborator may obtain new IP/files for patent(s) and CTC develops new skills or improves expertise in areas that could be subsequently applied to other CTC projects.

SOURCES OF SUPPORT

CTC was established with a core grant of £70 million from the UK Technology Strategy Board (TSB). This initial funding is for a period of 5 years. Following the initial 5-year period, CTC anticipates having access to multiple sources of funding, including:

- £10 million per annum in continuing grants from UK TSB from 2019 onwards
- £10 million per annum from other grant sources
- £10 million per annum from industrial research and sponsored studies to permit continuing operations at an annual budget of £30 million.

Besides the above funding plans, CTC plans continue to seek additional support from the UK Government, as it demonstrates progress of its annual plan(s) and ability to meet or exceed Key Performance Indicators (KPIs) and identified new gaps. One example of an additional gap assessment and funding thereof was recently announced as part of The Chancellor of the Exchequer's presentation of the 2014 Budget to UK Parliament on March 19, 2014, outlining the establishment of a £55 million UK Cell Therapy Manufacturing Centre managed by CTC that, once operational in 2016–2017, will provide vital large-scale manufacturing facilities, helping UK to retain manufacturing activity, attract inward investment and boost exports (2).

ASSESSMENT

In a recent report, Life Sciences UK (a partnership between the Bio Industries Association, Association of the British Pharmaceutical Industry, Association of British Healthcare Industries, and British In vitro Diagnostics Association, representing the human healthcare industry in the UK) provided an analysis of the delivery of the UK Government's 2011 Strategy for UK Life Sciences (spanning a series of 13 initiatives across the Life Sciences sector ranging from translation of academic research to uptake of innovation and commissioning). In this analysis, the Cell Therapy Catapult was commended as being one of eight (of the 13) initiatives that had made notable progress towards the actions and commitments set out in the original strategy.

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http://www.bioindustry.org/newsandresources/bia-news/government-made-progress-delivering-strategy-uk-life-sciences/

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OVERVIEW

EPFL was reorganized as a national university in 1969 as the second of two Swiss Federal Institutes of Technology; the other is ETH Zurich. EPFL is located in Lausanne, on the shores of Lake Geneva at the foot of the Alps. Its main campus houses more than 11,000 students, researchers, and staff. With more than 350 laboratories and research groups on campus, EPFL is one of Europe's most innovative and productive scientific institutions. It is ranked in the top three in Europe and top 20 worldwide in many scientific rankings. EPFL consistently attracts the best researchers in their fields (EPFL 2014).

FUNCTIONAL FOCUS

The Institute of Bioengineering (IBI) provides an interface between the life sciences and engineering. With an emphasis on fundamental research and understanding of basic biological mechanisms, IBI aspires to transform knowledge into clinical applications. Some of the

technological areas that are particularly relevant to the WTEC mission include: delivery of small molecule drugs, proteins and DNA; design of synthetic and biosynthetic biomaterials for bionanotechnology, biomaterials-assisted immunotherapy; functional tissue engineering: interventional and diagnostic biomedical micro-devices and image processing tools; biosensors and neuro-electrodes; soft bioelectronic ("electronic skin") and brain–machine interfaces, and biotechnology for therapeutic protein production.

RESEARCH & DEVELOPMENT ACTIVITIES

Prof. Stéphanie Lacour presented some of her work on soft bioelectronic interfaces, which brings together advances in bioengineering and neuroscience. She and her colleagues are moving toward personalized neuroprosthetics through a walk-again initiative, a bionic hand, rehabilitation after strokes, and human-computer confluence, as well as hearing and vestibular research. Her mission is to improve biocompatibility and enhanced functionality of hybrid interfaces between man-made devices and biological systems. Her Laboratory for Soft Bioelectronics Interfaces (LSBI) intends to develop electronic systems adapted to the human body and aligned to medical needs. Some technologies under development include: soft polymers, electronic materials like thin films and nanowires, and dry-patterning and MEMS-like fabrication technologies. Her group seeks to discover the engineering, computational, and neuroscience principles employed by the nervous system, and to exploit these insights to create materials and devices that will help develop enabling technologies to revolutionize healthcare in neurological diseases.

Dr. David Hacker reviewed the EPFL Protein Expression Core Facility (PECF), which he manages. The PECF was established to provide low-cost recombinant proteins for researchers. The PECF offers several services including large-scale transient expression of recombinant proteins in transfected mammalian cells. In addition, the PECF will adapt existing recombinant cell lines or hybridomas to growth in serum-free suspension culture for the large-scale production of recombinant proteins or monoclonal antibodies. Its suspension cultivation system is for mammalian and insect cells, using a serum-free medium in 2 mL to 10 L cultures in orbitally-shaken containers. He demonstrated the transfection of mammalian cells using polyethylenimine for DNA delivery, transient protein production in Sf9 cells, their Piggybac Transposon System, assembly of gamma-secretase, PB-generated gamma secretase cell lines with purified recombinant gamma-secretase, and recombinant antibody production using stable CHO DG44 pools. He addressed issues for scale-up of animal cell lines in orbitally-shaken containers for K562 myelogenous leukemia cells, hybridomas, and human immortalized lymphoblastoid cell lines. The core facility also produced proteins in *E. coli* cultures up to 20 L, using simple affinity purification.

ASSESSMENT

The Institute of Bioengineering (IBI) is one of the major innovation centers in Europe advancing biotechnology and bioengineering. LSBI is taking an interesting interdisciplinary approach to integrate technological advances in materials science and electronics to create artificial skin and ultra-compliant neural electrodes. As these hybrid devices are at the cutting edge of the field of biomedical devices, manufacturing issues are not at the forefront of concerns at the moment. Another direction of innovation is the development of recombinant biomaterials for a variety of therapeutic applications including immunotherapy and regenerative medicine. Recombinant polymers enjoy the advantage of precise control in composition, molecular weight, and molecular structure. Production of these polymers in an academic institution has proved challenging. The in-house support of EPFL with PECF bodes well for the development of these innovative biomaterials technologies.

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Edinburgh Enterprise and Edinburgh BioQuarter

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OVERVIEW

Edinburgh Enterprise is part of a national economic development authority that covers all disciplines on the way from basic discovery to commercial and government use. Mr. Henderson identified an overarching objective of Edinburgh Enterprise in helping to form the Edinburgh BioQuarter on the campus of the University of Edinburgh: the creation of an entire "ecosystem" to support the development of new therapies to treat disease and loss of function. Edinburgh BioQuarter occupies a central structure on the campus in close contact with the Scottish Center for Regenerative Medicine (SCRM). Edinburgh BioQuarter occupies 2½ floors in Building Nine with the ability to manufacture cells under GMP conditions by Roslin Cells (see the separate site visit report on Roslin Cells for additional details).

Edinburgh BioQuarter functions on an Academic Medical Centre complex that combines outstanding biomedical research from the University of Edinburgh with the clinical expertise of NHS Lothian and a seasoned team of industry professionals, all based at the BioQuarter campus three miles from the center of Edinburgh City.

FUNCTIONAL FOCUS

The functional focus of Edinburgh BioQuarter is to bring together clinicians, patients, scientists, R&D facilities, clinical trial facilities, an academic teaching hospital, and industry/commercialization expertise at a single campus (Figure B.2). Edinburgh BioQuarter carries out no research and development itself, but provides an environment and administrative expertise that facilitates the translation of research and development activities by the participating scientific organizations and companies to clinical and commercial practice.

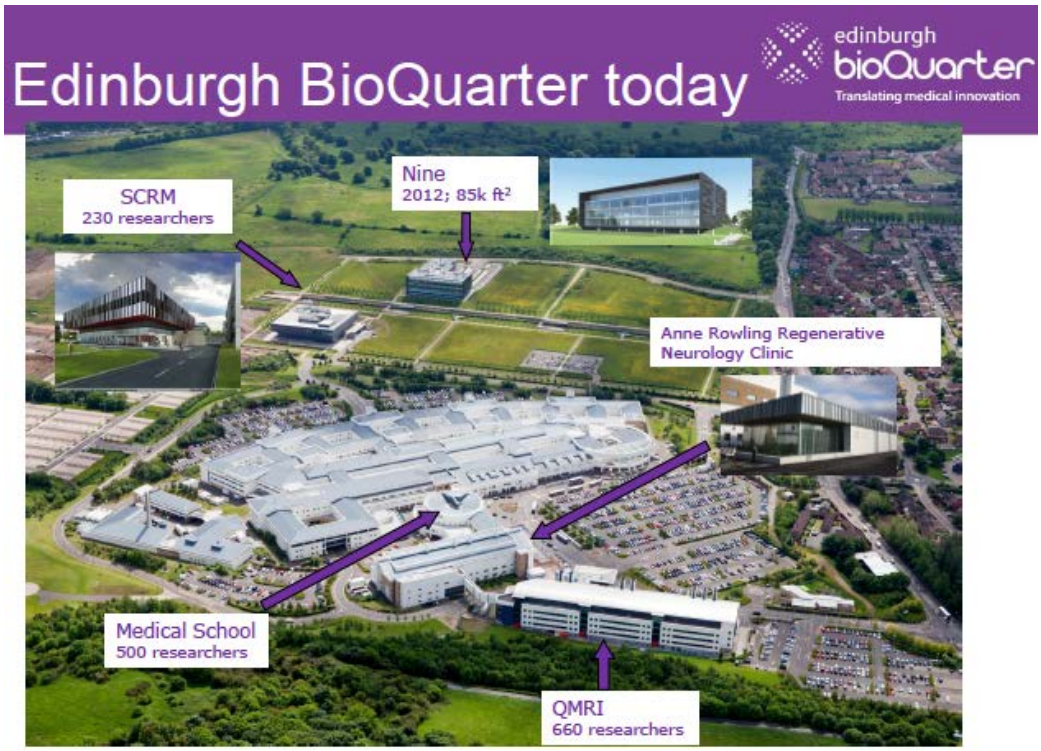

Figure B.2. Aerial photo showing the principal institutions of the Edinburgh BioQuarter (courtesy of Edinburgh BioQuarter).

TRANSLATION

The wide range of capabilities concentrated on the BioQuarter campus creates a powerful center for translation of medical research in cell therapy into clinical practice. There are currently 960 hospital beds and 1,300 researchers on the campus. Longer range planning anticipates more than 1,500 hospital beds and 2,000 researchers by 2016. The campus also offers an imaging center with PET, MRI, and CT-slice scanners and a Medicines and Healthcare Products Regulatory Agency (MHRA)-accredited Phase I clinical trials unit in addition to Advanced Therapy Medicinal Products (ATMP) GMP manufacturing capability for stem-cell expansion and manipulation. The site also is home to the Medical Research Council (MRC) Scottish Centre for Regenerative Medicine (SCRM), a leading center developing understanding of stem cell manipulation, differentiation, and function. SCRM has on-going collaborations with Biogen Idec on the study of Multiple Sclerosis and motor neuron disease in concert with the Anne Rowling Regenerative Neurology Clinic. Other collaborations include those with AstraZeneca, GlaxoSmithKline and Eli Lilly.

Edinburgh BioQuarter, working with the Stem Cell Intervention Framework, has overseen investment of £90 million since 2004. Nine start-up companies that have been launched at this writing (Table B.4).

SOURCES OF SUPPORT

Edinburgh BioQuarter and SCRM receive funding from the Medical Research Council (MRC), The United Kingdom Stem Cell Foundation, Cell Therapy Catapult, and the Wellcome Trust.

Table B.4. Companies Developed with the Aid of Edinburgh BioQuarter (courtesy of Edinburgh BioQuarter)

No.	Company	Description	Status
1	NeuroOrg	Consultancy	Launched
2	ipSOX	Insulin pump accessories	Launched
3	I2eye Diagnostics	Peripheral vision testing device	Launched
4	Coolgenics	Stem cell preservation	Launched
5	Cytomos	Benchtop Mass Spectrometers	Launched
6	Pharmatics	In silico drug screening	Launched
7	Aquila BioMedical	CRO – specialist disease models	Launched
8	Edinzyme	Reagent supplier	Launched
9	Edinburgh Molecular Imaging	Optimal Molecular Imaging	Launched
(10)	(NeurocentRx)	(Re-profiled Pain Therapeutics)	(March 14)

ASSESSMENT

All of the participants agreed that the nature of cell therapy development and regenerative medicine is inherently multidisciplinary. Edinburgh BioQuarter and its consortium members lower the barriers to cross-disciplinary research, development and commercialization.

Edinburgh BioQuarter has similarities to the Cell Therapy Catapult (CTC; London) but with a singular focus on Scotland and particularly Edinburgh. Its purview is broader than CTC with its focus on commercialization and funding to carry research through to full commercialization. With more than 15 spin-off companies since its inception in 2004, it compares itself with the Karolinska Institute with more than 60 spin-offs across its entire history.

The Scottish Centre for Regenerative Medicine maintains a GMP capability to develop clinical cell therapies (with Roslin Cells) from stem cells targeting clinical application for liver, brain, blood, and cardiovascular disease intervention. They are close to completing Phase 2 liver therapy in mouse models that will lead to human trials. They are developing imaging capabilities that will provide insight into how tissue repair progresses, leading to the essential metrics and analytics of tissue repair. At this point, the programs in liver therapy are limited to children by supply of hepatocytes. Expansion of cell therapy approaches is needed to extend the program to adults.

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OVERVIEW

The Department of Biosystems Science and Engineering (D-BSSE) is a multidisciplinary department of ETH Zurich located in Basel, Switzerland. The research of the D-BSSE faculty is broadly concerned with the rational engineering of biological systems. The ETH D-BSSE Zurich was conceived in 2002 and launched in 2007. It was founded with the aim of achieving collaboration and cooperation across administrative divisions, infrastructure, and scientific pursuits. It is home to over 250 researchers and 100 Ph.D. students. The research output of ETH Zurich in the area of complex biological systems is comparable to many leading U.S. institutions, including Harvard, MIT, and UC-Berkeley. Annual research expenditures are approximately 30 million euros. The D-BSSE is a relatively new department. It is the third youngest department within ETH Zurich and the second smallest department. It is also the only ETH department located in Basel. One of the key drivers in locating the department in Basel is the proximity to the robust pharmaceutical industry there. Other geographic circumstances were factored into this decision.

FUNCTIONAL FOCUS

Research activities within D-BSSE include the discovery and development of new methods and technologies for biological systems analysis. Furthermore, there is a visible strength in developing ways to engineer (program or reprogram) biosystems. The core areas of research include synthetic

biology, technological developments to study complex biosystems, and applications of complex biosystems. The systems biology pillar focuses on the detailed comprehensive and quantitative analysis of biological systems across all regulatory levels. The research area of complex biosystems focuses on the development of microsystems for miniaturization, parallelization, manipulation, and modeling of biological systems. Applications of these research efforts include the use of cellular systems for a range of industries including biochemical processing. The faculty members who conduct these research activities have broad expertise in experimental biology, theory, and engineering.

There are many opportunities for advancing the research program within the D-BSSE. Key initiatives include both increasing and decreasing complexity in biological systems. Representative examples of increased complexity include systems analysis with multiscale and multiparametric analysis. Research activities focused on managing complexity in bioengineered systems include novel design strategies, scalable mathematical models, and the development of orthogonal bioprocesses. There is also a broad interest in developing targeted interventions such as cell-based therapies, personalized medicine, the design of rational interventions, and optogenetics. Finally, there is a robust interest in the construction of large scale synthetic systems including integration of automation in design and fabrication of biological systems.

RESEARCH & DEVELOPMENT ACTIVITIES

The D-BSSE includes many thought leaders across a wide range of competencies including bioprocess engineering, bioelectronics, and synthetic biology.

Sven Panke directs the Bioprocess Laboratory within D-BSSE. This laboratory is broadly focused on three key areas: screening methods for more robust and effective catalysts; integrated separation processes to overcome thermodynamic limits; and reaction networks for one-pot synthesis of fine chemicals. These efforts blend several classical approaches (e.g., separations processes, microencapsulation techniques, directed evolution) with novel techniques (e.g., synthetic biology.

Andreas Hierlemann directs the Bioengineering Laboratory (BEL) at D-BSSE. This group is focused on the integration of microsensors and microsystems for use in biological applications. The core competencies include circuit design and device fabrication for use in chip-based electrophysiology and neuroscience. Leveraging CMOS technology for this application is particularly advantageous for use in physiology measurements.

Yaakov Benenson directs the Synthetic Biology Laboratory, which engages in bottom-up engineering of biological information processing. This group addresses grand challenges in designing complex biological systems using a system (versus *ad hoc*) approach for potential applications *in vitro* and *in vivo* for use in cancer therapy, immunotherapy, and bioproduction.

Martin Ehrbar is group leader at the University Hospital Zurich and directs the laboratory of Cell and Tissue Engineering. This laboratory has broad interests in the development of functional hydrogels for the formation of 3D-structured microenvironments and the controlled release of cell-instructive bioactive molecules. Clinical applications include development of biomaterials for endovascular devices, materials for bone regeneration, materials for fetal membrane regeneration and tissue sealants.

TRANSLATION

There are notable commercialization efforts that are being pursued by the D-BSSE. In the course of the Department's still short history, five spin-off companies have been founded with great success. For example, a start-up company entitled InSphero AG was founded in 2009 to translate microscale tissue structures. This award-winning start-up company opened an office based in the United States shortly after its inception.

SOURCES OF SUPPORT

The D-BSSE can draw funding support from many sources. Representative sources include the NanoTera and SystemsX.ch (Swiss National Initiatives), European Research Council (ERC), European Union framework programs, Marie-Curie, the Swiss National Science Foundation, and internal grants within ETH. Specific granting mechanisms from the ERC include advanced and starting grants and "Proof-of-Concept" (PoC) grants. The latter represents a translational granting mechanism to perform research toward developing a product that has a high potential for commercialization. The ERC PoC grants last for 1 year in the amount of 150k euros, which is approximately 10% of the amount of a standard ERC grant. Some investigators have been funded from U.S. foundations including the NIH via an R01 granting mechanism. The department was also recognized as a co-leading house of the National Center for Competence in Research (NCCR) on Molecular Systems Engineering. This stream of funding is designed to bring together core expertise in a key technical area in a long-term (up to 12-year, up to ≈30 million-euro) project.

There are world class facilities in place at the D-BSSE to support the research activities. For example, the genetic sequencing core has an Illumina 2000 sequencing core and a teaching/learning instrument as well. There are robust and highly sophisticated automated cell culture facilities to support the research activities of the faculty. Three automated cell culture systems and liquid handling systems are supported by a highly skilled and mature technical staff. The microscopy suite is also highly sophisticated and capable of single cell handling, live cell imaging, laser scanning confocal imaging. Custom microscopy setups also support microfluidic cell culture and electrophysiology. Moreover, there is a fully equipped microtechnological clean room.

ASSESSMENT

The D-BSSE at ETH Zurich in Basel is a highly sophisticated and well-funded research institute with world class faculty, facilities, and trainees. The product of this research has worldwide impact in fields such as microfabrication, systems biology, synthetic biology, and bioprocessing. The D-BSSE is a highly innovative and collaborative department within ETH Zurich. The proximity of academic research facilities near the established pharmaceutical industry in Basel will provide enormous opportunities for collaborative academic-industrial partnerships. It is projected that the D-BSSE will forge many novel basic discoveries and inventions that can be leveraged to develop many technologies that will underpin advances in biomanufacturing.

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OVERVIEW

EUFETS is a provider of integrated product development and cGMP manufacturing services for advanced cell and gene therapy products, viral vectors, and messenger RNA therapeutics (used for example as tumor vaccines). It was founded in 1997 as spin-off from local hospital Bone Marrow Transplant Unit, was acquired as a wholly-owned subsidiary of Fresenius Biotech GmbH in 2001, was subsequently divested into a private company, and was acquired by BioNTech AG (Mainz, Germany) in June 2009.

FUNCTIONAL FOCUS

EUFETS operates certified GLP laboratories for product and process development and a licensed cGMP facility for clinical commercial manufacture of mRNA, viral vectors, cell therapy products, and *ex vivo*, gene-modified cell therapy products. EUFETS works with academic investigators, emerging biotechnology companies developing cell and gene therapy products, and with large pharmaceutical companies.

RESEARCH & DEVELOPMENT ACTIVITIES

EUFETS activities encompass process, product and analytical development; product characterization; and manufacturing & fill/finish operations for the following product types:

- Viral Vector Products (for Retrovirus and Lentivirus vectors) - including vector construction; development of producer cell lines; generation and characterization of Master Cell Bank and Working Cell Bank; and cGMP production of viral vectors

- Cellular Products - including processes and technology platforms for cell isolation, culture, expansion, differentiation, & characterization of primary immune, stem, and somatic cells; and cGMP manufacture of cell therapy and *ex vivo* gene-modified cell (gene) therapy products
- Messenger RNA Therapeutics - including design of nucleotide templates, and *in vitro* enzymatic transcription for cGMP manufacture, fill/finish, and characterization of mRNA therapeutics and vaccines

TRANSLATION

To date, EUFETS has produced more than 1,000 cGMP product lots in its 500 m^2 cGMP facility, which consists of 9 clean room suites of class A/B (class 100) and one suite of class C (class 10,000) for multipurpose, multiproduct concurrent manufacturing. EUFETS has also been involved in numerous studies employing cell therapy and *ex vivo* gene-modified cell therapy in Europe. Publicly disclosed products manufactured at EUFETS facilities are listed in Table B.5.

Table B.5. Publicly Disclosed Products Manufactured by EUFETS (courtesy of EUFETS GmbH).

Indication	Clinical Trial Phase	Country	EUFETS Contribution
Various	n.a.	Germany	Manufacturing and QC of >1000 products (leukapheresis, bone marrow, cord blood, CD34+ stem cells, donor lymphocytes)
Leukemia	GvHD suicide gene therapy Phase I/II	Germany	Manufacturing and QC of retroviral vector batch & gene modified T cell products
ADA SCID	Phase I/II stem cell gene therapy trial	UK	Manufacturing and QC of retroviral vector batch
CGD	Phase I/II stem cell gene therapy trial	Germany, UK & Switzerland	- Process development - Manufacturing and QC of retroviral vector batch & gene modified stem cell products
HIV	Phase I/II T-cell and phase I/II stem cell gene therapy trial	Germany	- Process development & preclinical evaluation - Manufacturing and QC of retroviral vector batches & gene modified T-cell products
Cancer	8 Phase I/II T-cell gene therapy trials	UK, Netherlands & Australia	Manufacturing and QC of retroviral vector batches
Wiscott Aldrich Syndrome	Phase I/II stem cell gene therapy trial	Germany	Manufacturing and QC of retroviral vector batch & gene modified stem cell products

SOURCES OF SUPPORT

EUFETS is a revenue-generating subsidiary of BioNTech AG based on provision of contract services to its parent and customers. Their current customer base includes an approximately equal number of industrial and academic groups. However, the industry customers account for majority (approximately 80%) of the capacity demand/volume (product lots) and revenues. In addition, EUFETS has also obtained funding through European Consortium (FP7)-funded grants for clinical manufacture of cell and gene therapy products in support of European clinical trials.

ASSESSMENT

EUFETS has developed an efficient operational process for streamlined translation of novel cell and gene therapy products, viral vectors, and mRNA therapeutics/vaccines. EUFETS developmental capabilities, core competencies, cGMP facilities and modular operations thereof permit it to concurrently manufacture multiple and complex ATMP (cell therapy and gene-modified cell therapy) products within a relatively small facility, with appropriate levels of procedural control and with a small staff of approximately 70 employees. EUFETS represents one model of successfully leveraging automated platform technologies, modular facility design, and operating procedures to develop, manufacture, characterize, and deliver cost-effective ATMP

products throughout Europe (and beyond). Progression of ATMP products into more advanced stages of development and larger clinical trials (and commercial markets) will require novel approaches to integrate manufacturing platforms into an assembly-line type process to permit up-scaling to meet projected market demand while significantly reducing COGS for manufacture and delivery. The foundational elements for such up-scaling efforts appear to be in place at EUFETS, but the integration into commercial manufacturing processes will require further investments and innovations. EUFETS has decided to keep its business focused on the continuum from preclinical through late-stage clinical manufacturing and transfer to commercial manufacturing—without making any investments (unlike other CMOs) in building infrastructure for commercial manufacturing based on current (inadequate) "state-of-art" manufacturing processes.

More recently, EUFETS indicated that it had seen new interest, involvement and support for Large Pharmaceutical companies in the Cell and Gene Therapy product development efforts in Europe. EUFETS is currently working with a large pharmaceutical company on gene-modified, targeted T-cell immunotherapies for oncology.

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OVERVIEW

Founded in 2005, Fraunhofer Institute for Cell Therapy and Immunologies (Fraunhofer IZI) in Leipzig, Germany is one of the seven Fraunhofer Institutes in Germany in life sciences. Its mission is to develop innovative solutions and technologies for improving health. The institute focuses on applied science research to support the development of new products. It has 284 staff members (89% scientific staff). The operating budget in 2013 is 13 million euros, and 40% of the projects are from industry. Its main building has 2,300 m^2 of lab and office space, including 750 m^2 of GMP space. The second extension of the main building is under construction. It also has core cell and molecular biology laboratories. With one of the largest facilities in cell-based therapies in Europe,

Fraunhofer IZI is a leading European institution in developing cell based therapies and has a GMP facility that is one of the largest (top 3) GMP facilities in the cell therapy sector in Europe.

FUNCTIONAL FOCUS

Fraunhofer IZI is a research institute focusing on applied research projects with industry partners. It supports development efforts of its partners by providing them with new technologies, product candidates, and problem solutions. The main part of Fraunhofer IZI's research revenue is derived from contracts with industry and from publicly funded research projects with the industry.

RESEARCH & DEVELOPMENT ACTIVITIES

Research and development focus areas are:

- Cell based therapies, stem cell technologies, and regenerative medicine
- Immunology, immunological diseases, and vaccine technology
- Drug discovery, target validation, and preclinical development
- Diagnostics, biomarkers, and biobanks

TRANSLATION

It has 109 industry clients, 95 academic partners, 50 non-university partners, and 25 clinical partners.

SOURCES OF SUPPORT

The Fraunhofer IZI supports 50-70 projects per year, of which 14-15% receive support from government agencies and 84-85% are supported by contracts and grants from industry.

ASSESSMENT

- The GMP facility at Fraunhofer IZI has small units for specific customers; these are more cost effective compared with large units.
- Costs of goods are a major challenge. For now, most of companies in cell therapies are small companies, and the costs for manufacturing, quality control, and logistics are too high. Health insurance companies cannot pay for the high cost of cell-based therapies. Costs must be reduced.
- As for automation, Dr. Emmrich indicated that currently no company can afford to have fully automated processes in cell-based therapies. The "handmade" processes are very costly and have high variability. In 5-10 years we will see a switch to automated processes.
- Fraunhofer IZI recruits students and fills academic positions from various sources. In Germany, universities are designed for training people for industry, so they are not well prepared for special requirements or fields experiencing rapid change because of continuous innovation. There is a need to promote Ph.D. level research. It is also difficult to find people who have experience and training in GMP and policies.
- There are not enough examples of cell-based therapies in the clinic for health insurance companies to evaluate. Fraunhofer IZI has continuous discussions with insurance companies for processes and products being developed.

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http://www.izi.fraunhofer.de/fraunhofer-izi.html?L=1

Fraunhofer IZI at a glance. [PDF]

Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB)

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Date Visited: March 3, 2014

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OVERVIEW

The Department of Cell and Tissue Engineering at Fraunhofer IGB, headed by Dr. Schenke-Layland and Dr. Kluger, focuses on 3D tissue models that can serve as alternatives to animal testing. The Institute also has an interest on developing transplants, with an emphasis on the development of tissue engineering products that are GMP-friendly. The Institute addresses the tissue engineering paradigm from biomaterials synthesis to bioreactor design and to clinical translation. Fraunhofer IGB employs a staff of 292 at the end of year 2012, with ~90% of the staff scientific researchers.

FUNCTIONAL FOCUS

The Department of Cell and Tissue Engineering focuses on three areas: 1) biomaterials and *in vitro*-test systems; 2) bioreactors, bio-imaging and cardiovascular systems; and 3) GMP-production of cell-based products. It has an emphasis on optimizing and functionalizing surfaces for cell-substrate interactions, implantation, and as scaffolds for tissue engineering.

RESEARCH & DEVELOPMENT ACTIVITIES

The tissue engineering approach of the Institute follows the conventional paradigm of cell isolation, cell expansion (possibly in 3D scaffold), and followed by *in vitro* (drug testing) or *in vivo* (implantation) applications.

The research areas highlighted are development of materials for bone replacement, natural human extracellular matrix (ECM) proteins, and synthetic blood vessels. More information on specific projects include:

- Human skin model. It includes a melanoma and pigmented skin model for *in vitro* cytotoxicity testing and drug development.
- Raman spectroscopy and multiphoton imaging to non-invasively monitor the phenotypic development of cultured cells. The spectroscopic and autofluorescence signatures may indicate phenotypes such as state of differentiation, apoptosis, and ECM secretion. If successful, such technology would be important for the biomanufacturing process in both non-invasive monitoring and quality control.
- Cardiovascular tissue engineering/disease-in-a-dish model. The approach is to identify the most suitable cell source (autologous, adult stem cells, iPSC), identify the valvular ECM, and mimic this ECM by biomaterials design and fabrication such as hydrogel synthesis and electrospinning.

The Institute has a significant effort on developing bioreactor systems to augment its tissue engineering research. This effort is aided by mathematical modeling and tissue-specific considerations to optimize the bioreactor design with respect to mass transport and biomechanical cues. The goal is to create a biomimetic microenvironment with physiologically relevant flows to promote optimal tissue development.

There also appears a strong synergism with the Fraunhofer Institute for Manufacturing Engineering and Automation (IPA) at Stuttgart. To assist the 3D tissue development, IPA designs automated systems for cell manipulation and cell culture. Presumably IPA is also involved in novel bioreactor designs. The Department of Cell and Tissue Engineering and IPA are housed under the same roof to facilitate close collaboration.

TRANSLATION

The Institute has 10 clean rooms of class A-D according to the EU GMP Guideline. It has a total area of 216 m^2. Its capability includes:

- Clinical network for tissue sampling
- Cell isolation from primary tissues
- Cell amplification and characterization
- 3D tissue engineering & bioreactor technology
- Process development, validation and approval by the authorities

Its technology transfer activities include:

- Production of collagen: manufacture of collagen for research purposes
- Production of Vasograft™: coating of PTFE vascular grafts with autologous endothelial cells

- Production of Ixmyelocel-T: autologous bone tissue based on adult stem cells
- Production of CaReS®: matrix-based autologous chondrocyte implant

SOURCES OF SUPPORT

Its funding support comes from the Fraunhofer Gesellschaft, European Commission, Federal Ministry of Education and Research (BMBF), German Research Foundation (DFG), and industry-sponsored projects in roughly equal proportion. The Department of Cell and Tissue Engineering also collaborates with the California Stem Cell Initiative, receiving funding from the California Institute for Regenerative Medicine (CIRM) at UCLA.

ASSESSMENT

The Department of Cell and Tissue Engineering at the Fraunhofer IGB has a long track record of bioreactor development for cell culture. This has been applied to the construction of 3D tissue models for drug evaluation, with the skin model being the most advanced. Its collaboration with IPA appears to be highly complementary. IPA can recruit students with backgrounds in mechanical engineering, electrical engineering, physics, and informatics to do a master's thesis with the cell and tissue engineering researchers. Together they are in a strong position to advance the field of biomanufacturing with respect to cell-based delivery and therapy.

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Imperial College London

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Other Attendees: **Ken Pierce** (research coordinator for Stevens)
Jean-Phillipe St-Pierre, Ph.D., University of Toronto
Tommy Paschuck, Ph.D., Northwestern University
Joe Steele, Ph.D. student
Andrea Serio, Ph.D., Edinburgh
Rob Chapman, Ph.D., University of Sydney (Chemistry)
Leslie Chow, Ph.D., Northwestern University (materials science)

OVERVIEW

Imperial College London is a science-based institution with a reputation for teaching and research. It was founded in 1907 and presently has over 14,000 students from some 120 nations. The *Times of London* overall ranks it third in Europe, behind only Oxford and Cambridge, founded a bit earlier (Imperial 2014).

FUNCTIONAL FOCUS

The Biological Systems Engineering Laboratory (BSEL) is an interdisciplinary laboratory that associates the principles of process systems engineering with advanced experimental techniques to provide solutions to biological and medical problems. BSEL research areas include: mammalian cell culture bioprocessing, tissue engineering, stem cell technology, and biosynthesis of organic materials. It seeks to link the underlying biology with suitable experimental systems and advanced

mathematical models in order to develop better approaches to improve current technologies in the fields studied (BSEL 2014).

The Stevens Group[3] uses transformative bioengineering approaches to overcome limitations in current materials in: biosensing and regenerative medicine. They focus on understanding and engineering the biomaterial interface using innovative designs and state-of-the-art materials-characterization methods (Stevens 2014).

RESEARCH & DEVELOPMENT ACTIVITIES

The Stevens Group seeks bio-inspired designs for regenerative medicine and biosensing. Their work on engineering materials at the interface between the medical and natural sciences. Among others, its research areas include: multizonal synthetic cartilage scaffolds (Steele and St-Pierre), biological response to binding-peptide functionalized scaffolds (St-Pierre and Chow), biomaterials and devices to model the nervous system *in vitro*, particularly in the use of stem cells to study and treat neurodegenerative diseases (Stevens 2014b).

Athanasios (Sakis) Mantalaris from the BSEL presented a talk on stem cell bioprocessing, which focused on their work to develop a perfusion bioreactor for the physiological culture and targeted differentiation of embryonic stem cells. Their cell culture vessel was designed based on an extensive model, and has been successful in producing osteogenic constructs, as tested in a study to restore holes in the cranium of rabbits (Mantalaris 2014a).

Another BSEL presentation was on the development of a bio-inspired blood factory for personalized healthcare. This project is funded by a European Research Council Advanced Grant. The BSEL team included Profs. Mantalaris and Pistikopoulos, Drs. Panoskaltsis, Misener, Rende, Velliou, and Pefani, plus several graduate students. Bio-inspired features in their system include the first polymeric scaffold 3D hollow fiber bioreactor and the first 3D bioreactor with cell harvesting. In Project 1, their bioreactor produced red blood cells with correct: surface markers, shape, and oxygen carrying capacity. In Project 2 a model was developed to optimize bioreactor operation. In Project 3 a hollow fiber bioreactor was used to experiment with long-term growth in a cytokine-free culture for acute myeloid leukemia (AML). Project 4 deals with heterogeneity in the cell cycle and phase progression, including experiment and modeling for leukemia cell lines. Project 5 is a systems level study of personalized medicine in cooperation with the North West London Hospitals Trust for the provision of bone marrow samples and data from patients diagnosed with AML. The retrospective optimization results demonstrated that continuous doses of some types of chemotherapy are preferred, within bounds of clinically relevant doses and schedules, with plans to translate the optimization model into a prospective clinical trial in the near future (Mantalaris 2014b).

TRANSLATION

The Stevens Group cooperates with Prof. Kevin Shakesheff at the University of Nottingham in the one of the four hubs launched in the UK to deliver cross-UK research in regenerative medicine. Prof. Shakesheff has launched two spin-out companies: Critical Pharmaceuticals, Ltd. and RegenTech, Ltd. to commercialize such research findings. The personalized medicine results from the BioBlood project are being translated into clinical trials.

SOURCES OF SUPPORT

Engineering and Physical Sciences Research Council (EPSRC), Biotechnology and Biological Sciences Research Council (BBSRC), Medical Research Council (MRC), Wellcome Trust,

[3] Prof. Molly Stevens, FRENG, Department of Bioengineering, was not present, but others presented work from her lab.

Department of Trade and Industry, National Institute for Health Research/National Health Service (NIHR/NHS), and European Research Council (ERC).

ASSESSMENT

These labs are doing exemplary research and development that can lead to improved biomedical manufacturing. They pointed out that animal trials are especially challenging in the UK, but the UK National Health Service helps with set-up and support of clinical trials where relevant to the health and wealth of the nation. ICL seemed to be less directly involved in the latter stages of the innovation process, like manufacturing, than some of the other sites visited. They do have translation arrangements with other organizations, which can help.

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INFARMED

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OVERVIEW

The Autoridade Nacional do Medicamento e Produtos de Saúde I.P. (INFARMED, National Authority of Medicines and Health Products, IP) is the national regulatory authority of Portugal. INFARMED works under the supervision of the Ministry of Health of Pharmacy and Medicines and is organized into several sections under an Executive Board (Figure B.3). INFARMED evaluates, authorizes, regulates and controls human medicines as well as health products namely medical devices, homeopathic products and cosmetics. The primary goal of the institute is to ensure the quality, safety and efficacy of medicines and the quality, safety and performance of health products in order to avoid the risks of their use while ensuring adequate standards of public health and consumer's protection.

FUNCTIONAL FOCUS

INFARMED is the central regulatory agency for biomedical, pharmaceutical, and cosmetic products in Portugal. They participate in a variety of regulatory and quality assurance activities including, but not limited to the following activities:

- Research, evaluation, and authorization of medicine and pharmaceutical products
- Quality, safety and efficacy control of medicines
- Setting guidelines and ensuring that companies maintain Good Clinical Practices (GCPs) during the evaluation of clinical trials and post market study
- Licensing, auditing and inspection of manufacturers, wholesalers and pharmacies ensuring the respect for the rules applicable to each operator namely Good Manufacturing Practices (GMPs), Good Distribution Practices (GDPs) and Good Pharmacy Practices (GPPs)

Many of these activities are actively being performed in the context of biomanufacturing including cell-based therapies. INFARMED utilizes a centralized strategy for regulation and approval of many products. For example, INFARMED routinely relies on a panel of experts residing within the EU member states to provide guidance in the ultimate decisions. This strategy was noted as a viable approach to improve the efficiency and help satisfy the overall mission of the agency.

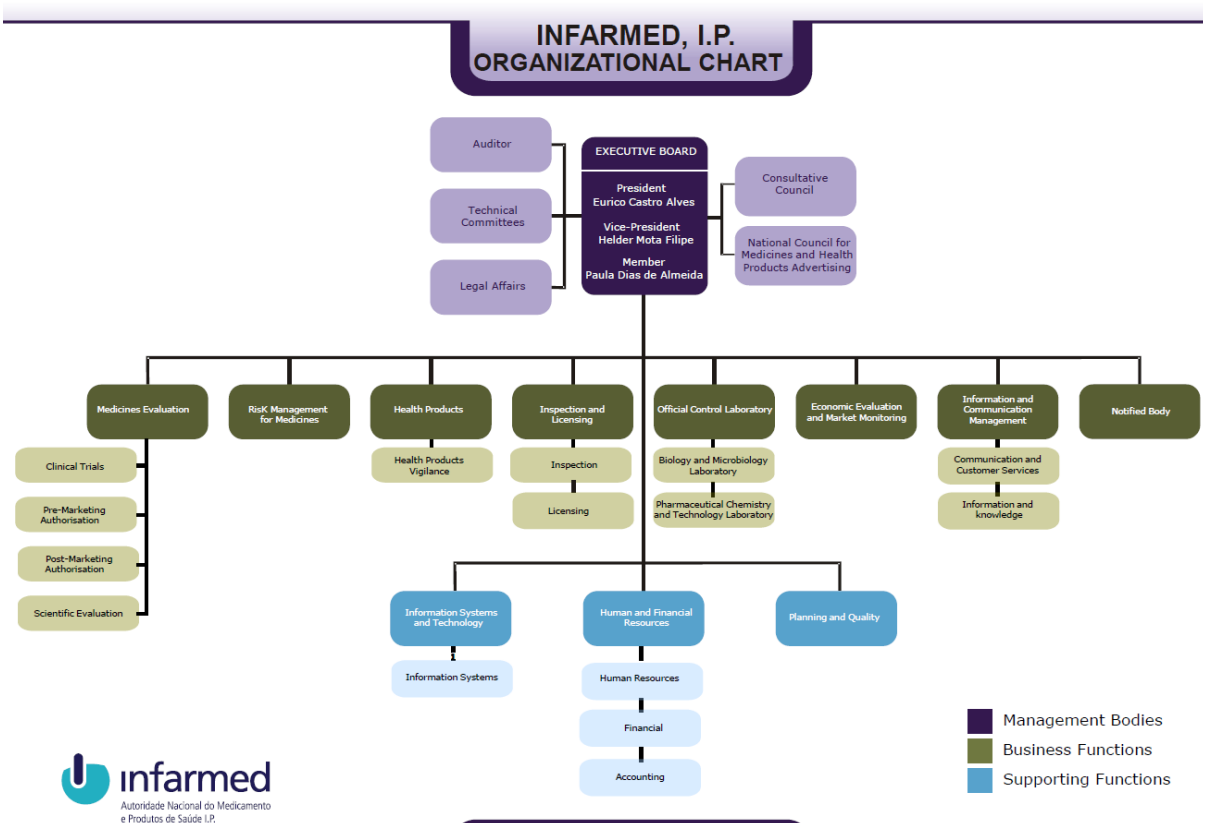

Figure B.3. INFARMED organization chart (courtesy of INFARMED).

Coordination with Other Regulatory Agencies within the EU

INFARMED coordinates the regulatory tasks within the framework set forth by the European System of Medicines. INFARMED ensures the equitable representation and participation in the various evaluation and supervision bodies and activities of the European Medical Agency (EMA) of the European Commission and of the European Network of Medicines and Health Products Authorities.

INFARMED also conducts regulatory functions at several levels including a scope that encompasses both Portugal and Europe. INFARMED works within the National Competent Authority on medicines and health products and also collaborates with the Reference Laboratory on the Quality Control of Medicines within the scope of the Network of Official Medicines Control Laboratories (OMCL).

RESEARCH & DEVELOPMENT ACTIVITIES

As a regulatory agency, INFARMED does not conduct an active research program. There are, however, several key examples of educational programs. One prominent program is a joint Ph.D. program between the National Science Foundation of Portugal (Fundação para a Ciência e a Tecnologia; FCT) and international partners. This training program focuses on entrepreneurship and encompasses collaborative partnerships with four universities. As of 2014, the first phase of

this collaboration was active. The second phase of this project will kick off shortly thereafter and focus on expanding the depth with existing university partners.

Along these lines, another FCT-lead initiative aims to forge collaborations with the National Institutes of Health (NIH) within the United States. This program, called the NIH-FCT Research Fellow Program, awards Portuguese researchers of exceptional scientific ability with post-doctoral fellowships to conduct research in one of the 1200 laboratories of the NIH campuses within the NIH Intramural Research Program. While the latter program may not be directly relevant to the mission to INFARMED, formal collaborations and outreach that are funded by FCT may serve the interests of regulatory agencies.

TRANSLATION

INFARMED is a regulatory agency with the primary responsibility of working with companies to ensure the safety of a broad range of medical products.

SOURCES OF SUPPORT

To fulfill its responsibilities in the various fields the Institute counts with 330 staff members of which more than 50% has an educational background in either the pharmaceutical sciences, biomedical sciences, or related scientific discipline.

ASSESSMENT

As the primary regulatory body for medical products within Portugal, INFARMED play an integral role in advancing biomanufacturing capabilities within the country. The policies and procedures set forth by INFARMED are seen as favorable for expanding research activities in this space. The openness and cooperation by INFARMED was noted as a key strength. The focus on expanding international collaboration and cooperation is also a beneficial aspect of the organization.

Institute of Experimental and Technologic Biology (IBET) and ITQB

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OVERVIEW

The Instituto de Technologia Quimica e Biologicia (ITQB) is a technology research institute associated with the Universidade Nova de Lisboa and located in Oeiras, near Lisbon, Portugal. It has had associated laboratory status since 2011. The primary missions of ITQB are to conduct scientific research and to engage in post-graduate training (Ph.D. and master's students only). The primary research focus is in the field of molecular biosciences, with the aim of addressing societal challenges in: (1) health and disease and (2) biological resources and sustainable development. The ITQB consists of 54 laboratories and more than 470 researchers. More than 200 members hold a Ph.D. and there are more than 150 Ph.D. students.

ITQB interacts very closely with the Instituto de Biologia Experimental e Technologia (IBET), a private, non-profit research institute. The primary goal of IBET is to translate primary knowledge produced by the ITQB and other academic partners plus its own science and technology knowledge to the private sector. This includes commercialization activities within Portugal and the EU at large. The IBET has a large suite of facilities to support these activities. These include 16 full size laboratories (75 m^2) and a pilot plant that is 2,400 m^2 in size. The staff of IBET (85) is significantly smaller than ITQB, but it should be noted that many members with primary appointments in ITQB are also affiliated with IBET. With respect to primary appointments within IBET, there are 24 associates with Ph.Ds. IBET carries out research work with over 30 Portuguese and 50 international companies, over 30 of which are in the biopharma area.

FUNCTIONAL FOCUS

The focus on health and disease research at ITQB involves the following: infection, immunity, antimicrobials, gut microbiota, and advanced protein therapies. The focus on biological resources and sustainable development is primarily on the following research activities: food security, plant sciences, green energy, renewable bioenergy, and bioremediation. The focus on these research areas is based on the traditional strengths in agriculture and plant sciences that have been established in Lisbon for many decades. Several key core facilities exist at ITQB/IBET to support this functional focus. Many of the facilities are clustered around research activities involved in therapeutic protein characterization. These dedicated facilities include NMR, mass spectroscopy, and X-ray crystallography. Other facilities are dedicated for plant sciences and technology. These facilities include greenhouses and growth chambers. IBET adds strong science and technology competences in biopharmaceuticals, in particular advanced therapy medicinal products (ATMPs) and food and wellness.

RESEARCH & DEVELOPMENT ACTIVITIES

The ITQB has a strong dedication to educational programs. There are active programs in the molecular biosciences including foci in biocatalysts, biotechnology, and sustainable chemistry. The educational programs are supported by a network of institutions including the University of Lisbon. The educational programs are well-established and mature. Students take core courses, specialize in a certain area, and then complete a thesis. These students are supported by individual fellowships through the Portuguese federal funding agency (FCT). There are also active masters programs in medical microbiology, science communication, and biochemistry for health. The ITQB has a dedicated program in integrating science with society. These include active outreach programs, integration of art and science, and interaction with students at primary schools.

TRANSLATION

Beyond IBET, carrying out over 6 million euro a year of translational work, there is also a for-profit corporation on site to assist in translation and commercialization. GenIBET Biopharmaceuticals is a for-profit company that is partially owned by IBET (45%). GenIBET consists of 1,000 m^2 total space, over 500 m^2 of which of dedicated GMP, fully compliant space

with four suites (bacterial, viral, cell culture, and fill and finish). They are also working with the FDA to get a facility to supply materials for prospective use in Phase III trials. A key competency for GenIBET is bioreactors that range from 100mL to 50L and the necessary downstream and formulation equipment up to fill and finish suite and are compatible with a variety of cell-based formats including encapsulation, microcarriers, and cryopreservation.

At IBET there are a wide range of core technical competencies. Animal cell technologies include a strong focus on bioprocess development, including cell therapies and the production of viral vectors for gene therapy and viral vaccines. These activities are transferable to GenIBET (http://www.genibet.com) for cGMP compliant production. There are also key activities in stem cell bioengineering including human embryonic stem cells (hESC), human mesenchymal stem cells (hMSCs), and induced pluripotent stem cells (iPS). Lastly, there is a strong focus on 3D cell culture including primary cells, stem cells, and cancer cells for tissue models for research and preclinical trials. Central to these activities is a pilot plant that is designed to process proteins, cells and viruses using a variety of organisms including bacteria, yeast, mammalian cells, and insect cells. This infrastructure is designed to produce preclinical grade therapeutic agents at gram scale. This production facility is GLP certified to facilitate translation and carries out the necessary technical runs when the process is transferred to GenIBET for cGMP compliant production. There is a strong analytic infrastructure. Also GMP certified, to complement the focus on regulatory and commercialization. Analytical laboratory facilities include chromatography, mass spectroscopy, and proteomic characterization suites (X-ray facilities).

SOURCES OF SUPPORT

The primary source of funding for research activities at ITQB is federal grants. At IBET, competitive European grants and European and U.S. company contracts are the norm, as IBET does not get direct governmental support (budget). There are also many international cooperative research partnerships to help support these activities. For example, the MIT-Portugal program is a strong interaction that can bolster research and training activities.

ASSESSMENT

Taken together, IBET and ITQB are well-positioned to conduct translational research in biomanufacturing with a specific focus on bioprocessing for acellular and cell-based therapies. The infrastructure is impressive and is a great asset to the organization. Perhaps the most exciting aspect of this program is the well-established process development competencies leading to GMP facilities. The multifaceted strategy for translation is commendable as well. Strong interactions between ITQB, IBET, and GenIBET will be instrumental in achieving their collective vision.

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www.ibet.pt
www.itqb.unl.pt
www.genibet.com/

Instituto Superior Técnico, University of Lisbon; Cell2B Advanced Therapeutics, SA

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OVERVIEW

The Instituto Superior Técnico (IST) is the largest and best known school in Portugal that is focused on the education of engineers and scientists. There are 12,000 students across multiple campuses. There are approximately 2000 students at the campus in Taguspark, a location in the suburbs of Lisbon. Most of the remaining approximately 10,000 students participate in engineering

programs with facilities in the city center of Lisbon. The primary postgraduate educational and research activities in the Taguspark are focused on bioengineering, including stem cell engineering.

FUNCTIONAL FOCUS

The primary research focus in life sciences and technologies on the Taguspark campus of IST is stem cell engineering and regenerative medicine (Dos Stantos et al. 2014, Rodrigues et al. 2014, Rodrigues et al 2011, and Fernandes, Diogo, and Cabral 2013). The Institute of Bioengineering and Biosciences (IBB), which is directed by Joaquim M.S. Cabral, is positioned within the IST. The current projects of the site focus on the following activities: manufacturing of stem cell-based therapies; multiscale strategies for human pluripotent stem cell bioprocessing; *ex vivo* gene therapy for regenerative medicine; tailoring biomaterials to support stem cell cultivation; and *ex vivo* expansion of mesenchymal stem cells using GMPs. The latter research thrust focuses on expansion of cord blood progenitors for use in combination products.

RESEARCH & DEVELOPMENT ACTIVITIES

The research, developmental, and educational programs are very strong within the IST/IBB. The cornerstone program is an international Ph.D. program that promotes the emergence of thought leaders in academia, hospitals, and industry. Representative frameworks include the goal of product development, clinical translation, and promoting new business ventures to commercialize the product. A typical challenge with most biomedical engineering programs is the broad technical depth that is required. The IST/IBB is well-positioned because of its foundations in the traditional discipline of chemical/biochemical engineering. The program consists of core courses in stem cell biology, stem cell engineering, translational research, and clinical applications. The program also offers elective courses in tissue engineering materials, animal cell technology, gene therapy, mechanisms of disease, drug delivery and cell biomimetics, and nanobiotechnology and nanomedicine. After the completion of formal coursework, the students participate in two 8-week periods that provide them with practical experience in the laboratory.

TRANSLATION

Cell2B Advanced Therapeutics, SA is a private company that had its origin in a successful research partnership between the University of Lisbon and hospitals within Portugal. Cell2B was founded in 2011 with initial seed funding support provided by angel investors within Portugal. It has expertise in stem cell engineering and works closely with the IST for process development studies. Formal collaborations include a project in which the IST works with Cell2B. The initial activities of Cell2B include organizational activities in preparation to launch a Phase I/II trial. The initial efforts focus on a cell-based therapy for treating acute graft-versus-host disease (GvHD) in up to 40 patients. Cell2B also leverages GMP facilities within IST for process development studies.

SOURCES OF SUPPORT

Many of the research activities within the IST/IBB (and other institutes) are funded through a 3 million euro grant to fund translational research activities in cell-based therapies. Students participating in IST/IBB are funded in part through a block grant that supports 10 students per year (40 students at any one time). This program is funded through the Fundacao Ciencia e Technologia (FCT), the Portuguese equivalent of the National Science Foundation (NSF). There are also numerous funding and collaborative research opportunities with partners such as Cell2B. One notable feature of the educational program at IST/IBB is the focus on biomanufacturing. Students may select this track, which is embedded within the existing program. Therefore, some of the students in the program have the option of pursuing a specialized curriculum in biochemical/biomanufacturing engineering. Similar to other peer institutions with Portugal, the IST/IBB is eligible for many cooperative funding agreements with international agencies including

those located within the United States. One representative example of these interactions includes a program sponsored by the NIH, which funds post-doctoral fellows who perform research in the United States. One notable challenge in establishing long-term partnerships with U.S.-based funding agencies such as the NSF is the periodic turnover and diverse interests of the program directors. These programmatic challenges render it difficult to establish stable funding streams for long-term coordinated research partnerships. That being said, one notable success in this area has been a broader effort to forge external partnerships with foreign universities. A five year program with the goal of installing robust training programs was recently completed. A second phase of five years has been started with a newfound focus on entrepreneurship and commercialization. This educational program will serve the broader goals of IST/IBB perfectly.

ASSESSMENT

The IST/IBB is well-positioned to be an international leader in research and training programs in the field of biomanufacturing. The strategy of clustering engineering activities that are nominally biologically-focused within the Taguspark campus is noted. Although the rationale for this decision is clear, the logistical separation of the two campuses may potentially limit the impact and scope of organic collaborations that arise from spontaneous interactions. The translational activities are appropriately focused. The initial successes of Cell2B have established a framework for future translational efforts as well. Training and educational programs are also identified as exciting aspects of this organization as it continues its activities.

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OVERVIEW

Linkoping University (LiU) is a research university in central Sweden that has approximately 27,000 undergraduate students and 1400 research students. There are three campuses. The main campus is located in the rural region of Linkoping and is home to 18,000 students. There are also secondary locations including a university hospital that has 3000 students and an additional campus

in Norrköping that focuses on technology and natural sciences. The latter has excellence in chemistry, physics, engineering, and visualization technologies. The Norrköping campus is also home to the Center for Organic and Bio-electronics (OBOE). LiU is a relatively new campus, which first started as a branch of Stockholm University in 1967 and was granted university status in 1975. LiU is identified as a world leader in collaborative and dynamic interdisciplinary research programs. For example, researchers at LiU are credited with the invention of the application of surface plasmon resonance for protein binding measurements. Furthermore, LiU is home to many pioneering graduate research programs. This tradition of innovation in both teaching and research within LiU can be attributed in part to the relatively recent history of the university.

FUNCTIONAL FOCUS

The functional focus of LiU is on advancing applied science and technology for solving societal challenges. Representative research thrusts on the main (technology) campus are clustered into groups such as applied physics, biosensors and bioelectronics, nanotechnology, and electrical engineering. A non-exhaustive list of areas of research excellent in applied physics includes molecular physics, applied optics, and materials science. The Biosensors and Bioelectronics Centre, led by Anthony Turner, has pioneered many advances in continuous glucose monitoring. The CeNano group is led by Kajsa Uvdal and has made many seminal contributions in nanotechnology and nanoengineering. Strategic research areas in the Health Science Campus include regenerative medicine, neurosciences molecular imaging, cancer, and digestive diseases.

RESEARCH & DEVELOPMENT ACTIVITIES

All three campuses of LiU have a collective interest and excellence in the research area of biosensors and bioelectronics. The overarching goal is to harness fundamental research for bioelectronics for applications in medicine, drug discovery, and large scale sensor manufacturing. Specific examples of this research thrust includes biosensors for use in glucose sensors, integrated printable biosensor instruments, multimodal biosensing platforms (Watson-Crick base pairing combined with thermal annealing of DNA strands), and polymerization methods for molecular imprinting. The robust and mature effort in biosensors complements the broader focus on distributed medicine. Distributed medicine has dedicated focus areas on personalized medicine and diagnostics.

The Integrative Regenerative Medicine Center (IGEN) is led by May Griffith. IGEN is dedicated to bridging the translational gap between basic science and clinical need by bringing regenerative medicine products to market. The IGEN is grounded in some of the traditional strengths of the LiU campus, including biomaterials and nanoparticles for therapeutic delivery and bioimaging. IGEN also brings to bear a valuable skill set in stem cells with a dedicated focus on engineering *in vitro* microenvironments. There are several active research programs that are focused on clinical translation. The cornerstone program is biomimetic materials that enable organ regeneration (e.g., cornea regeneration). An early version the technology is a corneal implant composed of recombinant cross-linked collagen that has been successfully evaluated in the clinic. New clinic studies of new generations of collagen-based materials and self-assembling peptides are the current focus. There is significant infrastructure to support these research activities, including three GMP facilities: one Class A clean room is dedicated for tissue handling while two mini-clean rooms are dedicated for handling biomaterials constructs and stem cells, respectively. Additional facilities of interest include a CyTOF system for diverse characterization of stem cell populations (>100 parameters including surface receptors and ligands) at a throughput rate that is comparable to many FACS sorters. A large animal facility equipped with *in vivo* confocal microscopy and ultrasonic imaging capabilities are available for GLP testing.

Magnus Berggren leads the laboratory for Organic Bioelectronics (BiOE), which is located at the LiU campus in Norrköping. This laboratory is supported by the Acreo Institute, which contains a pilot-scale facility that is capable of producing 100,000 devices for each process batch. The BiOE

uses electronics to sense and actuate excitable living systems. The representative example of this research includes brain-machine interfaces. The key scientific challenge is the conversion of electronic signals into chemical signals to achieve a broader array of functions. Specific projects in the BiOE include reversible control of protein adsorption, electrochemical control of heparin, electrochemical transistors, and controlled release strategies for neuropathic pain. These research activities are designed to build upon the traditional strengths in biosensors, biomaterials, and medical sciences.

The Center for Medical Image Science and Visualization (CMIV) is directed by Anders Persson. The CMIV employs 90 researchers and 40 Ph.D. students with a wide range of basic, applies, and clinical research activities in imaging. Research foci include flow visualization in cardiovascular systems using MRI. The operation of the expansive imaging infrastructure is funded by a combination of equipment grants and usage fees by patients from hospitals. Equipment purchases are funded by a combination of infrastructure grants from the federal government and private foundations such as Sweden's Knut and Alice Wallenberg (KAW) foundation.

TRANSLATION

There are several internal organizational elements that encourage the translation of fundamental discoveries and primary knowledge into commercial products. The Forum Scientium is a doctoral program to bridge the medical and physical sciences. Another program, initiated in 2009, is Linkoping Initiative in Life Science Technologies (LIST). It focuses on the development of future healthcare solutions, stimulates interest in new technologies for healthcare, and reinforces long-term collaboration between faculty at LiU with areas of excellence in transformative research areas. For example, one collective research area of interest is distributed healthcare, a model for treating patients that relies on remote diagnostics and treatment options. Finally, there is an organization called AgoraLink that brokers collaborations between LiU and industrial partners.

LiU actively participates in several joint R&D partnerships between academia and industry including StemBANCC, M3C (measurement, modeling, and control), and Biomechatronic Design through Carl-Fredrik Mandenius and his research division of biotechnology. StemBANCC is a large scale European academic-industrial partnership in the area of stem cell research. The goal of StemBANCC is to create a stem cell bank that is derived from 600-1000 donors. This effort supports 34 partners with over 50 million euros over 5 years (Mandenius et al. 2011). This program is funded using a consortium-type model in which 50% of the funds are provided by the European Commission and the balance comes from the European Federation of the Pharmaceutical Industries and Associations (EFPIA). The broader motivation of these activities is to create standard protocols for handling and expanding stem cells. The specific contribution of LiU is the application of microfluidics for use in miniaturization of cell-based assays. The M3C is a collaborative network between European universities and biotech companies dedicated to the measuring, monitoring, modeling and control applications in biotechnology and bioengineering such as microbioreactors, process analytical technology (PAT) and biomanufacturing. . The M3C network promotes research activities between the partners such as joint grant proposals, expert panels, policy documents and training courses (Mandenius and Titchener-Hooker 2013). Finally, the Biomechatronic Design effort combines aspects of methodology for design of biotechnology products such as biosensors, biotechnology equipment and whole biomanufacturing processes (Mandenius and Björkman 2010).

SOURCES OF SUPPORT

LiU has a total annual operating budget of 510 million USD, divided between education (45%) and research activities (55%) via external partnerships. Within LiU, the Department of Physics, Chemistry and Biology has an annual operating income of approximately 70 million USD with roughly 45 million in annual research expenditures. There are several unique funding mechanisms that are available to researchers within LiU. For example, there are seed grants available through the IGEN. These grants are designed to fund one post-doctoral fellow for two years to work on a

high-risk/high-reward project. This program is viewed as a key granting mechanism to stimulate new collaborations and catalyze novel interdisciplinary research directions. In contrast to many other European countries, there are notable opportunities for researchers at LiU (and other research universities within Sweden) to acquire monies from private foundations. One such example is the KAW, a private foundation that can assist investigators in acquiring large equipment to improve research infrastructure. Although it seems that this opportunity will be drawing down, it has supported many research activities within LiU including the Center for Medical Image Science and Visualization (CMIV). Another such example is VINNOVA, a Swedish accelerator fund that is dedicated toward funding ideas that hold promise for commercialization.

LiU is and has been eligible for many other joint EU-based funding mechanisms such as Horizon 2020, the EFPIA, and the European Cosmetic Industries Association (Cosmetics Europe, formerly COLIPA). The last of these is a fund dedicated to developing *in vitro* alternatives to animal testing. At present, Cosmetics Europe funds 50 partners in the amount of 50 million euros in the SEURAT program for stem-cell derived lineages for cosmetic product testing.

ASSESSMENT

LiU is recognized as a world leader in research in many important research areas that serve as pillars for biomanufacturing. Centers of excellence in biosensors, surface sciences, devices, materials, and applied physical sciences underpin these activities. The relatively recent history and collaborative philosophy leaves it well-positioned to undertake innovative and interdisciplinary projects in biomanufacturing. The diverse funding streams that are available to LiU are particularly noted. For example, internal fellowships to fund high risk, high reward projects are advantageous. The unique combination of a clinical/translational focus with on-site manufacturing capabilities is also intriguing. There is infrastructure present to support both translational projects in regenerative medicine and large scale organic electronic device fabrication. This is seen as a competitive advantage if strategically leveraged for specific applications in biomanufacturing such as the fabrication of biosensors, tissue arrays, etc. It may be possible to support these activities by recruiting faculty members with additional clinical expertise in the area of implantable medical devices. The funding opportunities and student training programs are viewed as exemplary. Taken together, LiU is projected to function as a thought leader in many research activities that are both directly and indirectly related to biotechnology, medical devices, and biomanufacturing.

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OVERVIEW

Loughborough University has established a "Centre for Biological Engineering" (CBE) shared between the Department of Chemical Engineering, The Wolfson School of Mechanical and Manufacturing Engineering and the Department of Electronic and Electrical Engineering. The Centre has a 650 m^2 facility including a suite of Class 2 laboratories for microbial, animal, and human cell growth, a bioelectrical facility and an analytical suite to service all laboratories. Access

to the facility laboratories is through controlled zones that allow isolation of the individual units. A GMP specification suite, the Cell Therapy Manufacturing Facility, is available to support production of clinical materials. Equipment includes cell culture bioreactor systems, an automated cell culture platform, FACS, as well as other imaging and analytical equipment. Class rooms and meeting rooms are located adjacent to the laboratories. The design of the CBE facility supports late stage, GMP, process research and development and integrates with the impressive focus of Loughborough in manufacturing science and engineering.

The CBE was home to the Engineering and Physical Sciences Research Council (EPSRC) Grand Challenge grant in Regenerative Medicine (remedi; £7M), and is home to its successor, the EPSRC Centre for Innovative Manufacturing in Regenerative Medicine (£5.3M) and the Doctoral Training Centre in Regenerative Medicine, (DTC; £6.1m) which funds the training of 60 Ph.D. students over 6 years (now renewed for a further 5 intakes). The DTC is held jointly with Keele and Nottingham Universities, but is led from Loughborough.

Loughborough is also a key center for sports and exercise medicine and is home to the National Centre for Sports and Exercise Medicine (http://www.lboro.ac.uk/research/ncsem-em/). Part of this initiative seeks to apply the lessons learned in elite sports, in the context of "exercise is medicine", to public health. Research and development along this focus supports their wellness and rehabilitation agenda. Approximately £7 million of funding in the area of rehabilitation and regeneration, supports senior faculty across the campus. Professor Mark Lewis, Head of the School of Sports and Exercise Science, leads a research focus on muscle tissue engineering in concert with a national military medicine rehabilitation program.

FUNCTIONAL FOCUS

The Wolfson School of Mechanical and Manufacturing Engineering and the Centre for Biological Engineering provide a unique interface between the life sciences and engineering that is focused on manufacturing sciences, engineering and regulatory affairs. Its research ranges from basic biological mechanisms to complex components of manufacturing on the path to delivery of regenerative medicine to the patient.

RESEARCH & DEVELOPMENT ACTIVITIES

At the time of the WTEC team's visit Professor David Williams was the director of the Loughborough-led EPSRC Centre for Innovative Manufacturing in Regenerative Medicine and he had recently been awarded an OBE (Officer of the British Empire) for services to science and engineering in the 2014 Queen's Birthday Honours List. The director role has since been handed over to Nick Medcalf.

The interaction of engineering with molecular and biological science establishes concepts, commitments, and practice that create the basis for scale-up and scale-out in the earliest advances from the laboratory bench. Novel approaches to scale-up of mammalian cell culture improve reliability and open the potential to manufacture both autologous and allogeneic cell therapies beginning in the clinical theater; concepts that promise to revolutionize manufacture of cell therapies. Current areas of research focus include:

- Bioprocess engineering:
- Tissue engineering and regenerative medicine
- Automated tissue and cell culture
- Cell based therapies
- Microbial fermentation and animal cell culture
- Analytical cytology and non-invasive monitoring
- Microfluidic lab-on-a-chip devices

- Systems biology
- Bioelectrical engineering

TRANSLATION

Loughborough's unique trademark is its substantial partnerships with industry, government and the professions, toward translation of research innovations to clinical and commercial products. Nearly 70% of Loughborough University's research is carried out in collaboration with external partners; compared to the national average for universities of 20%. Research at Loughborough cuts across the full spectrum of translation from research to clinical and commercial delivery.

SOURCES OF SUPPORT

Loughborough University receives support from a wide variety of public and private sources, including: Loughborough Research School of Health and Life Sciences development funds, Pfizer, TiGenix, NHS Blood and Transplant, Cell Medica, Biolatris, and EPSRC. The East Midlands Development Agency and the UK Technology Strategy Board funded the design, construction and commissioning of the Cell Therapy Manufacturing Facility.

ASSESSMENT

Loughborough University, through biological sciences, chemical engineering, mechanical engineering, and other associated disciplines, is active in defining the next generation of clinical and commercial products in regenerative medicine. Across all of these disciplines, the common thread is making these therapies in the right place at the right time in an affordable way. Loughborough is committed to creating structure from the spark of innovation to the end-game of clinical practice and commercial supply. They are defining and delivering manufacturing and regulatory science that facilitates clinical and commercial production of products; finding and defining the technological platforms at the biology-manufacturing engineering interface that enable new generations of products; and leading the development of standards. Loughborough is one of the best examples of integration of these disciplines that we observed in our studies.

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OVERVIEW

MolMed is the leader in cell and gene therapy in Europe focused on research, development, and clinical validation of innovative therapies to treat cancer and genetic orphan diseases. It was established in 1996 as a joint venture between Boehringer Mannheim and Science Park Raf to provide cell therapy services, and had a stock offering in 2008 (shares now worth €160 million). MolMed takes an integrated strategy that develops both drugs that effectively reducing the tumor mass in the acute stage of cancer and highly selective therapies to eliminate the residual disease. With 118 employees (of which two-thirds are staff scientists), MolMed is located in the San Raffaele Biomedical Science Park, which houses the San Raffaele Research Hospital and San Raffaele Scientific Institute, the largest and most important private research center in Italy. This location allows MolMed to complement its own R&D resources with the cutting-edge scientific, technological and clinical resources at the San Raffaele Biomedical Science Park.

Related to the visit to MolMed, the WTEC panel members also visited the San Raffaele Telethon Institute for Gene Therapy (TIGET), and talked with Dr. Ferrari Giuliana. TIGET has been collaborating with MolMed and developed the retroviral vector for gene delivery, as well as the first *ex vivo* gene therapies based on HIV vectors.

FUNCTIONAL FOCUS

MolMed's functional focuses are:

- Two innovative technologies: recombinant proteins and cell & gene therapy
- In-house GMP-based manufacturing of cell and gene therapy products
- Identifying oncology indications that require new therapy options
- Improving clinical and pharmaceutical approaches, independently or with partners

RESEARCH & DEVELOPMENT ACTIVITIES

The R&D activities at MolMed are focused on two core competencies:

- Recombinant proteins
  - NGR-hTNF: Tumor vascular targeting agent. Doses of 0.8 μg/m^2 systematically show antitumor activity
  - After a 2.5-year follow-up time, there is a more than 50% relative reduction in risk of death
  - High unmet medical needs and low competition: no drugs registered for second-line treatment or in Phase III development
  - Development of commercial-scale manufacturing ongoing for liquid and lyophilized formulations
- Cell and gene therapies
  - TK cell therapy: Patient-specific cell therapy product for high-risk leukemia. It is a cell-based therapy enabling bone marrow transplants from partially compatible donors, in absence of post-transplant immune-suppression, now in Phase III for treating high-risk acute leukemia.
  - Unmet clinical needs: ~50% of patients as candidates to hematopoietic stem cell transplant (HSCT) miss a fully matched donor; without a transplant, high-risk leukemia patients have extremely low survival rate
  - TK is a cell therapy product, based on the use of genetically engineered donor T cells administered to patients after hematopoietic stem cell transplants in order to improve anti-leukemic activity of the graft and to accelerate immune reconstitution.
  - The onset of reactions mediated by such lymphocytes against healthy tissues of the patients - known as graft-versus-host disease (GvHD) - has been reported so far in 28 patients and has always been rapidly and completely controlled thanks to the TK technology, without post-transplant immune-suppression. No adverse events correlated to the use of TK cells were ever reported in these studies.
  - Projects with Third Parties: Working with third parties, MolMed uses its GMP facilities for the development and production of patient-specific cell & gene therapies for rare genetic diseases. MolMed has constantly growing revenues (+27.5% in FY2013 vs. FY2012) and strategic opportunities on this.

TRANSLATION

- Recombinant proteins
  - NGR-hTNF: For non-small cell lung cancer, Phase II completed. For treating pleural mesothelioma, Phase II completed and in preparation for Phase III. For treating ovarian cancer and soft-tissue sarcomas, proof of efficacy in randomized Phase II trials, long-term safety data established, pivotal Phase III results expected in 2014
- Cell and gene therapies
  - TK: Molmed has been making its own commercialization efforts. Phase II completed and conducting Phase III now. Expected filing for conditional approval in EU in 1st Quarter of

2014 based on proof of efficacy, established long-term safety data and high unmet medical need for patients lacking HLA-matched donor
- Automation of cell manufacturing, process ongoing

SOURCES OF SUPPORT

- Large amount of capital (~€160 million) through IPO. 51.63% of shares from strategic investors; 48.37% free float
- Contracted projects from third parties:
  - In 2011 Telethon Foundation signed a 8.3 m€ agreement for the development of six gene therapies for rare genetic diseases, including MLD, WAS, β Thal, MPS I, GLD and CGD.
  - In 2011 and 2013 GlaxoSmithKline signed two agreements for the development of the ADA-SCID commercial production process and for the production of the experimental therapy for ADA-SCID for compassionate use.

ASSESSMENT

MolMed is a gene therapy pioneer in Europe. Its successes are based on four factors: know-how, dedicated in house facility, track record, and IP position.

MolMed has a great vision towards the next generation fully automated production platform. The advantages of such a system include:

- Flexibility: process and devices easy to adapt to different cell processing applications magnetic separation of different cell types as well as customized cell processing protocols
- Scalability: allows to increase production scale to target large indications
- Feasibility: scientific and regulatory experience and know-how

There is a high potential for the future expansion of the automation process to the whole *ex vivo* gene therapy platform.

The technology developed by MolMed for *ex vivo* genetically engineered TK-T cells has proven to be technically feasible without safety problems. Based on this technology, MolMed has now developed a complete technological platform for *ex vivo* gene therapies including retroviral vector (RVV) for treating TK and ADA-SCID, and lentiviral vector (LVV) for treating MLD, WAS, β Thal, MPS I, GLD, and CGD.

The success of MLD and WAS gene therapies produced by MolMed includes the progress in scientific research, as demonstrated by the first *ex vivo* gene therapies based on HIV vectors developed through partnership between MolMed and TIGET. MolMed team participated in the work that led to the publication in *Science* of results obtained on the gene therapies for MLD and WAS (Biffi et al. 2013, Aiuti et al. 2013).

Concerning educational opportunities, there is a lack of well-trained engineers for biomanufacturing in Italy. There are 20,000 engineering students in Italy every year, but only 4,000 or so working in bioengineering or the related fields. The reason why so few are working in this field is that there is the need to have both engineering and biology training. Currently very few bioengineers are specifically trained in biomanufacturing. One possibility is to have a "biotechnology 3+2 education," with B.S. in biology (3 years) plus an M.S. in engineering (2 years) or manufacturing (design), and having project-based internship in a company. Students in biomanufacturing will need to learn a broad range of topics, including robotics, automated systems, regulatory issues, process engineering, QC, GMP, and standards. Students need training for the automation process, with skills in computer engineering, cell testing, sensors, remote-controlled ELISA, imaging, and other areas. It would be attractive if the universities in the United States

would send students to MolMed for 6 months or a year for internship. MolMed does this now with students from local universities.

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http://www.molmed.com

PharmaCell B.V.

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6167 RD Geleen
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Date Visited: March 5, 2014

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Hosts:

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OVERVIEW

PharmaCell was established in January 2005 with the mission to contribute to the cell therapy industry by establishing itself as center of excellence in Europe to provide development and manufacturing services for advanced therapy medicinal products. PharmaCell's Maastricht facility was first GMP certified in 2006 and has been inspected by the Dutch Authorities (IGZ) on a regular basis since then. In October 2013, PharmaCell announced that it has entered into an agreement with Dendreon Corporation to be the Contract Manufacturing Organization (CMO) for the European commercial production of its recently approved cellular immunotherapy product Provenge® (autologous peripheral blood mononuclear cells activated with PAP-GM-CSF or sipuleucel-T) for treatment of prostate cancer.

In January 2014, PharmaCell acquired the commercial cGMP manufacturing facility of TiGenix NV located at the Chemelot site in Sittard-Geleenand, and the contract to manufacture TiGenix's

ChondroCelect® product for cartilage repair in the knee, the first EMA approved cell-based product in Europe currently being sold across Europe.

FUNCTIONAL FOCUS

PharmaCell is integrating its operations at the two cGMP manufacturing sites and its Development Laboratory (Maastricht), with a total staff of approximately 65, as it continues to serves its customers from preclinical through marketed cell therapy products by providing quality and flexibility in:

- Designing a Technology Transfer project to the requirements of the clients
- Providing Process Development and Process Optimization services for developing commercializable manufacturing processes
- Structuring a manufacturing capacity program in line with patient recruitment and market demand, and having project teams and clean room available around the clock (24 hours x 7 days)

They also provide a long-term perspective and continuity through all phases of clinical development (Figure B.4).

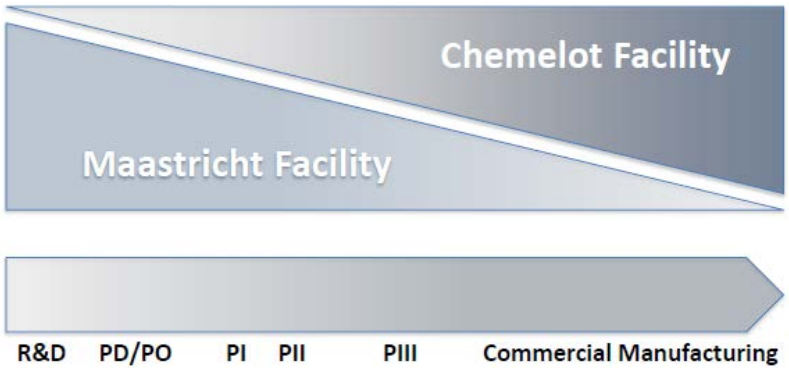

Figure B.4. Continuum of functional activities through integrated operations in two facilities (courtesy of PharmaCell).

The WTEC panel met with the Mr. Alexander Vos (CEO), Dr. Soenke Brunswieck (Director, Business Development) and Mr. Arjan Roozen (Director, Operations), Mr. Yogesh Krishan Dave (Director, Quality Assurance and Qualified Person), and Mr. Stefan Dullens (Manufacturing Manager, TiGenix NV). Mr. Vos provided an overview of PharmaCell's business and operation. Mr. Dullens led a tour of the cGMP commercial manufacturing facility and the processes/systems with the manufacture of TiGenix's ChondroCelect® product.

RESEARCH & DEVELOPMENT ACTIVITIES

PharmaCell's activities span the continuum from preclinical product development to manufacture of marketed ATMP products (Figure B.5).

During the facility tour, PharmaCell explained that the process of manufacturing for TiGenix's autologous products actually starts at the time of collection of patient biopsy material in the hospital. TiGenix has developed and validated biopsy collection procedures, and physician training program thereof. Furthermore, they provide the hospital sites with a pre-packaged kit for collection, formulation, and transport of patient biopsy material to the commercial cGMP manufacturing facility in Chemelot. At the Chemelot facility, the incoming biopsy materials undergo incoming material inspection and established Quality Control checks prior to being accepted for initiation of manufacture of patient-specific product lot.

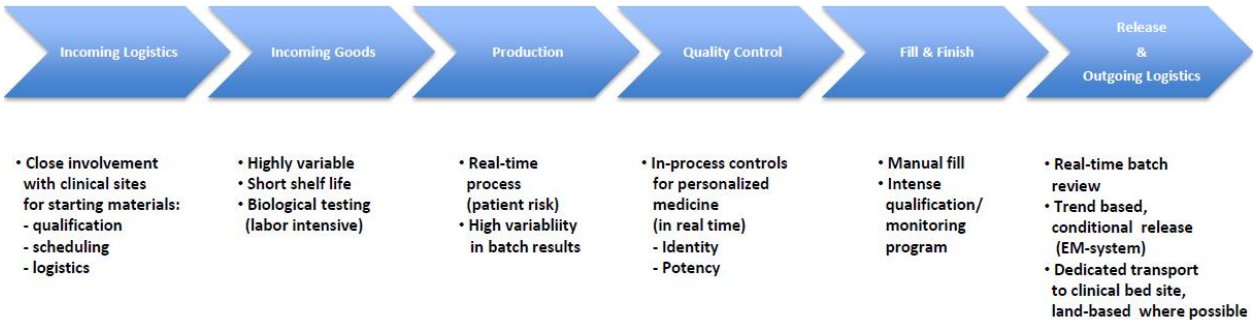

Figure B.5. Cell therapy manufacturing process chain (courtesy of PharmaCell).

The manufacturing processes/systems encompass all aspects and activities associated with manufacturing, testing, and release of patient-specific cell therapy products (Figure B.6).

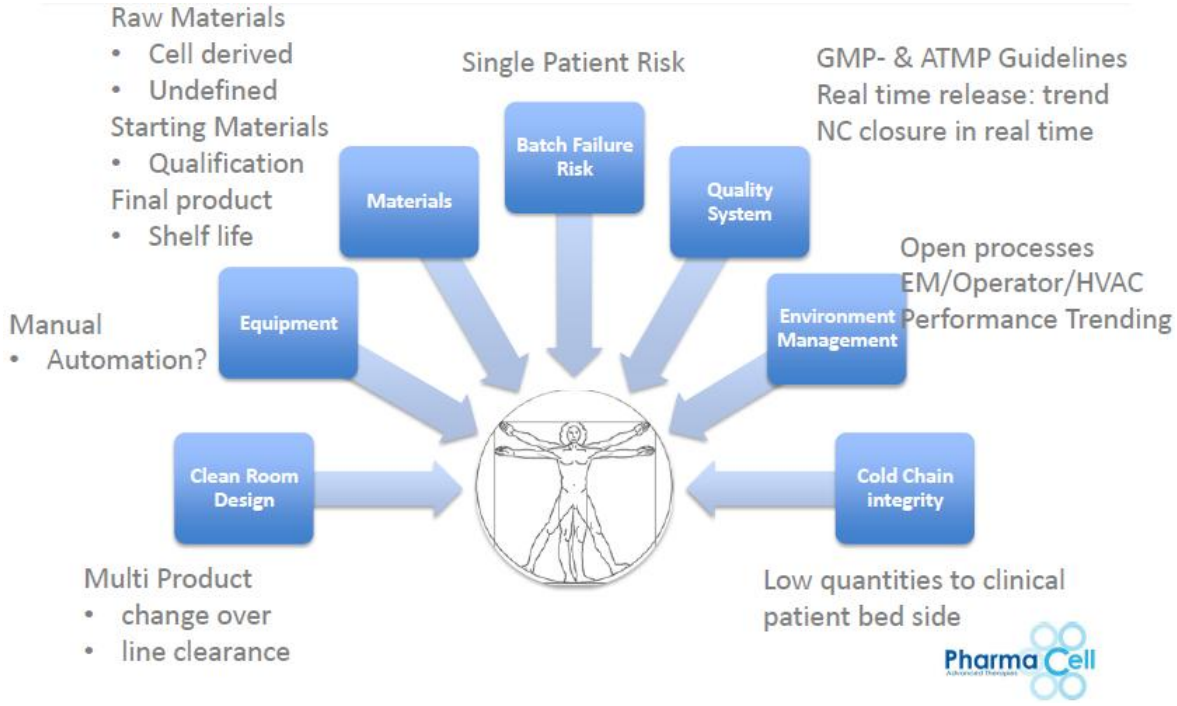

Figure B.6. Process considerations for cGMP manufacture of patient-specific cell therapy products (courtesy of PharmaCell).

TRANSLATION

PharmaCell is the commercial manufacturing partner for two of the three EMA approved cell-based products commercially marketed in Europe: (1) Dendreon's cellular immunotherapy product Provenge® (autologous peripheral blood mononuclear cells activated with PAP-GM-CSF or sipuleucel-T) for treatment of prostate cancer and (2) TiGenix's ChondroCelect® product, for cartilage repair in the knee, the first EMA approved cell-based product in Europe currently being sold across Europe

In addition, PharmaCell also supports preclinical development and clinical manufacture of other cell therapy products. The company's developmental activities also encompass participation in two international consortium of European Strategic Technology Development Projects funded through FP7 grants entitled (1) SMARTCARE (Smart Micro Tissues for Cardiac Regeneration), and (2) BALANCE (Bio Artificial Liver). The company's preclinical development and process scale-up

activities have encompassed a strategic partnership with Merck Millipore for translating a T-flask culture process into a 200L single-use disposable bioreactor process while concurrently reducing the COGS associated with up-scale manufacturing by 65% and generating sufficient product to treat 200 patients from a single lot.

SOURCES OF SUPPORT

PharmaCell is a revenue-generating business through provision of process development and clinical/commercial manufacturing services for cell therapy products in Europe. Additionally, PharmaCell has received grant funding through its participation in FP7 funded European Consortium Development Projects.

ASSESSMENT

Currently, PharmaCell has the prestige and responsibility of being the commercial manufacturing partner for two of the three EMA approved cell-based products commercially marketed in Europe.

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Achieving Solutions for Cell Therapy Manufacturing. Presentation to WTEC panel on March 5th, 2014. [PDF]

Roslin Cells

Site Address:	Scottish Centre for Regenerative Medicine 5 Little France Drive Edinburgh BioQuarter Edinburgh, Scotland EH16 4UU, UK
Date Visited:	March 4, 2014
WTEC Attendees:	S. Drew (report author), M.V. Peshwa, K. Ye, H. Ali
Hosts:	**Janet Downie** Chief Operating Officer janet.downie@roslincells.com Tel.: +44(0) 131 658 5182 **Kevin Bruce** Head of Development Operations kevin.bruce@roslincells.com Tel.: +44(0) 131 658 5180

OVERVIEW

Roslin Cells is a private company, established in 2006 as spin-off from the world-renowned Roslin Institute and is a member of The Roslin Foundation and University of Edinburgh. The company employs 25 scientists, engineers, and technicians. They had an annual operating turnover of £2 million in 2013-2014. Roslin Cells is supported by the Scottish National Blood Transfusion Service, and by Scottish Enterprise.

Roslin Cells is Medical and Health Regulatory Authority (MHRA) licensed for imp manufacture (cell therapies and cell banks) and is licensed by the Human Tissue Authority (HTA) and Human Fertilization and Embryo Authority (HFEA). They are ISO9001:2008 accredited.

Roslin Cells is situated on the University of Edinburgh campus at Nine BioQuarter (Figure B.7), together with the Royal Infirmary of Edinburgh, the Anne Rowling Regenerative Neurology Clinic, the Queens Medical Research Institute, the University Medical School, and the Scottish Center for Regenerative Medicine (SCRM). They operate 7 clean room suites in concert with the Scottish National Blood Transfusion Service and can accommodate derivation of human embryonic stem cells (hESC), induced pluripotent stem cells (iPS); developing master cell banks, allogeneic cell therapies, and autologous cell therapies. Roslin also has an area designed for containment of higher risk materials and radioisotope labeled analytics. The campus offers many resources to facilitate development of cell therapies including a state-of-the-art imaging facility.

FUNCTIONAL FOCUS

The main objective of Roslin is to support the development of new cell therapies and manufacture ATMP products for European Union (EU) clinical trials. Their GMP-grade pluripotent stem cells target to support drug discovery and development and associated clinical research programs. Immediate access to the hospital allows Roslin Cells to manufacture iPS and other tissues from procurement through to derivation and delivery of fully characterized cells.

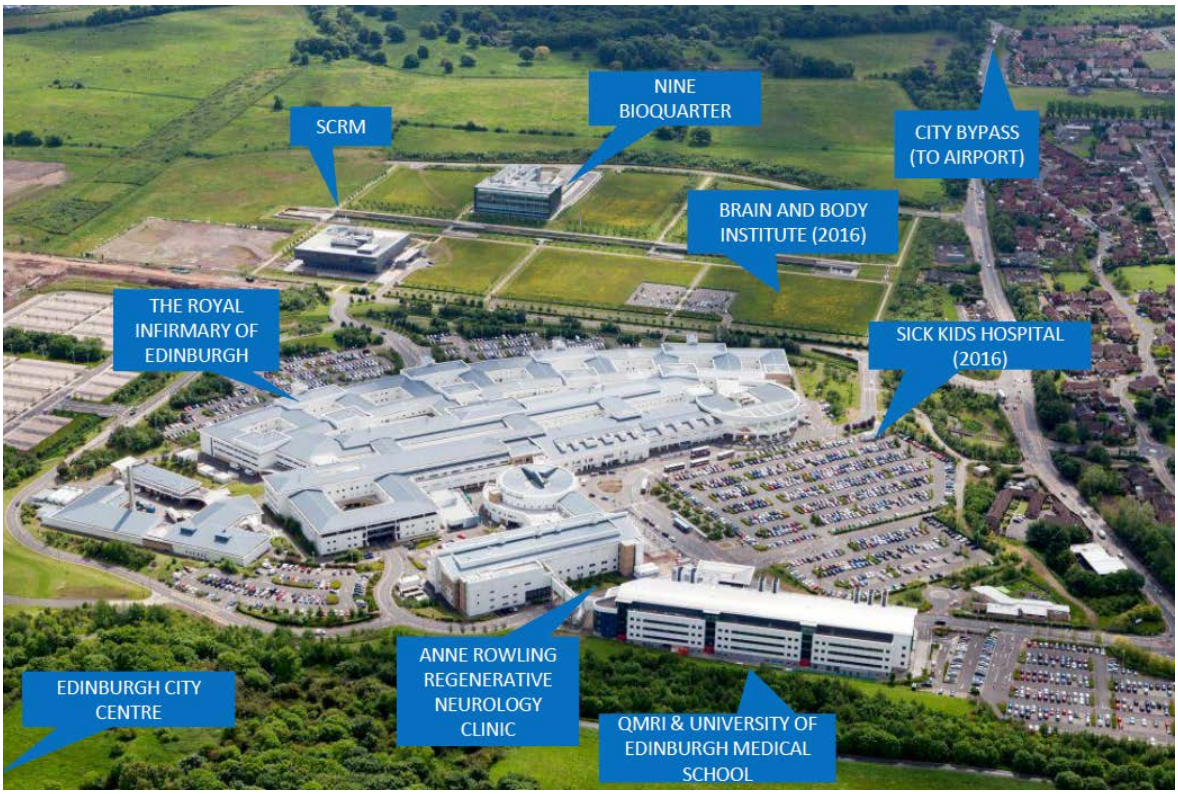

Figure B.7. Aerial view of the facilities near Roslin Cells, which is located in Nine BioQuarter (courtesy of Edinburgh BioQuarter).

RESEARCH & DEVELOPMENT ACTIVITIES

Roslin Cells Ltd. provides cells and cell-related materials to research and development efforts across the world and participates in the scientific evaluation of experimental data. Research and development at Roslin Cells extends the technology for manufacture of cells under Good Manufacturing Practice conditions.

TRANSLATION

Roslin Cells has a professional, skilled team, with industry experience and expertise spanning cell culture, cell biology, Quality Management (QM) and Good Manufacturing Practice (GMP). They offer a portfolio of services in their GMP facilities that range from tissue procurement to cell banking, processing, manufacturing, testing, storage, and distribution for organizations wishing to develop or manufacture of Advanced Therapy Medicinal Products (ATMPs) in Europe. Their capabilities include GMP services to support the development of cell-based therapies and production of new clinical grade pluripotent stem cells for use in research and therapy.

SOURCES OF SUPPORT

Medical Research Council (MRC), UK Stem Cell Foundation (http://www.ukscf.org/), Cell Therapy Catapult, Wellcome Trust

ASSESSMENT

Roslin Cells is the implementation of the “everything is multidisciplinary” concept articulated by Andrew Henderson of the Scottish Enterprise coordinating group. The company often begins working with the scientists and clinicians at the University of Edinburgh as early as the first few

publications on disease etiology and conceptual cell therapy intervention. They introduce the needs for GMP and develop a dialog on analytics that moves from the scientist's understanding of cell function to the measurements that will link efficacy to the manufacturing endpoint.

The company embraces the role of manufacturing to reduce variability and increase consistency across inherently variable starting materials and their biology. Their work with the Scottish National Blood Transfusion service provides a particularly strong, well-established and well-understood translational platform to products as a basis for working with innovators toward non-blood or non-bone marrow transplant cell therapies.

Roslin Cell's facilities operate 4 Class B clean suites that can accommodate two projects each in a single room. Where open manufacturing process are required, the manufacturing room is operated with a single product/patient. Sequencing between batches is by wipe-down cleaning with no VHP or similar sterilization required. This seems to be a more liberal interpretation of EMA or MRC requirements than was indicated by the University College London team of laboratories.

Roslin is now operating at 50% of capacity with half of their activity supporting commercial customers. They estimated Quality Control testing and characterization costs at 20 to 30% of overall cost of goods, not including long-term testing or routine testing for product release.

As with most of the groups that we visited, Roslin Cells lamented the lack of standardized criteria for choosing cell isolates and uniform colonies and for analytics for cell and cell function characterization. They did not describe their work to address this gap but did indicate that they are providing materials for the European Bank for induced Stem Cells (EBiSC; a consortium of 26 partners conceptualized by Pfizer Ltd Cambridge and managed by Roslin) that may be a platform for better standardization.

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Scottish National Blood Transfusion Service

Site Address:	University of Edinburgh BioQuarter Campus Edinburgh EH16 4UX
Date Visited:	March 4, 2014
WTEC Attendees:	M.V. Peshwa, K. Ye, S. Drew (report author)
Host:	**Prof. Marc Turner** Medical Director and Director of Cell Therapy Catapult marc.turner@ed.ac.uk marc.turner@nhs.net

OVERVIEW

The Scottish National Blood Transfusion Service (SNBTS) has a long established track record of exemplary performance in the collection, identification, and characterization of blood, bone marrow and related cell types. They are a primary tissue banking site with many years of experience in stem cell purification, differentiation, and cell therapy manufacture. They interface with the clinic to provide blood transfusions and bone marrow transplant materials across the EU. They are at the forefront of clinical analysis leading to the manufacture of cells (blood, mesenchymal stem cells, and other cells) under rigorous GMP conditions for cell therapy. Their integrated manufacturing approach ranges from collection of cells through cell manipulation and purification to packaging, storage, and shipping. The work of the SNBTS was recognized as standard-setting across all of the sites that we visited.

FUNCTIONAL FOCUS

The SNBTS enables research and development on human blood, including embryonic stem cells, broadly throughout Europe through access to their cell types and internal research.

RESEARCH & DEVELOPMENT ACTIVITIES

Research within the SNBTS reaches broadly from genetic and metabolic function in blood and bone marrow to differentiation of embryonic stem cells and on to methodology for collection, handling, and storage of blood cells and related biological materials. Professor Marc Turner is a central researcher, principal investigator, and prolific author with many of his scientific colleagues across the world.

ASSESSMENT

Professor Marc Turner identified a goal of the SNBTS as bridging the distinct mind-sets of discovery and development leading from the basic science underlying clinical disease to the manufacture of end products that treat disease. Blood transfusion and bone marrow transplant represent the most established and oldest forms of cell therapy, but other forms of cell therapy hold great promise. Professor Turner spoke of the difficulty in understanding, monitoring, and control of cell variation during growth and expansion. The field of cell therapy must understand the differentiation of cell types and their control before manufacturing of cell therapies can mature. Sources of investment exist to help this growth, but traditional large pharmaceutical companies

seem cautious. SNBTS has the advantage of a well-established presence in the field of cell therapy and some of the resources to pursue applications toward broader applications. The panelists and participants ask if other cell therapies can learn valuable lessons for product development from the blood/cord blood/bone marrow transplant industry. Perhaps the most enlightening concept for this comes from the realization that manufacturing these products is fundamentally different than the manufacture of pharmaceuticals or biologics. In this example of cell therapy through blood and other tissues collection, manufacturing starts at the medical theater where tissue collection to meet GMP requirements for manufacture must occur; and continues, sometimes through limited expansion, to small, unitized volumes of product delivered nearly directly to individual patients. Some of the tools for product and process analytics already exist in this industry, but fundamental understanding of unit-to-unit variation still seems to be lacking and manufacturing control is largely a matter of great precision in the unit operations.

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Sistemic UK

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Host:	**Jim Reid** Chairman and CEO jim.reid@sistemic.co.uk Tel. (UK): +44 141 946 9682; (U.S.): 1-617-523-3272

OVERVIEW

Sistemic Ltd. was formed in 2009 to leverage microRNAs (miRNAs) as indicators of cell phenotype, developing biomarkers of metabolic activity, and the potency and quality of cell therapy agents. In humans, the miRNAs are a set of approximately 2,600 ncRNAs that regulate around 60% of the total gene expression within a cell.

FUNCTIONAL FOCUS

As the Sistemic web site states: "Sistemic is a development-stage biomedical company focused on the analysis of the information content of microRNA and other non-coding RNAs for applications in the development of cell therapies and drug repositioning." (http://www.sistemic.co.uk/)

RESEARCH & DEVELOPMENT ACTIVITIES

Sistemic has correlated miRNA patterns with distinct phenotypes. Early studies demonstrated that although cells passaged for varying times were thought to be identical with the starting phenotype, they elaborated different miRNAs that correlated with subtle differences in phenotype. With more than 400 individual cell profiles analyzed, the company believes that miRNAs can be key measurements/monitors of cell phenotypic purity, evolving differentiation of cell types, and may support the development of direct or surrogate standardized assays for potency.

TRANSLATION

Sistemic believes that miRNAs, termed kmiRs™, identified in their studies, are good candidates for profiling phenotype and cell differentiation from initial research to commercialization of cell therapies. CEO Jim Reid presented several examples of this:

- Key marker microRNAs (kmiRs™) predictive of mesenchymal donor stem cell performance
- The use of kmiRs™ for monitoring product purity in terms of percent of contaminating cells

- Identification of kmiR™ markers predictive of MSC donor performance in standard differentiation assays, demonstrating their utility for optimal donor selection (including optimization of directed differentiation to hematopoietic cells from adult HSCs or hESCs)
- Sistemic's analytic system, SistemQC™ identified kmiRs™ as candidate potency markers distinguishing efficacious from non-efficacious cells
- Sistemic has developed surrogate potency markers for cellular immunotherapy products using SistemQC™ to assess kmiRs™ that may be representative signatures of cellular immunotherapy-suppressed T cells

SOURCES OF SUPPORT

Revenue from sales of services.

ASSESSMENT

Sistemic's vision portrays a research-to-commercialization universe of cell therapy that could be monitored, controlled and quality assured across a standardized array of microRNAs that are cell-type and phenotype specific. The data are statistically compelling that miRNAs have this potential and Sistemic kmiR™ markers and analytic systems are currently under evaluation by several companies leading development in the cell therapy space. The field of cell therapy awaits compelling Phase 2 clinical outcome data and the use of miRNAs may be able to facilitate the advance to Phase 2 by establishing reproducible metrics for cellular homogeneity and metabolism. If the correlations continue to prove strong, expression of key miRNAs could become comparative measurements for manufacturing consistency and reliability. Once established as a member of the standard analytic arsenal for cell therapy manufacture, they might also support directed optimization through principal component analysis for process variables. Mr. Reid also spoke of using miRNA to monitor the purity of mesenchymal stem cells as a platform to develop these concepts, with manufacturers, to achieve robust, flexible, standardized products and processes where inherent variability can be measured quantitatively.

Though participants in the discussion believe that miRNAs are ubiquitous in mammalian cells, and may be present in other living systems (e.g., prokaryotes) it was not clear whether the correlation to individual cell phenotype has been as well established as it has for differentiating mammalian cells. If those correlations can be established for other biological systems (such as the manufacture of monoclonal antibodies, other mammalian proteins or even proteins and metabolites from other organisms), the use of novel microRNA signatures could have even broader application as a manufacturing development tool. The panelists believe that examples of miRNA variation resulting from pH, CO_2, and temperature fluctuations within human cell systems have already been demonstrated.

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TAP Biosystems, Ltd.

Site Address:	York Way Royston, Hertfordshire SG8 5WY, UK
Date Visited:	March 10, 2014
WTEC Attendees:	D. Applegate, S. Drew (report author)
Host:	**Dr. Rosemary Drake** Chief Scientific Officer rosemary.drake@tapbiosystems.com Tel.: +44 (0) 1763 227213

OVERVIEW

TAP Biosystems, Ltd.,[4] formerly The Automation Partnership (TAP), is a long-standing supplier of high-level automation for high-throughput screening (HTS), compound management, and cell culture. Their new branding recognizes a further evolving range of benchtop systems and consumables to improve productivity in diverse areas such as bioprocessing, vaccine production, biobanking, cell therapy, and *in vitro* cell testing, in addition to automation of the traditional compound stores and HTS systems that are at the core of many major pharmaceutical drug research programs.

The company's new initiatives improve production of protein-based therapeutics, the scale-out (adding more nodes to test and development systems) of physiologically-relevant cell models to better predict drug behavior, and automated systems that allow cost-effective scale-out and scale-up of cells for use in regenerative medicine. TAP Biosystems provides cell-processing systems, combined with novel sensing technologies and associated consumables used in their systems.

They target reproducibility of the biological processes through reduction in systems and component variability. They have demonstrated their ability to reduce process and product variability and increase overall process reliability across decades of interaction with the pharmaceutical domains of discovery, development, and manufacturing.

FUNCTIONAL FOCUS

TAP Biosystems is a provider of equipment and automation to the regenerative medicine, biologics, and pharmaceutical industries. They also provide strategic counsel on integration of automation into proprietary systems for manufacture and the consumable materials associated with TAP Biosystem devices.

RESEARCH & DEVELOPMENT ACTIVITIES

TAP Biosystems has developed two automated systems for process optimization: the development of cell growth media and cultivation conditions that lower the variability of process conditions by standardizing materials, volumes, and process control in bioreactors. Their approach allows the scale-out of bioreactors to support statistical study of environmental variables in cell culture and

[4] In 2013 TAP Biosystems was acquired by Sartorius Stedim Biotech Group (http://www.sartorius.com/).

cell differentiation. TAP's automated system pairs 15 ml or 250 ml bioreactors with a workstation that provides temperature, dissolved oxygen and pH monitoring, agitation control, and data collection (http://www.tapbiosystems.com/tap/cell_culture/ambr.htm#).

The ambr™ 15 system (Figure B.8) mimics the characteristics of classical bioreactors in 10-15 ml individual vessels by using disposable micro bioreactors, controlled by an automated workstation enabling rapid evaluation of multiple bioreactor cultures for media and process condition optimization. The ambr workstation controls 24 or 48 disposable bioreactors, offering parallel processing and evaluation of multiple experiments in an automated bench-top system. Each disposable bioreactor operates with closed loop control of pH and dissolved oxygen with independent control of O_2 and CO_2. Automated liquid handling for reactor set-up, feeds, base addition, and sampling are available through the workstation base. The entire unit can be placed into a laminar flow hood, allowing aseptic operation, and can be integrated with external or third-party instrumentation (e.g., the Vi-CELL® cell viability analysis; Beckman Coulter). The ambr 15 and ambr 250 systems are designed to mimic the performance of individual 5 to 10 liter bioreactors under highly reproducible process control (Nienow et al. 2013).

Figure B.8. Ambr 15 disposable bioreactor (courtesy of TAP Biosystems Ltd.).

TAP Biosystems has also developed methodology and process platforms for evaluation of three-dimensional tissue structures that mimic *in vivo* tissue function and differentiation/growth. The RAFT (Real Architecture For 3D Tissue) system is based on three dimensional concentrated collagen hydrogels encapsulating viable cells to form metabolically active analogs of normal tissues (Figure B.9).

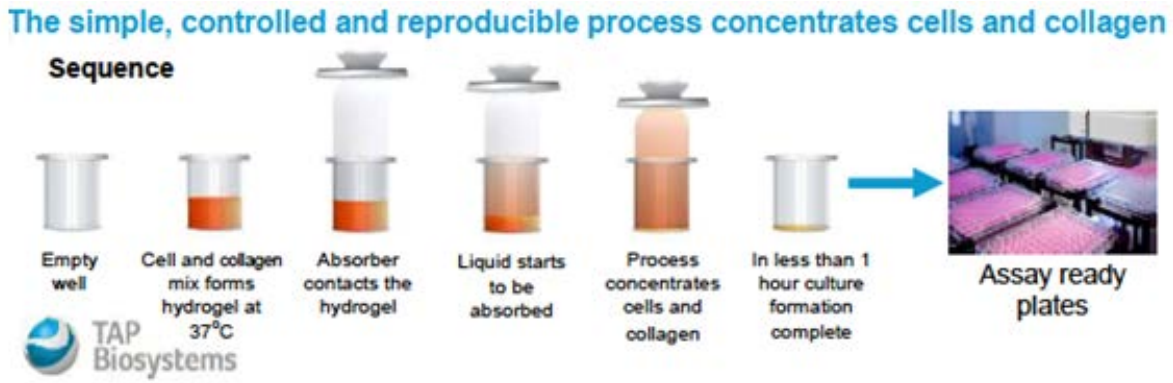

Figure B.9. RAFT (Real Architecture For 3D Tissue) system (courtesy of TAP Biosystems Ltd.).

RAFT cell cultures can be formed directly in a micro-well plate or cell culture insert for studying cell movement, migration, wound healing, and angiogenesis. A range of analytical techniques can be used, including direct imaging of the active 3D tissue culture. (http://www.tapbiosystems.com/tap/cell_culture/RAFT.htm). As an off-shoot of TAP's development of the RAFT system, they discovered ways to configure topology on the surfaces of collagen matrices, showing in one study that the surface topography influenced alignment of human primary muscle of RAFT cultures within the structural grooves (confidential pers. comm.). Further, working with Professor Julie Daniels (University College London), they have shown that their system can mimic the architecture of corneal limbal epithelial stem cell niche (Levis et al. 2013). The collagen RAFT architecture topography creates stable crypts, which are preferentially populated with cells expressing stem cell markers.

ASSESSMENT

TAP Biosystems is a service and equipment company with significant insight into the advancing arena of cell therapy. Their equipment and methods are recognized and used by many of the companies and academic laboratories that we visited during this study.

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University College London

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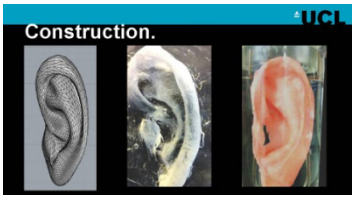
London, WC1E 7JE, UK



Date Visited: March 3, 2014

WTEC Attendees: S. Drew (report author), H. Ali, D. Applegate, M.V. Peshwa

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OVERVIEW

UCL was established in the heart of London in 1826 to open up education to students of any race, class or religion. UCL was also the first [British] university to welcome female students on equal terms with men. Now there are 29,000 students at UCL from more than 150 countries, and more than a third of these are graduate students. Based on the nationwide Research Assessment Exercise, UCL won the largest funding allocation from the UK research councils in 2013 (£135 million) (UCL 2014).

FUNCTIONAL FOCUS

Prof. Titchener-Hooker introduced the UCL Department of Biochemical Engineering, which focuses on the translation of scientific discoveries in biology and medicine into clinical and commercial practice. It is the largest academic department of this type in the world, with more than 100 doctoral students. Thus it was well suited to be a host for the WTEC panel on biomanufacturing (UCL-DBC 2014). The department also collaborates with 12 other UCL units, and researchers from two of these made presentations (Titchener-Hooker 2014).

RESEARCH & DEVELOPMENT ACTIVITIES

Prof. Seifalian from the Division of Surgery & Interventional Science presented a wide variety of research themes and results, under the title "Nanotechnology: Driving the future of organ development." Their applications of nanotechnology were particularly interesting (Jameson, Seifalian et al. 2007). The image at the top of this site report is from this presentation, showing a human ear as an obviously successful result from their work in organ development. They and their partners were the first to create successful human trachea, noses, bypass grafts, and lachrymal ducts, using nanoparticles, nanocomposites, and stem cells, and they have many other organs at the clinical stage in their labs (Seifalian 2014).

Dr. Daniels reviewed the R&D being done at the Cells for Sight Therapy Unit. They have a number of exciting research themes under way to use cell therapy to restore vision. A review article on their work has been well received (Daniels, Dart, Tuft et al. 2001). They have a small GMP manufacturing facility in-house to put these therapies in to practice. They also cooperate with TAP Biosystems in Royston, UK (which the WTEC panel also visited) for expansion and differentiation of cells and broader dissemination of the results. In particular they have licensed their RAFT (Real Architecture for 3D Tissues) patented technology exclusively to TAP. This is based on plastic compression of type I collagen, which can be used for therapy of the corneal epithelium, the corneal stoma, and corneal endothelium, which can treat a large number of causes of vision impairment (Daniels 2014).

TRANSLATION

Dr. Lowdell described UCL's very extensive good manufacturing practice (GMP) facilities for clinical trials. They had more than a dozen cell therapy products under test, some in cooperation with companies like Cell Medica. An example was the autologous stem cell-derived, cell seeded biocompatible structure for tracheal transplants. One very inspiring paper with results from several of our hosts was (Elliott et al. 2012). Dr. Lowdell's presentation is accessible through the references, below (Lowdell 2014).

Prof. Pankhurst reviewed the program of the UCL Institute of Biomedical Engineering (IBME), whose slogan is "Innovation, Translation, Impact." Their institute leads the UK in most highly-cited publications; one paper alone (Pankhurst 2003) has 2300 citations in the Web of Science. Their work is closely coupled to clinical practice via the world's largest academic health science centre—UCL Partners. The IBME MedTech Accelerator seeks to be the world's fastest and most cost-effective deliverer of medtech-derived patient benefits (Pankhurst 2014).

Dr. Wall reviewed the main themes of the Department of Biochemical Engineering: manufacturing innovation, and clinical translation. One important result was reported in Wall et al. (2009). He outlined the unique UCL approach of bioprocessing of cells for therapy, which provides whole bioprocesses from development of cells and their isolation, through bioprocessing to delivery to the patient (Wall 2014).

SOURCES OF SUPPORT

There are dozens of funding sources for the Department of Biochemical Engineering. The presentation by Prof. Titchener-Hooker lists them, including amounts. The other UCL units attending the meeting listed these sources:

- Wellcome Trust, MRC, EPSRC, TSB, NIHR, EU, Javon Trustees, Royal Free Charity, GOSH Charity, Action Medical Research, Healing Foundation (Seifalian)
- NHS, Technology Strategy Board, Fight for Sight, Aniridia, MRC, Moorfields Eye Hospital, Moorfields Eye Charity (Daniels)

ASSESSMENT

UCL has very impressive GMP facilities. They have the resources to pursue multiple approaches to solve a problem. Their approach is academic, of course, but they are attracting a lot of funding and many industrial partners. UCL is a holistic landscape of research and its translation to clinical and commercial reality, allowing it to be efficient in developing and promoting single products.

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University of Leeds - Nanomanufacturing Institute

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Date Visited: March 7, 2014

WTEC Attendees: G. Bao, K. Leong, M.V. Peshwa, K. Ye (report author), P. Foland

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OVERVIEW

The University of Leeds is the second largest university in the UK, with 33,600 students. It was founded in 1904 and is one of the members of Russell Group (the UK's Ivy League). It is a research intensive university and founding member of the World University Network. The university hosts the largest teaching hospital in Europe with 2400 beds. The university is home for a number of UK Centers of Excellence for Clinical Trials, including Oncology, Cardiovascular, Musculoskeletal Diseases, Dentistry, and Molecular Medicine.

The Leeds Nanomanufacturing Institute was established in 2005 (Leeds 2014). Prof. Terry Wilkins is the CEO of the Institute. He also serves on the EU Manufacturing Advisory Board to strategize the education and industrial work for manufacturing. There are 250 researchers in the institute.

FUNCTIONAL FOCUS

The Leeds Nanomanufacturing Institute has 264 companies in a partnership benchmarked as Europe's premier academic center for nanoparticle engineering. It focuses on nano-medicine, -diagnostics, -theragnostics, -surgery, and risk analysis of nanoparticles in the environment.

RESEARCH & DEVELOPMENT ACTIVITIES

The institute's R&D activities include both basic and translational research on engineering, medicine and health, mathematics and physical sciences, and business—with a goal of translating lab discoveries into industrial products through collaborative research involving its 250 researchers and 260 partner companies. Four themes—medicine and health, consumer and industrial, nanoelectronics and quantum information, and innovation and business management—are actively pursued. The institute has a number of semi-scale and pilot plants that are capable of batch and continuous manufacturing of nano-mediated or enabled products. For example, the institute has developed a technology for scaling up liposome nanocapsules. The technology realizes uniform-production of liposome nanoparticles with 46 ± 2 nm in a batch or continuous manner. It can manufacture 100 g per batch.

Its nano-enabled laparoscopic surgery technique uses super-paramagnetic iron oxide nanoparticles. The attachment of these nanoparticles to target tissues allows for fluorescence-imaging guided tumor resection. The institute also focuses on developing ABCD nanoparticle-based label-free multi-analyte sensor chips for improving spintronics sensitivity and serum biomarker profiling. The institute has also developed ABCD nanoparticle-based imaging agents, RNAi drugs for minimal-invasive fluorescence magnetic-nanoparticles guided surgery.

Another example of the products developed by the institute is peptide-aptamer based *in vitro* clinical diagnostic nanobiosensors. The idea is to insert an aptamer such as pep9 in a protein scaffold (STM), which a cysteine group binds to an electrode. The binding of the peptide aptamer specifically to protein CDK2 that is involved in cell cycles leads to changes in the impedance between electrode and fluid changes, offering *in vitro* clinical diagnostics.

The institute manufactures various different types of therapeutic nanoparticles. These nanoparticles consist of four elements:

- A-components − non-classical drugs (APUs) for delivery into cells and intracellular trafficking
- B-lipid components − protection against short short-term degradation
- C-stealth − biocompatibility polymer layer components and delivery to target cells
- D-biological targeting of ligand components

The ABCD-enabled nanodrugs are less toxic and more effective.

The institute is developing a number of scale-up/scale-out membrane reactors for manufacturing targeted drug delivery nanocapsules. These bioreactors can be operated in a batch or continuous manner.

SOURCES OF SUPPORT

The institute has €114 million of funding for four years to support its research and education program. It also received €5 million from EU Marie Curie Action project to support its management of innovation through Leeds Business School.

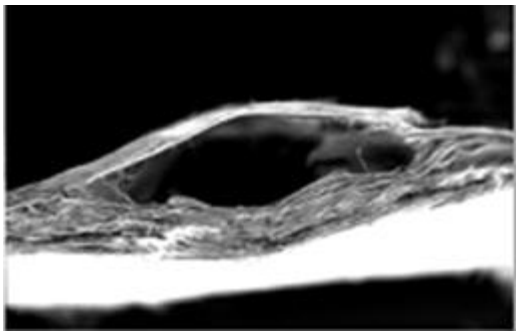

Figure B.10. Electrospun collagen with hyaluronic acid nanorods shaped into a scaffold.

ASSESSMENT

The institute is an excellent example of integrating academic research with industrial R&D collaborative research. It serves a gateway to innovation. Its semi-technical scale and pilot plants offer excellent infrastructures for testing and validating preclinical products, which are critical components of biomanufacturing.

The institute organizes numerous meetings and serves as a platform for companies to interact with researchers at Leeds University. As a result, industrial partners get access to investigators, basic research, and clinicians. The leading edge scientists at the university help attract bio-pharmaceutical companies to partner with the institute, forming an excellent ecosystem for the institute to grow.

The institute also has both M.S. and Ph.D. programs in manufacturing partnership with industry. Students spend a substantial amount of time in industry and bring ideas back to the university to work out prototype system and devices. The institute also offers a biomanufacturing minor for undergraduate students.

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University of Würzburg

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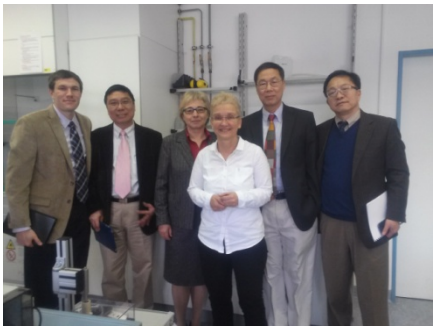

Date Visited: March 3, 2014

WTEC Attendees: G. Bao, C. Bettinger, K. Leong, K. Ye (report author), P. Foland

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OVERVIEW

The Julius-Maximilians University of Würzburg was founded in 1402. Fourteen Nobel laureates have taught at the university over its 600 years of history. Conrad Röntgen, who discovered X-rays at the university in 1895, is perhaps the most famous of these. The town and university were almost completely obliterated in a bombing raid in 1945, but were quickly rebuilt. Today, it ranks among the top universities in the Academic Ranking of World Universities list. The Tissue Engineering and Regenerative Medicine lab cooperates with several sister labs, and with an alliance with Fraunhofer institutes (IPA, IPT, and IZI) to lead the evolution of human tissue engineering; from expensive manual based production; to increasingly convenient factory-produced tissue (http://igb.fraunhofer.de/en/competences/tissue-engineering/tissue-models/skin-from-the-factory.html).

FUNCTIONAL FOCUS

Dr. Walle's bioreactor lab is part of the University of Würzburg Lehratuhl Tissue Engineering and Regenerative Medicine. Her lab is focused on manufacturing individualized skin products through scale-out. They have developed a variety of automated mini-bioreactors that produce skin tissue for wound healing and other medical use. The extensive research and testing through collaboration between biologists, cell and molecular biologists, and mechanical engineers has led to the development of these bioreactors. The creation of these robotic bioreactor operational systems represents a revolutionary process for manufacturing biomedical products to meet unmet needs in modern medicine.

RESEARCH & DEVELOPMENT ACTIVITIES

The Institute has a large shared lab space, a 3D printing core lab, and a GMP facility. The goal is to house engineers and biologists at one building to bring biological discoveries to bioproducts. They have developed a number of vascularized tissue products including 3D skin, livers, and other organs using automated bioreactors (Figure B.11). These tissue products have been extensively tested and validated at the university hospital, which is a few blocks away. The lab also focuses on developing tissue products for drug testing and for risk assessments.

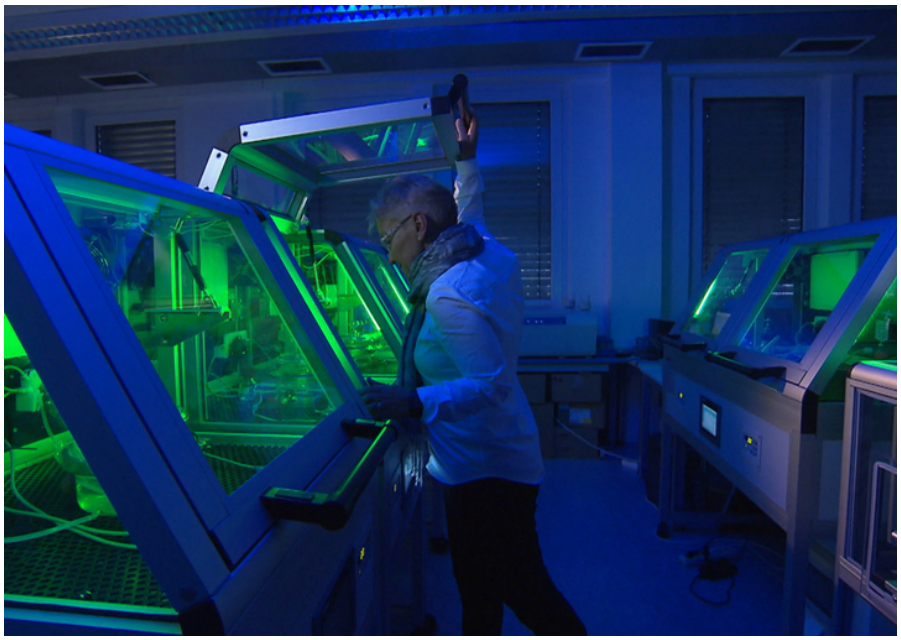

Figure B.11. Prof. Dr. Walles at a bioreactor (from http://www.term.ukw.de/bilder-aus-der-forschung.html).

The lab manufactures these tissue products in a GMP facility for use in wound healing and drug screening. All production processes are automated by designing a robotic operation system. All samples are bar-coded. For example, they have developed a cell sampling and collecting automated system for processing a patient's skin biopsy. This automated system can control thickness of the skin biopsy and control the chopping speed and force to prepare single cell suspension, which is then used for growing 3D scaffolds skins for treating patients.

Another example is the manufacturing human tracheal patch for tracheal reconstruction. To manufacture these 3D vascularized human patches, they decellularize porcine jejunal segment and reseed SMC and fibroblasts into the porcine jejunal segment along with endothelial cells. The SMC and fibroblasts are automatically prepared from human samples. The perfusion of cell culture medium in and out of the bioreactors promotes the formation of vasculatures within these decellularized scaffolds. Impedance spectroscopy is used to monitor the maturation of the tissues by measuring changes in electrical resistance of epithelial tissues.

TRANSLATION

The University of Würzburg uses its alliances with the other labs to have access to more advanced and sophisticated lab structure and equipment and to process essential parts of skin tissue engineering. The lab also has nine international patents pending and is searching for business partners to introduce products to the market.

SOURCES OF SUPPORT

University Of Würzburg receives a percentage of its funding from the government and some from the industry.

ASSESSMENT

This is a truly multidisciplinary team consisting of biologists, biomaterials scientists, mechanical engineers, and clinicians. The university hospital is nearby, so the lab has direct access to patients, samples, treatment, and clinical trials. Researchers learn from each other and work collaboratively on integration. The lab focuses on scaling-up using bioreactors dealing with different samples. They have modularized bioprocesses using robotic mechanisms. This is a unique type of thinking about manufacturing in order to standardize the process using an imaging approach.

The University of Würzburg's bioreactors are essential to produce factory-made skin tissue. They have a unique approach employing three-dimensional imaging to achieve the goal that has been recognized for a decade: a cheaper, more efficient way to replace the process of manual skin tissue processing.

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APPENDIX C. SITE VISIT REPORTS – ASIA

Reports are listed in alphabetical order by organization name.

Biomedical Research Institute and Dankook University

Site Address:	Joint Institute for Regenerative Medicine Kyungpook National University School of Medicine Daegu, Korea
Date Visited:	May 27, 2014
WTEC Attendees:	S. Drew, G. Bao (report author), K. Leong, M.V. Peshwa, A. Sambanis, H. Ali
Hosts:	**Prof. Jeong-Ok Grace Lim** Biomedical Research Institute Department of Biomedical Science, Vice-Director Tel.: +82 53 420 5447 jolim@knu.ac.kr **Sangmee Ahn** Department of Nanobiomedical Science Dankook University smahn@dankook.ac.kr **Hae-Won Kim** Department of Nanobiomedical Science Dankook University kimhw@dku.edu

OVERVIEW

The lunch discussions with Prof. Grace Lim and associates were focused on DGMIF (Daegu-Gyeongbuk Medical Innovation Foundation), a global medical R&D hub, which is located near Daegu City, South Korea. The DGMIF will have a space of 1,030,000 m^2, including 475,623 m^2 for research and R&D facilities. The construction started in Oct 2011 and is expected to finish in 2038. The basic idea is to construct a Medivalley, a global R&D hub of high-tech medical industry, shifting the R&D center away from Seoul.

FUNCTIONAL FOCUS

The DGMIF consists of the following major facilities and functional units: New Drug Development Center, Medical Device Development Center, Laboratory Animal Center, Clinical Drug Manufacturing Center, Communication Center, and High-tech Clinical Trial Center.

In the therapeutics area, there are two major efforts: small molecule drug (through chemical synthesis) and cell-based therapeutics. A GMP facility for cell-based therapeutics (collecting and processing patient cells) will be developed, which will be associated with local hospitals.

RESEARCH & DEVELOPMENT ACTIVITIES

- Small molecule drug discovery and development, with cancer, metabolic and CNS disorders as target diseases
- Medical devices, especially IT-based and imaging-based devices for disease diagnosis and therapeutics
- Cell-based therapeutics, especially that related to stem cell research, biomaterials and regenerative medicine
- Will establish a Laboratory Animal Center to support new drug and medical device development

TRANSLATION

Will establish a clinical drug manufacturing center to produce and supply clinical trial materials in compliance with the global GMP standards, and provide support for scale-up and process optimization. So far, two companies in cell-based therapeutics have moved in, and DGMIF is recruiting more companies.

SOURCES OF SUPPORT

The total project cost is about US$4.6 billion (national government US$1.1 billion, Local government US$0.9 billon, private capital US$2.6 billion).

ASSESSMENT

Although still under construction, the DGMIF is a huge undertaken and will have a significant impact to the R&D efforts related biomanufacturing and to the development of high-tech medical industry in South Korea.

SELECTED REFERENCES

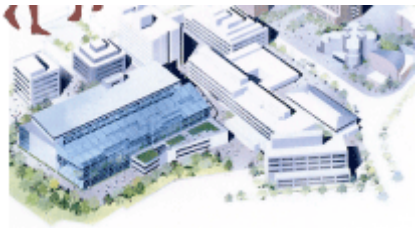
http://www.medivalley.re.kr/eng/

CellSeed, Inc. and Tokyo Women's Medical University (TWMU)

Site Address:

CellSeed, Inc.
Katsura-Bldg, 4F, 3-61, Haramachi,
Shinjuku-ku, Tokyo, 162-0053, Japan
http://www.cellseed.com/company-e/

[CellSeed also has locations in Europe.]



Visit Hosted at:

Tokyo Women's Medical University (TWMU)
Institute of Advanced Biomedical Engineering and Science
8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666 Japan
http://www.twmu.ac.jp/english/

Date Visited:

May 28, 2014

WTEC Attendees:

S. Drew, G. Bao, K. Leong (report author), M.V. Peshwa, A. Sambanis, H. Ali

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OVERVIEW

The visit included both Tokyo Women's Medical University and CellSeed. Our host, Teruo Okano, is a founder and director of the board of CellSeed, Inc., which licenses technologies and patents from TWMU. The missions of the two organizations are best summarized by these statements from their websites:

> TWMU is a medical university with over one hundred years of history, which now has a modern and sophisticated educational, clinical and research environment. The uniqueness of TWMU derives from the founder's strong volition to establish women's professionalism for women patients. Her conviction, sincerity and compassion, enlightens our commitment for medical services and care we provide to our clients.
>
> CellSeed is a biotechnology innovator focused on novel surface and cell culture products. CellSeed is dedicated to providing innovative solutions for tissue-engineering through development of novel cell harvest methods and three-dimensional living tissue replacement products for "Cell-sheet therapy" and regenerative medicine. We are committed to providing solutions for patients suffering from certain incurable diseases. Our products seek to increase and improve human quality of life through technology innovations in cell culture and manipulation to achieve replacement tissue constructs.

FUNCTIONAL FOCUS

The Institute of Advanced Biomedical Engineering and Science, under the guidance of Professor Teruo Okano, bridges across many disciplines to apply biomaterials for biomedical research applications. Their work develops and applies advances in micro-domain structured polymers, stimuli-responsive polymers, hydrogels, and polymeric micelles to cell engineering, tissue engineering, and artificial organs. Tailored substrate polymers allow facile expansion of cultured cells as viable and confluent cell layers. Multiple layers can be engineered into regenerative tissue for disease intervention.

RESEARCH & DEVELOPMENT ACTIVITIES

Professor Okano and his colleagues at TWMU have developed techniques for the endoscopic transplantation of cultured autologous oral mucosal epithelial cell sheets to tissues underlying esophageal cancer. The complex, sometimes multilayer cell sheets are placed on the exposed basement tissues following endoscopic submucosal dissection. The epithelial cell sheets successfully prevent esophageal stricture (narrowing of the esophagus that causes difficulty swallowing) after surgery. Epithelial cell sheets cultured from oral mucosal tissue on temperature-responsive polymer beds can be recovered after expansion by simply changing the temperature of the support system. At low temperature, the thermosensitive polymer undergoes a change in characteristics to become hydrophilic, thereby releasing the cell sheet with its attached extracellular matrix proteins. The recovered cell sheet can then be transplanted to the wound site without the use of adhesive material. This regenerative procedure has promoted the epithelialization of the ulcerated area safely and effectively and is advancing endoscopic treatments in regenerative medicine.

Professor Teruo Okano is the inventor of the cell sheet technology, which forms the foundation of many of the tissue engineering products developed by CellSeed. His research group has succeeded in harvesting cultured cells as viable and confluent cell layers by modifying the temperature-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm), layered onto the surface of ordinary polystyrene tissue culture dishes. Based on this temperature-responsive surface, they have proposed a new concept of "cell sheet engineering" which introduces an alternate path for tissue and organ regeneration, using only manipulated cell sheets. These cell sheets have found applications ranging from tissue-engineered cornea to cardiac patch and to skin tissue engineering.

TRANSLATION

TWMU, and particularly the Institute of Advanced Biomedical Engineering and Science, have demonstrated world-class excellence in shepherding discoveries and innovations in science through development of essential engineering systems to prototype clinical applications. They have invented novel automated cell culture systems for manufacture of clinical supplies and reduced these inventions to practice for supply of preclinical and clinical materials.

Professor Okano is a founder of CellSeed, a company created to commercialize the innovations of the Institute of Advanced Biomedical Engineering and Science. He is currently a member of the Board of Directors of CellSeed.

SOURCES OF SUPPORT

Significant funding support from government sources enable strong collaboration and exchange of technical knowhow between scientists and engineers in academe and in industry. CellSeed is a successful spin-off of Professor Okano's laboratories and provides an undisclosed level of support. Other members of the biotechnology industry (e.g., Hitachi) also support the TWMU initiatives in regenerative medicine.

ASSESSMENT

Professor Okano has demonstrated an extraordinary ability to integrate disciplines across the entire spectrum, from metabolic research to materials and cell engineering to clinical application, to development of effective autologous cell systems for regenerative cell therapy. His approach to translation of science and engineering insights into commercial and clinical practice is among the very best that we observed in our studies. His laboratories and colleagues demonstrate a level of smooth integration of disciplines that may be unparalleled in this young discipline of regenerative medicine. The panel is very impressed with the high level of automation in manufacturing cell sheets. This company serves as a model on how government, industry, and academia can come together to make a significant impact on biomanufacturing for tissue-engineering product development.

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Mitani, G., M. Sato, M. Yamato, M. Kokubo, T. Takagaki, G. Ebihara, T. Okano, and J. Mochida. 2014. Potential utility of cell sheets derived from the anterior cruciate ligament and synovium fabricated in temperature-responsive culture dishes. *Journal of Biomedical Materials Research Part A* 102(9):2927-2933.

Ohki, T., M. Yamato, T. Okano, and M. Yamamoto. 2014. Regenerative medicine: tissue-engineered cell sheet for the prevention of post-esophageal ESD stricture. *Gastrointestinal Endoscopy Clinics of North America* 24(2):273-281.

Technology to create a vascular network (Gradual lamination method) English Video: http://youtu.be/zMESSovxubo

Center for iPS Cell Research and Application (CiRA), Kyoto University

Site Address:	53 Kawahara-cho, Shogoin, Sakyo-ku Kyoto, 606-8507, Japan https://www.cira.kyoto-u.ac.jp/e/
Date Visited:	May 29, 2014
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Hosts:	**Dr. Ayaka Nakauchi** International Public Communications Office Tel.: +81-75-366-7005 a-nakauchi@cira.kyoto-u.ac.jp **Prof. Junya Toguchida** Deputy Director, Principal Investigator Department of Cell Growth and Differentiation Tel.: +81-75-366-7000 toguchida-g@cira.kyoto-u.ac.jp
Other Attendees:	**Jun Takahashi** Deputy Director **Prof. Koji Eto** **Prof. Haruhisa Inoue**

OVERVIEW

The Center for iPS Cell Research and Application (CiRA) at Kyoto University was established in 2010, led by Professor Shinya Yamanaka, with the goals of establishing basic iPS cell technology, securing the intellectual property rights associated with this technology, building a stock of iPS cells for use in regenerative medicine, carrying out preclinical studies, working toward clinical studies, and contributing to the development of therapeutic drugs using patient-derived iPS cells. In 2006, Prof. Yamanaka and his associates published a seminal paper on generating iPS cells from mouse fibroblasts by using only 4 genes (Sox2, Oct3/4, Klf4 and c-Myc), which led to his Nobel Prize for Physiology or Medicine in 2012, shared with Dr. John Gurdon. Currently CiRA has 30 principal investigators and about 300 employees and students, with research space of about 12,000 m^2. A second research building is under construction (expected to open in March 2015), with a total research space of 5,400 m^2. The 2012 total budget for CiRA was about 46 million USD. Since Prof. Yamanaka was out of town, Prof. Junya Toguchida gave an overview of CiRA, and presented the research of Profs. Jun Takahashi, Koji Eto, and Haruhisa Inoue, who also joined the discussions. The WTEC panel members toured the research labs and learned about the Facility for iPS Cell Therapy (FiT), a GMP facility.

FUNCTIONAL FOCUS

CiRA has five functional focus areas:

- Reprogramming Science (11 principal investigators)

- Cell Growth and Differentiation (8 principal investigators)
- Clinical Application (6 principal investigators)
- Fundamental Cell Technology (3 principal investigators)
- iPS Cell Ethics (2 principal investigators)

RESEARCH & DEVELOPMENT ACTIVITIES

Among the major accomplishments of CiRA are the following:

- Sought to build a cell stock that covers 30 to 50 percent of the Japanese population within 5 years by building a cooperative relationship with the Japanese Red Cross Society for recruitment of HLA-homozygous donors and through umbilical cord blood bank operations
- Carried out laboratory animal experiments that will lead to clinical research into Parkinson's disease
- Succeeded in developing a highly efficient method of differentiating human intermediate mesoderm from iPS cells and reproducing the three-dimensional structure of renal tubules, thus taking the first step toward the regeneration of the kidney
- Showed the successful creation of disease models of chronic infantile neurological cutaneous and articular (CINCA) syndrome, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease

TRANSLATION

Translational activities at CiRA are carried out in the Department of Clinical Application, mostly through collaborations with medical schools and hospitals in Japan (including the Graduate School of Medicine and University Hospital at Kyoto University). CiRA has biomanufacturing capabilities, as demonstrated by the establishment of a GMP facility.

SOURCES OF SUPPORT

The 2012 total budget was about 4.6 billion Japanese yen (~46 million USD), with 28% directly from the Cabinet Office of the Japanese government (FIRST grant), 51% from other public research grants, 9% from the Grants-in-Aid for Scientific Research (Japan Society for the Promotion of Science), 8% basic operating funds (Kyoto University), 3% from private sector grants, and 1% from the iPC Cell Research Fund.

ASSESSMENT

CiRA is a world-class research institute focusing on iPS cell research and applications. It is the first major research institute in the world dedicated to leading iPS cell research and pursuing the potential applications of iPS cells through both fundamental and applied research. CiRA's research and educational efforts are carried out through its close ties with Kyoto University's Institute for Integrated Cell-Material Sciences, Graduate School of Medicine, and University Hospital.

SELECTED REFERENCES

PowerPoint presentation
CiRA brochure

Global Stem Cell & Regenerative Medicine Acceleration Center (GSRAC), and Ajou University School of Medicine

Site Address:	C-806, Jeongseok B/D., 366 Seohae-daero Jung-gu, Incheon 400-712 Korea http://www.gsrac.org/ http://medicine.ajou.ac.kr
Date Visited:	May 26, 2014
WTEC Attendees:	S. Drew, G. Bao (report author), K. Leong, M.V. Peshwa, A. Sambanis, H. Ali
Hosts:	**Dr. So Ra Park** Director of GSRAC ymkang@gsrac.org Tel.: +82 32 889 5023 **Prof. Byoung Hyun Min** Department of Orthopedic Surgery Ajou University School of Medicine Wonchon-dong, Youngtong-gu, Suwon Gyeonggi, Korea, 442-749 bhmin@ajou.ac.kr
Other Attendee:	**Dr. David Kim**

OVERVIEW

The Global Stem Cell & Regenerative Medicine Acceleration Center (GSRAC) was established in November 2011 by the Ministry of Health and Welfare (MOHW) of the Korean government, with Prof. So Ra Park of Inha University as the director. With 14 full-time staff members, GSRAC is a nonprofit think-tank commissioned by the MOHW, with the goal of establishing collective intelligence to solve technological, regulatory and commercial challenges in translate innovative stem cell and regenerative medicine related technologies into clinical practice. During dinner discussions, Dr. So Ra Park and Dr. David Kim at GSRAC and Prof. Byoung Hyun Min at Ajou University School of Medicine explained to us how GSRAC works, and the challenges they have. The GSRAC has established a database on projects in stem cell research and regenerative medicine worldwide, which is accessible in person by researchers in South Korea. The GSRAC plans to have this database accessible on-line. The GSRAC also provides input/advice to the MOHW on the development of new research programs in the area of stem cell research and regenerative medicine.

FUNCTIONAL FOCUS

GSRAC collects information from industry experts as well as scientists and researchers in academia, and analyzes the issues and strategic options facing policy makers and the foresail communities. The functional focus areas at GSRAC include:

- Policy and National Strategic R&D Projects – Developing a technology roadmap for stem cell research and regenerative medicine and analyzing issues in the regenerative medicine industry and developing strategies for enhancing its competitiveness
- Information Gathering and Analysis Projects – Analyzing global trends and compiling a database on regenerative medicine worldwide
- Human Resources Training Projects – Providing practical training with respect to the administration and commercialization of new findings in stem cell research and regenerative medicine, and providing consultation and training on intellectual property right management
- Performance Review and Analysis Projects – Developing a performance analysis system and providing support for project performance review
- Technology Commercialization Projects – Helping clients develop research and business development (R&BD) strategies, providing assistance for the commercialization of new technologies and research findings

RESEARCH & DEVELOPMENT ACTIVITIES

The activities at the GSRAC support two R&D efforts in South Korea. The Global Stem Cell & Regenerative Medicine Initiative is a strategic research program with a particular focus on translational and clinical researches to accelerate the delivery of stem cell & regenerative therapeutics to patients. The GSRAC strives to hammer down all the roadblocks to converting this emerging technology into medical products curing currently untreatable diseases.

The Stem Cell and Regenerative Therapeutics Roadmap is a strategic move to design specific technology pathways and solutions to meet near and long-term goals of promoting the advance of the science and technology in stem cell research and regenerative medicine and their translation into medical practice.

TRANSLATION

The GSRAC supports translational efforts in stem cell research and regenerative medicine through the following activities:

- Support for R&BD strategy development
- Support for business model design
- Support for technology commercialization

SOURCES OF SUPPORT

One hundred percent of the support is provided by the Ministry of Health and Welfare, Korea Government.

ASSESSMENT

GSRAC collects information from experts in academia and industry, analyzes the issues and strategic options facing policy makers, researchers and entrepreneurs in different communities in stem cell research and regenerative medicine, and provides input/evaluation/advice to the government. This is a rather unique organization and the services provided are quite valuable.

SELECTED REFERENCES

http://www.gsrac.org/eng_about/?PHPSESSID=3caeb2bc0864631a9b1df7697708f9dd

Guangzhou Institutes of Biomedicine and Health (GIBH), Chinese Academy of Sciences

Site Address: 190 Kai Yuan Avenue, Science Park
Guangzhou 510530, China
http://english.gibh.cas.cn/

Date Visited: July 24, 2014

WTEC Attendees: C. Bettinger, T. Conway, C. Stewart, K. Ye (report author)

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OVERVIEW

The Guangzhou Institutes of Biomedicine and Health (GIBH) combine several component institutes, centers, and labs into a single unit, here called the Institute. It was established by the Chinese Academy of Sciences (CAS) in partnership with the City of Guangzhou and Guangdong Province in 2003. The Institute is located in a newly constructed campus in the Guangzhou Science Park. Its facility includes five buildings, each containing state-of-the-art, fully equipped research laboratories. It employs approximately 400 research staff members mentoring about 250 students. The Institute is focused on drug discovery and the advancement of new therapeutics. It consists of five departments: The Division of Stem Cell Biology and Regenerative Medicine, The Division of Chemical Biology, The Division of Infection and Immunity, The Division of Public Health, and The Division of Drug Discovery (GIBH 2014).

FUNCTIONAL FOCUS

The Drug Discovery Division serves as a focal point for translating basic discoveries into therapeutic agents for disease treatment. It has achieved several important milestones since it was established in 2009. These milestones include formation and integration of project teams to enable the advancement of drugs, characterization of core technology groups to support drug discovery, development of a sustainable pipeline of projects with novel intellectual property, establishment of key international partnership for the co-development of drugs, recruitment of international experts with discovery experiences to enable effective drug discovery, provision of technical services and internal expertise to local universities and companies in Southern China, and licensing of intellectual property and drugs matured in the division to pharmaceutical companies in Guangzhou for advanced clinical development. It is expected to complete its eighth milestone in 2014-2015, i.e., advancing a new drug candidate into clinical trials.

RESEARCH & DEVELOPMENT ACTIVITIES

The division developed a number of integrated core technologies for automated drug screening, as illustrated in Figure C.1. The integrated technologies include high-throughput screening (HTS), structure biology and crystallography, pharmacokinetics (PK), biomarkers, medicinal chemistry, and biotherapeutics. The HTS core is capable of automatically screening thousands of compounds

per week in various enzyme- and cell-based assays. The structure biology core allows for optimizing lead compounds using computer-aided design as well as virtual screening. The PK core is integrated to determine the PK and safety of the lead compounds. The biomarker core is used to validate the efficiency and safety of the drug in preclinical setting. The medicinal chemistry core is intended to design, synthesize and formulate of new drugs, whereas the biotherapeutic core is intended to test the drugs for disease treatment.

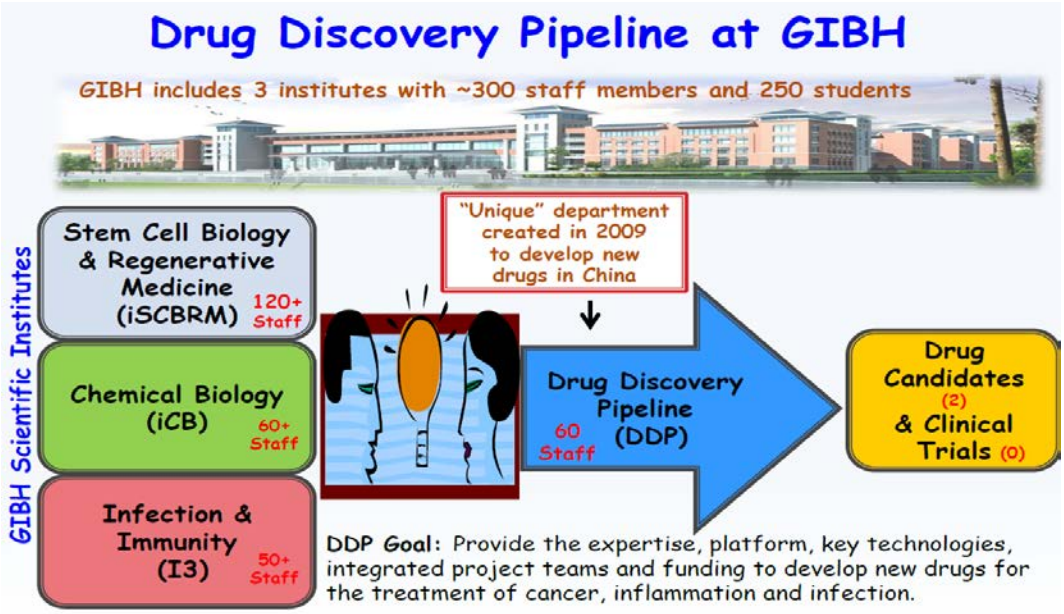

Figure C.1. The organization of the Drug Discovery Division at Guangzhou Institutes of Biomedicine and Health (courtesy of GIBH).

The Core Lab has supported discovery of more than 100 different drug screening assays against disease targets in cancer, diabetes, inflammation, arthritis, influenza, hepatitis C, and other diseases. It has helped screen 23 protein kinases, 12 proteases, 11 deacetylases, 6 anti-inflammatory targets, 6 GPCRs, and 13 other protein targets. It has created a comprehensive internal compound library that includes many novel molecules made at GIBH (approximately 4500). The lab adds almost 10,000 compounds a year to the library through either synthesis by the GIBH or acquisition from other groups. The library will be used for drug screening at GIBH.

The structural biology core has developed various protein expression platforms that include *E. coli*, baculovirus, pichia pastaris and mammalian cell protein expression systems for drug screening. It has also been equipped with a new supercomputer platform with a total peak performance of 1.2 Tflops, plus 192 GB memory and 10 TB storage for virtual drug screening and biological macromolecule modeling. The PK core lab has analyzed more than 260 drugs for their pharmacokinetics, 85 drugs for their drug-plasma protein binding capability, 120 drugs for their metabolic stabilities, 91 drugs for their drug-drug interaction, 21 drugs for their Caco-2 assays, 7 drugs for their blood-brain barrier penetration, 32 drugs for their toxicity in zebra fish, and 8 drugs for their hepatocyte toxicity in rodents. The Medicinal Chemistry core lab has prepared several thousands of new chemicals targeting pathways in cancer, arthritis, Alzheimer's disease, diabetes, flu, malaria, pain, and other diseases. The analytic core provides services for advanced biochemical assays including zatasizer, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectroscopy (LC-MS), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), element analyzer, Prep, NMR, IR spectrometer, X-ray diffraction, polarimeter, and a melting point machine. The biotherapeutics core is equipped for discovery of protein-based therapeutics. The lab is now developing a recombinant protein of ADAMTS-13 for the treatment of thrombotic thrombocytopenic purpura. The lab is also

equipped with transgenic animal facilities for creating diseased animal models. The lab has so far created nine gene targeting vectors and four corresponding embryonic stem cell clones. The biomarker lab has been focused on cell-based screening, protein-protein interaction, and chromosome and chromatin assays.

TRANSLATION

One of the missions of the GIBH is to translate basic studies into drug discoveries. The following drugs have been discovered and been used for clinics:

- D824, a new Bcr-Abl inhibitor for the treatment of chronic myeloid leukemia resistance. GIBH is now partnering with Shunjian Pharmaceuticals, Inc. to market this drug.
- GIBH-130 for Alzheimer's disease treatment. This drug blocks neuro-inflammation by targeting active microglia cells in the brain. The drug has been formulated as a tablet for oral dosing. GIBH is partnering with South China Center for Innovative Pharmaceuticals to commercialize the drug.
- GIBH-104 for treating inflammation and acute pain. GIBH is seeking partner companies to market the drug.
- GIBH-117 for antimalarial viruses. GIBH is in the process of translating it to the market.
- R001/2, which is a siRNA-based drug treating osteoarthritis.
- ADAMTS13, a protein-based drug for treating thrombotic thrombocytopenic purpura
- DDR1, for lung cancer therapy.

GIBH is working with its partner companies to translate all these new compounds into drugs.

SOURCES OF SUPPORT

GIBH is a part of the Chinese Academy of Sciences, which provides core funding. The National Natural Science Foundation of China also contributes. GIBH has received more than 300 research awards totaling CNY600 million.

ASSESSMENT

GIBH plays a critical role in the R&D base for biomedicine and is an integral component of the development of science and technology in Southern China. Its research projects have generated 205 patents and 327 publications. GIBH is highly multidisciplinary and integrated. The research team includes researchers from all over the world, with different backgrounds in science, medicine, and engineering. The highly integrated drug screening core facilities not only provide services to the entire institute, but they are also actively involved in drug screening. Quite a few new drugs have been identified at these core facilities. The Institute has translational research built into its mission and has actively sought for and partnered with commercial entities for translating their drug discoveries into drug products. The automated drug screening platforms play key role in drug discovery at GIBH. However, the protein expression, PK study, biomarker discovery, and chemical design have not yet been automated. The automating of these steps will further expedite its drug discovery, making advanced biomanufacturing critical to the success of the entire field.

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Pan, G.J., Z.Y.Chang, H.R. Scholer, and D. Pei. 2002. Stem cell pluripotency and transcription factor Oct 4. *Cell Research* 12(5):321-329.

Japan Tissue Engineering Co., Ltd. (J-TEC)

Site Address:	6-209-1 Miyakitadori Gamagori Aichi 443-0022, Japan
Corporate Headquarters:	Japan Tissue Engineering Co., Ltd. 6-209-1 Miyakitadori Gamagori Aichi 443-0022, Japan Tel.: +81-533 662020 Fax: +81-533 662019 jtec-info@jpte.co.jp http://www.jpte.co.jp/english
Date Visited:	May 29, 2014
WTEC Attendees:	H. Ali, G. Bao, S. Drew, K. Leong, M.V. Peshwa (report author)
Host:	**Ken-ichiro Hata**, DD.S. and Ph.D. Managing Director, R&D Department
Other Attendees:	**Mayumi Miura** Senior Manager, Corporate Control Division Manager, Business Strategy Department

OVERVIEW

J-TEC was established in February 1999 by NIDEK (Gamagori, Japan) and in 2004 built cGMP Manufacturing Facility to focus on the regenerative medicine products business. Currently J-TEC is a public company, traded on JASDAQ, with annual sales (2014) of ~JPY1 billion. J-TEC is the only company in Japan that has commercially marketed cell therapy/tissue engineering based products approved by the Pharmaceutical and Medical Devices Agency (PMDA), the FDA-equivalent regulatory authority in Japan.

The WTEC panel's visit was hosted by the managing director and three managers. The panel was also provided with a facility tour through the cGMP Manufacturing Facility and various Quality Laboratories and Logistics & Production Planning areas.

FUNCTIONAL FOCUS

The company's primary source of revenue is from sale of two commercial products: JACE (J-TEC Autologous Cultured Epidermis) for treatment of severe burns, and JACC (J-TEC Autologous Cultured Cartilage) for traumatic cartilage defects and Osteochondritis Dissecans (OCD) of knee, excluding Osteoarthritis (OA); both products being approved as Medical Devices. JACE received marketing authorization in Oct 2007, and JACC received marketing authorization in April 2013.

J-TEC is currently seeking regulatory guidance on its third product: Autologous Cultured Corneal Epithelium. Additionally, the JACE product is in advanced clinical trial for additional indications

of Epidermolysis Bullosa and giant nevi. J-TEC is the only biotechnology company in Japan to have received QMS (Quality Management System) and GMP approved manufacturing facilities for cell-based product manufacturing. They currently have more than 200 employees.

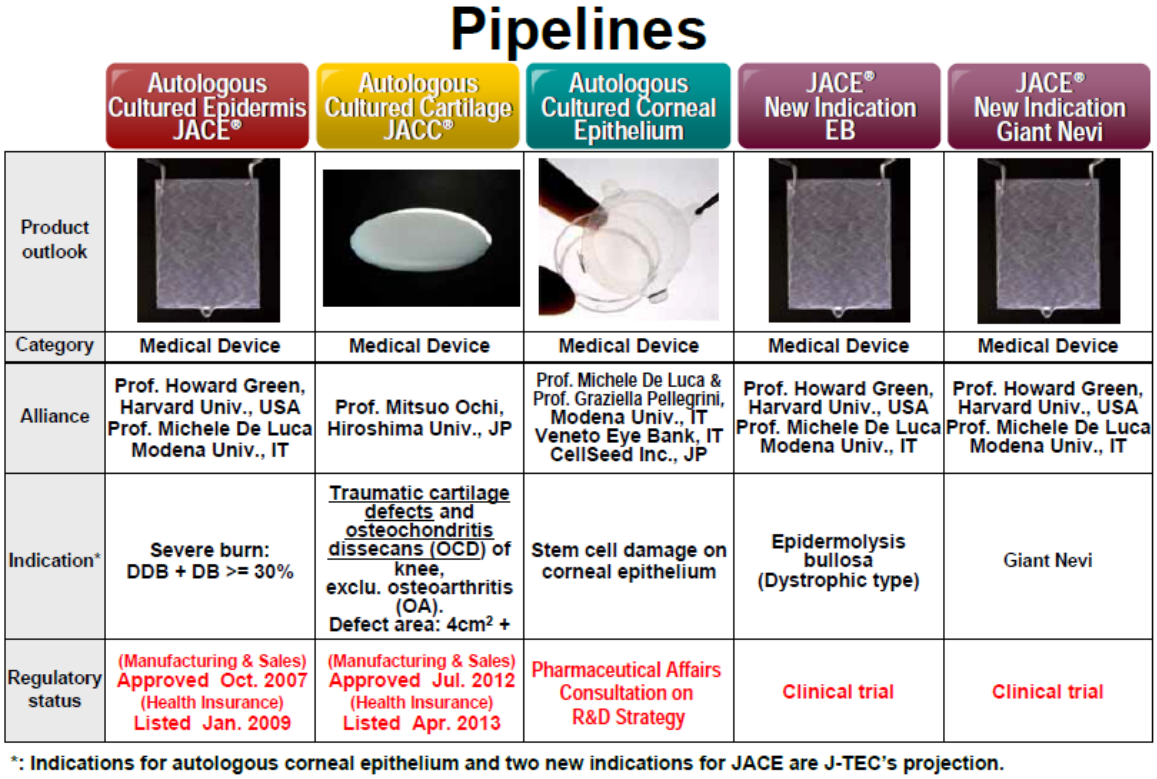

Figure C.2. J-TEC's Tissue Engineered Medical Products (TEMP) pipeline (courtesy of J-TEC).

RESEARCH & DEVELOPMENT ACTIVITIES

R&D activities encompass providing scientific review expertise on cell therapy products and undertaking preclinical, translational, and analytical development to support clinical and regulatory strategy for development and testing of human clinical trial and commercially marketed TEMP Products. J-TEC's facilities are well-equipped, have modular laboratories, plus space for meetings and networking. The facility design is intended to promote collaboration and innovation.

The JACE Product was approved in Oct 2007 and consists of autologous cultured epidermis (approximately 3–5 cell layers). This product is similar to Epicel Product that is marketed in the United States as a humanitarian use device by Genzyme Biosurgery (acquired in April 2014 by Aastrom Biosciences, Inc.; subsequently renamed as Vericel Corporation). Manufacturing takes approximately 3 weeks of *ex vivo* culture and requires one CO_2 incubator to be dedicated to a single patient's product. The product is shipped at 10–25 °C temperature and has a shelf life of 56 hours.

TRANSLATION

Reimbursement for JACE was approved in Jan 2009 (almost 15 months after product approval) and pays JPY 314,000 (~ US$3,140) per 8' x 10' sheet (covers approximately 0.5% of total body surface); with a maximum of 20 sheets allowed per patient. Any additional sheets required to treat a patient need to be provided by J-TEC at its own cost (with no reimbursement coverage permitted). This was a significant challenge for the company because the approved indication requires more than 20 sheets to be manufactured and delivered for use in treatment.

In the initial years following reimbursement approval, approximately only 40% of the products supplied by the company were being adequately reimbursed, with 60% of the products exceeding the 20 sheet limit; with 25 hospitals being part of the commercial network. Following ongoing dialogue and education of the reimbursement agency, in 2012 reimbursement was expanded to permit coverage for up to 40 sheets per patient. Currently, there are over 200 hospitals that provide JACE treatment and reimbursement covers approximately 94% of the products supplied (with the 40 sheet limit); with J-TEC providing products for the remaining 6% patients at its own cost.

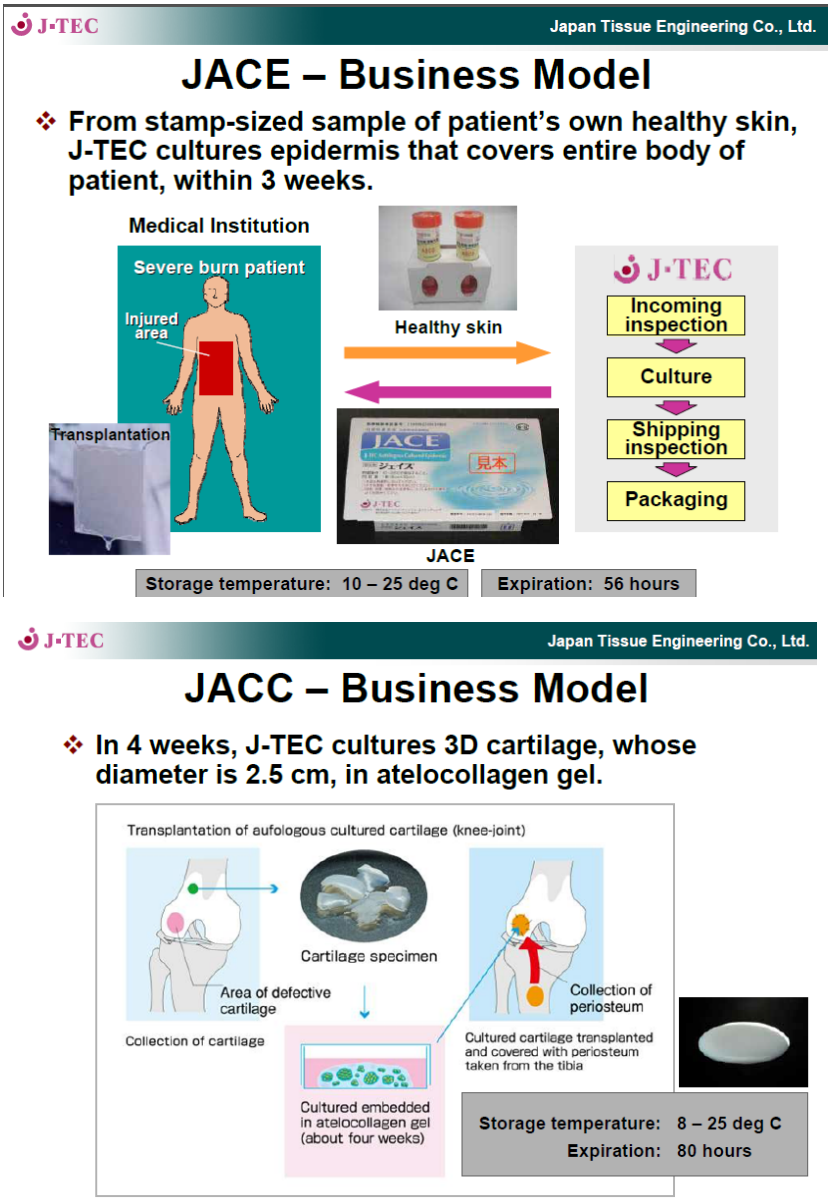

Figure C.3. JACE and JACC product manufacturing and logistics infrastructure (courtesy of J-TEC).

SOURCES OF SUPPORT

J-TEC is a public company, traded on JASDAQ. In its most recent non-consolidated summary of Financial Report for the 3rd Quarter of FY2014 (issued January 30, 2015), the company forecasts annual sales for FY2014 (April 01, 2014–March 31, 2015) to be JPY1,275 million (~US$10.7 million).

J-TEC has a strategic partnership with Fujifilm, which owns approximately 40% of the company. In the immediate future J-TEC plans to: (1) become operationally break-even in 2015, (2) build market penetration for its approved products, and (3) develop new Tissue-Engineered Medicinal Products and introduce them into market rapidly through the "conditional approval" provisions of the new Regenerative Medicine Law (effective November 2014).

Furthermore, in partnership with Fujifilm, J-TEC has established a joint venture in China and plans to expand into other Asian markets.

ASSESSMENT

J-TEC has built core competency and fully integrated value chain to discover, develop, manufacture, sell, and conduct post-marketing surveillance of TEMP Products in Japan, in compliance with ordinances and notifications released by the Health, Labor and Welfare Ministry. J-TEC has developed a quality management system which complies with the Pharmaceutical and Medical Device Act, and ISO9001:2008. This system is constantly being improved to enable us to offer high-quality products of outstanding efficacy and reliability.

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OVERVIEW

The Korea Institute of Science and Technology is a national R&D institute with a mission to bring a better quality of life for all people by preparing for the future. KIST was founded in 1966 as the first Korean science and technology research institute, and has been a model for other Korean institutes. KIST employs more than 2000 researchers, and has several branches abroad. In addition to the biomedical institute that WTEC visited, KIST includes institutes on brain science, convergence of materials, technology policy, and green cities, plus divisions on future convergence research and the research themes of Korea's national agenda (KIST 2014; Wikipedia 2014).

The KIST Biomedical Research Institute (BRI) was established in 2011 to promote the national agenda on healthcare and welfare for a better quality of life. The institute is committed to integrating the engineering and biomedical sciences with the clinical sciences to lead biomedical innovation. To accomplish this, the institute develops core technologies, and also focuses on translational research for clinical applications. Its vision is to be recognized as a global leader in biomedical research and to be a frontier national institute improving quality of life for the elderly and disabled. BRI has 381 personnel organized into three centers: bionics (with 23 faculty), biomaterials (13), and theragnosis (16) (BRI 2014).

FUNCTIONAL FOCUS

The research in the BRI Center for Biomaterials under Dr. Seok is most aligned with the WTEC study scope. Their mission is personalized treatment for recovering injured tissues and organs. One of their teams focuses on regeneration by personalized and timely tissue engineering aided by analysis. Topics include nanomaterials, biomaterials for tissue engineering, microenvironment control, drug delivery for regeneration, and stem cell therapy. Their second team focuses on replacement, that is, biomaterials for replacement and medical devices for replacement surgery. Their specific topics include biocompatible metals, ceramics, and polymers; surface modification; biologic functionalization, materials–body interfaces; and materials and devices for surgery.

Another interesting program is the Global siRNA Carrier Initiative (GiRC) under the leadership of Dr. Ick Chan Kwon. The mission is to develop effective nonviral siRNA nanocarriers that can realize the full potential of RNAi discoveries. A particular focus is to develop injectable formulations that can be administered intravenously for a wide range of therapeutic applications. The program leverages on the molecular imaging expertise of KIST to visualize biodistribution and *in vivo* targeting efficiency of these carriers. Five leading researchers on nonviral siRNA delivery in the United States are invited to join a similar-sized research team at KIST to innovate in nanocarrier development. This can serve as a model for effective international collaboration.

Also in BRI, the Bionics Center under Dr. Kang is involved in R&D on the convergence of biology and mechatronics, including neuro-robotic rehabilitation and computer-assisted surgical systems. The Center for Theragnosis under Dr. Yang is particularly concerned with personalized medicine and the development of theragnosis technology, which is the combination of diagnosis and therapy. This includes molecular imaging, molecular diagnostics, and nanomedicine.

RESEARCH & DEVELOPMENT ACTIVITIES

While a broad range of BRI activities was presented, only some details of the R&D of the biomaterials center will be summarized here. In particular the approach of their replacement team includes: 1) platform materials fabrication using metals, ceramics, polymers, and hybrid materials with precision processing; 2) bio-functionalization via nano/micro patterning on surfaces, loading and release control of drugs, proteins, and genes, plus cell and tissue compatible environment control; and 3) innovative medical devices including orthopedic implants, stents for cardiology and urology, and hydrogel patches. The work directed toward replacements of tissues and organs is being led by Drs. H.K. Seok, D.K. Han, Y.C. Kim, J.K. Joung, H.J. Jeon, and D.G. Han.

TRANSLATION

BRI has an extensive program for translation of its findings into clinical research, including a new Translational Research Program (TRP), which is intended to serve as a role model for M.D.-Ph.D. collaboration as a contribution to Korea's medical industry. It includes a partnership with an MD for all BRI faculty by working on the same research grants. Nine projects in partnership with the Samsung medical center were started in 2014.

SOURCES OF SUPPORT

BRI's budget in 2013 was $31.9 million. Of this, about half ($16.1 million) was extramural funding from the National Research Foundation (NRF), the Ministry of Science, ICT [Information and Communications Technology], and Future Planning (MSIP) program of the Korean Government.

ASSESSMENT

BRI conducts excellent basic research focused on areas of practical importance with a view towards translation and commercialization, although the success stories in this area are still limited. They have good international collaborations, convergence of biomedical, electronics, mechanical and biomaterials research.

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OVERVIEW

MEDINET was established in October 1995 with the objective of establishing a new medical support business in preventive medicine. In April 1999, following construction of the first cell processing center (CPC) at the Seta Clinic, MEDINET started providing its current core business, immuno-cell therapy (ICT) total support service, to Seta clinic's patients at the Seta clinic. In May 2003, MEDINET established its research and development Center (MEDINET Medical Institute) in Tokyo. Subsequently, in October 2003, MEDINET [TSE # 2370] was listed as public company on MOTHERS Board of Tokyo Stock Exchange.

The WTEC panel met with the Dr. Ryuji Maekawa (Member of Board of Directors and Head of R&D Division and MEDINET Medical Institute) at the MEDINET Medical Institution (Tokyo). Dr. Maekawa gave the panel members a tour of the R&D facilities and provided an overview of MEDINET's R&D strategy and approach to ICT product development. This was followed by technical presentations by three researchers: M. Takahara, K. Miki, and M. Muto on selected ICT products. Dr. Maekawa facilitated and accompanied the panel members to MEDINET's cGMP Facility located at Shin-Yokohama. At the Shin-Yokohama location, Mr. Daisuke Noguchi (Manager, Cell Processing Center) led the panel members on a tour of the Cell Processing Center and walked them through the Patient Lobby of the adjacent Seta Clinic Center; and subsequently Dr. Yoshimi Toda (Global Business Development Specialist, R&D Planning & Administration Section, Business Strategy & Planning Department) provided the panel members with a business and corporate overview of MEDINET.

FUNCTIONAL FOCUS

MEDINET is a R&D oriented biotechnology company supporting development, manufacture, and patient treatments using novel immuno-cell therapy (ICT) products, with intensive experiences and advanced technologies. Immuno-cell therapy has been validated as means for restoring immune balance in cancer patients (Noguchi et al. 2014, Hosoi et al. 2014). ICT products consist of two broad categories for treatment of cancer:

- Tumor-antigen non-specific therapies (passive immunotherapies) comprising:
  - αβ T-cell therapy
  - γδ T-cell therapy
  - Natural killer (NK)-cell therapy
- Tumor-antigen specific therapies (active immunotherapies) comprising dendritic cell (DC) vaccine therapy generated using either:
  - Electroporation of autologous tumor lysate
  - Co-incubation with patient specific HLA class I restricted, tumor antigen associated protein derived, peptide(s)

In both instances, the dendritic cells are activated by supplementation of zoledronic acid to cell culture medium. The way these therapies work is illustrated schematically in Figure C.4.

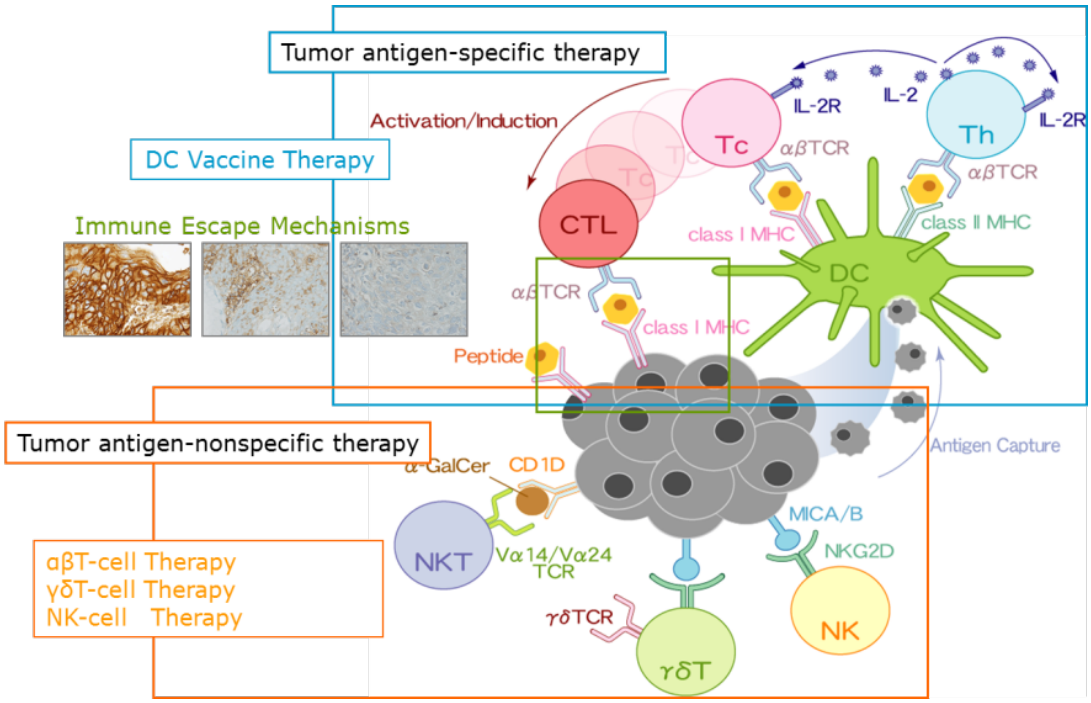


Figure C.4. Immuno-cell therapy products (courtesy of MEDINET).

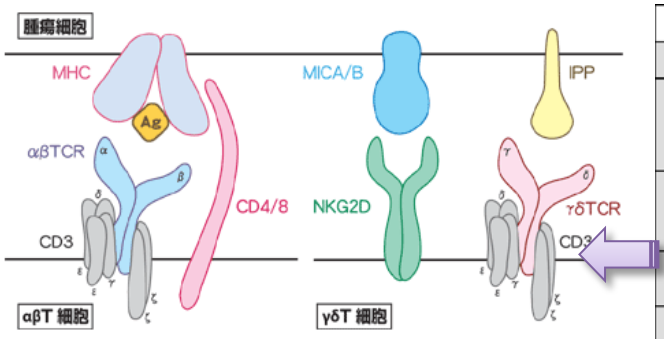
RESEARCH & DEVELOPMENT ACTIVITIES

MEDINET Medical Institute has been largely instrumental in conducting preclinical development of novel immuno-cell therapies and translating these to commercial delivery through MEDINET's ICT Total Support Service offering. MEDINET had originally commenced operations providing non-antigen-specific T-cell expansion as αβ T-cell Therapy. In October 2007, MEDINET commenced providing γδ T-cell Therapy (Figure C.5) to expand its ICT product offering beyond the initial product offering of αβ T-cell Therapy (which recognizes antigenic peptide bound to HLA molecules) to also include non-HLA restricted passive immunotherapy that recognizes MIC-A/B antigens and IPP expression on tumor cells through recognition via NKG2D receptor and γδ-TCR.

More recently, new ICT Products have been introduced as follows:

- August 2007 - Commercial offering of dendritic cell vaccine therapy with zoledronic acid.
- August 2008 - Launch of an enhanced potency Dendritic Cell Vaccine Therapy comprised of electroporation of patient's tumor lysate coupled with Zoledronic Acid treatment.
- November 2012 - Expansion of commercial product portfolio to also include NK-cell Therapy as an additional product through its Total Support Services offering
- April 2014 - Announcement that partner medical institution will commence treatment of patients with MACS® GMP PepTivator® WT1-pulsed dendritic cell vaccines.
- May 2014 - Announcement of licensing of sendai virus technology for use with dendritic cell vaccines from DNAVEC Corporation (Ibaraki, Japan), for development of a potential future commercial ICT Product

Researchers from MEDINET Medical Institute (MMI) provided scientific overview, manufacturing process, and mechanism of action on selected ICT product (Table C.1).



	αβT-cell	γδT-cell
Ratio in Blood	65～75%	1～5%
Distribution	Blood, Lymphatic Tissue	Blood, Epithelium, Lymphatic Tissue
Receptor	αβTCR CD3 CD4(orCD8)	γδTCR CD3 NHG2D
Capture Antigen	MHC-Peptide	MIC A/B IPP
MHC-restricted	○	-
Cytotoxicity	○	○

Figure C.5. Comparison of characteristics of αβ T-cells and γδ T-cells (courtesy of MEDINET).

Table C.1. Product Presentations Provided by MEDINET Medical Institute Researchers

Product/Topic	MMI Researcher
γδ T-cells for effective immunotherapy of cancer (Kondo et al. 2011, Yoshida et al. 2011, Sakamoto et al. 2011, Wada et al. 2014)	Mr. Muto
Adjuvant effect of Zoledronic Acid on dendritic cells (DC) (Nieda et al. 2003, Takahara et al. 2008)	Mr. Takahara
DC Vaccine electroloaded with autologous tumor lysate (Hosai et al. 2008, Wolfraim et al. 2013)	Mr. Takahara
Induction of both OVA-specific CD4+ and CD8+ T cells by using PepTivator OVA-pulsed DCs in mouse model	Mr. Miki

TRANSLATION

MEDINET's current core business is to provide immuno-cell therapy total support services to their 8 contracted and 61 allied medical institutions for treatment of cancer patients at these clinical centers under Medical Practitioners Act. The eight contracted institutions in Japan are:

- Four Seta Clinic Centers (Tokyo, Shin-Yokohama, Osaka, and Fukuoka)
- Tokyo University Hospital 22nd century medical and Research (Tokyo)
- Translational Research Center of Kanazawa University Hospital (Ishikawa)
- National Hospital Organization, Osaka National Hospital (Osaka)
- Center for Advanced Medical Innovation Kyushu University (Fukuoka)

The contracted medical centers are responsible for patient interactions and treatment. They conduct medical exams, determine disease status of patients, write prescriptions for use of ICT Products for patient treatment, and make clinical assessments of effectiveness of treatment with ICT Products. MEDINET serves capacity as an "in-house cell processing center" for these contracted medical centers and manufactures ICT Products for the contracted Clinics and receives royalty revenue from the clinics for providing them with such manufacturing services. Figure C.6 depicts the responsibilities under the Medical Practitioners Act and organization of business operations between MEDINET and the clinics.

All of MEDINET's ICT Products are delivered as fresh formulations, have a 24-hour shelf life, and are administered by intravenous infusion. The connection of the CPC to the clinical site permits delivery of fresh formulations with short shelf life. The CPC at Shin-Yokohama, comprised of

three Class 10,000 suites can operate at maximal throughput capacity to manufacture 1,200 ICT Products monthly; employing the current SOP-controlled manual manufacturing process without the need for investment in process automation. It is currently operating at a capacity of 400 ICT products per month in servicing approximately 100 cancer patients per month treated at the co-located Seta Clinic Center. MEDINET currently does not have any ongoing efforts to automate the manufacturing process.

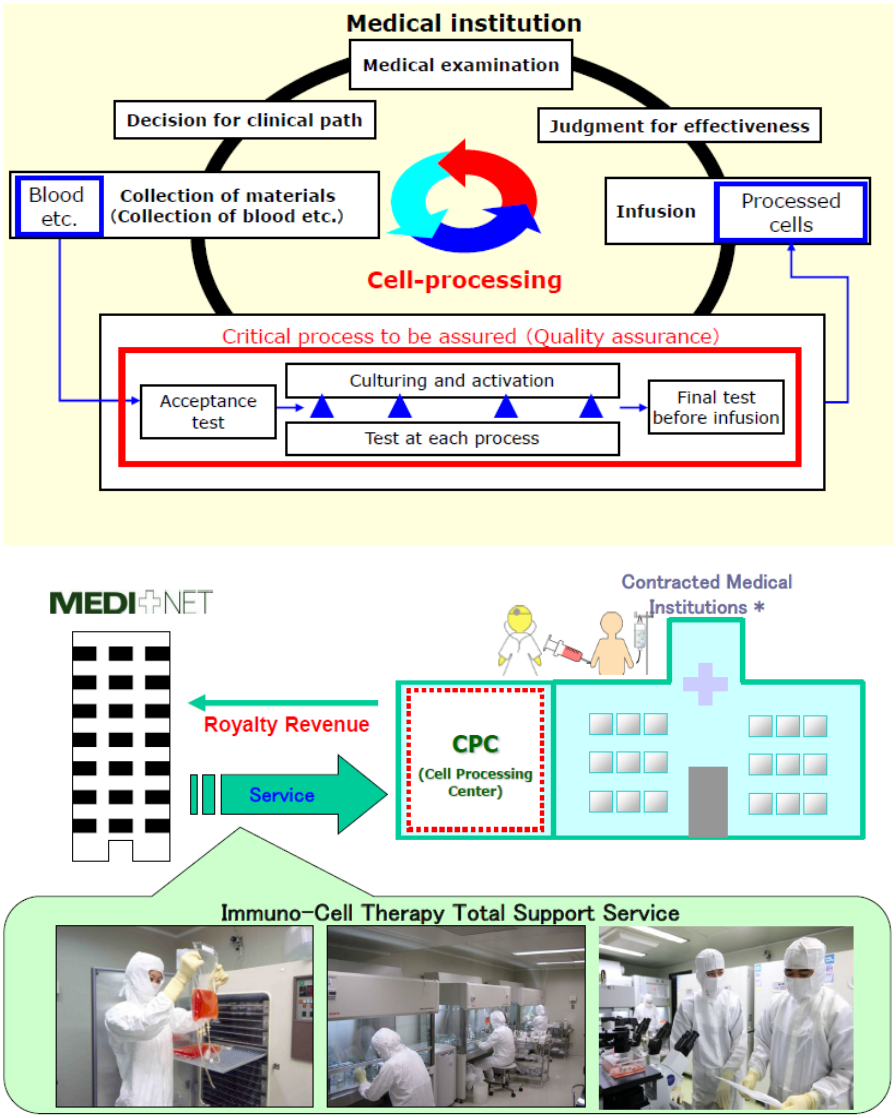


Figure C.6. Responsibilities and organization of business operations under the Medical Practitioners Act (courtesy of MEDINET).

SOURCES OF SUPPORT

In October 2003, MEDINET was listed as public company on MOTHERS Board of Tokyo Stock Exchange. It is traded under the ticker symbol TSE # 2370, with an approximate market capitalization [as of June 12, 2014] of JPY25.9 billion (approximately US$259 million).

The company's latest financials are detailed in the most recent [Release Date May 08, 2014] Consolidated Financial Report for two quarters (Oct 2013-March 2014) of the company FY2013 (October 2013-September 2014) projections (Suzuki 2014). Net sales for first two quarters of

FY2013 were JPY998.5 million (approximately US$10 million). The company reported Cash and Cash Equivalent of JPY7.05 billion (approximately US$70 million) as of March 31, 2014.

Shared Research, Inc. (Tokyo, Japan), Japan's leading Equity Research firm, in the most recent research report [dated June 06, 2014] estimates MEDINET's FY2013 [ending September 2014] total sales as JPY2.27 billion (approximately US$ 23M), representing 3.6% CAGR over FY2012 sales; and near break-even operations with a net income (loss) of JPY975,000) (approximately US$10,000) (Shared Research 2014).

ASSESSMENT

MEDINET has built its ICT Total Support Services business to provide novel products to 8 contracted medical centers and 61 allied medical institutions throughout Japan. MEDINET Medical Institute has continued to develop new ICT Products based on understanding of immune mechanism of action to provide a portfolio of products to treat patients through the entire continuum of disease stages and progression. These new products have been developed in close collaboration with scientific collaborators, clinical investigators, and treating physicians; and have been successfully implemented into GMP manufacturing to ensure cost-effective manufacture and delivery in a non-reimbursed environment under the Medical Practitioners Law in Japan. By far, MEDINET is the world leader in terms of its experience in terms of number of products manufactured and patients treated. Furthermore, MEDINET has built a successful commercial business operation providing ICT product manufacturing services to contracted medical institutions. Having built this fundamental core competency, MEDINET has an opportunity to build value for its stakeholders by leveraging the changing regulatory environment in Japan. MEDINET has a demonstrated ability to be nimble and expand its core business competencies to set up a new CMO business and a new cell medicine products business with a diverse product portfolio of internally (MEDINET Medical Institute) developed products as well as one in-licensed product (in phase III clinical trials in the United States) and one out-licensed product (for clinical/commercial development in Europe).

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OVERVIEW

MEDIPOST was established in 2000 as a Private Cord Blood Bank in South Korea. MEDIPOST is a public company, traded on KOSDAQ under the ticker symbol 078160.KQ, with an approximate market capitalization [as of June 04, 2014] of KRW428.26 billion (~US$428 million).

MEDIPOST's core business activities encompass four different market segments:

- private cord blood banking
- human umbilical cord blood derived mesenchymal cell therapeutics (hUCB-MSC cell drugs)
- nutritional supplements
- cosmetics

MEDIPOST has also established a wholly-owned subsidiary, MEDIPOST America Inc. (Rockville, MD), to conduct clinical development of stem cell drugs outside South Korea.

The WTEC panel met with the Dr. Antonio S.J. Lee (CEO & Managing Director of MEDIPOST America Inc.), Mr. Hyukjun Nam (General Manager of R&D Center GMP Facility/Quality Assurance Manager) and Ms. Yun-Mi Kim (Assistant Manager, Business Development). Dr. Lee provided the Panel with a business, scientific, and clinical overview. Mr. Nam led the panel on a tour of the cGMP facility.

FUNCTIONAL FOCUS

The company's primary source of revenue is the private cord blood banking business, which generated ~US$30 million in annual revenue in 2013. The company has approximately 180,000 units of cord blood in storage. This accounts for ~45% of private CB banking market in South Korea, where the potential market is ~9% of natural births. The private CB has been used as practice of medicine for treatment of oncology and cerebral palsy patient. In addition to CB banking, MEDIPOST has for many years invested in R&D efforts to develop hUCB-MSC as platform for development of allogeneic cell drugs.

RESEARCH & DEVELOPMENT ACTIVITIES

For manufacture of the hUCB-MSC platform products, the starting cord blood (CB) source is donations (individuals who do not sign up for private banking) that are tested for compliance with established donor eligibility screening, infectious disease screening, and mother's medical history. Donated CB is collected in validated collection kits (the same kit used for private CB banking business) and is shipped to the MEDIPOST GMP Facility in Guro-go for manufacture of cell drugs (Figure C.7).

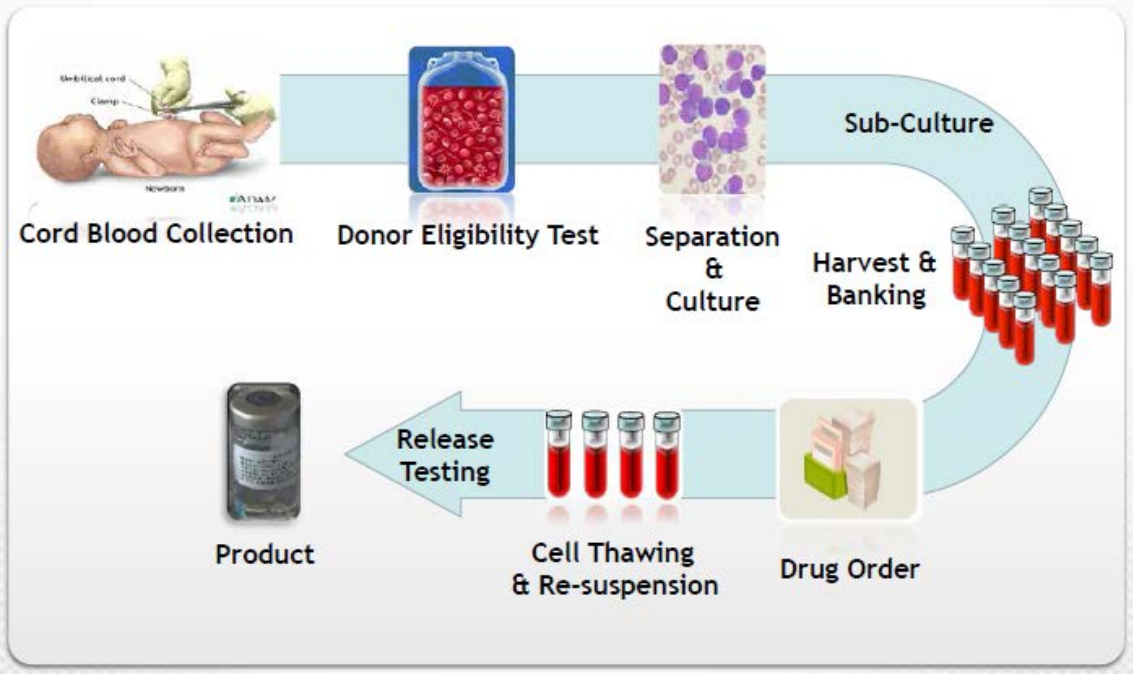

Figure C.7. Process of manufacture of hUCB-MSC cell drugs (courtesy of MEDIPOST).

MEDIPOST's Guro-go GMP facility consists of two separate production areas:

- CARTISTEM Production Area, consisting of five class 10,000 clean room suites equipped with class 100 BSCs for open cell processing. This production area was inspected and licensed for clinical manufacturing in August 2006 and for commercial manufacturing in January 2012.

- Multifunctional Production Area, consisting of additional 5 class 10,000 clean room suites equipped with class 100 BSCs for open cell processing. This production area was inspected and licensed in January 2014 to provide additional capacity for CARTISTEM, PNEUMOSTEM and NEUROSTEM manufacturing.

The facility occupies approximately 1,200 m^2 of space with approximately 770 m^2 of dedicated production space. All quality control testing is performed in house. The facility employees 38 FTEs, approximately evenly divided between manufacturing and QA/QC activities.

There is a separate GMP facility used for processing and storage of CB samples collected for the private cord blood banking business. In its experience with collecting approximately 180,000 units of CB for private banking, MEDIPOST has detected a collection related contamination rate of approximately 0.5%, providing metrics for validating the collection and shipping process of donated CB units intended for use in manufacture of hUCB-MSC products.

TRANSLATION

CARTISTEM received marketing authorization approval in South Korea from MFDS in January 2012. It is the first ever allogeneic cell therapy product approved by a national regulatory agency anywhere in the world.

The basis for commercial licensing approval in Korea was a 103 patient, randomized phase III trial comparing CARTISTEM to microfracture (as control). In the intent-to-treat (ITT) population of 103 patients, 50 patients received CARTISTEM and 53 patients were treated with microfracture. Patient demographics are depicted in Figure C.8.

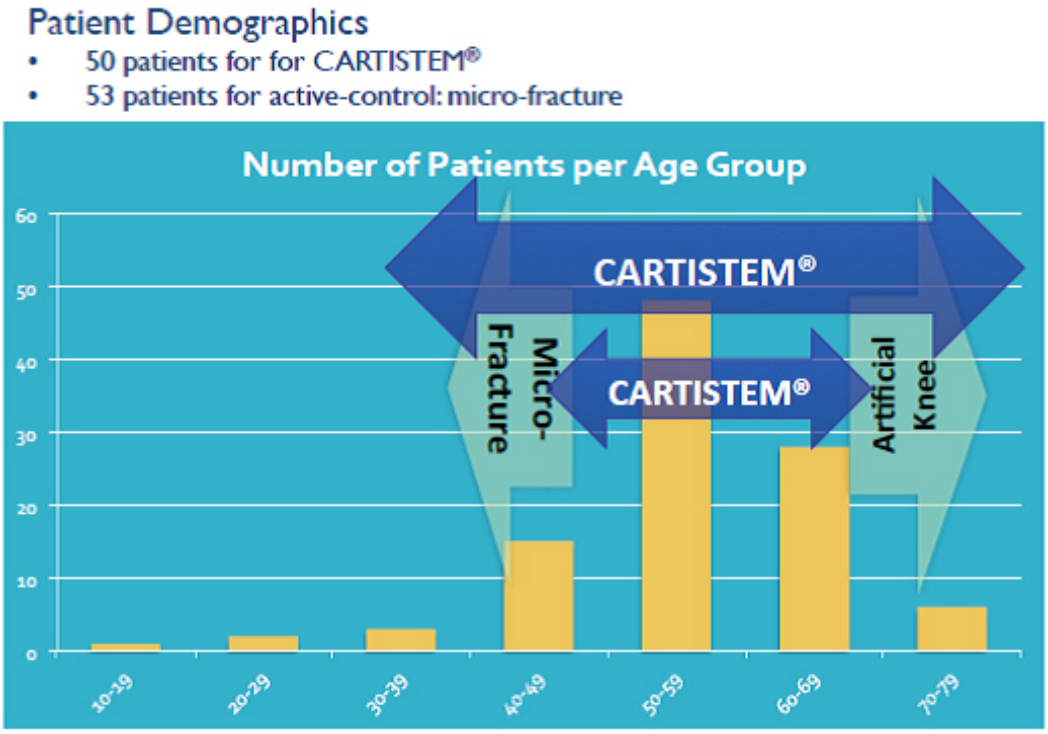

Figure C.8. Phase III trial patient demographics and current standard of care (courtesy of MEDIPOST).

Patients were followed for one year by articular cartilage-specific, T2-weighted MRI imaging (primary end point). Results for primary end point from the Phase III trial are depicted in Table C.2. Additionally, cartilage biopsy was performed on patients with acute lymphoblastic leukemia at 1 year follow-up, and samples were analyzed by immunohistochemistry (IHC) for Safranin-O and Type II collagen expression. Additional functional testing (secondary end points) comprised 100

mm VAS Evaluation, IKDC Score, and WOMAC scale. Long-term safety was assessed by cartilage-specific MRI (T2 mapping) at 3 years post treatment.

Although primary efficacy was statistically significant, the improvement in objective outcomes of CARTISTEM treatment versus microfracture was approximately 10% in the ITT population over all age groups. Retrospective subset analysis indicated that efficacy of CARTISTEM therapy was significantly superior to microfracture in patients >50 years of age (Table C.3), a positive outcome for older patients who have previously not been considered suitable for treatment.

Following approval in January 2012, MEDIPOST has entered into a sales & marketing relationship with Dong-A Pharmaceuticals and does not engage in any direct marketing itself. There are currently approximately 160 hospitals and private clinics prescribing CARTISTEM. To date treated >1,200 patients [1,148 patients as of April 2014] have been treated using CARTISTEM. MEDIPOST has made a commitment to provide MFDS with 600 patient post-marketing surveillance data, an effort that is currently ongoing.

Table C.2. Primary Efficacy Outcome Measure in ITT Population for Phase III Study (courtesy of MEDIPOST)

	Treatment Group	Control Group
Therapy Administered	CARTISTEM	microfracture
Intent-to-Treat (ITT) Population	50	53
Number of Evaluable Patients	43	46
Patients with Primary Efficacy	42/43	33/46
p-value (Treatment Effect)		0.008
Objective Improvement	60 ± 17%	50 ± 23%

Table C.3. Primary Efficacy Outcome Measure in ITT Population and Age-Group Subsets for Phase III Study (courtesy of MEDIPOST)

	ITT Population	Age Group Subsets		
	10-80 years	50-60 years	60-65 years	>65 years
Treatment Group	42/43	21/22	5/5	7/7
Control Group	33/46	13/21	4/8	5/6

SOURCES OF SUPPORT

In 2005, MEDIPOST launched its Initial Public Offering (IPO) on the KOSDAQ market. It is traded under the ticker symbol 078160.KQ, with an approximate market capitalization (as of June 04, 2014) of KRW428.26 billion (~US$428 million). The company's latest financials are detailed in the most recent (Effective Date March 31, 2014) 10-Q filing posted on May 30, 2014 (MEDIPOST 3/31/2014).

In addition to previous private and public financing, the company also generates revenues from its private cord blood banking business and has received approximately US$26+ million from 17 grants obtained from multiple South Korean government agencies.

ASSESSMENT

MEDIPOST has built an allogeneic umbilical cord blood stem cell therapy platform business with one approved product for cartilage regeneration that is commercially marketed in Korea and a pipeline of products in earlier-stages of clinical development for multiple other regenerative

medicine applications. These products leverage the core R&D competencies, manufacturing operations, and delivery infrastructure of MEDIPOST to evaluate potential ability of transient immune modulatory and trophic factor-based biological function of the allogeneic hUCB-MSC platform for stimulating endogenous regeneration of other organs/tissues.

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OVERVIEW

The vision of NanoCarrier is to contribute to the betterment of human health and to medical progress by creating new drugs utilizing nanotechnology for the improvement of patients' quality of life. NanoCarrier's goal is to be an innovative and leading biotech company, unrivalled in the cancer field.

FUNCTIONAL FOCUS

NanoCarrier's core technology, micellar nanoparticle technology, was proposed by professors Kazunori Kataoka of the University of Tokyo and Teruo Okano of Tokyo Women's Medical University (also visited on this trip). They demonstrated that when drug-encapsulating micellar nanoparticles were intravenously administered, the particles could function as stable drug carriers in the bloodstream, and that they accumulated in cancerous tissues (Figure C.9). NanoCarrier researchers hope that, if the efficacy and safety of drugs are further improved by utilizing their micellar nanoparticle technology, they will be able to contribute to the advance in medication of cancer and other diseases. (NanoCarrier 2014).

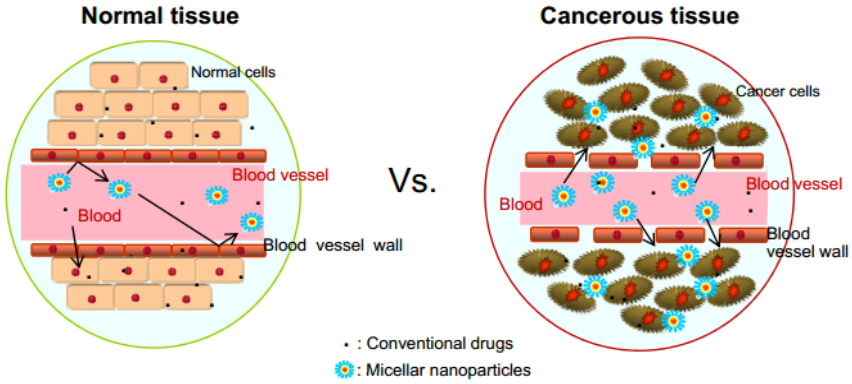

Figure C.9. Concept of treatment of cancer with micellar nanoparticles (courtesy of NanoCarrier).

RESEARCH & DEVELOPMENT ACTIVITIES

NanoCarrier seeks to use nanomedicine to add significant value to existing drugs and improve the patient's quality of life by reducing the side effects of chemotherapy. NanoCarrier trademarked systems include:

- NanoCap - improves the solubility of drugs
- Medicelle - improves a drug's retention in the bloodstream
- NanoCoat - enhances targeting ability to a specific locus
- ADCM (antibody/drug-conjugated micelle) - enhances the amount of drugs effectively targeted to a locus

The NanoCarrier concept is based on a Trojan horse strategy, with phases of delivery, penetration of the target cell, and treatment with a large amount of the drug released at once. The characteristics of the technologies include: (1) controlled release, which increases bloodstream retention and reduces side effects and (2) passive targeting via accumulation of micellar nanoparticles in cancerous tissue.

NanoCarrier's projects are the most advanced polymer-based nanomedicines now in clinical development. Those in Phase III trials include:

- NK105, a polymer nanocarrier containing Paclitaxel, which is intended to treat metastatic or recurrent breast cancer. This is being done by a partner, Nippon Kayaku, under a license from NanoCarrier.
- NC-6004, a micelle intended for treatment of pancreatic cancer. This is a co-development with Orient Europharma Co., Ltd. This approach applies micellar technology to Cisplatin, which is widely used in chemotherapy, but which has serious side effects with conventional treatment.

There are many other candidates in earlier stages of R&D, including active targeting approaches via ADCM, which is part of their longer range plan. They also seek to expand application of these oncology solutions to other medical fields, and even to cosmetic areas like hair restoration.

TRANSLATION

NanoCarrier is focused on translation via close relationships with partner manufacturing companies, including Nippon Kayaku, Calando Pharmaceuticals, BIND Therapeutics, and Cerulean Pharmaceuticals.

SOURCES OF SUPPORT

NanoCarrier Co. Ltd. is largely funded by investors seeking financial growth in biotechnology. Its 40 million shares are traded on the Tokyo Stock Exchange with a range of JPY900– JPY3000 per share over the last year. Sales in the fiscal year ending in March, 2014 were JPY472 million (much of it from licensing), but net loss that year was JPY1,113 million.

This year NanoCarrier is expanding into a new three-story research and administration building in Kashiwonoha, Chiba. They are also planning a satellite research lab addition for brain delivery drugs near Tokyo Haneda Airport in 2015.

ASSESSMENT

Judicious use of nanocarriers with effective intracellular delivery capability can enhance the efficacy of many drug gene therapies, with cancer therapy as one prominent application. NanoCarrier has innovated in developing polymer diblock-based micelles to deliver a wide range of biological cargoes. They demonstrated an understanding of the hurdles and potential they face, but are working on collecting information about control of the surface characteristics of their micelles that could expand their utility to regenerative medicine. They are subcontracting all of their manufacturing. NanoCarrier is a leader in nanomedicine commercialization.

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OVERVIEW

The National Natural Science Foundation of China (NSFC) was established on February 14, 1986, under the jurisdiction of the State Council, one of the major funding agencies for basic research in China. Its mission includes:

- Support basic research
- Identify and foster talented researchers
- Strengthen international cooperation
- Promote socioeconomic development

NSFC is administered by its council, which consists of the president, vice presidents and council members. Currently the council is headed by one president and six vice presidents. Figure C.10 shows the WTEC panel with the NFSC staff who hosted our visit.

FUNCTIONAL FOCUS

Some characteristics of the NSFC research programs include:

- General program (individual curiosity-driven researches)
- Key program (researches dealing with the exploration of key scientific issues with a certain research goal and scale)

- Major program (multidisciplinary and comprehensive researches on strategic key scientific issues)
- Major research plan (an integrated cluster of projects with unified objectives or orientations to be carried out concertedly by excellent research teams)
- International (regional) joint research program (joint academic activities between mainland Chinese scientists with researchers from other countries and regions)

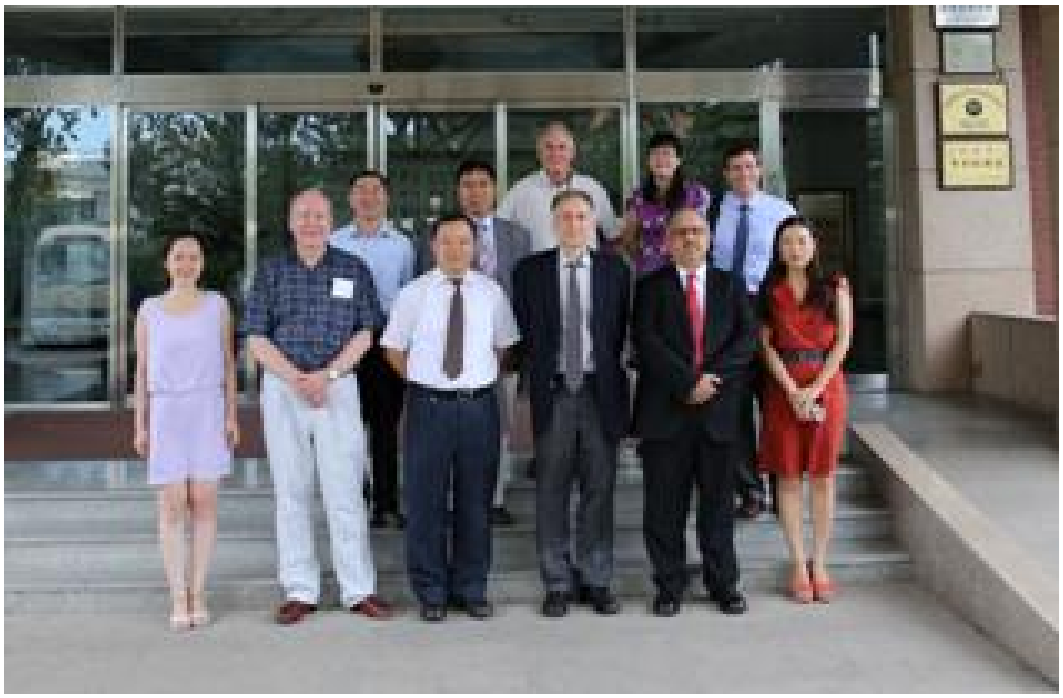

Figure C.10. WTEC panel members and NSFC hosts.

Talent Training Programs

NSFC administers a variety of talent training programs including:

- Young Scientists Fund
- Excellent Young Scientists Fund
- National Science Fund for Distinguished Young Scholars
- Fund for Creative Research Groups
- Fund for Less Developed Regions
- Joint Research Fund for Overseas Chinese Scholars and Scholars in Hong Kong and Macao
- Research Fund for International Young Scientists

Research Support

The research support programs include:

- International (Regional) Exchanges Program
- Research Program of National Major Research Instruments and Facilities
- Programs of Joint Funds

International Cooperation

The underlying principles that guide NSFC program execution are:

- Promote international cooperation in basic research in consideration of the national and international science development
- Promote substantial joint research
- Enhance research quality and strengthen talent training
- Equity and mutual benefit

RESEARCH & DEVELOPMENT ACTIVITIES

The proposal evaluation process employed by NSFC is shown in Figure C.11.

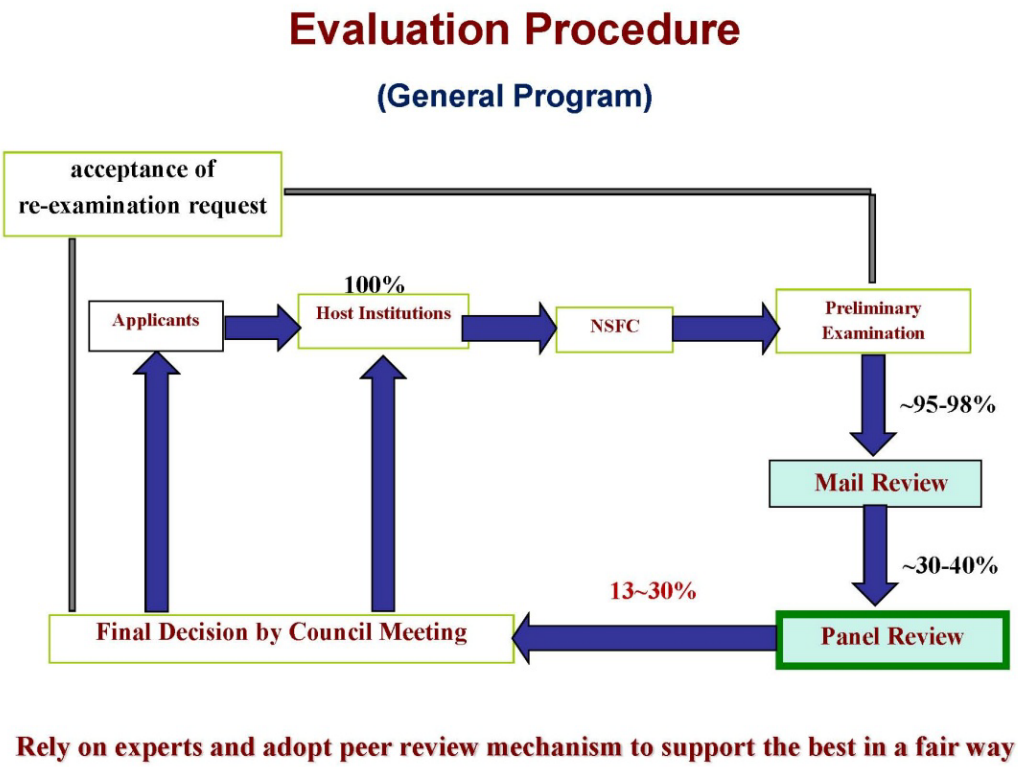

Figure C.11. NSFC proposal evaluation process (courtesy of NSFC).

The categories of international cooperation projects that NSFC funds include:

- MoU-based Programs (joint research, joint exchange, workshops, etc)
- Key International Joint Research Projects
- Research fellowship for international young scientists
- Sino-German Center for Research Promotion

The distribution of expenditure on joint research programs between NSFC and its partner organizations during 2013 by scientific discipline is shown in Figure C.12.

ASSESSMENT

NSFC evaluates more than 150,000 proposals per year (Figure C.14).

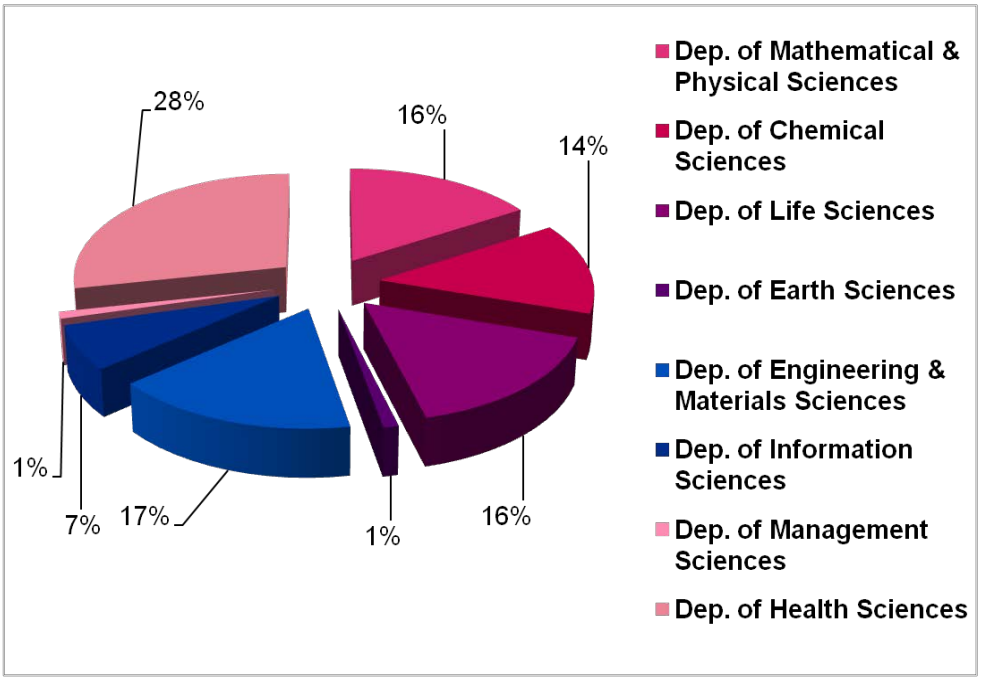

Figure C.12. Distribution of joint program funding by scientific discipline (courtesy of NSFC).

SOURCES OF SUPPORT

The total NSFC funding per year has been growing rapidly in recent years (Figure C.13).

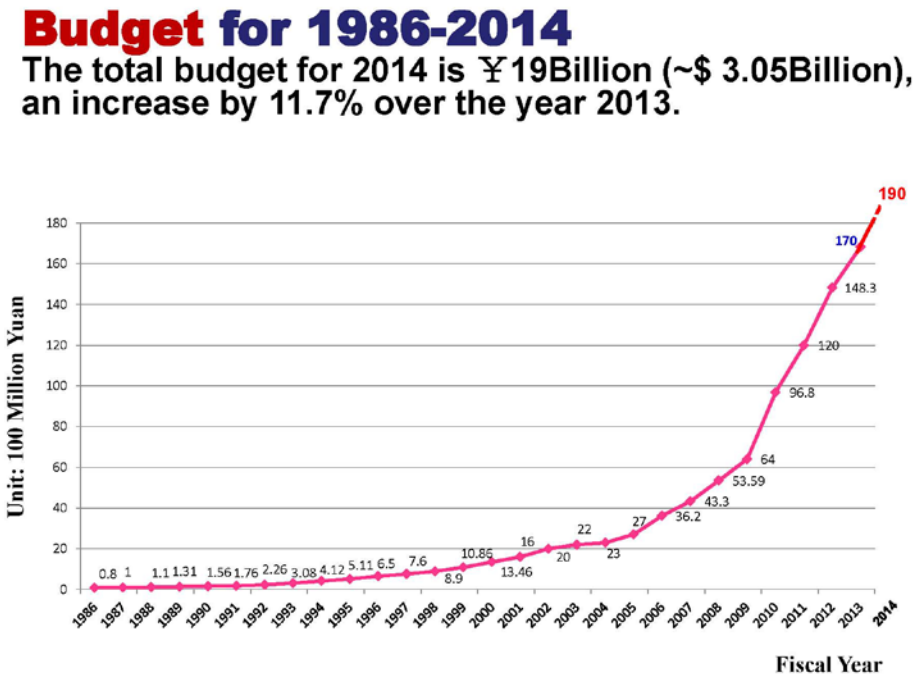

Figure C.13. Budget of the National Science Foundation of China (courtesy of NSFC).

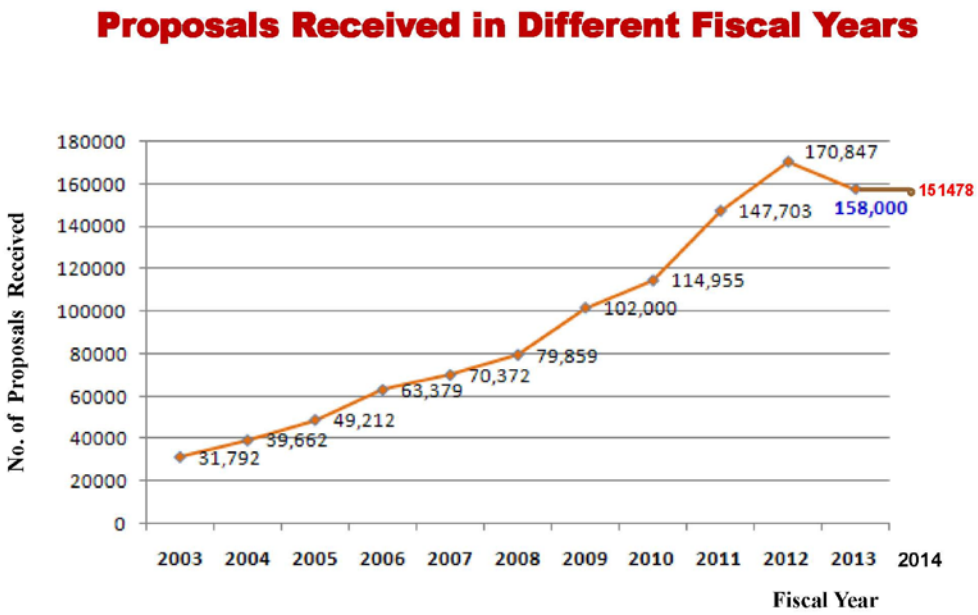

Figure C.14. Proposals received by the NSFC, 2003-2014 (courtesy of NSFC).

In addition NSFC has an extensive network of cooperation including 72 cooperative agreements or memoranda of understanding with institutions in 36 countries and regions (Figure C.15).

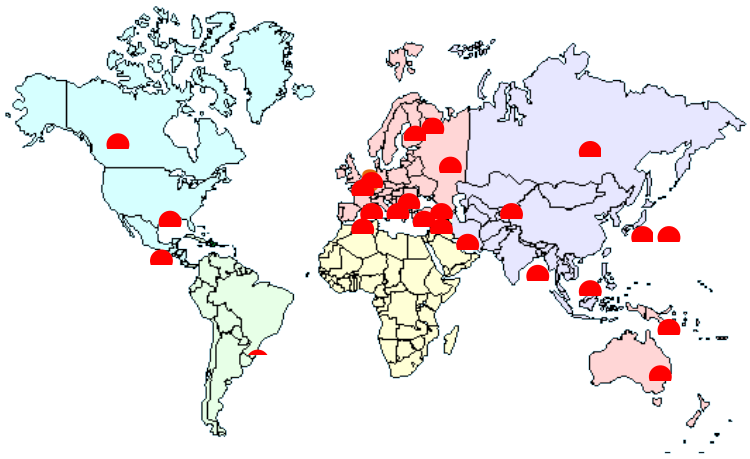

Figure C.15. Locations of organizations having cooperative agreements or memoranda of understanding with the NSFC (courtesy of NSFC).

Overall, NSFC appears to be a very efficient and effective organization for funding fundamental research in China, including international collaboration programs.

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OVERVIEW

The School of Life Sciences is located at Peking University (PKU), a world renowned international research university with expertise in the natural sciences. The school was established in 1952 by merging three departments of biology. The precursor departments include one established in 1923 at Yenching University, one established in 1925 at Peking University, and one established in 1926 at Tsinghua University. The School currently enrolls over 500 undergraduates and 400 graduate students. Undergraduates focus on coursework during the first 3 years, which is followed by practical research in their fourth year. Graduate students participate in a vibrant research program as they progress towards the Ph.D. degree in various areas of the life sciences. The School of Life Sciences is composed of approximately 60-70 independent investigators with a broad skill set in developmental biology, molecular biology, protein engineering, and genetics. Four professors are members of the Chinese Academy of Sciences.

FUNCTIONAL FOCUS

Research activities within the School of Life Sciences at PKU include a wide range of activities including biochemistry and molecular biology, plant biology, physiology, neurobiology, cell biology, developmental biology, behavior science, bioinformatics, evolutionary biology, conservation biology, ecology, and genetics. The School of Life Sciences at PKU is also home to several centers of excellence. This site hosts the State Key Laboratory of Protein and Plant Gene Research and the State Key Laboratory of Biological Membranes and Membrane Biotechnology. PKU is also home to the Key Laboratory of Cell Proliferation and Differentiation of the Ministry of Education.

RESEARCH & DEVELOPMENT ACTIVITIES

The School of Life Sciences at PKU comprises many thought leaders across a wide range of competencies including genetics and molecular biology. Many of the professors are encouraged to

initiate collaborations with other institutions within China including the Chinese Academy of Sciences (CAS) and National Institute of Biological Sciences (NIBS).

Dr. Bo Zhang is a graduate of the PKU in the Department of Biology (now called the School of Life Sciences) and has acquired a background in cell biology. Dr. Zhang leads a group of 12 Ph.D. students and an associate professor. This size is typical of most labs in the School of Life Sciences at PKU. Research labs can vary from approximately 5-25 members, most of which are Ph.D. students at PKU. Dr. Zhang's current research focuses on the use of genetics and genomic tools for modeling human disease in zebrafish. Most of these tools involve retroviral vectors for random insertional mutagenesis. Additional novel tools have also been explored including zinc finger nucleases, TALEN, and CRISPR/Cas9 system. Dr. Zhang has pursued many active international collaborations including productive interactions with scientists in the United States. These interactions include collaborations with Dr. Shuo Lin (UCLA) and Dr. Shawn M. Burgess (NIH, National Human Genome Research Institute).

Research Achievements

The laboratory of Bo Zhang has made several key achievements over the last decade. These achievements include rapid screening of random mutants (Wang et al. 2007). Dr. Zhang has also made seminal discoveries regarding the role of Kctd10 in cardiac morphogenesis (Tong et al. 2014) and TALEN assembly (Huang et al. 2011). The latter discovery is notable because Dr. Zhang provides these constructs to other laboratories throughout the world. These interactions have led to more than 10 publications.

Dr. Wensheng Wei directs a molecular biology and genetics laboratory. Dr. Wei has a background in genetics and started his independent career at PKU in 2007. The focus of Dr. Wei's lab is using the combination of technology and biology to address grand challenges in disease and infection. A key thrust is the focus on high-throughput screening methods for identifying targets for CRISPR/Cas9 (Zhou et al. 2014). Dr. Wei also has an active program in identifying receptor-ligand function in the context of *Clostridium difficile* infection. Genetic manipulation of HeLa cells can reduce the binding of Toxin B to membrane receptors and serves as a potential treatment with therapeutic potential.

TRANSLATION

The School of Life Sciences at PKU is heavily focused on basic research. As such, there are limited direct commercialization efforts. The most likely scenario is for clinical translation.

SOURCES OF SUPPORT

The School of Life Sciences at PKU draws substantial funding support from many sources. Representative sources include competitive grants that are administered through the National Natural Science Foundation of China (NSFC) and the Ministry of Science and Technology (MoST). Federal funding programs from the NSFC accounts for one-third of research funding. The remaining funding comprises direct funding from the federal government (one-third) and direct funding from the university (one-third) to support the development of junior faculty. There are some notable restrictions on the expenditures of federal grants that are administered by the NSFC and MoST. For example, up to 10% of the funds can be allocated to support students and technicians. The remaining 90% of the funds can be used for materials and supplies, travel, and small equipment. The average (small) grants are in the amount of $100k to $200k total over four years. Larger grants can be in the range of $500k to $600k total over five years. Other notable funding sources include large multinational corporations with an interest in basic research (e.g., Astra Zeneca, Roche).

ASSESSMENT

There are world class facilities installed within the School of Life Sciences at PKU to support concomitant research activities. The laboratory for each principal investigator (PI) is well equipped with equipment to support molecular biology, microscopy, and cell culture experiments. In addition to the individual research facilities, there are abundant core facilities, including a genome sequencing core, an advanced microscopy core, and extensive infrastructure to support basic research on zebrafish. The latter includes an elaborate system of pumps, tanks, and hardware to support large scale zebrafish culture.

The School of Life Sciences at PKU in Bejing, China is a highly sophisticated and well-funded research institute with world class faculty, facilities, and trainees. The product of this research has the potential to impact biomanufacturing across a wide range of high profile facets. The School of Life Sciences is highly innovative and collaborative department within PKU. PKU has the potential to forge many novel basic discoveries in biology that can be leveraged to develop many biomedical technologies that will underpin advances in biomanufacturing.

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SITE VISIT IMAGES

Figure C.16. Prof. Chris Bettinger of the WTEC panel and Prof. Bo Zhang of PKU.

Figure C.17. Example of the microscopy facilities at the School of Life Sciences, PKU.

Shanghai Jiao Tong University (SJTU)

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Date Visited: July 25, 2014

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OVERVIEW

Shanghai Jiao Tong University (SJTU 2014) is one of the most respected universities in China, and is rising in esteem on the world stage. Indeed, SJTU itself pioneered worldwide rankings of universities by objective indicators, and its index, *Academic Ranking of World Universities*, rivals that by the *Times of London* and others (ARWU 2014). While American universities dominate the top 100 places, SJTU comes in at about 120 in this "Shanghai" ranking.

The SJTU Institute of Bone and Joint was founded in 1986. It consists of three branches: the Shanghai Key Laboratory of Orthopaedic Implants, the Laboratory of Orthopaedic Cell and Molecular Biology, and the Engineering Research Center of Digital Medicine and Clinical Translation. The Shanghai Key Laboratory of Orthopaedic Implants is housed in the 9th People's Hospital, SJTU School of Medicine. The Laboratory of Orthopaedic Cell and Molecular Biology is situated in the Institute of Health Science, Shanghai Institutes for Biological Sciences, China Academy of Sciences, whereas the Engineering Research Center of Digital Medicine and Clinical Translation is supported by the Ministry of Education and is located at the Xuhui Campus, SJTU.

RESEARCH & DEVELOPMENT ACTIVITIES

Shanghai Key Laboratory of Orthopaedic Implants

This laboratory was founded by Professor Kerong Dai in 1986. It was one of the pioneer institutions focused on biomechanics of musculoskeletal systems. The laboratory is well known internationally for its research in orthopedic implants and training. The lab includes a number of multidisciplinary teams consisting of orthopedic surgeons, life science investigators, and engineers. The lab has been actively carrying out clinically oriented medical research on orthopedic translation, with considerable achievements in optimization design and application of artificial joints, stem cell-based therapy for bone repair and regeneration, development and evaluation of functional bone substitutes, the mechanism and prevention of periprosthetic osteolysis, osteoporotic fractures, fracture healing, and others (see Figure 4.3)

The lab also focuses on translating lab discoveries into clinical products by developing individualized artificial joints and bone allografts using 3D printing technologies. These implants have been approved by SFDA for commercialization and clinical application. The lab provides technique service and support for other research institutes and enterprises in Shanghai through collaborative research, training, and joint R&D.

The Shanghai Key Laboratory for Orthopaedic Implants won the second prize of Shanghai Science and Technology Progress Award, the third prize of Shanghai Medical Advancement Award. The lab has applied and received 22 national patents. The lab has published over 250 papers in international and domestic journals. In addition, the lab has received a number of awards for training talent young clinical researchers. These training programs included the Program for New Century Excellent Talents in University, New Century Hundred, Thousand, Ten Thousand Talent Project, Shanghai Cultivation Program for Academic Leaders, Shanghai Science and Technology Committee Rising-Star Program and Rising-Star Tracking Program, Shanghai Pujiang Talent Program, Shanghai Dawn Program and Dawn Tracking Program, and China Scholarship Council Program for Constructing High-level Universities, among others.

Laboratory of Orthopaedic Cell and Molecular Biology

This laboratory was jointly founded by the Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences and the Medical School of SJTU in 2004. The focus of the lab is to solve key scientific problems in clinical treatments, in particular in bone, cartilage, and tendon repair and regeneration. The laboratory is also focused on studying the pathological mechanisms of joint disease and intervention, and the development and application of gene delivery systems with new nanometer scaffold materials.

Laboratory for Tissue Stem Cells and Directional Differentiation

This laboratory is more focused on fundamental study of bone marrow mesenchymal stem cells, cartilage stem cells and tendon stem cells, and their directional differentiation potential to bone, adipose, cartilage and tendons. The lab has not only developed a platform for isolating and culturing these tissue stem cells, but made also discoveries in the effect of signal transduction system, epigenetic regulation and mechanical stimulation on lineage-specific differentiation of these tissue stem cells. Representative achievements include the discovery of (1) the roles of different transcription factors in directional differentiation of BMSCs, (2) the mechanical and cell growth environment regulation of BMSC's osteogenic differentiation, (3) isolation and differentiation of tissue stem cells from the cartilage and tendons, and (4) stem cell enrichment and its clinical application.

The Engineering Research Center for Digital Medicine and Clinical Translation

This center is supported by Chinese Ministry of Education. It is the first national center focusing on innovative translational research and development of digital medicine. It was established in 2006 and certified by the Ministry of Education in 2011. The mission of the center is to advance the critical and core technologies in digital medicine, serving as the bridge connecting scientific research with clinical application and industrial development, encouraging the top-ranking of medical industry of China through application of new engineering technologies licensed under independent intellectual property rights. The major research directions of the center include customized medical implants and biomechanics, mobile and digital medical information, surgical navigation and training, and orthopedic rehabilitation technology.

The center has established a number of international collaborative research projects with its partner institutes around the world such as the Rehabilitation Institute, University of Toronto; Medical Center at Leiden University; Research Center for Frontier Medical Engineering, Chiba University in Japan; the University of Western Australia School of Medicine; and the University of Leeds.

These collaborative research projects have been supported by both the Ministry of Science and Technology and the program for Shanghai Municipal Projects of International Cooperation. The center has successfully held the International Congress on Orthopedic Advanced Techniques and Clinical Translational Research for seven years, actively promoting the development of digital medicine technology and clinical translational research in China.

The Engineering Research Center for Digital Medicine and Clinical Transplantations currently consists of seven principal investigators, including Prof. Kerong Dai, Prof. Dongyun Gu, Prof. Chengtao Wang, Prof. Jinwu Wang, Prof. Le Xie, Prof. Lixu Gu, and Prof. Yun Luo. The Engineering Research Center for Digital Medicine and Clinical Transplantations has been awarded more than twenty prizes, including the Second Prize of National Invention, the Second and Third Prize of National Scientific and Technological Progress Award, and other science and technology progress awards. Three of the achievements have obtained the medical equipment registration certificates and been translated successfully to clinics. In the last five years, the center has made a number of scientific and technology achievements. The intelligent patient lifting and handling device (RoboNurse), led by Prof. Dongyun Gu and jointly conducted by the Toronto Rehabilitation Institute, provides a significant approach to safely moving patients and effectively reducing occupational nursing injury. In the new field of mobile digital medicine, the center has successfully developed computer-aided fracture diagnosis and a clinical treatment decision support system on PC and mobile platforms. This system has been widely applied and promoted by nearly 400 hospitals in 29 provinces and regions in China. In the field of minimally invasive technology, the team led by Prof. Chengtao Wang and Prof. Yun Luo has made a number of scientific research achievements and realized clinical translation, through industry-academy-research cooperation.

The Engineering Research Center for Digital Medicine and Clinical Transplantations has published more than 200 research papers and received 48 patents.

SOURCES OF SUPPORT

During 2006-2010, the Shanghai Key Laboratory of Orthopaedic Implants received 73 grants totaling CNY38.5 million (including four from the National 863 Program, two from the National 973 Program, and seventeen National Natural Science Foundation awards).

During the past five years, the Engineering Research Center for Digital Medicine and Clinical Transplantations has received 90 research grants with the total funds of over CNY58 million including five projects supported by the National 863 Program, two projects supported by the National Key Technology Support Program, seven subprojects supported by the National 973 Program, and 30 projects supported by the National Natural Science Foundation of China.

ASSESSMENT

Overall, the Institute is an excellent example of translational medicine. Its research infrastructure spans from fundamental stem biology to 3D bioprinting and orthopedic implant banks. The panel was particularly impressed by the individualized implants such as 3D printed joints that have been invented through collaborative research among industries, universities and hospitals. This industry or hospital-driven research model will be a good example of promoting advanced biomanufacturing, which is the focus of this study.

The panel was also impressed by its activities in training talented young clinical scientists. Due to its nature of multidisciplinary study, the Institute has actively engaged in training young generation of clinical scientists by acquiring funding from all levels from central government to local city support. One of the critical challenges that we have always encountered is to the lack of adequate mechanism to attract and train clinical scientists on industry or hospital-driven research. The training model developed by the Institute offers a good solution to this challenge.

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Soochow University and BioBay (Suzhou Industrial Park)

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OVERVIEW

Soochow University is a comprehensive research university located in Suzhou, in Jiangsu Province, a 2-3 hour drive from Shanghai. Suzhou is a dynamic city ranked as a top-4 city in China (economy) and the number 1 innovation city in China (*Forbes* 2012). The city is over 2500 years old and has historical economic significance related to the silk industry. Soochow University was founded in

1900 by Methodist American missionaries and is one of the most rapidly growing universities in China. It was recently selected as one of the 14 universities under the Plan 2011 from the Ministry of Education in China with a specific focus on nanotechnology. Soochow University is home to several notable firsts: the first Chinese university of western-style education; the first Chinese university to publish an academic journal; the first Chinese university to provide postgraduate education; and the first Chinese university to confer the first master's degree in Chemistry in 1917.

The BioBay (Suzhou Industrial Park Bio&Nano Technology Development Co.) is an industrial development project area located in Dushu Lake Science and Education Innovation District within Suzhou, China. The mission of BioBay is to serve as a resource for translating biomedical technologies and serve as a resource for regional economic development. BioBay is essentially an innovative science and technology carrier for development of the emerging biological industry and the nanotechnology industry. The resources include a project incubator, accelerator, industrialization area, administrative office, and living facilities. BioBay also provides many services including, regulatory application and filing, industry-university-institute interfacing, investment-financing interfacing, business promotion, human resources recruitment and training, business registration, firefighting and environment protection registration and filing, laboratory safety management, environment monitoring, and other professional services. BioBay is a regional center of innovation and hosts many scientific events to spur these activities.

FUNCTIONAL FOCUS

Soochow University has several colleges that participate in research activities that are closely related to biomanufacturing, including the College of Electronics and Information Engineering, the College of Chemistry, Chemical Engineering, and Materials Science, and the Institute of Functional Nano & Soft Materials. Contributions from these colleges and institutes include notable advances in polymers, soft materials, and nanomaterials for biomedical applications.

RESEARCH & DEVELOPMENT ACTIVITIES

The WTEC panel met with several members of the Soochow University faculty (Figure C.19), four of whom explained aspects of their research programs.

Professor Hong Chen

Prof. Chen is the Dean of the College of Chemistry, Chemical Engineering, and Materials Science. He conducts an active research program in the area of polymeric biomaterials and biointerface science for applications in biosensors, anti-fouling coatings, and other types of medical devices.

Professor Zhiyuan (Bill) Zhong

Prof. Zhong runs the Biomedical Polymers Laboratory at Soochow University, which has a dedicated focus towards the use of biodegradable nanoparticles for use in targeted drug delivery. The key principle of ongoing materials design strategies include working on strategies to manage mutually exclusive properties of chemical stability (to reduce burst release) and promote intracellular release. These technologies could have a great impact for cancer therapies. One example of this approach is reduction-sensitive dextran that is reversibly cross-linked with lipoic acid, a naturally occurring disulfide-bearing molecule (Wei et al. 2012). Other applications include the design and synthesis of self-assembled functionalized cross-linked hyaluronic acid (HA) nanoparticles for targeting CD44+ cancer cells, core-cross-linked pH-sensitive degradable micelles, gold nanorods for photothermal-triggered release, and stimuli-responsive polymersomes for controlled release. Prof. Zhong also maintains a leadership role within the international community of controlled release scientists as evidenced by his organization of a biannual symposium on Innovative Polymers for Controlled Delivery in Suzhou Industrial Park. Prof. Zhong's work is recognized by awards such as the Bressel Research Award and an ACS Young Investigator Award.

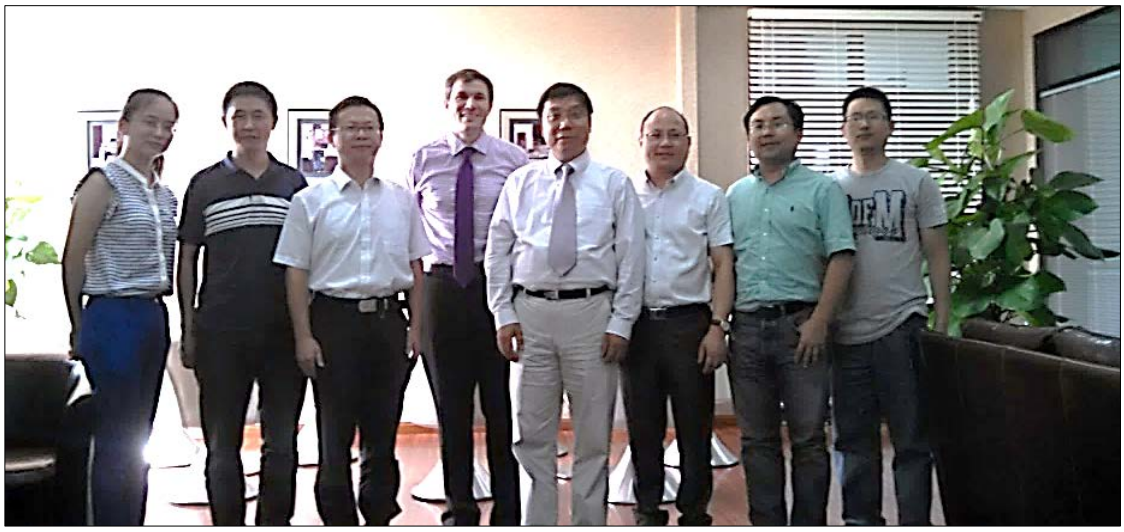

Figure C.18. WTEC panel members with representatives from Soochow University.

Professor Jian Liu

Prof. Liu is a member of the Institute of Functional Nano & Soft Materials (FUNSOM) within Soochow University. The FUNSOM conducts interdisciplinary research in a number of emerging areas. Representative projects include the use of graphene for multimodal imaging and photothermal therapy, transition metal dichalcogenides (MoS_2) for *in vivo* imaging, and conducting polymers for near-IR imaging agents for multimodal imaging and cancer combination therapy. Other projects include the synthesis and evaluation of silicon nanoparticles and silicon nanowires for tumor ablation. The functional effects of nanomaterials interactions with biological systems are also being studied. Prof. Liu manages a vibrant research program in the use of reduced graphene oxide to improve charge injection for applications in neural stimulation and cell micropatterning.

Professor Xinjian Chen

Prof. Chen is a member of the College of Electronics and Information Engineering. He conducts bioimaging research across a wide range of areas including the segmentation, registration, and visualization of many kinds of imaging data including optical coherence tomography, computer tomography (CT), and MRI. One representative example of this exciting research program is in retinal image analysis to predict the progression of macular degeneration.

TRANSLATION

The primary mechanism for translation is BioBay, a development project located within the Suzhou Industrial Park (SIP) Bio&Nano Technology Development Company. The BioBay hosts dozens of companies on a 0.8 km^2 campus within the SIP (Figure C.27). There are many centralized resources that can help companies that are housed within BioBay. For example, BioBay provides centralized, high-throughput drug screening capabilities in addition to chemical and physical analysis. These capabilities are in line with the goals of many of the companies. The focus and objectives of the companies within BioBay vary. Biomedical technologies represented include medical devices, pharmaceuticals, biomedical instrumentation, and bio-nanomaterials. The vast majority of these companies is still in the start-up phase and have between 10 and 50 employees. They have access to GMP-certified facilities that can support a variety of biomedical products ranging from cell-based therapies to implantable devices. BioBay receives substantial financial support and infrastructure advancement from the local government within Suzhou Industrial Park. BioBay is a key component of local strategic investment to maintain Suzhou as a key innovation hub within China.

Figure C.19. Jerry Xu (BioBay) highlights some key companies that are located on the campus.

SOURCES OF SUPPORT

Soochow University is highly active in terms of high impact publications, patents, and grants. The sources of research funding include a variety of both federal and local funding. A non-exhaustive list of the funds includes the following:

- National Key Basic Research Funding
- NSFC (Retinal Age-Related Macular Degeneration)
- One thousand Young Talents Project
- Jiansu Provincial Innovation Project
- Soochow University Distinguished Professor Funding

In addition to formal funding mechanisms, BioBay supports translation activities by providing a number of resources including funds, equipment, consulting teams, and incubator space for companies. These resources are packaged together in pre-negotiated agreements. BioBay also operates a modest venture fund, financed by the local government, which provides additional monies for start-up companies.

ASSESSMENT

Soochow University is a top-tier research institute in Southern China. Its stature as a key research institution within China is increasing rapidly due to strategic local investment. Soochow builds upon historical expertise in the chemical and physical sciences. The university will likely contribute to the worldwide effort in biomanufacturing by synthesizing novel polymers, inventing new nanomaterials, and devising new processes for scalable materials preparation with increased control. Soochow University has well-developed educational programs to train the next generation of students who can contribute to these potential activities that are related to biomanufacturing. Soochow University maintains close relationships with BioBay, a regional incubator for biomedical technologies. The close proximity of Soochow University and BioBay is seen as a tremendous advantage to advance biomanufacturing technologies within the region.

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OVERVIEW

Sungkyunkwan University (SKKU) School of Medicine was established in 1997 with funding from Samsung. Students are recruited from among high school graduates and undertake clinical training at Samsung Medical Center (SMC). The School of Medicine is an independent unit within SKKU, which also has schools of Engineering, Natural Science, Life Sciences and Biotechnologies, and Information and Communication Engineering. It is a cofounder of the Samsung Advanced Institute for Health Sciences & Technology (SAIHST), South Korea's multidisciplinary convergence center for education and research in health sciences and technology. According to its website, the medical

school currently has a total of 220 undergraduate students, 292 graduate students, and 535 faculty members.

FUNCTIONAL FOCUS

The School of Medicine is organized into two divisions. The Basic Medical Science Faculty includes individual departments for anatomy, physiology, molecular cell biology, and social and preventive medicine. The Research Institutions division includes the Medical Research Institute, the Samsung Biomedical Research Institute (SBRI), and the Animal Experiment Center.

The Biomedical Research Institute promotes a wide range of academic research projects including exchanges with domestic and international partners. The institute also hosts conferences, workshops, and lectures. The mission of SBRI is to support the university's research capacity and serve as a facilitator of biomedical industrialization efforts. The Animal Experiment Center was designed to meet U.S. National Institutes of Health criteria as well as those of the Institute of Laboratory Animal Resources.

RESEARCH & DEVELOPMENT ACTIVITIES

Chul-Won Ha

Dr. Ha's research interests are regenerative medicine, stem cells, and biopharmaceuticals. His lab focuses on musculoskeletal regenerative medicine using adult mesenchymal stem cells. Dr. Ha's team invented the world's first allogenic stem cell therapeutics for the treatment of articular cartilage defects in osteoarthritis (CARTISTEM) in partnership with MEDIPOST Corporation. More recently, the lab has been working with other research groups to develop methods for isolating highly potent adult stem cell populations as well as developing stem cell-based therapeutics for osteoarthritis and cartilage regeneration.

Jong Wook Chang

Dr. Chang's research interests include regenerative medicine, biopharmaceuticals, and neuroscience. His lab, the Stem Cell Therapeutics and Engineering Laboratory, is researching the use of mesenchymal stem cells (MSCs) that have been isolated and cultured from human adipose tissue and bone marrow for use in treatments for neurodegenerative diseases, based on the reported capability of MSCs to secrete soluble factors of significant therapeutic efficacy. A major goal of Dr. Chang's lab is to uncover the mechanisms of MSCs in disease models, and to apply this knowledge to develop second-generation MSC therapeutics.

Jae Wook Ko

Dr. Ko's research focus is biopharmaceuticals. His group, the Department of Clinical Pharmacology and Therapeutics, is in the process of proceeding to early phase clinical trials with a new drug candidate that was developed using *in vitro* animal experiments. The department is also researching a variety of personalized medicine applications for pharmacogenomics, including optimal drug therapy consults and modeling/simulation. The department participates in a wide range of interdisciplinary collaborations, and is gradually assuming a leadership role in coordinating them. The focus of the department's Clinical Trial Center (CTC) is to translate bench research into clinical applications and to identify unmet clinical practice needs.

Yeup Yoon

Dr. Yoon's research focuses on molecular and cellular biology, molecular oncology, and antibody engineering. His Molecular Oncology Laboratory is focusing on the development of biomarkers for cancer diagnosis and treatment, particularly in the form of novel antibodies; functional and clinical validation; and the identification of underlying molecular and cellular mechanisms.

TRANSLATION

The Stem Cell Therapeutics and Engineering Laboratory (Jong Wook Chang) intends to continue applying the principles of good manufacturing practice (GMP) to expand the production and cultivation of MSCs.

SOURCES OF SUPPORT

SKKU School of Medicine receives funding from Samsung and may also receive funding through the University's budget.

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Sun Yat-Sen University

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Other Attendees from SYSU Faculty:	**Chang-Qing Yi, Qian-Ying Gao, Xintao Shuai, Qingtang Zhu, Ye-Lin Huang**, and **Qi Zhang**

OVERVIEW

Sun Yat-Sen University (SYSU) is among the top 10 universities in China and the premier comprehensive research university in Southern China. The School of Medicine was founded in 1866 and SYSU was founded in 1924. There is a focus on polymer chemistry and polymer engineering at SYSU in the Natural Sciences and Engineering Departments including polymer chemistry, polymer physics, inorganic chemistry, and condensed matter physics. There are also strengths in the medical sciences including ophthalmology, oncology, neurology, pharmacology, toxicology, and general surgery. The total research budget for SYSU is approximately CNY1.3 billion.

FUNCTIONAL FOCUS

There are several important focus areas of basic research being performed in SYSU. The Advanced Polymer Materials for Biomedical Application at SYSU includes faculty members from the Schools of Chemistry and Medicine as well as clinical hospitals within SYSU. These research areas

are categorized into three discrete efforts: (1) synthesis of polymers for use in biomedical applications; (2) nanomaterials for theragnostics; and (3) materials for tissue engineering and regenerative medicine. This research center originated from efforts in polymer research and education during the 1960s.

The Center for Stem Cell Biology and Tissue Engineering is a research unit that was founded in 2003 and is led by Prof. Bruce Lahn and Prof. Andy Peng Xiang. This center is located across three different sites: (1) a molecular and cellular biology laboratory within the north campus of SYSU; (2) a site for non-human primates located 80km from SYSU; and (3) a facility designed for manufacturing of stem cells for clinical studies which is located at the Third Affiliated Hospital of Sun Yat-Sen University.Other active research interests include studying self-renewal and multi-differentiation of stem cells, and stem-cell-based therapy.

RESEARCH & DEVELOPMENT ACTIVITIES

The WTEC panel met with several members of the SYSU faculty, six of whom explained aspects of their research programs (Figure C.21).

Figure C.20. Members of the SYSU delegation, including Professors Xi Ping Zhu and Qi Zhang.

Professor Xintao Shuai

Professor Shuai leads a research group in polymeric biomaterials in the School of Chemistry and Chemical Engineering at SYSU. He led a research group at Case Western University as recently as 2005. His group currently focuses on functional polymeric materials for controlled release and imaging (Figure C.22), including pH-sensitive materials, dual-sensitive materials for intracellular release, and ultrasound-sensitive carriers.

Professor Xiaolin Liu

Professor Liu is a member of First Affiliated Hospital with SYSU and is interested in neural tissue engineering. Specific projects include designing tissue engineered nerve graft, repairing radial nerve defects using acellular allografts, repairing human nerve defects, manufacturing of PLGA nerve conduits, and 3D printing of artificial neural scaffolds.

Professor Changqing Yi

Professor Yi has an emerging research program in the development of food safety and disease related research including active programs in assessing nanomaterial toxicity, chemical biology, and *in vitro* biosensors.

Figure C.21. Prof. Shuai describes recent SYSU research in the area of polymeric nanomaterials.

Professors Xiaolin Liu and Qingtang Zhu

The research group led by Professor Liu (presented by Prof. Zhu) is focused on neural tissue engineering and regeneration of peripheral nerves (Zhang, Qui, and Liu 2009, Zeng et al. 2011). Their laboratory focuses on translational research efforts through corporate partnerships with Guangzhou ZhongDa Medical Equipment Company. Clinical trials are organized through the Sun Yat-Sen University Medical Center.

Professor Qianying Gao

Professor Gao is an ophthalmologist who is interested in designing vitreous substitutes. He has developed a silicone-based foldable polymer device to be inserted into the vitreous. This device is currently in Phase II clinical trials. This device may also be used as a fixation device for retinal prosthesis or as a matrix for controlled release technology. These devices are being evaluated in Zhongshan Ophthalmic Center, which is a State Key Lab of Ophthalmology and the number 1 ophthalmology center in China.

Professor Andy Peng Xiang

Professor Xiang directs the stem cell biology and tissue engineering center, which was established in 2003 and accredited as a Key Laboratory in 2008. Efforts in stem cell and regenerative medicine include the following specific areas: gene occludome project, tissue regeneration, iPS and human disease models, development of interspecies chimera, and gene editing of non-human primates.

TRANSLATION

There are notable efforts and resources dedicated to commercialization within SYSU. The GMP-approved Stem Cell Facility is the most prominent of these features. It is located in the 3rd Affiliated Hospital of Sun Yat-Sen University, which works closely with a stem cell bench research facility located in the SYSU Medical School. Another key aspect of translation includes the extensive non-human primate facility that is located within driving distance of the north campus of SYSU. The non-human primate facility utilizes disease models to study gene editing via TALEN and CRISPR/Cas9 systems. Both of these impressive facilities position SYSU to be a world leader in biomanufacturing. Other translational activities include close collaboration with large corporations. One prominent example is neural tissue engineering through a partnership with Guangzhou ZhongDa Medical Equipment Company. ZhongDa Corporation has active collaborations for biologically-derived nerve grafts.

SOURCES OF SUPPORT

There are many possible sources of funding and support from federal and regional government agencies. A partial list of these organizations follows:

- National Natural Science Foundation of China
- Guangdong Natural Science Foundation
- Guangzhou Science and Information Technology Bureau
- Sun Yat-Sen University (Start-up Funds)
- Funding Scheme for Key Laboratory
- Funding from the Guangdong Province

Other funding sources include the National High Technology Research and Development Program of China, the National Basic Research Program of China, the 985 Program of SYSU, and 973 grants that are consortium grants administered by MoST.

ASSESSMENT

Sun Yat-Sen University in Guangzhou, China is a top-tier research institute in Southern China. SYSU is well funded, with world class faculty, facilities, and trainees. SYSU is building on historical expertise in polymeric materials and a leading medical school to forge innovations in biomaterials for controlled release, tissue engineering, and other biomedical applications. SYSU uses funding from many organizations to support these multidisciplinary research activities. The focus on clinical translation, a strong hospital, productive collaborations with local corporations, and GMP facilities for cell-based therapies are noted strengths. These advantages leave SYSU well-positioned to contribute to the worldwide effort on increasing biomanufacturing capabilities.

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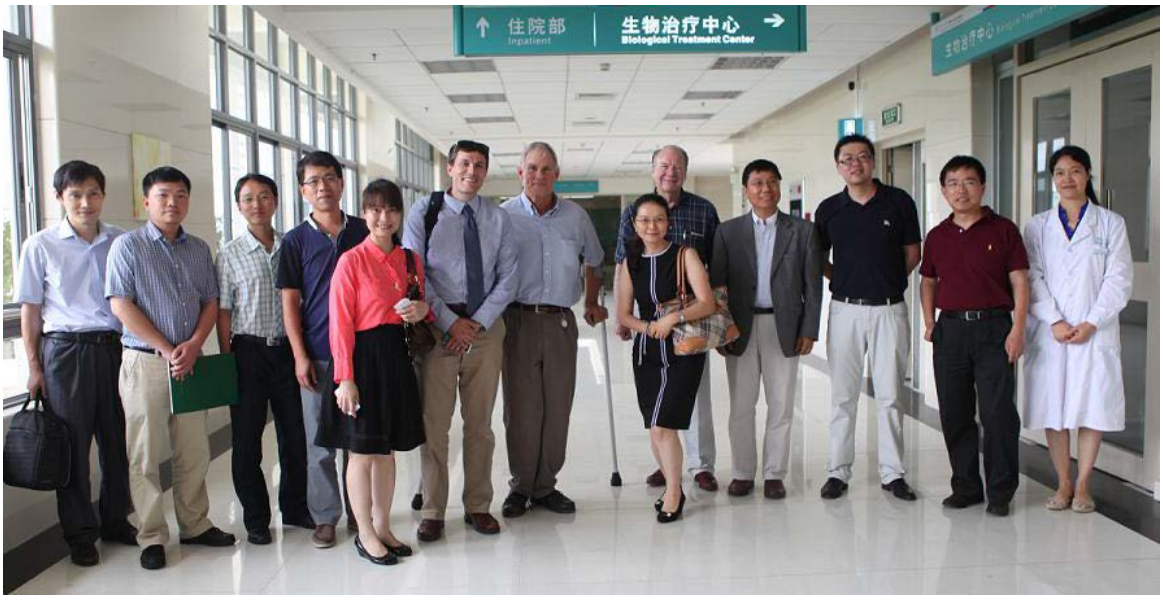

Figure C.22. WTEC panel members with the SYSU delegation (courtesy of Qi Zhang, SYSU).

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OVERVIEW

Tracing its origins to the formation of biotechnology research laboratory in April of 1967and being subsequently spun-out from being the biotechnology division of Takara Shuzo Company, Takara Bio was officially established in April 2002 and became a publicly listed company in 2004. Today, Takara Bio is a conglomerate with issued capital of JPY15 million (September 2013); that is 60% owned by Takara Holdings; and organized into three separate business units:

- Bio-Industry Business Unit: Largest of the three business units, with 50% of revenues coming from sales outside Japan; sells research reagents (including enzymes, cloning systems, vectors, etc.); scientific instruments (including PCR and mass spectroscopy Instruments); and performs

contract research services (including NextGen sequence analysis, gene expression analysis, epigenetic analysis, and custom manufacturing of iPS cell lines)

- AgriBio Business Unit: Manufactures and markets Health Foods, Agar Drinks, and Mushroom products
- Cell & Gene Therapy Business Unit: Focused on Cell and Gene Therapy Product development, manufacturing, and commercialization

FUNCTIONAL FOCUS

In its Cell & Gene Therapy Business Unit, Takara Bio has continued to build core competency through both academic collaborations and acquisitions of technologies from other commercial entities. Key academic collaborations include:

- Development of recombinant human fibronectin (CH-296 fragment) with Indiana University
- Gene Therapy program in clinic for treatment of HIV/AIDS with University of Pennsylvania
- Adeno-associated Virus (AAV) technology platform with Oregon Health Sciences University
- Induced Pluripotent Stem (iPS) Cells with University of Kyoto

Key commercial technology acquisitions include:

- Clontech business from BD BioSciences (United States)
- HF10 Oncolytic Virus Gene Therapy from M's Science (South Korea)

These experiences have shaped and provided for establishment of core competencies in (1) cell-related technologies and competency in cell handling, cell culture, cell characterization, and biological production; (2) competency in design and operation of cGMP Facilities for viral vector production and cell manufacturing; and (3) technology and capability related to analytical characterization and genomic analysis. The company leverages these core competencies in providing CDMO services to its partners and in design, development and clinical translation of its proprietary gene-modified cell therapy products.

RESEARCH & DEVELOPMENT ACTIVITIES

Having built a business on platform of tools, reagents and CDMO business, Takara Bio acquired worldwide rights to RetroNectin® through a collaborative development effort with Professor David Williams at Indiana University, and has subsequently embarked on development of proprietary gene therapy therapeutic product programs with key milestones as follows:

Herpes simplex virus-based thymidine kinase (HSV-TK) gene therapy for treatment of Leukemia in October 2005; currently in joint Phase I clinical trials in Japan and South Korea for use as concurrent donor lymphocyte therapy in haplo-identical transplantation.

HSV-mutant oncolytic virus program following acquisition of HF10 gene therapy for solid cancers from M's Science (South Korea) in October 2010; currently in clinical research studies at Mie University and Nagoya University.

United States Investigational New Drug (IND) filing for MazF gene therapy for treatment of HIV/AIDS in March 2012; MazF encodes for *Escherichia coli* RNAse enzyme that cleaves single stranded RNA (such as messenger RNA) to destroy translational machinery. This product is currently in Phase I/II Clinical Trials at University of Pennsylvania and Drexel University for Tat promoter controlled viral vector based gene delivery into CD4 T-cells; the hypothesized mechanism being that when HIV infects CD4 T-cells; expression of Tat protein from HIV will activate MazF enzymatic activity within the infected cells whereby it will cleave all single stranded RNA in infected cells thus preventing replication of the HIV virus.

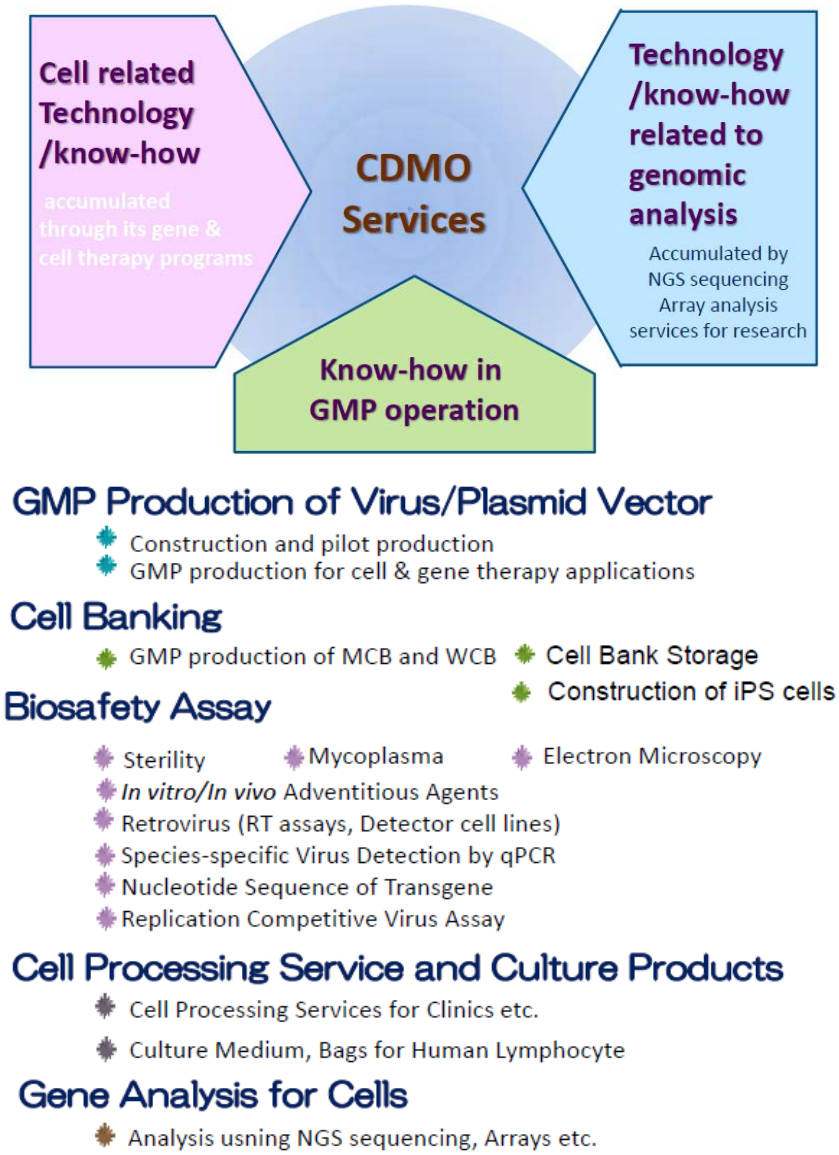

Figure C.23. Core competencies and key services of CDMO business in cell and gene therapy products (courtesy of Takara Bio, Inc.).

IND filing for T-cell receptor (TCR) gene therapy for solid cancers in Japan in March 2014. The program is currently targeting an HLA-A4 restricted peptide epitope from MAGE antigen, with other targets being evaluated for clinical studies in Japan from NY-ESO-1 antigen and WT-1 antigen. In addition, Takara Bio has developed its own proprietary vector system which in addition to delivery of TCR also includes *in situ* production of siRNA directed against endogenous TCR in the transduced cells suppressing endogenous TCR expression and re-directing T-cells specifically to the specified tumor target.

Initiation of Phase II U.S. clinical trials for HF10 gene therapy for treatment of solid cancers in April 2014.

Initiation of *in vitro* studies using retrovirus transduced chimeric antigen receptor (CAR) T-cells targeting CD19 antigen (CAR molecule with CD3-zeta and CD28 intracellular signaling domain obtained under CDA from Memorial Sloan Kettering Cancer Center, New York) in collaboration with Prof. Keiya Ozawa (Professor & Chairman, Department of Hematology, Tokyo

University). Future development studies will (1) expand to other B-cell antigens (example: CD20) and (2) combine expression of CD19 or CD20 CAR with other suicide genes (example: thymidine kinase, or iCaspase).

TRANSLATION

Takara Bio has worldwide exclusive rights to RetroNectin® (CH-296 fragment of recombinant human fibronectin) which has been used in over 60 human clinical trial protocols. RetroNectin® is manufactured in microbial (*E. coli*) fermentation and has been marketed as cGMP grade reagent for *ex vivo* use in human gene therapy trials since February 2013. It has been licensed for clinical and commercial use by four commercial entities, including GlaxoSmithKline (UK) and MolMed (Italy).

Takara Bio's proprietary gene-modified cell therapy products are currently in various stages of clinical research and clinical trials as depicted in Figure C.25.

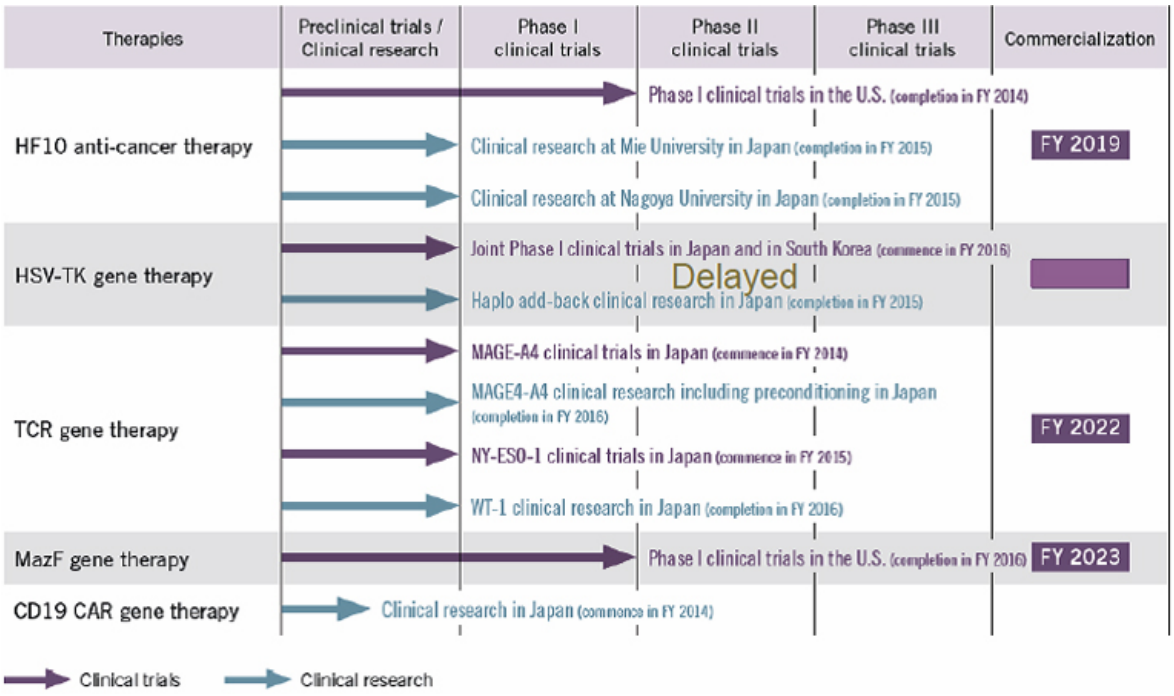

Figure C.24. Development stage and projected milestones for Takara's gene-modified cell therapies (courtesy of Takara Bio, Inc.)

SOURCES OF SUPPORT

Takara Bio became a public listed company on the MOTHERS Board (Tokyo, Japan) in December 2004. Today, Takara Bio is a conglomerate with issued capital of JPY15 million (September 2013), and is 60% owned by Takara Holdings. Takara Bio reported Net Sales of JPY24,000 million (~US$212 million) in FY2014; with Net Operating Income of JPY1,954 million (~US$17 million) with total R&D Expense of JPY3,026 million (~US$27 million).

ASSESSMENT

Through different stages of its evolution, Takara Bio has emerged from being a biotechnology reagents and instrument provider, to a CDMO organization, to an enabler of gene therapy products, to a therapeutic developer, and manufacturer of viral vectors and gene-modified cell therapy products. The ability to successfully build a tools business, use revenues to make investment to

move up the value chain to a CDMO business, and subsequently repeat the same to further move up the value chain in becoming a developer of gene-modified cell therapy products demonstrates success in execution and evolution.

Takara Bio is the leading commercial gene-modified cell therapy and gene therapy company in Asia with multiple programs in clinical development and core competency and facility infrastructure to drive translational development of gene-modified cell therapy products. The WTEC panel benefitted from observing the new cGMP manufacturing facility, thereby being able to visualize "what it takes" to build a fully-integrated ecosystem and infrastructure to promote development and manufacture of all critical materials, viral vectors, and analytical methodologies in development of gene-modified cell therapy products. The WTEC panel also learned of innovative manufacturing-driven opportunities for successful business execution in building a long-term stable, foundation for development novel gene-modified cell therapy products.

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Tsinghua University

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OVERVIEW

Tsinghua University was established in 1911, originally under the name "Tsinghua Xuetang." The school was renamed "Tsinghua School" in 1912. The university section was founded in 1925. The name "National Tsinghua University" was adopted in 1928. After the founding of the People's Republic of China, the University was molded into a polytechnic institute focusing on engineering in the nationwide restructuring of universities and colleges undertaken in 1952. Since China opened up to the world in 1978, Tsinghua University has developed into a comprehensive research university. At present, the university has 14 schools and 56 departments with faculties in science, engineering, humanities, law, medicine, history, philosophy, economics, management, education, and art. The Department of Mechanical Engineering (DME), founded in 1932, is one of the earliest departments in an engineering field in Tsinghua University as well as one of the most historic engineering departments in China. The Bio-manufacturing Center, located within the DME, is an interdisciplinary program in which living cells, biologics, proteins, and biomaterials are used as basic building blocks for fabrication of *in vitro* biological structures and cellular systems with application to biology, tissue engineering, disease pathogeneses study, drug test and discovery, and cell/tissue/organ-on-a-chip devices.

FUNCTIONAL FOCUS

The Biomanufacturing Research Center in the Department of Mechanical Engineering at Tsinghua University, directed by Professor Wei Sun, strives to create a leading research center for conducting cutting-edge research education and innovation in the emerging interdisciplinary field of biomanufacturing, exploring a new paradigm of modern engineering and manufacturing for innovative application in bioengineering and health sciences, biology and biomedicine, and promoting the development of the field of advanced biomanufacturing.

RESEARCH & DEVELOPMENT ACTIVITIES

The Biomanufacturing Research Center is conducting research activities in the following areas:

- Bioprinting of heterogeneous tissue constructs and *in vitro* cellular function study
- 3D printing for cell microenvironment reconstruction with application to regulation of stem cell and IPSC cell function
- Encoded biological model: design, 3D printing and *in vitro* reconstruction of biological function
- 3D printing of *in vitro* tumor model and tumorigenesis characterization
- Precisely controlled cell assembly for construction of three-dimensional *in vitro* biological model
- Study on mechanism of three-dimensional structural formation of tissues/organs with biomaterials
- Design and manufacture of personalized tissue scaffolds and implants
- Printing *in vitro* cell models for 3D biology, pathology and pharmacology study
- Integration of bio-, micro- and nano-fabrication technology
- Novel 3D cell printing process and equipment development
- 3D cell printing for cell/tissue/micro-organ-on-a-chip and advanced medical diagnostic devices

TRANSLATION

The projects presented at the meeting were primarily basic research. Commercialization of the work was not part of the discussion.

SOURCES OF SUPPORT

Neuroimaging studies being conducted at Tsinghua University include biomanufacturing of *in vitro* biomimetic tissue (Zhao et al. 2014), constructing a vascular tree (Yue et al. 2006), engineering of myocardial tissue (Zhang et al. 2012), generation of hESC-derived liver-like tissues (Yao et al. 2014), and construction and transplantation of a tissue engineered corneal graft (Xiao et al. 2014). Funding sources for these studies included the National Science Foundation of China, the Ministry of Science and Technology, the Youth Fund of the National Natural Science Foundation of China, and a First-class National Postdoc grant.

ASSESSMENT

The Biomanufacturing Research Center in the Department of Mechanical Engineering at Tsinghua University, is conducting a variety of projects related to tissue engineering, basic stem cell applications and 3D printing for cell microenvironment reconstruction. The university is known for its high standard for academic excellence and this is exhibited by the quality of faculty in the Biomanufacturing Research Center and their advanced level of research at all levels of biomanufacturing. Their goal of creating a leading research center for conducting cutting-edge research, education and innovation in the emerging interdisciplinary field of bio-manufacturing is being realized.

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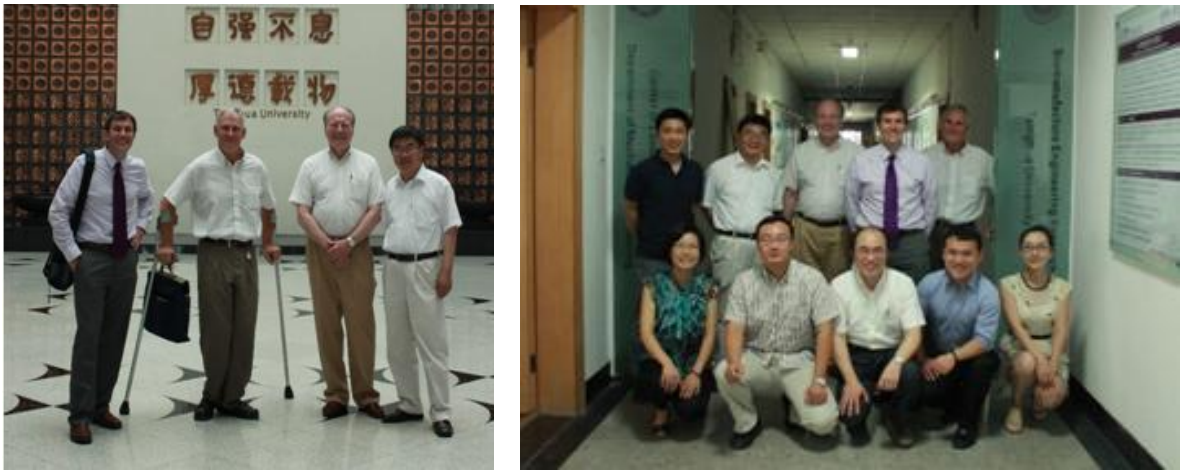

Figure C.25. WTEC panel members with researchers at Tsinghua University.

APPENDIX D. GLOSSARY OF ABBREVIATIONS AND ACRONYMS

2D	two-dimensional
3D	three-dimensional
3DP	three-dimensional printing
AAV	adeno-associated virus
ACS	American Chemical Society
ADA-SCID	adenosine deaminase-severe combined immunodeficiency disease
ADCM	antibody/drug-conjugated micelle
ALS	amyotrophic lateral sclerosis
ATMP	advanced therapy medical products
ATP	adenosine triphosphate
ATS	Advanced Tissue Sciences (La Jolla, CA)
AVIF	alternating viscous and inertial force
BCRT	Berlin-Brandenburg Centre for Regenerative Therapies
BiOE	Organic Bioelectronics (Sweden)
BMI	brain–machine interfaces
BRI	Biomedical Research Institute (KIST, Korea)
BSEL	Biological Systems Engineering Lab-oratory (Imperial College London, UK)
CAS	Chinese Academy of Sciences
Cas	CRISPR-associated [proteins]
CB	cord blood
CBE	Centre for Biological Engineering (Loughborough University, UK)
CBET	Chemical, Bioengineering, Environmental, and Transport [Systems] division of U.S. NSF
CDMO	contract development and manufac-turing organization (also called CMO)
CGD	chronic granulomatous disease
cGMP	current good manufacturing practice
CiRA	Center for Induced Pluripotent Stem Cell Research & Application (Kyoto University, Japan)
CMIV	Center for Image Science and Visualization
CMIV	Center for Medical Image Science and Visualization (LiU, Sweden)
CMO	Contract Manufacturing Organization
CNY	Chinese Yuan (currency, also known as renminbi)
COGS	cost of goods sold
CPC	cell processing center
CQAs	Critical Quality Attributes
CRISPRs	clustered regularly interspaced short palindromic repeats
CRO	contract research organization
CT	computed tomography
CTA	clinical trial authorization (Europe)
CTC	Cell Therapy Catapult, Ltd. (UK)
D-BSSE	Department of Biosystems Science and Engineering (ETH, Basel, Switzerland)
DGMIF	Daegu-Gyeongbuk Medical Innovation Foundation (Korea)
DHAP	dihydroxyacetone phosphate
DME	Department of Mechanical Engineering (Tsinghua University, China)
EFPIA	European Federation of Pharmaceutical Industries and Associations
EMA	European Medicines Agency

EPFL	Ecole Polytechnique Fédéral de Lausanne (Switzerland)
EPSRC	Engineering and Physical Sciences Research Council (UK)
ERC	European Research Council
ETTC	Edinburgh [Scotland] Technology Transfer Center (UK)
EU	European Union
FACS	fluorescence-activated cell sorting
FCT	Fundacao Ciencia e Technologia (Portugal's funding agency for science and technology)
FiT	Facility of iPS Cell Therapy (Japan)
FP7	Seventh Framework Programme of the European Consortium
FTE	full-time equivalent [employee]
FUNSOM	[Institute for] Functional Nano & Soft Materials (Soochow University, China)
GCP	good clinical practice
GDP	good distribution practice
GIBH	Guanzhou Institutes of Biomedicine and Health [Chinese Academy of Sciences]
GLD	globoid cell leukodystrophy (Krabbe's disease)
GLP	good laboratory practice
GMP	good manufacturing processes
GOSH	Great Ormond Street Hospital [Children's] Charity (UK funding agency)
gRNAs	CRISPR guide RNAs
GSCRAC	Global Stem Cell & Regenerative Medicine Acceleration Center (Korea)
HDR	homology-directed repair
HEPA	high-efficiency particulate arrestance [filtration]
hESC	human embryonic stem cell
HLA	human leukocyte antigen
HPLC	high-performance liquid chromatography
HSCs	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant
HSV	herpes simplex virus
HTA	Human Tissue Authority (UK)
HTS	high-throughput screening
hUCB-MSC	human umbilical cord blood-derived mesenchymal stem cell (cell drug products)
HVJ	hemagglutinating virus of Japan
HZG	Helmholtz-Zentrum Geesthact (Germany)
IBB	Institute of Bioengineering and Biosciences (Portugal)
IBET	Instituto de Biologia Experimental e Technologia (Technologic Institute of Experimental Biology, Portugal)
IBET/ITQB	Technologic Institute of Experimental Biology/Institute of Chemical and Biological Technology (Portugal)
IBI	Institute of Bioengineering (EPFL Lausanne, Switzerland)
IBME	Institute for Biomedical Engineering (University College London)
ICL	Imperial College London
ICT	immuno-cell therapy
IGB	[Fraunhofer]-Institut für Grenzflächen- und Bioverfahrenstechnik (Institute for Interfacial Engineering and Biotechnology, Germany)
IGEN	Integrative Regenerative Medicine Center (LiU, Sweden)
IHC	immunohistochemistry
IIPA	[Fraunhofer]-Institut für Produktionstechnik und Automatisierung (Institute for Manufacturing Engineering and Automation)

IKDC	International Knee Documentation Committee [Score]
IL2RG	interleukin 2 receptor gamma [mutations]
IND	Investigational New Drug [filing with U.S. FDA]
INFARMED	Autoridade Nacional do Medicamento e Produtos de Saúde I.P. (Portugal)
iPSC	[human] induced pluripotent stem cell
IST	Instituto Superior Technico (Portugal)
ITQB	Instituto de Technologia Quimica e Biologia (Institute of Chemical and Biological Technology, Portugal)
ITT	intent-to-treat [population of patients]
IZI	[Fraunhofer]-Institut für Zelltherapie und Immunologie (Institute for Cell Therapy and Immunology, Germany)
JACC	J-TEC Autologous Cultured Cartilage
JACE	J-TEC Autologous Cultured Epidermis
JPY	Japanese Yen (currency)
J-TEC	Japan Tissue Engineering Co., Ltd.
KIST	Korea Institute of Science and Technology (Korea)
KPIs	Key Performance Indicators
LIST	Linkoping Initiative in Life Science Technologies (LiU, Sweden)
LiU	Linkoping University (Sweden)
LSBI	Laboratory for Soft Bioelectronics Interfaces (EPFL Lausanne, Switzerland)
LVV	lentiviral vector
MEA	methyl acrylate
MEMS	microelectromechanical
MHRA	Medicine and Healthcare Products Regulatory Agency (UK)
MIT	Massachusetts Institute of Technology
MLD	metachromatic leukodystrophy
MMI	MEDINET Medical Institute (Japan)
MOHW	Ministry of Health and Welfare (Korea)
MoST	Ministry of Science and Technology (China)
MPS I	mucopolysaccharidosis I (Hurler, Hurler-Scheie, Scheie syndrome)
MRC	Medical Research Council (UK)
MRI	magnetic resonance imaging
MSC	mesenchymal stem cells
MSIP	Ministry of Science, ICT, and Future Planning (Korean funding agency)
NCCR	National Center for Competence in Research (Switzerland)
NGR-hTNF	aminopeptidase N ligand-human tumor necrosis factor (therapy for non-small cell lung cancer)
NHEJ	non-homologous end joining [DNA repair pathway]
NHS	National Health Service (UK)
NIBS	National Institute of Biological Sciences (China)
NIH	National Institutes of Health (United States)
NK	natural killer [cells]
NMR	nuclear magnetic resonance
NRF	National Research Foundation (Korea)
NSF	National Science Foundation (United States)
NSFC	[Natural] National Science Foundation of China
OBOE	[Center for] Organic and Bio-electronics (LiU, Sweden)
OCD	Osteochondritis Dissecans

ODA	Orphan Drug Act (U.S.)
PAP-GM-CSF	prostatic acid phosphatase fused with granulocyte-macrophage colony-stimulating factor (sipuleucel-T) (a blood mononuclear cell activator)
PCR	polymerase chain reaction
PECF	Protein Expression Core Facility (EPFL Lausanne, Switzerland)
PEDOT	poly(3,4-ethylenedioxythiophene)
PEGDA	polyethylene diacrylate
PEI	polyethyleneimine
PET	positron emission tomography
PIPAAm	poly(N-isopropylacrylamide)
PK	pharmacokinetics
PKU	Peking University
PLA	polylactic acid
PLX	Placental eXpanded [cells therapy]
PMD	Pharmaceutical and Medical Device [Act](Japan)
PMDA	Pharmaceutical and Medical Devices Agency (Japan)
PNI	peripheral nerve interfaces
PNIPAAm	poly(N-isopropylacrylamide)
PoC	Proof-of-Concept [grants] (European Research Council)
PPy	polypyrrole
PRINT	Particle Replication in Non-wetting Templates
PSS	poly(styrene sulfonate)
PVBC	poly(vinylbenzylchloride)
QC	quality control
QM	quality management
R&D	research and development
RAFT	Real Architecture for 3D Tissue (growth platform from TAP Biosystems Ltd.)
SBRI	Samsung Biomedical Research Institute (Korea)
SCID-Xl	X-linked severe combined immunodeficiency disease
SCRM	Scottish Centre for Regenerative Medicine (UK)
SEURAT	Safety Evaluation Ultimately Replacing Animal Testing (Europe)
SJTU	Shanghai Jiao Tong University
SKKU	Sungkyunkwan University (Korea)
SMC	Samsung Medical Center (Sungkyunkwan University, Korea)
SMEs	small and medium sized enterprises
SNBTS	Scottish National Blood Transfusion Service (UK)
SIP	Suzhou Industrial Park (Shanghai, China)
SYSU	Sun Yat-sen University
Tal	transcription activator-like [effector nucleases]
TALENs	transcription activator-like [effector nucleases]
TAP	The Automation Partnership (now TAP Biosystems Ltd.)
TCR	T-cell receptor
TEMP	tissue-engineered medicinal product
TGA	Therapeutic Goods Administration (Australia)
TGF	tissue growth factor
TIGET	[San Raffaele] Telethon Institute for Gene Therapy (Milan, Italy)
TK	thymidine kinase [cell therapy]
TPP	Therapeutic Product Profile
TRL	Technology Readiness Level
TRP	Translational Research Program
TSB	Technology Strategy Board (UK)
TWMU	Tokyo Women's Medical University
UCL	University College London

UCLA	University of California at Los Angeles
UK	United Kingdom
VAS	visual analogue scale [evaluation]
VHP	vaporized hydrogen peroxide (type of sterilization)
VINNOVA	Sweden's Innovation Agency
WAS	Wiskott-Aldrich syndrome
WOMAC	Western Ontario and McMaster Universities Arthritis Index [scale]
WTEC	World Technology Evaluation Center, Inc.
ZFNs	zinc-finger nucleases

APPENDIX E. QUESTIONS FOR HOST RESEARCH ORGANIZATIONS

The following list of questions developed for the panel's tour of leading research organizations in Europe and Asia is intended to help you understand the study objectives and to guide conversations during the site visits. The number of questions reflects our interest in learning about your work and exemplary accomplishments. The panel does not expect detailed answers to each of these questions. Please feel free to examine the list and determine which questions would be most appropriate for you and your organization. Our aim is for the discussion on issues related to these questions to lead to a productive exchange of views that will benefit research programs worldwide.

1. **Grand Challenges of/for Biological Engineering & Manufacturing**
   a. Please identify what you feel are the grand challenges in the field of Biological Engineering & Manufacturing.
   b. What engineering or technological advances must be made to achieve these grand challenges, and what are the roadblocks to achieving these advances?

2. **Development Issues Related to Biological Engineering & Manufacturing**
   a. Please state the major objectives of your laboratory or organization's research and development efforts with respect to Biological Engineering & Manufacturing. What therapeutic indications or specific target populations can benefit from your research?
   b. What are three key accomplishments of your laboratory in the past ten years? How have these results related to Biological Engineering & Manufacturing from (a) a research & development perspective, (b) a manufacturing perspective and (c) translational applications perspective? If possible, please provide copies of written reports, manuscripts, patents or patent applications, and other references that provide additional details and non-confidential information.
   c. What percentage of your work is being directly applied to translational or manufacturing applications vs. basic research and engineering development efforts? What clinical partners or user groups do you work with to test your technology? What performance and quality of life measures are you using to assess safety/toxicity and biological activity (potency) of the systems? More specifically, how many INDs/CTAs have you been involved in? What class of therapies, using what type of cells, for treating which indications? What regulatory agencies reviewed the application? What were key learnings from these interactions that impacted clinical manufacturing plans? Did you have to access develop, in-license, or evaluate new technology solutions to support these efforts from a manufacturing perspective or from a analytical/characterization perspective?
   d. How do you balance basic research and technology translation? Which is easier to pursue in today's funding environment?
   e. How important is interdisciplinary research and/or technology transfer to the success of your organization, in terms of available funding mechanisms, generating high impact publications, establishing new training programs or departments, receiving recognition/awards, attracting research talent, clinical translation or product commercialization? What stifles innovation and/or technology translation to commercialization?
   f. Given your accomplishments, what would you have done differently, if you were start all over again?
   g. Does any of your research have involvement in any way with the military or veterans?

3. **Technology Transfer, Commercialization, and Regulatory Issues**

   a. Are there any commercial products resulting from your research?

   b. Have you filed patent applications or in other ways commercialized/monetized your efforts in Biological Engineering & Manufacturing areas? In which countries? Have any patents been licensed? Are the patents critical for commercialization? Have any provided economic or commercial benefit to your organization or to the community?

   c. What human subjects and regulatory approvals are required for your research or commercial development activities?

   d. Are you in collaborating, partnering, co-development or licensing relationships with other Organizations? Please provide examples of such efforts or interest in future explorations.

4. **Funding – Government and Commercial Sponsorship**

   a. What are your current funding mechanisms for Biological Engineering & Manufacturing?

   b. To what extent do these mechanisms involve government, private, and commercial sources?

   c. Are funding mechanisms typically single-investigator, multi-investigator, or multi-institutional? In your opinion, what do you think works best in your country?

5. **International Collaborations and Comparisons**

   a. What do you see as the strengths of Biological Engineering & Manufacturing programs relative to those in the U.S., and vice versa?

   b. If relevant, identify research and development areas worth exploring as future collaborations with U.S. science and technology programs.

6. **Training and Education**

   a. What types of training programs in Biological Engineering & Manufacturing exist at your institution or in your local/regional community?

   b. To what extent do these training programs involve industry, government, the private sector or the local community?

   c. Are there new or emerging programs sponsored by the government or private sector to advance Biological Engineering & Manufacturing in your community? Are there opportunities for the NSF to partner with these programs?

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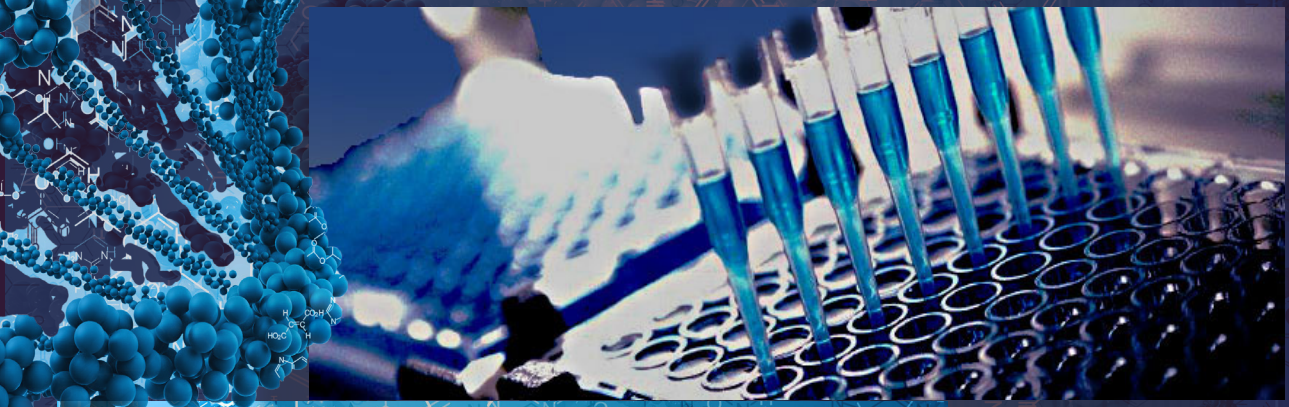

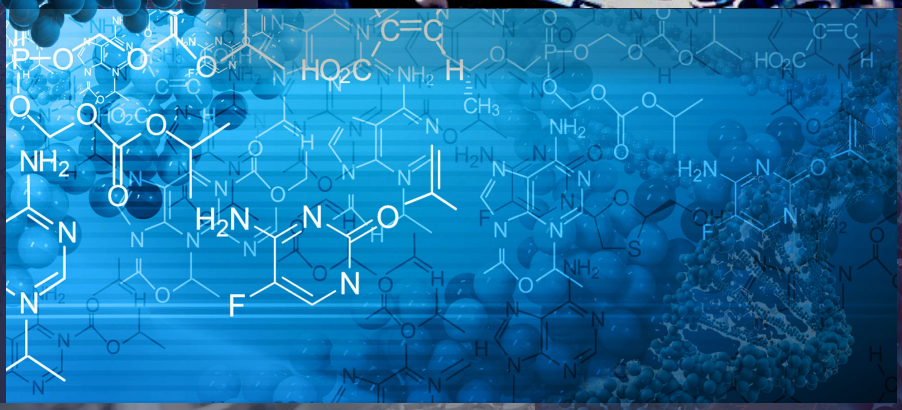

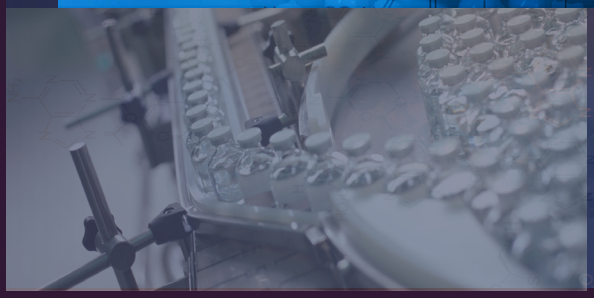

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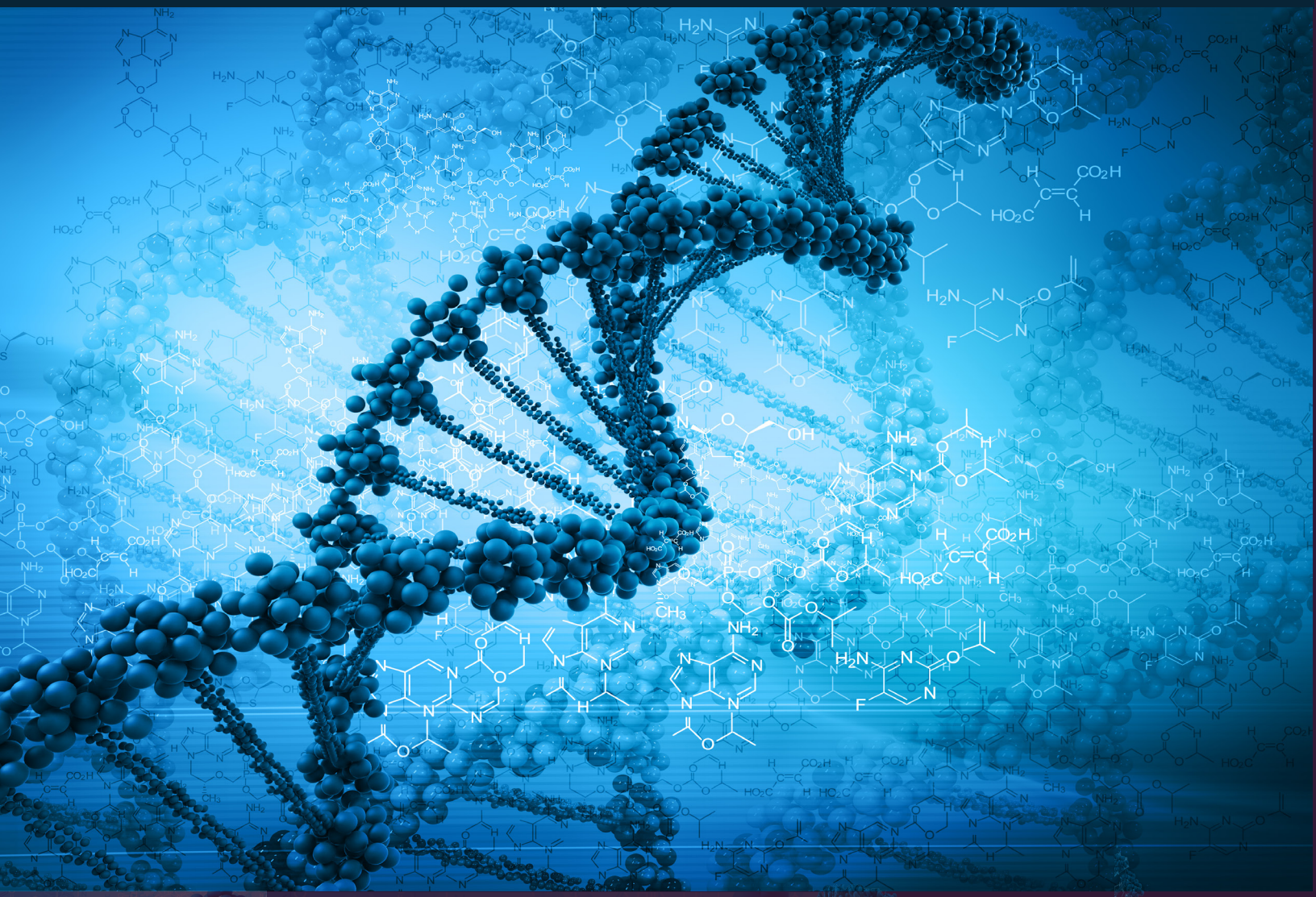

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