TISSUE ENGINEERING: Part A
Volume 20, Numbers 19 and 20, 2014
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.tea.2013.0468

A Global Assessment of Stem Cell Engineering

Jeanne F. Loring, PhD,[1] Todd C. McDevitt, PhD,[2] Sean P. Palecek, PhD,[3] David V. Schaffer, PhD,[4] Peter W. Zandstra, PhD,[5] and Robert M. Nerem, PhD[6]

Over the last 2 years a global assessment of stem cell engineering (SCE) was conducted with the sponsorship of the National Science Foundation, the National Cancer Institute at the National Institutes of Health, and the National Institute of Standards and Technology. The purpose was to gather information on the worldwide status and trends in SCE, that is, the involvement of engineers and engineering approaches in the stem cell field, both in basic research and in the translation of research into clinical applications and commercial products. The study was facilitated and managed by the World Technology Evaluation Center. The process involved site visits in both Asia and Europe, and it also included several different workshops. From this assessment, the panel concluded that there needs to be an increased role for engineers and the engineering approach. This will provide a foundation for the generation of new markets and future economic growth. To do this will require an increased investment in engineering, applied research, and commercialization as it relates to stem cell research and technology. It also will require programs that support interdisciplinary teams, new innovative mechanisms for academic–industry partnerships, and unique translational models. In addition, the global community would benefit from forming strategic partnerships between countries that can leverage existing and emerging strengths in different institutions. To implement such partnerships will require multinational grant programs with appropriate review mechanisms.

THE STUDY REPORTED HERE provides a summary of a global assessment of stem cell engineering (SCE) that was performed in 2011–2012. This yearlong study was conducted by the six authors of this review at the request of scientific officers from the National Science Foundation (NSF), the National Institutes of Health (NIH), and the National Institutes for Standards and Technology (NIST) and managed by the World Technology Evaluation Center (WTEC).

Over the last 15 years, our knowledge of stem cell (SC) biology has increased, seemingly at an exponential rate. The result is that there is an ever-increasing array of stem cells, which includes pluripotent stem cells both embryo derived and induced, and various types of variably defined and validated adult tissue-derived stem cells. A few years ago reprogramming to pluripotency was heralded as ''a significant breakthrough,'' and last year the key scientists whose reprogramming work resulted in this technology shared the award for the Nobel Prize in Physiology and Medicine. The advent of reprogramming has provided insight into cell lineage boundaries, and cell fate conversion has emerged as an important activity in the research community. Also, engineers have become increasingly involved in stem cell biology and translation, participating in new fundamental discoveries and leading efforts into applications in biotechnology and medicine.

A key goal of regenerative medicine (RM) and bioengineering is the quantitative and robust control over the fate and behavior of individual cells and their populations, both *in vitro* and *in vivo*. Central to this endeavor are SCs, which can be functionally defined as undifferentiated cells of a multicellular organism that balance the capacity for sustained self-renewal with the potential to differentiate into specialized cell types. SCs promise a renewable source of human tissue for research, pharmaceutical testing, and cell-based therapies. Fulfilling this promise will require not only the precise control of SC self-renewal and differentiation, but also imposing this control on the formation of more functionally complex tissue-like structures.

Engineering approaches to understanding and controlling SC fate,[1] that is, SCE, will be required at multiple stages during the development and implementation of RM-based

[1]Director, Center for Regenerative Medicine, the Scripps Research Institute, LaJolla, California.
[2]Department of Biomedical Engineering, Georgia Tech and Emory University, Atlanta, Georgia.
[3]Department of Chemical and Biological Engineering, University of Wisconsin, Madison, Wisconsin.
[4]Department of Chemical Engineering, University of California, Berkeley, Berkeley, California.
[5]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada.
[6]Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia.

therapies. For example, optimizing SC growth is fundamental for efforts to generate the quantities of cells (pluripotent cells and their derivatives for drug screening or somatic cells such as mesenchymal stem cells [MSCs] for therapy) that are projected to be required. Similarly, the rigorous control of differentiation, including the functional stabilization of stem cells and their progeny in formulations that can be used to discover RM drugs or treat disease, requires fundamental technological developments. Finally, the translation of these essential technologies into products that can be commercialized will require cost effective and robust cell generation and delivery strategies.

Concurrent with the advances in stem cell biology, two new ''streams of thinking'' have emerged. One of these is that of interdisciplinary research. The 2009 National Academies report[2] entitled ''A New Biology for the 21st Century'' makes the point that achieving the deeper understanding of biology necessary to address the major problems that society is facing will require not just breaking down the ''silos'' within biology itself, but incorporating chemists, computational researchers, engineers, mathematicians, and physicists into basic biological research. Further, only through such an integration of disciplines will it be possible to address major societal problems. There is no area of biology where this might be more true than that of stem cells.

A second ''stream of thinking'' that has emerged is that of translational research. Not only are Federal agencies in the United States interested in fostering the translation of benchtop science into a variety of commercial/clinical applications, but this also has become a priority for many states. Furthermore, activities in the United States simply ''mirrors'' what is taking place in the rest of the world. Thus, a global assessment of SCE such as that reported here is timely and warranted.

What is SCE? As defined for the purposes of this study, it is not just tissue engineering (TE) and RM, but rather the application of engineering fundamentals to stem cell biology and to the translation of the science into applications. The term stem cell bioengineering was first coined in 2001,[1] and it encompasses basic stem cell research, models and tools, enabling and scalable technologies, stem cell biomanufacturing, and the development of stem cell-based applications and products. It is in this context that this global assessment was conducted and that is reported here.

A preliminary workshop on Stem Cell Research for RM and TE was held at NSF on February 1–2, 2007. It was sponsored by NSF and also by the NIH, and it was facilitated by WTEC. The workshop speakers presented an overview of the research activities in North America. The workshop confirmed the increasing convergence of these research areas in the drive toward clinical solutions that will address the deterioration of various human tissues and organs impacted by injury or disease. The workshop revealed that, although substantial research has been accomplished, there was much to be done to meet expectations for improvement in human health and for commercial success. It was also clear that there was much to be learned abroad as other nations have been making rapid progress.

In May 2010, the NSF and others funded the Second International Conference on Stem Cell Engineering in Boston, Massachusetts. The conference emphasized how research in stem cell biology and engineering can combine to aid in the development of stem cell therapeutics and bioprocesses. The goal of the conference was to accelerate progress toward innovative solutions to basic and translational problems in RM. Topics emphasized how quantitative approaches could yield an increased understanding of the biological mechanisms that underlie stem cell fate choices, cancer stem cells, induced pluripotent stem (iPS) cells, technologies to study stem cell function, and the development of bioprocesses to culture stem cells for commercial applications. This conference not only provided background for this study, but was followed by the Third International Conference on Stem Cell Engineering held April 29–May 2, 2012 in Seattle, Washington.

Scope of the Study

The purpose of this study was to gather information on the worldwide status and trends in SCE, that is, the interface of engineering with the world of stem cells. The study panelists gathered hands-on information on SCE activities abroad that will be used by the U.S. Government to inform the direction of current and future programs. The intent of this study was to critically analyze and compare the research in the United States with that occurring in Asia and in Europe, to identify opportunities for collaboration, and to suggest ways to refine the thrust of U.S. research programs. To realize the intended benefits, this study focused on a range of issues in which the R&D occurring worldwide will best inform both our own government programs and our research community of the challenges, barriers, and opportunities in SCE. The study panel developed and refined the scope of the study, with the guidance of the sponsors. The scientific areas of focus for this study thus included the following:

- Understanding and controlling the signals that regulate cellular responses
- Formulating biomaterial scaffolds and the tissue matrix environment
- High-throughput screening and microfluidics
- Real-time, nondestructive phenotyping
- Systems-based quantitative analysis
- Computational modeling approaches
- Scalable expansion and differentiation
- Biomanufacturing and bioprocessing
- Targeted delivery of stem cells

Beyond the technical issues, the report on which this review is based also addresses the following broader issues:

- Mechanisms for enhancing international and interdisciplinary cooperation in the field
- Opportunities for shortening the lead time for deployment of new SCE technologies emerging from the laboratory
- Long range research, educational, and infrastructure issues that need to be addressed to promote better progress in the field
- Current government R&D funding levels overseas compared to the United States, to the extent data are available

Study Process

The study was conducted by the authors of this review, managed by the WTEC (http://wtec.org), and sponsored by the NSF, the National Cancer Institute at NIH, and NIST.

There were many components to the process used in this global assessment of SCE. These start with the knowledge provided by each of the panelists, knowledge not only about activities in the United States, but also the awareness each of them had about activities in other parts of the world. To this foundation the following four components were added:

- Site visits in Asia and Europe
- Workshops in Atlanta and in Seoul, Korea
- Participation in the third International Conference on Stem Cell Engineering
- Virtual site visits

The process began with a ''kick-off'' meeting at the NSF in Arlington, Virginia on June 22, 2011. This was followed by a series of conference calls that led to the Asia site visits, which occurred November 13–19, 2011. The Asia site visits were carried out with the panel divided into two teams, one that conducted site visits in China and the other site visits in Japan. The countries and the institutions visited are listed in Table 1.

The next event in the study was a workshop held at Georgia Institute of Technology, December 15–16, 2011 on the topic of ''Stem Cell Biomanufacturing.'' This workshop was financially supported by Georgia Tech and the Emory University Woodruff Health Sciences Center, as well as WTEC and the British Consulate in Atlanta. There were ~40 participants from academia and industry who were not only from the United States, but also Ireland, Japan, Korea, and the United Kingdom.

On January 17, 2012 a workshop was held in Seoul, Korea on SCE. This workshop did not involve the panel, with the exception of the chair of the WTEC panel who was co-organizer of this meeting. The workshop was held at the Korean Institute of Science and Technology (KIST) and hosted by Professor Soo Hyun Kim. More than 100 participants attended, and this meeting provided the opportunity to assess SCE activities in Korea.

The European site visits took place February 26–March 3, 2012. The panel was again divided into two teams, and the countries and the specific institutions visited are listed in Table 2.

The next component to this study was the participation of the panel in the third International Conference on Stem Cell Engineering held in Seattle, Washington, April 29–May 2, 2012. At this conference the organizers provided the opportunity for a town hall meeting with discussion taking place not only among the panelists, who were seated up front and were each asked to make brief opening comments, but also with members of the audience.

Three weeks later, a workshop was held at the NSF in Arlington, Virginia. At this 1-day event on May 24, 2012, the WTEC panel had the opportunity to deliver a series of oral presentations reporting on their assessment of activities globally. There were ~60 people in attendance; however, the audience was in fact much larger as the workshop was webcast, with 69 sites and an estimated 200 people around the world watching the presentations (www.SCEC.gatech.edu/global-assessment).

In addition to the above components, there were other mechanisms referred to by the panel as virtual site visits. This included site visits where information was gathered solely through the internet and/or by e-mail exchange. The term virtual site visit was also used for a site visit where only one panel member visited. The institutions/organizations that were assessed through virtual site visits are listed in Table 3.

There were some countries with active SCE activities that were not visited because the WTEC panelists believed that, through a variety of interactions, they already had a reasonable idea of what was going on in that particular country. One example is Ireland where, because of the close relationship between Georgia Tech and several universities in Ireland, considerable knowledge of stem cell activities already existed. Furthermore, Dr. Frank Barry from the National University of Ireland, Galway participated in the Atlanta workshop in December 2011. Another example is the United Kingdom. Here again there is a close relationship between Georgia Tech and Imperial College London. Furthermore, Dr. McDevitt has visited both Cambridge University and Loughborough University, and both Loughborough University and University College London were represented at the Atlanta workshop. A third and final example is Israel. The WTEC panel chair was supposed to visit this country at the end of March 2012; however, for

Table 1. Sites Visited in Asia

China	Academy of Military Medical Sciences, Tissue Engineering Research Center
China	Chinese University of Hong Kong (CUHK)
China	Fudan University, Zhongsan Hospital
China	Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences
China	Institute of Biophysics, Chinese Academy of Sciences
China	Institute of Zoology, Chinese Academy of Sciences
China	National Natural Science Foundation of China (NSFC)
China	National Tissue Engineering Center, Shanghai Jiao Tong University School of Medicine
China	Peking University, The College of Life Sciences
China	Shanghai Jiao Tong University, School of Medicine
China	State Key Laboratory of Bioreactor Engineering
China	Tongji University School of Medicine
China	Tsinghua University, School of Medicine
Japan	Keio University, Yagami Campus
Japan	Kyoto University–CiRA (Center for iPS Cell Research and Application)
Japan	Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
Japan	Osaka Univ. at TWMU (Kiro)
Japan	RIKEN Institute, Kobe
Japan	Tokyo Women's Medical University (Kano)
Japan	University of Tokyo, Hongo Campus, Department of Biomedical Engineering
Japan	University of Tokyo, Hongo Campus, Laboratory of Cell Growth and Differentiation
Japan	University of Tokyo, Komaba Campus, Research Center for Advanced Science and Technology
Japan	University of Tokyo, Komaba II Campus, Institute of Industrial Science
Japan	University of Tokyo, Shirokanedai Campus

iPS, induced pluripotent stem.

Table 2. Sites Visited in Europe

France	Institute for Stem Cell Therapy and Exploration of Monogenic Diseases (I-STEM)
Germany	Berlin-Brandenburg Center for Regenerative Therapies
Germany	Fraunhofer Institute for Immunology and Cell Therapy
Germany	Institute for Medical Informatics and Biometry (IMB), Dresden University of Technology (TUD)
Germany	Life&Brain Center, Bonn
Germany	Lonza Cologne GmbH
Germany	Max Planck Institute for Molecular Biomedicine
Netherlands	Netherlands Initiative for Regenerative Medicine
Netherlands	Leiden University Medical Center
Sweden	Karolinska Institute and Karolinska University Hospitals
Sweden	Lund University Biomedical Centre (BMC)
Sweden	University of Uppsala
Switzerland	Basel Stem Cell Network (BSCN), University Hospital Basel and University of Basel
Switzerland	Laboratory of Stem Cell Bioengineering (LSCB), Ecole Polytechnique Fédérale de Lausanne (EPFL)
Switzerland	Swiss Center for Regenerative Medicine (SCRM), University Hospital Zurich, and University of Zurich

personal reasons it was necessary for him to cancel the trip. Still, because of the active participation of Israeli scientists and engineers in North American stem cell meetings, the WTEC panel believed that they had a reasonable idea of activities in Israel.

Finally, it must be noted that Canada has a particular concentration of SCE activity. A critical component in the establishment and growth of the Canadian SCE effort has been the availability of funding targeted specificity at bringing stem cell biologists and bioengineers together on both basic and translational research teams. Perhaps the best example of this funding strategy is the Canadian Stem Cell Network (SCN) (www.stemcellnetwork.ca), a federally funded National Center of Excellence (NCE) that has, over the last 13 years, invested over $42 million (not including partner cash and in-kind contributions) in interdisciplinary projects. These projects have in a number of cases been led by bioengineers, and the work has benefited from this intimate interdisciplinary collaboration. The outcomes of the SCN are significantly greater than one would expect given the financial investment [962 peer-reviewed articles, of which 21% appeared in high impact journals (impact factor >10), 399 patent applications, 60 issued patents, and 43 licenses granted]. SCN-supported intellectual property has catalyzed the growth or launch of 11 start-up biotechnology companies, and, critically, the SCN has also brought together teams around these basic discoveries and translational technologies to initiate nine phase I or II stem cell-based clinical trials. Globally across the SCN $\sim$20% of these activities have involved at least one engineer and one biologist/clinician. The Canadian government has continued to foster this interdisciplinary (and now multi-sectorial) activity with the recent funding of the Centre for the Commercialization of Regenerative Medicine (www.ccrm.ca) that will be discussed later in this chapter.

Table 3. Individual and "Virtual" Site Visit Reports

Australia	Stem Cells Australia
Iran	Royan Institute for Stem Cell Biology and Technology (RI-SCBT)
Korea	Workshop on Stem Cell Engineering
Korea	MEDIPOST, Co., Ltd.
Korea	Pharmicell Co., Ltd.
Portugal	Stem Cell Bioengineering Laboratory, Instituto Superior Técnico (IST)
Portugal	Instituto de Engenharia Biomédica (INEB)
Singapore	Bioprocessing Technology Institute
Singapore	National University of Singapore (NUS)

It should be noted that the countries selected for visits and the specific institutions that were visited were chosen based on the knowledge of the panel members. For each institution visited, there is a site visit report that is contained in the full report. Furthermore, although the WTEC panel was able to see much of the stem cell activities going on around the world, they certainly did not see everything. If funding had permitted, the WTEC team easily could have spent 2–3 weeks in both Asia and Europe, could have visited Australia, perhaps even India, and could have site visited activities in the Middle East. Even so, one can make the argument that the process outlined above in terms of the various components, as well as the knowledge base that each panelist had coming into this study, provided for what we believe is a fairly accurate global assessment of the current state of the field of SCE. It is from this that the principal findings to be discussed next were derived.

Results

In this section, the principal findings will be summarized. This section has been organized into four parts, representing the four major chapters of the full report. There then are separate sections with some additional comments on translational models, education, and opportunities for collaboration, a brief summary of the status in the countries where an assessment of SCE was conducted, a brief section on government policy, and conclusions.

Engineering and Physical Sciences Principles in Stem Cell Research

During development and throughout adulthood, stem cells reside within specialized regions of tissue referred to as stem cell niches.[3–5] These niches regulate stem cell behavior by presenting them with rich signaling information, in the form of soluble molecules; extracellular matrix (ECM) proteins, glycosaminoglycans, and proteoglycans; growth factors and morphogens that may be soluble or immobilized to the ECM; and cues presented from the surface of neighboring cells.

It is well recognized by stem cell biologists and engineers that soluble components of the cellular microenvironment

play important roles in regulating stem cell function and fate. Accordingly, many approaches that have been developed for controlling stem cells involve serial or combinational application of a small number of factors, in many cases inspired by knowledge from developmental biology, to guide cell fate decisions. However, it is becoming increasingly recognized that biophysical in addition to biochemical cues provide key regulatory information.

In general, engineers are educated to conduct both analysis and synthesis. Through analysis, one can identify key components of highly complex systems and understand how these collectively interact to enable function. For example, one can investigate how collections of biochemical and biophysical cues are integrated by a cell to regulate downstream responses (e.g., quiescence, proliferation, self-renewal, differentiation, migration, or apoptosis), an area where engineers are making strong contributions.[6–10]

One theme that has emerged in particular is the development of novel systems that allow an investigator to pursue analysis by synthesis, that is, the creation of new technologies and experimental systems that better enable basic investigations. One example is the development of bioactive hydrogels whose biochemical and mechanical properties can be tuned. Aided by such a system, in landmark work Engler and Discher demonstrated that the lineage outcome of differentiating MSCs is strongly influenced by substrate stiffness, such that cells developed into neuron-like cells on soft polyacrylamide gels, myoblasts on intermediate stiffnesses, and osteocytes on harder substrates.[11] Subsequent work has found that neural stem cells,[12] muscle stem cells,[13] and mouse and human embryonic stem cells (hESCs)[14,15] are also mechanosensitive. In addition to the static stiffness of a material, other mechanical inputs such as cyclic strain[16] and fluid shear[17] have been found to be important for stem cell self-renewal or differentiation.

Other biophysical properties of tissues may regulate cell function, and building upon lithography technologies developed for silicon materials processing in the electronics industry, methods have been developed to pattern bioactive surfaces with interesting properties. McBeath *et al.* used microcontact printing to pattern adhesive islands of different sizes onto a surface.[18] When MSCs were seeded onto these substrates, it was found that large 10,000 μm^2 islands permitted cell spreading and promoted osteogenic differentiation, whereas small 1024 μm^2 islands that did not enable substantial cell spreading promoted adipogenic differentiation. Recent work has indicated that stem cell sensitivity to both mechanical and shape cues may be regulated by common transcriptional effectors, such as YAP and TAZ.[19]

In addition to microenvironmental properties that alter cell shape on the micron scale, topographical cues—such as the organization of the ECM into fibers—offers a cell with features that can modulate its shape at the nanometer scale. Such topographical cues are considered to provide features intermediate between a 2D and a 3D microenvironment, and they can be synthetically generated by several techniques, including electrospinning, self-assembly of materials, and lithography-based methods. For example, one study explored the effects of electrospun fibers of polyethersulfone with different dimensions on the behavior of adult NSCs, and they found that fibers of small dimension (283 nm) promoted oligodendrocyte specification, whereas larger fibers (749 nm) increased neuronal differentiation.[20] In addition, it has been shown that MSCs are sensitive to topographical cues on the 100–1000 nm scale, likely through the direct involvement of focal adhesion proteins.[21]

Increasing numbers of studies have identified additional engineering and physical principles that regulate stem cell behavior, including, for example, electric fields. In early work, Radisic *et al.* subjected neonatal cardiomyocytes to a square wave electrical field to emulate the natural electrophysiological environment of the heart. Cells became aligned with the direction of the field, exhibited a substantial increase in contractile amplitude, and expressed higher levels of various cardiac protein markers compared with nonstimulated cells.[22] Subsequent work has further explored the effects of electric fields on other cell and stem cell behaviors. In addition, mass transport limitations can both pose challenges and provide opportunities for engineering stem cell behavior. For example, it is well established that spatial gradients of soluble cues, formed with the aid of diffusion, help pattern the formation of complex tissues.[23] Also, atmospheric oxygen levels are often considerably higher than levels in organs and tissues due to solubility and transport limitations, and tuning oxygen levels therefore provides an opportunity to better control cell function.[24]

Analytical engineering approaches can thus contribute to the understanding of both the biochemical and biophysical features of complex cellular microenvironments. Furthermore, this basic information can be applied toward the synthesis of engineered niches for stem cells that provide the necessary input signals to guide the desired output cell behaviors. The resulting engineered culture systems and biomaterials can then be harnessed for stem cell expansion, differentiation, and implantation in numerous clinical applications.

High Throughput Screening, Microfluidics, Biosensors, and Real-Time Phenotyping

As has been pointed out, there are a number of challenges in engineering the stem cell microenvironment. These include identifying the factors that regulate stem cell fate and understanding the combined effects of different cues, constructing culture systems that apply the desired cues, and ultimately developing processes that allow one to direct the fate of stem cells by precise spatial and temporal presentation of such physicochemical signals. These challenges can be addressed by integration of microscale technologies to control the microenvironment and monitor cell fate into cell culture platforms.

High throughput screening (HTS) to discover mechanisms of stem cell regulation and identify compounds that control stem cell fate is an area where engineers and the engineering approach can make significant contributions. HTS does not require *a priori* knowledge of stem cell regulation, and thus it offers the potential to identify factors that regulate stem cell fate, to improve upon lead compounds, to optimize presentation of soluble and immobilized cues, and to identify combinatorial interactions between regulatory signals. For example, HTS has been applied to identify small molecule compounds that increase osteogenesis in human MSCs,[25] promote self-renewal or direct

differentiation in hESCs,[26] and enhance reprogramming of somatic cells to a progenitor state.[27] HTS has also been adapted to immobilized configurations, enabling screening of combinatorial polymer libraries and peptides to identify substrates that support hESC self-renewal.[28,29] In addition to screening chemical compositions of biomaterials, HTS has also identified how material topography regulates MSC proliferation and differentiation[30] and how pattern size and shape regulate MSC differentiation fates.[31]

The application of HTS technologies to identify microenvironmental cues that regulate stem cell fates is typically limited by the throughput of stem cell analysis. Thus, to screen larger libraries and increase the odds of obtaining a hit, more reliable, faster, and less expensive methods to assess cell response to the compounds in the library must be developed. Dynamic, nondestructive approaches for monitoring cell state, such as enzymatic activity or fluorescent reporter concentration, will facilitate identifying the temporal regulation of stem cells by microenvironmental cues. In addition, screening relies on observing the behavior of a single stem cell or small number of stem cells, and population heterogeneity can result in a high frequency of false positives or false negatives. An understanding of the heterogeneous responses of stem cells to microenvironmental cues and strategies to account for these differences are needed to realize the potential of HTS in stem cell applications. In addition, an opportunity exists to expand HTS platforms beyond chemical and biomaterial libraries. Recent studies have identified important roles of microRNAs and long noncoding RNAs in developmental biology, including stem cell proliferation and differentiation as well as cell reprogramming.[32] Screening RNA libraries may identify new mechanisms of stem cell regulation and identify tools to control developmental programs in stem cells. Engineering platforms to screen cues such as intercellular contacts or mechanical forces in the context of a physiologically relevant chemical microenvironment would improve the power of stem cell screening platforms. Three-dimensional screening platforms, such as microwells or biomaterials scaffolds would enable identification of factors that regulate stem cell assembly and tissue formation or morphogenesis from stem cell sources.

Another technology that is taking on increasing importance in stem cell R&D is microfluidics. A variety of microfluidic systems have been developed, and such systems can spatially and temporally regulate the stem cell microenvironment with great precision, facilitate the analysis of the dynamic response of stem cells to microenvironmental cues, enable the isolation and analysis of clonal populations, and facilitate isolation of low abundance stem cells from a mixture of cells. Microfluidic culture systems have been used to identify and characterize the regulation of ESC self-renewal by both secreted paracrine factors and by physical shear forces.[33,34] Microfluidic technology also permits construction of highly parallel cell culture systems that enable clonal analysis of stem cells. Such systems have been applied to characterize heterogeneity in hematopoietic stem cell (HSC) responses to growth factors.[35] Additionally, the ability to construct patterns and spatial gradients of microenvironmental factors in microfluidics has been exploited to produce spatially distinct differentiation fates in MSCs.[36] Spatial and temporal control of fluid flow has also been applied to capture and release rare stem and progenitor cells from peripheral blood.[37,38]

To further realize the potential of microfluidics in SCE, reliable and inexpensive sources of relatively simple microfluidic devices must be available. In addition, collaborative efforts between researchers with expertise in device manufacture and stem cell researchers posing questions that can be addressed by these devices will be important. Better integration of stem cell culture and separations platforms with cell characterization is needed. With the advent of single cell gene expression analysis, the opportunity exists to use microfluidics to more deeply probe clonal differences in stem cell populations.

Stem cells have been used as a source of cells for construction of biosensors and *in vitro* tissue models. Using iPS cell technology, cells and tissues representative of a particular patient or disease state can be constructed.[39] Such *in vitro* biosensors are being employed in drug screening or drug toxicity testing applications. These integrated stem cell microtechnology systems are often referred to as organs-on-a-chip, and they need to be precisely designed to accurately model tissue and organ level function *in vitro*. Real-time functional analysis of the behavior of the system is an important aspect of organ-on-a-chip design. For example, a cardiotoxicity biosensor that integrated mESC-derived cardiomyocytes with an automated imaging system to monitor changes in cell contraction rate in response to pharmacologic agents has been reported.[40] Efforts to construct stem cell biosensors have only begun to realize the potential of stem cells in diagnostics and *in vitro* modeling applications. Advances in microscale fabrication technologies will enable construction of more physiologically relevant, 3D structured cell and tissue biosensors. If such systems can be engineered to provide in general a physiological environment, one that in many cases will need to be multicellular and have a three-dimensional architecture, these stem cell biosensors will at a minimum supplement animal testing in the development of a drug and perhaps even replace it.

Computational SCE

As noted earlier, engineering approaches to understanding and controlling SC fate are required at multiple stages during the development and implementation of RM-based therapies. Critically, implementing robust RM therapies, in even the simplest of tissues, will benefit from a predictive understanding of the molecular events that occur within individual SCs, and the role of the microenvironment (i.e., the SC niche) in perturbing these events. These molecular events are typically organized as cascades and include gene regulatory and intracellular signal transduction networks, cell–cell communication networks and the mechanical, electrostatic, biochemical, and cellular interactions that impinge on those networks.

Certainly understanding complex molecular processing in mammalian cells is a dominant endeavor in biomedical research. However, investigating these molecular and cellular events in SC is made significantly more challenging by additional features of SC biology (Table 4). The rarity and spatially heterogeneous distribution of individual SC requires that some degree of discrete and stochastic mathematics should be involved, as deterministic and continuous

Table 4. Features of Stem Cell Biology Relevant to Modeling Approaches

Stem cell property	*Biological impact*	*Modeling impact*
Rarity	Efferent signals diluted across many potential targets Behavior of other cells in population may overwhelm that of stem cells Stochastic responses within the small population may be important determinants of cell population behavior	Responses must be normalized for cell type ratios Spatial effects must be considered Deterministic or continuous models may not reflect underlying biology Experimental validation challenging Models must span many time and length scales Models of behavior dynamics must be utilized Widely varying space and time scales are important Population averages may not represent dominant outlier behavior
Metastability	Dynamic responses to exogenous signals Cells are rarely in equilibrium	Population averages may not be relevant to the individual stem cell
Heterogeneous spatial distribution	Developmental cues originate from diverse regions Afferent signals may vary widely	Heterogeneous populations of cells should be modeled and single cell behavior followed Spatial and temporal aspects should be considered
The stem cell niche is a heterogeneous and dynamic environment	Afferent signal will vary widely across the stem cell population Different types of molecular and biophysical signals need to be integrated	

methods such as ordinary differential equations may not accurately describe the underlying biology. The heterogeneous nature of the niche also requires that models include descriptions of the spatial interactions and gradients among various cell types and their environment to correctly predict how any cell or cell population will behave. Finally, the metastable nature of SC fate and the hierarchical organization of differentiation suggest that guiding cell fate along specific lineages involves balancing the dynamic differentiation process and feedback from progeny and the environment. Consequently, dynamic analysis across a wide range of timescales may need to be included in accurate system-wide modeling studies to understand the time evolution of the cell populations.

Modeling approaches in SCE can be segregated according to the cellular and molecular resolution at which the analysis is performed. In what follows, we will describe SC models at several of these levels that point to current trends and future opportunities for engineering to contribute to the understanding and control of SC biology.

A dominant use of modeling in SC biology is that of single cell analysis, likely due to the need to understand the endogenous mechanisms controlling SC fate, but also due to the lower degree of complexity required of biophysically realistic intracellular models when compared with those at a tissue or cell population level. In fact, one of the earliest computational studies using SC was the first to demonstrate that SC act randomly within a population, as individuals.[41] In this seminal Canadian study, it was established that the distribution of colony forming cells (CFCs) within mixed populations of cells derived from the splenic colonies of cells formed after mice were injected with hematopoietic cells followed a gamma distribution. After realizing this observation was consistent with a Markov birth–death process in which individual CFCs either proliferated to form two CFCs (''birth'') or underwent differentiation (''death'') at fixed probabilities, a Monte-Carlo model was created, which accurately fit the empirical data. This early article demonstrates an important feature of all useful models, namely that the model results were directly compared to the empirical data to validate the underlying hypothesis of the model. Among more recent single cell computational approaches to understanding SC biology, several sub-trends can be identified and include dynamic analyses of genetic and signal transduction networks including approaches that reduce networks to key components[42] or approaches that model the emergent behavior of the networks.[43,44] An important component of some of this single cell modeling is the coevolving development of new tools to test and validate model predations.[45]

The design and optimization of bioreactors and cell growth and differentiation systems is a subject of particular focus for bioengineers involved in SCE as it is of importance to the generation of large volumes of cells needed for both drug design and RM. Empirical and cell population dynamic models active research across the world and a number of research organizations in Asia visited by the WTEC panel specialized in these approaches. For example, the Bioprocessing Technology Institute (BTI) in Singapore, until recently led by Dr. Miranda Yap, study biomanufacturing systems focusing on the pharmaceutical and cell therapy industries. Recently, Dr. Steve Oh's group at BTI published studies in which microcarriers were used to increase yields of cardiomyocytes threefold over embryoid body controls,[46] and in which cell agitation due to shear stress in stirred cultures resulted in decreased pluripotency and an increase in differentiation-specific markers in a cell line-specific manner.[47] While the empirical studies provide a wealth of information regarding culture methodologies, a pair of articles from the Zandstra laboratory in Toronto,

Canada provides examples of the value of a combined *in silico* and experimental approach in the analysis of cell populations and the optimization of culture technologies. Motivated by the elusive nature of techniques capable of increasing human HSC numbers *in vitro*, Kirouac *et al.*[48] developed a simplified model of HSC differentiation in which cell fates were regulated by feedback from secreted molecule cell interaction networks among various cells from different points in the developmental hierarchy. Using this model, the authors were able to predict that negative feedback originating from more differentiated cells in the hierarchy is a principal regulatory mechanism controlling HSC fate. In a recent extension of this work, Csaszar *et al.* used these concepts to develop a strategy by which the controlled and specific inhibition of negative regulators of HSC differentiation allowed for global control of the cell population dynamics.[49] Overall, these articles demonstrate the productivity achievable when even simplified models and experimental efforts are tightly linked. By integrating math model outputs with validated stem cell assay outputs, the authors were able to predict the optimum culture method to maximize the yields of cell types of interest. Although this connection may not always be possible, especially for models of a more theoretical nature, the feedback between modeling and experimental efforts should function as an iterative process to maximize the effectiveness of each.

Far fewer computational studies have been performed specifically exploring the role of SC in whole tissues and development than have been performed for individual or SC populations. One area in which models have been utilized is in the study of the vertebrate gut, specifically the development of the intestinal crypt. A pair of recent articles from the University of Leipzig illustrates the use of models in this area, and highlights the importance of biomechanical forces in regulating cell fate and tissue morphogenesis. Motivated by the idea that the traditional view of hierarchical tissue organization in which SC differentiate into progenitors, which subsequently terminally differentiate may not be required to explain aspects of tissue development, the authors of these articles first developed a model to determine if cell–cell and cell–environment interactions alone could result in the self-organization of the intestinal crypt.[50] This multiscale model treated cells as elastic objects capable of growing, dividing, moving, and making contacts with other cells and the surrounding ECM. Cell fate was modeled to be dependent on the internal activity of the cell itself, along with that of its neighboring cells. Accordingly, individual cell differentiation, and therefore tissue development, was assumed to be dependent on the curvature of the crypt basal membrane and the types and number of contacts it makes to its surroundings. The critical prediction made using this model was that the robustness of this tissue to cell loss was made possible by the flexibility in the cell fate decision process—any population could be removed from the simulated crypt without impacting the long-term tissue organization because the cell fate transitions experienced by the progenitors in the model were reversible, and the development occurred within an externally imposed Wnt gradient.

As outlined in the few selected examples provided, computational modeling can provide a solid foundation on which to study and understand the complex system made up of the SC and its environment. One aspect that is clear from the analysis is that mathematical approaches are increasingly being used to gain fundamental insight into the mechanistic underpinnings of complex biological systems; many of these studies benefit from interdisciplinary approaches and data sharing and engineers are particularly well suited to take leadership roles in the area. Although great progress has been achieved in the 50 years since the first mathematical treatment of SC biology was published,[41] significant prospects remain for advancing the field of computational SCE. By consolidating efforts among modelers and experimentalists of various scientific backgrounds, we can expect to make more rapid progress in the years to come. This is especially true given the incredible rate at which new and larger data sets are generated, and novel technologies produced that allow for increasingly sophisticated questions to be addressed. With backgrounds spanning experimental biology, computer science, physics, chemistry, and design, bioengineers are in an envious position to rapidly and efficiently advance our knowledge, leading to innovations in fundamental biology and its clinical applications.

Stem Cell Bioprocessing and Biomanufacturing

There is a subtle, yet important distinction that exists between the use of the terms ''bioprocessing'' and ''biomanufacturing,'' despite the fact that they are often used interchangeably and refer to many overlapping activities. For the purposes of at least this discussion, ''bioprocessing'' refers to the development of systems for the scalable growth and differentiation of stem cells while ''biomanufacturing'' is the implementation of bioprocessing for stem cell production and commercialization. Traditionally, bioprocessing systems were devised to support the culture of cells at high density to produce concentrated batches of molecular factors secreted by the cells, thus the cells served simply as a vehicle to attain the end product. On the other hand, stem cell bioprocessing represents a fundamental paradigm shift whereby the cells themselves are defined as the product. Despite such a significant shift in the deliverable of the process, the currently used technologies and platforms for stem cell bioprocessing have remained largely unchanged. Stem cell biomanufacturing research and companies are starting from this current state of the art, but are looking forward to the development of new culture and sensor technologies to meet the large anticipated needs for stem cells in future RM therapies and screening platforms for drug development. The primary challenges to be addressed include the development of scalable culture systems, the incorporation of real-time monitoring and feedback control systems, and the development of robust, reproducible automated processes.

As noted above, current approaches to stem cell bioprocessing have started primarily by using existing formats and platforms originally optimized for biochemical engineering applications. However, in the biochemical processing industry, automated culture systems with in-line monitoring are frequently used for many, if not all of processing steps. In contrast, stem cell bioprocessing in its current form requires ''hands on,'' manual processing of the various culture steps. Automation of routine culture steps, such as re-feeding and passaging of cells, has been achieved for different

types of stem cells grown in conventional culture flasks,[51,52] but development of more sophisticated systems that significantly enhance throughput and facilitate scale-up and monitoring in addition to improving reproducibility have really only begun to be developed.

The strategy of scale ''up'' versus simply scale ''out'' is critical to the long-term success of commercialization of stem cell products. For adherent cells, dependence on a fixed amount of surface area for attachment and subsequent growth translates into a linearly proportional increase in surface area to increase cell yield. Thus given no other changes within such a system (e.g., media composition), at some point scaling out becomes impractical, and thus there is an impending need to transition to suspension culture formats for industrial scale-up manufacturing of stem cells and stem cell-derived products. For suspension culture, formats include microcarrier beads with adherent cells on the outside of solid beads or distributed throughout porous materials,[53,54] the use of cell aggregates either with or without materials,[55–60] and the microencapsulation of individual stem cells or aggregates.[57,61–63] Each of these configurations has inherent advantages and disadvantages that often complement one another, so it is unlikely that one format will be used for all types of stem cells, and hybrid variations of two or more combined formats might also be used. The eventual implementation of suspension culture formats makes the significant increase in the anticipated number of cells for *in vitro* screening platforms and regenerative therapies a much more feasible and attainable goal.

One of the most challenging and daunting issues is the multitude of parameters involved in bioprocessing systems that are capable of affecting cell growth and phenotype. As noted above, a complex combination of biophysical and biochemical environmental factors can influence stem cell phenotype and growth. Some such parameters, such as culture media composition and oxygen tension can be relatively easily and effectively scaled-up, whereas others, such as hydrodynamics, may not be as readily and directly scalable due to differences in vessel geometries and mechanisms of imparting media agitation to create well-mixed environments.[64,65] For process optimization to be ultimately achieved, the vast experimental space must be explored thoroughly, but current high throughput formats and screening platforms are inherently incapable of simulating numerous bioprocess parameters simultaneously. Thus, some efforts to ''scale-down'' prior to ''scale-up'' are likely necessary to perform such screening in a cost-efficient and experimentally feasible manner before proceeding to stem cell biomanufacturing in much larger volume systems.

Most of the focus thus far in stem cell bioprocessing has been on the upstream portions of amplifying the numbers of stem cells and stem cell-derived progeny, but as the quantities of cells and volume of media increases, so does the strain on downstream processes that are responsible for concentrating cell products in a readily deployable and stable format without compromising potency or viability. At small volumes or with numerous parallel processing of stem cell batches, current means of reducing and exchanging media volume and storing of cells (i.e., cryopreservation) are not severely limiting. However at some point, the challenge of efficiently separating cells, particularly during downstream processes, becomes the rate-limiting step. Cell sorting methodologies based on fluorescent signal detection or magnetic separation rely almost exclusively on antibody-detection, thus introducing a xenogenic element into the process.[66] For this reason, an increasing interest and effort has shifted to robust, label-free detection methods that can effectively delineate different populations of cells from varying degrees of heterogeneous starting populations.[67,68]

Finally, stem cell manufacturing infrastructure and facilities that accommodate the bioprocessing systems could also benefit from systems engineering analysis. More ''closed'' culture systems are needed in to ensure the sterility and safety of stem cell-derived products, particularly for cell therapies. In addition, reducing the physical ''foot print'' of systems and facilities, as well as designing interconnectable and exchangeable modular elements would benefit the economics and flexibility of stem cell bioprocessing systems. The majority, if not all, of current stem cell manufacturing practices suffer from a lack of feedback control mechanisms that relate to specific cell phenotypes. The introduction of feedback mechanisms provides new opportunities for meaningful real-time monitoring of stem cell cultures and potential application of machine learning-based algorithms and computational modeling to enhance stem cell bioprocesses.[69]

There thus are ample opportunities for engineers and systems engineering approaches to contribute to the further development of bioprocessing systems to advance the rapidly growing stem cell biomanufacturing industry. As already implied, cell manufacturing is really in its infancy. Furthermore, the number of cells required in a treatment is very much dependent on the specific disease or application. If we use as an example a procedure for the revascularization of the heart, it is estimated that as many as 10^{15} cells may be required annually.

Translational Models

For benchtop stem cell science to have an impact on patients and the treatment of disease and other disorders, it must be translated into clinical therapies and into commercialization. To do this successfully requires that a clinical need be addressed and that the treatment be economically viable. One of the important aspects of this global assessment was identifying some of the interesting models for this translation, four of which are listed below:

- Berlin Brandenburg Centre for Regenerative Therapies
- Cell Therapy Catapult in the United Kingdom
- Centre for Commercialization of Regenerative Medicine in Canada
- Tokyo Women's Medical University

The uniqueness of the Berlin Brandenburg Centre for Regenerative Therapies is that they do an opportunity analysis early in the development of a research project. There are three multidisciplinary platforms: basic science, biomaterials, and translation technology. Several of the groups within the Center are organized in a matrix structure, supporting the work of a particular host platform as well as those of other platforms by delivering basic technologies and principles. In addition, there is a Department of Clinical Development and Regulatory Affairs and a Department of Business Development. These support all projects within the center.

In the United Kingdom the Cell Therapy Catapult is one of seven such catapult initiatives established by the Technology Strategy Board of the United Kingdom government to create new industries. The Cell Therapy Catapult will support the development and commercialization of cell therapies and advanced therapeutics as well as the enabling technologies for manufacturing, quality control, and safety. It will be based in London, and it will be a center, independent of higher education institutions, but where academics, industry experts, clinicians, and regulatory experts can work together, focusing on the commercial development of innovative technologies.

In Canada, Dr. Peter Zandstra, a co-author of this review and a member of this WTEC panel, is the Chief Scientific Officer of the Centre for Commercialization of Regenerative Medicine. This is a federally incorporated, not for profit organization supporting the development of technologies that accelerate the commercialization of stem cells- and biomaterials-based products and therapies. The business strategy is to enable unique translational platforms that address key barriers in RM commercialization, integrate Canada's strength in stem cells and engage industry partners so as to make the center a global nexus for RM commercialization.

Finally, at Tokyo Women's Medical University Dr. Teruo Okano heads the Institute of Advanced Biomedical Engineering and Science, and he has provided the leadership to create a unique activity. The focus has been on cell sheet TE, and the institute has partnered with the Waseda University's Graduate School of Bioscience and Medical Engineering. In 2008, the Tokyo Women's Medical University-Waseda University Joint Institution for Advanced Biomedical Sciences (TWIns) opened. There also is a partnership with Professor Masahiro Kino-Oka from Osaka University to develop a tissue factory for cell sheet manufacturing.

While these four are primarily discussed in this report, there are of course other models for translation. One of these is the Global Stem Cell and Regenerative Medicine Initiative recently established by the Korean Ministry of Health and Welfare as part of a national Korean strategy to exercise global leadership in the stem cell and RM field. The operation and management of this initiative is being assisted by the Global Stem Cell and Regenerative Medicine Acceleration Center, whose activities include strategic planning, project design, performance assessment, global networking, and many other supporting activities. The major focus of this initiative is on translational research to accelerate therapeutic development, clinical research aimed at the delivery of treatments, and infrastructure development to speed up commercialization. As this initiative is brand new, the exact details are still somewhat unclear; however, it will be interesting to see how this activity in Korea develops.

It should be noted that there are a variety of clinical trials taking place using stem cells. Most of these trials are using human adult stem cells or progenitor cells and not hESCs, and such trials are taking place in a variety of different countries in Europe and also in Asia, for example, Korea. In the case of clinical trials taking place in the U.S. using hESCs, two examples are (1) the trials by Advanced Cell Technology treating macular degeneration and (2) the planned trial by Asterias Biotherapeutics. This latter trial was initiated by Geron, after a few patients stopped, the technology then acquired by Asterias, and now there is a plan to renew the trial. Also, in Japan there are efforts to launch a series of clinical trials using differentiated cells from iPS cells.

Finally, a key factor in the translation of benchtop stem cell science into products and therapies is the regulatory pathway through which the technology must go. Unfortunately, as important as this issue is, it was not part of the study reported here.

Education

It was clear to this WTEC panel that, for engineers to have a recognized and valued impact in biology, they need to comprehend and make significant contributions to the fundamental knowledge of biological mechanisms. Thus, for training programs to be successful, they need to understand basic biological principles and have what might be called a ''high level'' of biology, and this is certainly what is being done in the leading bioengineering programs in North America.

Outside of North America, an excellent example of a unique training program is that at Loughborough University in the United Kingdom The Doctoral Training Centre there was established with funding from the United Kingdom's Engineering and Physical Sciences Research Council and in partnership with Keele University and with the University of Nottingham. There are more than 50 PhD students in this program. The program introduces the students to the principles of bioprocessing and manufacturing and provides ''hands on'' research experiences with stem cells in existing and new, novel platforms. The intent is to train the future leaders of the cell biomanufacturing industry. What is unclear is the degree of interaction with some of the major stem cell biology centers in the United Kingdom. Such interaction and integration is necessary for engineering and biology to be developed and implemented together.

One of the outcomes of the Atlanta Workshop on Stem Cell Biomanufacturing was the agreement to establish an international school in the area of cell manufacturing. The initial offering of this school was April 28–May 4, 2013 in Portugal. The organization of the school was led by Professor Joaquim Cabral from the Instituto Superior Technico in Portugal with faculty from Loughborough University in the United Kingdom, and Georgia Tech.

Opportunities for Collaboration

Research today in general is very interdisciplinary in nature, and this is true of biology in general and the stem cell field in particular. This is certainly a theme for the National Academies report already referred to previously. As part of this, collaborations almost become a necessity. These might be with an investigator at one's own institution, somewhere else in the city, or even at a longer distance.

In today's world where research and the development of technology is conducted within the global community, collaborations can also exist between investigators in different countries. There are in fact a number of examples of global, international collaborations. One example of an international collaborative initiative is the International Stem Cell Forum (www.stem-cell-forum.net/). Under the auspices of this forum, the International Stem Cell Initiative (ISCI) was established. This initiative is an expert group working to

establish a global set of standardized criteria and techniques that will underpin the eventual development of applications for hESCs and human induced pluripotent stem (hiPS) cells in human medicine. Members of ISCI include the University of Sheffield in the United Kingdom, the University of Toronto in Canada, and the Bioprocessing Institute in Singapore. Another example of a collaboration is that of the Tissue Engineering Research Center at the Academy of Military Medical Sciences in China with Rice University. Finally, a third example is the partnership of Harvard's Wyss Institute with Charite Hospital in Berlin.

In fact, U.S. investigators need to cooperatively take advantage of the excellent activities in other countries, and it thus was encouraging for the WTEC panel members to see the hosts of the different sites visited being so open and very interested in the possibility of collaborating. What is needed, however, if we are to encourage international collaborations are government programs that foster such interactions. Included should be realistic levels of funding. Also, the review process needs to be one that uses a single review committee with membership from both of the countries sponsoring the program.

State of SCE Outside of North America

The full report contains the site visit reports for each institution. These site visit reports provide more detail than can be stated here; however, in the listing below for each country visited or in some other way assessed, the state of SCE is briefly characterized.

Europe sites

France: Observed some engineering involvement

Germany: Strong engineering involvement at the Berlin Brandenberg Centre for Regenerative Therapies and at the Fraunhofer Institutes

Ireland: A major stem cell center at NUI Galway with engineering involvement

Netherlands: A good integration of engineering with biology and medicine in NIRM

Portugal: Strong engineering involvement in bioprocessing

Sweden: Significant activity including translation into the clinic, some government funding, some engineering involvement of engineering and the physical sciences

Switzerland: Strong engineering and physical sciences involvement at EPFL and in Zurich (ETH) and Basel

United Kingdom: Major engineering activities, largely in bioprocessing and manufacturing

Asia Pacific sites

Australia: A new stem cell initiative with the involvement of some engineers

China: Excellent young investigators, massive investments by the government, high impact biology, engineers involved in more traditional roles

Japan: A leader in iPS cells, engineering integrated with biology and medicine at Tokyo Women's Medical University, other engineering activities more independent

Korea: Significant activities with major government funding, a number of startup companies, some engineering involvement

Singapore: Excellent BTI with engineering involvement

Other countries

Iran: A major stem cell research institute but limited if any engineering involvement

Israel: Considerable activities involving both biologists and engineers, also some commercial activities

Government Policies

From the assessment conducted, it is clear that many countries recognize the importance and value of investing in science and technology and are actually doing it. A list of such countries includes China where the R&D budget continues to be increased on an annual basis, Japan where it appears that the country has identified RM a key priority of their 21st century economy, Korea where there is a new global RM initiative, and Singapore that has invested heavily in scientists and the research infrastructure. This list also includes such European countries as Germany, the Netherlands, Sweden, and the United Kingdom. Taking the United Kingdom as a specific example, at the end of 2011 the British government launched a new strategy for United Kingdom Life Sciences. This comprehensive strategy includes significant new investments in life sciences research and in the development and commercialization of research. The Cell Therapy Catapult initiative is a part of this strategy. The British government, in spite of the global economic recession and a very significant United Kingdom budget deficit, is doing this because its goal is for the United Kingdom to be the global ''hub'' for the life sciences in the future.

In contrast, in the United States the budgets of the Federal agencies that support R&D are ''flat'' (decreasing when inflation is considered) with little indication that this situation will change soon. This problem is highlighted in a recent report entitled ''Leadership in Decline,''[70] which describes such a decline in life sciences in general, thus affecting the areas of stem cell research. A decline in research funding could threaten the U.S.'s globally acknowledged leadership in the development of enabling technologies, new clinical therapies, and other innovative stem cell-based applications, areas where engineers and the rigorous, systematic engineering approach can play a critical role in transforming the potential of stem cells into commercially viable and societally impactful products. In the light of this situation, the White House Office of Science and Technology Policy on April 26, 2012 released a National Bioeconomy Blueprint. This document outlines what are called five strategic imperatives that potentially will result in the generation of new markets and economic growth. These are as follows:

1. Support R&D that will provide the foundation for the future bioeconomy;
2. Facilitate the translation of research to the market;
3. Develop and reform regulations so as to reduce barriers and increase the speed and predictability of regulatory processes and thus reduce costs;
4. Update training programs and provide institutional incentives for student training for national workforce needs; and
5. Identify and support opportunities for the development of public–private partnerships and precompetitive collaborations.

With the exception of the third recommendation above that deals with regulatory issues that were not included in this study, the conclusions offered in the next section align with the above recommendations from the White House Office of Science and Technology.

Conclusions

From this global assessment of SCE as conducted by the WTEC panel, it is clear that engineers and the engineering approach with its quantitative, systems-based thinking can contribute much more to basic stem cell research than it has to date. As stated in the National Academies report on ''A New Biology for the 21st Century,'' to achieve the deeper understanding of biology required in this century there will need to be an integration of many disciplines into biological research, and this certainly includes engineering. Engineering analysis can be used to identify the critical components of highly complex stem cell systems and provide an understanding of how such components work together to regulate stem cell fate and function. Furthermore, computational models will be increasingly necessary in our efforts to achieve a better understanding of complex biological systems. In all of the above, engineers are in a position to take a leadership role.

In addition to the contributions describe above, engineers can and should take the lead in developing new, innovative enabling technologies. This includes HTS techniques, improved culture and differentiation systems, and *in vitro* models engineered to be more physiologic. The last of these include organ-on-a-chip models and also engineered *in vitro* tumor models that can lead to a better understanding of cancer.

Finally, for stem cell biomanufacturing there is an increasing need for advances in scaleable culture systems, techniques for real-time monitoring, and for the implementation of process automation. Computational models will again have an important role to play, in particular in the optimization of bioprocessing systems and the integration of feedback control.

In summary, this study identifies the needs and opportunities for an increasing involvement of engineers in the field of stem cells and related technologies. Although one might argue that the United States today has a leadership role, to capitalize on this and to build on the current existing momentum, and most importantly to accelerate the translation of benchtop research into various applications including clinical therapies and into commercialization, will require taking bold steps. The panel thus offers the following conclusions:

- The United States has a unique opportunity to maintain a leadership position in the stem cell field through the continued support of R&D that will provide a foundation for the generation of new markets and that will lead to economic growth.
- Because of the contributions that engineers can make in all areas of the stem cell field as illustrated by the global assessment reported here, this needs to include increased investment in engineering, applied research, and commercialization as it relates to stem cell research and related stem cell-based technologies.
- A major component in this could be that the government agencies that support R&D should establish a broad interagency program for SCE, one that provides grants to interdisciplinary teams that include engineers, computational researchers, and biologists as well as individuals from other disciplines.
- Another component that would be beneficial is the establishment of new, innovative mechanisms that support academic–industry partnerships and unique translational models that facilitate the translation of research into the private sector.
- To address national workforce needs, the development of training programs at universities and advanced short courses should be encouraged and supported by government agencies.
- Finally, in today's global economy and with the excellent activities taking place in other countries, the global community including the United States would benefit from forming strategic partnerships with other countries so as to leverage the existing and emerging strengths in institutions outside of the United States; to implement such partnerships will require binational grant programs with appropriate review mechanisms.

As noted in the previous section, these conclusions align with the National Bioeconomy Blueprint released by the White House Office of Science and Technology Policy. It is up to the government agencies to implement a plan based on the conclusions from this assessment study. Without the implementation of the above, however, this unique opportunity could be lost. In this case, it might be possible that the United States in the future will be relegated to the second tier of countries in this critical area of SCE and that other countries will become the acknowledged leaders. On the other hand, if implementation takes place in some form, and there is an urgency in doing so, then the United States can expect to continue to be in a leadership position and at the forefront in advancing the sciences, developing new, innovative enabling technologies and platforms that lead to clinical therapies, to commercializing the results of stem cell research, and to the generation of new markets and economic growth based on advances in the stem cell field. Some of the results from this will be as follows:

- Acceleration in the development of new drugs while at the same time reducing the costs of this development process.
- The development of cell therapies that address diseases and conditions of injury for which today there are no real treatment options available for patients in need, therapies that also are widely available.
- The growth of the 21st century bioeconomy in the United States and around the world with advances in our knowledge of stem cells and the translation of this into applications and products.

This has been the dream for at least 20 years; however, with the right strategy this can be realized and be the reality of tomorrow.

Acknowledgments

This study would not have been possible without sustained support from several U.S. Government program officers: Michael Reischman, Kesh Narayanan, Semahat Demir, Ted Conway, and Theresa Good of the National

Science Foundation, Anne Plant of the National Institute of Standards and Technology, and Larry Nagahara of the National Cancer Institute at NIH. We also wish to thank Joe Gold, Ron McKay, and Jon Rowley who served as consultants to this study. Most importantly we extend our sincere thanks to the foreign hosts for their hospitality and generosity in sharing with us their research results, their facilities, and their insights. This all contributed to making this study a success. Finally, we thank the WTEC staff. For those interested in seeing the full report on this global assessment of stem cell engineering, they should contact the World Technology Evaluation Center (WTEC) at 448 Murry Hill Circle, Suite 120, Lancaster, PA 17601 (Ph: 717-299-7130).

Disclosure Statement

No competing financial interests exist.

References

1. Zandstra, P.W., and Nagy, A. stem cell bioengineering. Annu Rev Biomed Eng **3,** 275, 2001.
2. National Research Council. A New Biology for the 21st Century. Washington, DC: The National Academies Press, 2009, 112 pp.
3. Schofield, R. Relationship between spleen colony-forming cell and hematopoietics stem-cell-hypothesis. Blood Cells **4,** 7, 1978.
4. Scadden, D.T. The stem-cell niche as an entity of action. Nature **441,** 1075, 2006.
5. Watt, F.M., and Hogan, B.L. Out of Eden: stem cells and their niches. Science **287,** 1427, 2000.
6. Keung, A.J., Kumar, S., and Schaffer, D.V. Presentation counts: microenvironmental regulation of stem cells by biophysical and material cues. Annu Rev Cell Dev Biol **26,** 533, 2010.
7. Guilak, F., Cohen, D.M., Estes, B.T., Gimble, J.M., Liedtke, W., and Chen, C.S. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell **5,** 17, 2009.
8. Discher, D.E., Mooney, D.J., and Zandstra, P.W. Growth factors, matrices, and forces combine and control stem cells. Science **324,** 1673, 2009.
9. Lutolf, M.P., Gilbert, P.M., and Blau, H.M. Designing materials to direct stem-cell fate. Nature **462,** 433, 2009.
10. Saha, K., Pollock, J.F., Schaffer, D.V., and Healy, K.E. Designing synthetic materials to control stem cell phenotype. Curr Opin Chem Biol **11,** 381, 2007.
11. Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. Matrix elasticity directs stem cell lineage specification. Cell **126,** 677, 2006.
12. Saha, K., Keung, A.J., Irwin, E.F., Li, Y., Little, L., Schaffer, D.V., *et al.* Substrate modulus directs neural stem cell behavior. Biophys J **95,** 4426, 2008.
13. Gilbert, P.M., Havenstrite, K.L., Magnusson, K.E.G., Sacco, A., Leonardi, N.A., Kraft, P., *et al.* Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science **329,** 1078, 2010.
14. Chowdhury, F., Li, Y., Poh, Y.C., Yokohama-Tamaki, T., Wang, N., and Tanaka, T.S. Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions. PLoS One **5,** e15655, 2010.
15. Keung, A.J., Asuri, P., Kumar, S., and Schaffer, D.V. Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. Integr Biol (Camb) **4,** 1049, 2012.
16. Saha, S., Ji, L., de Pablo, J.J., and Palecek, S.P. Inhibition of human embryonic stem cell differentiation by mechanical strain. J Cell Physiol **206,** 126, 2006.
17. Yamamoto, K., Sokabe, T., Watabe, T., Miyazono, K., Yamashita, J.K., Obi, S., *et al.* Fluid shear stress induces differentiation of Flk-1-positive embryonic stem cells into vascular endothelial cells *in vitro*. Am J Physiol Heart Circ Physiol **288,** H1915, 2005.
18. McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K., and Chen, C.S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell **6,** 483, 2004.
19. Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., *et al.* Role of YAP/TAZ in mechanotransduction. Nature **474,** 179, 2011.
20. Christopherson, G.T., Song, H., and Mao, H.-Q. The influence of fiber diameter of electrospun substrates on neural stem cell differentiation and proliferation. Biomaterials **30,** 556, 2009.
21. Yim, E.K.F., Pang, S.W., and Leong, K.W. Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. Exp Cell Res **313,** 1820, 2007.
22. Radisic, M., Park, H., Shing, H., Consi, T., Schoen, F.J., Langer, R., *et al.* Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proc Natl Acad Sci U S A **101,** 18129, 2004.
23. Turing, A.M. The chemical basis of morphogenesis. 1953. Bull Math Biol **52,** 153, 1990; discussion 19.
24. Koller, M.R., Bender, J.G., Miller, W.M., and Papoutsakis, E.T. Reduced oxygen tension increases hematopoiesis in long-term culture of human stem and progenitor cells from cord blood and bone marrow. Exp Hematol **20,** 264, 1992.
25. Alves, H., Dechering, K., Van Blitterswijk, C., and De Boer, J. High-throughput assay for the identification of compounds regulating osteogenic differentiation of human mesenchymal stromal cells. PLoS One **6,** e26678, 2011.
26. Desbordes, S.C., Placantonakis, D.G., Ciro, A., Socci, N.D., Lee, G., Djaballah, H., *et al.* High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Cell Stem Cell **2,** 602, 2008.
27. Xu, Y., Zhu, X., Hahm, H.S., Wei, W., Hao, E., Hayek, A., *et al.* Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc Natl Acad Sci U S A **107,** 8129, 2010.
28. Mei, Y., Saha, K., Bogatyrev, S.R., Yang, J., Hook, A.L., Kalcioglu, Z.I., *et al.* Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nat Mater **9,** 768, 2010.
29. Derda, R., Musah, S., Orner, B.P., Klim, J.R., Li, L., and Kiessling, L.L. High-throughput discovery of synthetic surfaces that support proliferation of pluripotent cells. J Am Chem Soc **132,** 1289, 2010.
30. Unadkat, H.V., Hulsman, M., Cornelissen, K., Papenburg, B.J., Truckenmuller, R.K., Carpenter, A.E., *et al.* An algorithm-based topographical biomaterials library to instruct cell fate. Proc Natl Acad Sci U S A **108,** 16565, 2011.
31. Ruiz, S.A., and Chen, C.S. Emergence of patterned stem cell differentiation within multicellular structures. Stem Cells **26,** 2921, 2008.

32. Tiscornia, G., and Izpisua Belmonte, J.C. MicroRNAs in embryonic stem cell function and fate. Genes Dev **24,** 2732, 2010.
33. Moledina, F., Clarke, G., Oskooei, A., Onishi, K., Gunther, A., and Zandstra, P.W. Predictive microfluidic control of regulatory ligand trajectories in individual pluripotent cells. Proc Natl Acad Sci U S A **109,** 3264, 2012.
34. Toh, Y.C., and Voldman, J. Fluid shear stress primes mouse embryonic stem cells for differentiation in a self-renewing environment via heparan sulfate proteoglycans transduction. FASEB J **25,** 1208, 2011.
35. Lecault, V., Vaninsberghe, M., Sekulovic, S., Knapp, D.J., Wohrer, S., Bowden, W., *et al.* High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. Nat Methods **8,** 581, 2011.
36. Zhang, Y., Gazit, Z., Pelled, G., Gazit, D., and Vunjak-Novakovic, G. Patterning osteogenesis by inducible gene expression in microfluidic culture systems. Integr Biol (Camb) **3,** 39, 2011.
37. Stott, S.L., Hsu, C.H., Tsukrov, D.I., Yu, M., Miyamoto, D.T., Waltman, B.A., *et al.* Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. Proc Natl Acad Sci U S A **107,** 18392, 2010.
38. Plouffe, B.D., Kniazeva, T., Mayer, J.E., Jr., Murthy, S.K., and Sales, V.L. Development of microfluidics as endothelial progenitor cell capture technology for cardiovascular tissue engineering and diagnostic medicine. FASEB J **23,** 3309, 2009.
39. Trounson, A., Shepard, K.A., and DeWitt, N.D. Human disease modeling with induced pluripotent stem cells. Curr Opin Genet Dev **22,** 509, 2012.
40. Kim, S.B., Bae, H., Cha, J.M., Moon, S.J., Dokmeci, M.R., Cropek, D.M., *et al.* A cell-based biosensor for real-time detection of cardiotoxicity using lensfree imaging. Lab Chip **11,** 1801, 2011.
41. Till, J.E., McCulloch, E.A., and Siminovich, L. A stochastic model of stem cell proliferatin, based on the growth of spleen colony-forming cells. Proc Natl Acad Sci U S A **51,** 29, 1964.
42. Glauche, I., Horn, K., Horn, M., Thielecke, L., Essers, M.A., Trumpp, A., and Roeder, I. Therapy of chronic myeloid leukaemia can benefit from the activation of stem cells: simulation studies of different treatment combinations. Br J Cancer **106,** 1742, 2012.
43. Huang, A.C., Hu, L., Kaufman, S.A., Zhang, W., and Shmulevich, I. Using cell fate attractors to uncover transcriptional regulation of Hl60 neutrophil differentiation. BMC Syst Biol **3,** 20, 2009.
44. Markert, E.K., Baas, N., Levine, A.J., and Vaszquez, A. Higher order Boolean networks as models of cell state dynamics. J Theor Biol **264,** 945, 2010.
45. Tay, S., Hughey, J.J., Lee, T.K., Lipniakci, T., Quake, S.R., and Covert, M.W. Single-cell NF-Kappa B dynamics reveal digital activation and analogue information processing. Nature **466,** 267, 2010.
46. Lecine, M., Tng, S., Choo, A.B., Reuveng, S., and Oh, S.K. Scalable platform for human embryonic stem cell differentiation to cardiomyocytes in suspended microcarrier cultures. Tissue Eng Part C Methods **16,** 1609, 2010.
47. Leung, H.W., Chen, A., Choo, A.B., Reuveny, S., and Oh, S.K. Agitation can induce differentiation of human pluripotent stem cells in microcarrier cultures. Tissue Eng Part C Methods **17,** 165, 2011.
48. Kirouac, D.C., Madlambayan, G.J., Yu, M., Sykes, E.A., Ito, C., and Zandstra, P.W. Cell-cell interaction networks regulated blood stem and progenitor cell fate. Mol Syst Biol **5,** 293, 2009.
49. Csaszar, E., Kirouac, D.C., Yu, M., Wang, W., Quiao, W., Cooke, M.P., Boitano, A.E., Ito, C., and Zandstra, P.W. Rapid expansion of human hematopoietic stem cells by automated control of inhibitory feedback signaling. Cell Stem Cell **10,** 218, 2012.
50. Buske, P., Galle, J., Barker, n., Aust, G., Clevers, H., and Loeffler, M. Comprehensive model of the spatio-temporal stem cell and tissue organization in the intestinal crypt. PLoS Comput Biol **7,** e1001045, 2011.
51. Ratcliffe, E., Thomas, R.J., and Williams, D.J. Current understanding and challenges in bioprocessing of stem cell-based therapies for regenerative medicine. Br Med Bull **100,** 137, 2011.
52. Want, A.J., Nienow, A.W., Hewitt, C.J., and Coopman, K. Large-scale expansion and exploitation of pluripotent stem cells for regenerative medicine purposes: Beyond the T flask. Regen Med **7,** 71, 2012.
53. Abranches, E., Bekman, E., Henrique, D., and Cabral, J.M. Expansion of mouse embryonic stem cells on microcarriers. Biotechnol Bioeng **96,** 1211, 2007.
54. Santos, F., Andrade, P.Z., Abecasis, M.M., Gimble, J.M., Chase, L.G., Campbell, A.M., Boucher, S., Vemuri, M.C., Silva, C.L., and Cabral, J.M. Toward a clinical-grade expansion of mesenchymal stem cells from human sources: a microcarrier-based culture system under xeno-free conditions. Tissue Eng Part C Methods **17,** 1201, 2011.
55. Fok, E.Y., and Zandstra, P.W. Shear-controlled single-step mouse embryonic stem cell expansion and embryoid body-based differentiation. Stem Cells **23**, 1333, 2005.
56. zur Nieden, N.I., Cormier, J.T., Rancourt, D.E., and Kallos, M.S. Embryonic stem cells remain highly pluripotent following long term expansion as aggregates in suspension bioreactors. J Biotechnol **129,** 421, 2007.
57. Kehoe, D.E., Jing, D., Lock, L.T., and Tzanakakis, E.S. Scalable stirred-suspension bioreactor culture of human pluripotent stem cells. Tissue Eng Part A **16,** 405, 2010.
58. Steiner, D., Khaner, H., Cohen, M., Even-Ram, S., Gil, Y., Itsykson, P., Turetsky, T., Idelson, M., Aizenman, E., Ram, R., Berman-Zaken, Y., and Reubinoff, B. Derivation, propagation and controlled differentiation of human embryonic stem cells in suspension. Nat Biotechnol **28,** 361, 2010.
59. Bratt-Leal, A.M., Carpenedo, R.L., Ungrin, M.D., Zandstra, P.W., and McDevitt, T.C. Incorporation of biomaterials in multicellular aggregates modulates pluripotent stem cell differentiation. Biomaterials **32,** 48, 2011.
60. Fluri, D.A., Tonge, P.D., Song, H., Baptista, R.P., Shakiba, N., Shukla, S., Clarke, G., Nagy, A., and Zandstra, P.W. Derivation, expansion and differentiation of induced pluripotent stem cells in continuous suspension cultures. Nat Methods **9,** 509, 2012.
61. Dang, S.M., Gerecht-Nir, S., Chen, J., Itskovitz-Eldor, J., and Zandstra, P.W. Controlled, scalable embryonic stem cell differentiation culture. Stem Cells **22,** 275, 2004.
62. Hwang, Y.S., Cho, J., Tay, F., Heng, J.Y., Ho, R., Kazarian, S.G., Williams, D.R., Boccaccini, A.R., Polak, J.M., and Mantalaris, A. The use of murine embryonic stem cells, alginate encapsulation, and rotary microgravity bioreactor in bone tissue engineering. Biomaterials **30,** 499, 2009.
63. Wilson, J.L., and McDevitt, T.C. Stem cell microencapsulation for phenotypic control, bioprocessing, and transplantation. Biotechnol Bioeng **110,** 667, 2013.

64. Kinney, M.A., Sargent, C.Y., and McDevitt, T.C. The multiparametric effects of hydrodynamic environments on stem cell culture. Tissue Eng Part B Rev **17,** 249, 2011.
65. Fridley, K.M., Kinney, M.A., and McDevitt, T.C. Hydrodynamic modulation of pluripotent stem cells. Stem Cell Res Ther **3,** 45, 2012.
66. Diogo, M.M., da Silva, C.L., and Cabral, J.M. Separation technologies for stem cell bioprocessing. Biotechnol Bioeng **109,** 2699, 2012.
67. Stringari, C., Sierra, R., Donovan, P.J., and Gratton, E. Label-free separation of human embryonic stem cells and their differentiating progenies by phasor fluorescence lifetime microscopy. J Biomed Opt **17,** 046012, 2012.
68. Singh, A., Suri, S., Lee, T., Chilton, J.M., Cooke, M.T., Chen, W., Fu, J., Stice, S.L., Lu, H., McDevitt, T.C., and Garcia, A.J. Adhesion strength-based, label-free isolation of human pluripotent stem cells. Nat Methods **10,** 438, 2013.
69. Phan, T.H., Saraf, P., Kiparissides, A., Mantalaris, A., Song, H., and Lim, M. An *in silico* erythropoiesis model rationalizing synergism between stem cell factor and erythropoietin. Bioprocess Biosyst Eng **36,** 1689, 2013.
70. Atkinson, R.D., Ezell, S.J., Giddings, I.V., Stewart, L.A., and Andes, S.M. Leadership in Decline: Assessing U.S. International Competitiveness in Biomedical Research. Washington, DC: The Information Technology and Innovation Foundation and United for Medical Research, 2012, 23 pp.

Address correspondence to:
Robert M. Nerem, PhD
Parker H. Petit Institute for Bioengineering and Bioscience
Georgia Institute of Technology
315 Ferst Drive, Northwest
Atlanta, GA 30332-0363

E-mail: robert.nerem@ibb.gatech.edu

Received: August 1, 2013
Accepted: December 23, 2013
Online Publication Date: February 28, 2014