Engineering Strategies, Opportunities, and Challenges for Tissue Repair and Regeneration: CIRM Workshop Summary and Recommendations

San Francisco, CA; January 12-13, 2012

TABLE OF CONTENTS

EXECUTIVE SUMMARY

CIRM organized a workshop in January 2012 to explore the role of tissue engineering and biomaterials development in regenerative medicine, and to determine whether areas of opportunity exist for CIRM to help further support and advance the field. This report summarizes the workshop proceedings and the recommendations made by the participants.

Tissue engineering (TE) refers to combining biological approaches to regenerative medicine with engineering and materials science. A consortium of federal agencies defined TE as "the use of physical, chemical, biological and engineering processes to control and direct the aggregate behavior of cells" (Multi-Agency Tissue Engineering Sciences Interagency Working Group, 2007). By this definition, TE encompasses a variety of tools and approaches for tissue growth, repair and/or regeneration, which enable treatment of diseases and injuries for which cell therapy alone may be inadequate.

Workshop participants identified a number of areas where CIRM could help advance tissue engineering. Those recommendations could be grouped into two general categories: 1) those that address communication and/or collaborative opportunities to advance the field more broadly and 2) those that target specific technical hurdles in the field.

The workshop participants recommended:

1. Support research using scaffolds pre-seeded with cells for transplantation as well as cell-free scaffold approaches.
2. Support the development of hydrogels that mimic natural tissue properties.
3. Develop biomaterials-based methods to model the niche in which stem cells reside *in vivo* to enhance and direct stem cell expansion *in vitro*.
4. Develop better quantitative tools for testing tissue engineered products.
5. Develop materials that mitigate immune response and enhance engraftment.
6. Help educate researchers about the translational considerations of cell-scaffold combination products.
7. Promote interaction between the stem cell and biomaterials communities
8. Facilitate dialogue with the FDA regarding regulation of tissue engineered products.

Recommendation #1: Support research using scaffolds pre-seeded with cells for transplantation as well as cell-free scaffold approaches **(see full report Page 12).** Cell-seeded scaffolds offer technology that protects and delivers cells to a target tissue and provides three-dimensional architecture to facilitate regeneration and/or repair. However, these scaffolds can present challenges to developing an off-the-shelf product. Acellular scaffolds, which can recruit the appropriate endogenous stem cells and their associated support cells, are an encouraging development in the tissue engineering field. The

success of these scaffolds relies on understanding and subsequently providing the proper matrix molecules, mechanical cues, and/or chemical cues.

Recommendation #2: Support the development of hydrogels that mimic natural tissue properties **(see full report Page 15)**. Hydrogels are aqueous networks of chain-like molecules and are a promising technological approach both for cell delivery and to enable endogenous repair processes. Their properties can be modulated to mimic and integrate with a range of tissue types. Hydrogels are likely to play an increasingly significant role in regenerative medicine applications.

Recommendation #3: Develop biomaterials-based methods to model stem cell niches *in vivo* to enhance and direct stem cell expansion *in vitro* **(see full report Page 18)**. A synthetic niche refers to the 2D or 3D microenvironment created by the integration of extracellular matrix molecules and/or growth factors and engineering technologies. These synthetic niches are needed to direct stem cell differentiation and expansion *in vitro* and in certain cases *in vivo*.

Recommendation #4: Develop better quantitative tools for testing tissue engineered products **(see full report Page 19).** Characterization methods are essential to understanding a product and are particularly important in the FDA's regulatory review process to enable clinical trials. This is especially the case for combination products, such as cells on a scaffolding material, which can be difficult to analyze and may behave differently than the individual components. Possible methods may include polarized light microscopy to determine tissue/fiber orientation and 3D imaging for evaluating repair processes.

Recommendation #5: Develop materials that mitigate immune response and enhance engraftment **(see full report Page 22).** Tissue engineering and the foreign body response are intricately linked. Almost all implants trigger a foreign body response, which leads to fibrotic capsule formation and/or phagocytic toxic byproduct accumulation at the implant site. Developing materials and devices that minimize this process is critical to successful implant engraftment.

Recommendation #6: Help educate researchers about the translational considerations of cell-scaffold combination products **(see full report Page 24).** Combination products can be particularly challenging to navigate through the regulatory process and advance to human clinical trials. Informing researchers regarding early considerations to avoid potential pitfalls can enable translation of tissue engineered products. CIRM has numerous programs designed to help educate researchers on aspects of facilitating research translation, ranging from webinars and other active educational outreach efforts to embedding translational considerations into CIRM's processes such as by mandating the development of a Target Product Profile with certain research award applications. These and other efforts with particular emphasis on combination products will be continued and, where possible, expanded.

Recommendation #7: Promote interaction between the stem cell and biomaterials communities **(see full report Page 25).** Participants discussed the importance of fostering collaboration between these disciplines to help develop innovative tissue engineered products that are aligned with biological systems applications and clinical needs. The materials science field has unique tools and technologies that can benefit stem cell research and other biological applications. Attendees saw opportunities for CIRM to help promote interaction and foster collaborations between these complementary fields. CIRM has encouraged collaborative work in its Requests for Application (RFAs) to include engineering based strategies on a project-by-project basis and sends representatives to attend conferences with both biomaterials and stem cell science emphases. Recent RFAs, such as Tools and Technologies III (RFA 13-05), have identified tissue engineering and biomaterials-based approached as priority areas and the new External Innovation Pilot Program (RFA 13-04) includes the ability to help bring transformative research, which might include tissue engineering technologies, into California from outside the state. However, significant opportunities to promote further interaction between these vital communities still exist.

Recommendation #8: Facilitate dialogue with the FDA regarding regulation of tissue engineered products **(see full report Page 29).** Products utilizing tissue engineering strategies continue to move towards clinical application with some products already in trials and/or on the market. Many of these products have multiple components, which can complicate the regulatory pathway. It is important for the field to maintain an understanding of the current regulatory process and a dialogue with the FDA. Attendees felt that CIRM could play a valuable role in facilitating these discussions. In fact, CIRM is already serving in such capacity and regularly hosts webinars (available at: http://www.cirm.ca.gov/our-funding/regenerative-medicine-consortium) and participates in roundtables with the FDA on topics which have included some that are important for tissue engineering research and development. CIRM will continue to seek opportunities to further implement this recommendation.

While some of the workshop's recommendations are already represented within CIRM's portfolio, the agency will seek opportunities to expand on our support of these identified needs. These research topics may be considered as areas of particular interest in forthcoming Requests for Applications (RFAs).

CHAPTER 1: INTRODUCTION

The promise of regenerative medicine is the complete, functional repair of human tissue damaged by disease or injury. This vision includes directing the integration of transplanted cells into damaged tissue, replacing damaged structures with new tissues and organs generated *ex vivo* using cells and materials, and regenerating damaged structures by recruiting endogenous repair mechanisms *in vivo*. Advances in stem cell biology are central to achieving this vision, but certain therapeutic approaches require additional breakthroughs in tissue engineering.

The mission of the California Institute for Regenerative Medicine (CIRM) is to support the development of cures and therapies for human disease based on stem cell science. Since a combination of human stem cells, engineered products, novel drug and protein therapies, and breakthrough surgical techniques may all be required to realize this regenerative potential, CIRM is looking for new opportunities to enable therapeutic development. CIRM organized a workshop in January 2012 to explore the role of tissue engineering and biomaterials development in regenerative medicine, and to determine whether this field is appropriately supported by CIRM. The goals of the workshop were to overview recent research advances in the field of biomaterials and tissue engineering to understand how tissue engineering is contributing to stem cell science and regenerative medicine, and to outline the pathway to the clinic for therapies utilizing tissue engineering. Participants, including representatives from academia, government, and industry, were asked to recommend actions that CIRM might take to advance activities in tissue engineering that would promote stem cell therapy development. Outcomes of the workshop included recommendations related to scientific and technological barriers as well as needs related to communication and collaborative functions.

Tissue engineering (TE) historically referred to combining biological approaches to regenerative medicine with engineering and materials science. The field has evolved, however, and a consortium of federal regulatory agencies (the Multi-Agency Tissue Engineering Sciences Interagency Working Group) defined TE more broadly as “the use of physical, chemical, biological and engineering processes to control and direct the aggregate behavior of cells” (Multi-Agency Tissue Engineering Sciences Interagency Working Group, 2007). TE thus encompasses a variety of tools and approaches, ranging from developing synthetic or bioengineered scaffolds that recruit or enhance the body’s natural repair processes, to optimizing transplantation with products that direct the migration and integration of cells into damaged tissue, to creating three-dimensional tissues seeded with cells and built *in vitro*. Combined with a more recent focus on stem cells spurred by CIRM’s commitment to advancing regenerative medicine, California is positioned to become a global leader in developing regenerative therapies that use tissue engineering principles. Participants in CIRM’s Tissue Engineering Workshop highlighted several areas that, with CIRM support, could capitalize on this competitive strength and result in breakthroughs in both science and medicine.

TE offers tremendous therapeutic potential; however, integrating advances in with stem cell biology with bioengineering, which is the application of engineering principles to solve biological problems, has been challenging. Bioengineers, materials scientists, stem cell biologists, and clinicians often work in segregated environments. Furthermore, developing tissue engineered products and translating these products from the research lab to the clinic presents unique scientific, procedural, analytic, and regulatory challenges that make development of TE products challenging. The workshop was designed to consider these multidisciplinary challenges and develop options that CIRM could draw upon when designing strategies to support the role of tissue engineering in regenerative medicine.

CIRM WORKSHOP: TISSUE ENGINEERING APPROACHES

CIRM promotes therapeutic development in regenerative medicine by including tissue engineering strategies and seeks find ways to increase the representation of TE projects in its funding portfolio. For example, tissue engineering strategies are represented in CIRM's Basic Biology, SEED, Comprehensive, New Faculty, Tools & Technology, Early Translation, and Disease Teams RFAs. Thus, CIRM invited world experts in the field to share their research and advice. Workshop participants included basic researchers designing new biologically-responsive materials, industry veterans who have created TE products, and clinicians who have treated patients using tissue engineered products.. Recent RFAs have identified tissue engineering and biomaterials-based approached as priority areas and the new External Innovation Pilot Program (RFA 13-04) includes the ability to help bring transformative research, which might include tissue engineering technologies, into California from outside the state. In this report we summarize the opinions presented at the Workshop and consider CIRM's role in promoting scientific advances in tissue engineering.

Discussion centered on the following areas of critical importance to the field:

1. **Creating three-dimensional (3D) engineered tissue.** TE will be essential in cases where the organ that needs to be regenerated or repaired has particular spatial characteristics or a defined 3D architecture. Although some techniques are in use already, further research is needed to help develop methods that enable engineered tissues with complex 3D structures that improve engraftment or are required for the appropriate recruitment and/or differentiation of cells and/or stem cells.

2. **Creating natural and artificial scaffolds.** Scientists are developing new customized scaffolds with characteristics including tolerance to stress, reduced immunogenicity, and the ability to recruit endogenous growth factors and/or stem cells. Furthermore, these scaffolds are being refined in terms of their molecular or protein composition to suit the local environment in which they will be used and support the healing processes and enable tissue repair. Scaffolds can be:
    a. derived from donor tissue;
    b. engineered from synthetic materials in a lab;
    c. generated using a combination of biological and engineered materials.

Scaffold materials science is an active area of research. There is; however, still a need to generate scaffolds with different physical and biological characteristics as might be required for specific clinical applications, as the workshop participants demonstrated for various tissue and organs, such as for the heart, lungs, cartilage, and intestine.

3. **Recruiting cells to tissue.** Cells must be seeded on an engineered scaffold *in vitro* or recruited *in vivo* to a tissue that needs repair. Therefore, understanding the biological basis of cell migration, differentiation, and organogenesis, and the interaction of cells with engineered materials, is essential for developing biologically relevant products.

4. **Developing clinically relevant products.** The engineering of scaffolds and basic research on the cellular responses to scaffolds are essential for developing tissue engineered products. However, these products must be driven by a clinical need. The specific clinical application will determine the target cells and characteristics required of the scaffold or other engineered product to be used. Furthermore, the success of any tissue engineered product will depend on its penetration into a clinical setting. It is therefore critical to support multidisciplinary collaborations between researchers interested in developing tissue engineered products and clinicians or other experts who understand the clinical setting into which this product will be delivered.

5. **Additional factors that contribute to the commercial success of TE products.** TE products can present unique challenges to successful therapeutic development, not only from a scientific standpoint but also because their development, delivery and market profile is very different from pharmaceutical products. The workshop considered several factors that impact therapeutic success of TE products, including gaining insurance acceptance, meeting market expectations and clinical need profiles, and ensuring practitioner buy-in. Each of these should be considered when evaluating the potential impact of a TE product under development.

6. **Regulatory considerations that contribute to the success of TE products.** Tissue engineered products can present challenges to meeting regulatory requirements, in particular if they include multiple components. Depending upon their composition and primary mode of action, tissue engineered products may be regulated as biologics, devices, or combination products, so the regulatory approval path is not always clear. Tissue engineering shares with other aspects of the regenerative medicine field the regulatory challenge that some clinical indications do not have adequate preclinical models to prove the safety and efficacy of a product. Clinical trials and commercialization plans should be designed bearing the regulatory environment in mind. The workshop participants' consensus was that investigators must work closely with the FDA and patients to develop an acceptable risk tolerance profile and an achievable regulatory path to approval if these products are to be successful.

CHAPTER 2: ACTIVE AREAS OF SCIENTIFIC DISCOVERY IN TISSUE ENGINEERING

CIRM's workshop included discussions on a range of tissue engineering approaches (summarized in Table 1) including injectable, cell-free scaffolds or a thin layer of cells that support endogenous tissue recovery, as well as complex three-dimensional structures that need to be fabricated from synthetic or biological materials. In the more complex cases, scaffolds are seeded with autologous or allogeneic cells, matured in culture, and then implanted in patients. Finally, researchers are combining bioengineering and stem cell biology to engineer 3D environments that affect the differentiation and function of groups of cells. The workshop presentations and discussions are summarized below.

Table 1. Summary of tissue engineering approaches presented at CIRM workshop.

Hydrogels	Cell-Free Scaffolds and/or Combination Products
Synthetic peptide-based hydrogel to improve cell delivery	Mixture of natural material-based hydrogel with and without pre-seeded chondrocytes for cartilage repair
Decellularized heart derived hydrogel to promote endogenous cardiac repair	Vascular graft made from synthetic polymer scaffold with autologous endothelial or bone marrow cells
ECM peptide-derived hydrogel to promote endogenous repair	Engineered trachea made from decellularized cadaver trachea reseeded with autologous cells
Growth factor-releasing hydrogel to improve recovery from SCI	Bioengineered gut made from a synthetic polymer scaffold with donor cells
Blended natural materials-based hydrogel for retinal disease	
Growth factor-encoding viral vector released from natural material-based hydrogel for cartilage repair	

A. Three–dimensional Cell Scaffolding for Tissue Repair

The CIRM workshop opened with a discussion of 3D cell scaffolding. Tissue engineered products have traditionally involved seeding cells on a scaffold, a structure capable of supporting 3D tissue organization and development. Scaffolds can be of biological or synthetic origin, or a combination of both. They can be conjugated to bioactive material such as growth factors or extracellular matrix components. They can be seeded with autologous or allogeneic cells, used without pre-seeding, and/or can recruit endogenous cells. Workshop participants discussed research needs and opportunities in materials development, product design, and integration of scaffolds with host tissue.

A common source material for scaffolds is extracellular matrix (ECM) derived from tissues. Decellularized natural scaffolds are generated by treating native tissues or complex organs with detergents to remove cellular material (a process called decellularization), leaving behind the underlying, acellular ECM scaffold, which can then be reseeded with healthy autologous or allogeneic cells (Atala, 2012; Badylak et al, 2012). This approach was recently applied clinically to engineer a replacement trachea by decellularizing a cadaveric donor trachea and reseeding it with autologous epithelial cells and chondrocytes from a patient with end-stage bronchial disease. Dr. Martin Birchall presented his experience conducting tracheal replacement therapy on a patient-specific basis in Europe. Tissue was produced from a human decellularized scaffold along with the patient's stem cells and successfully implanted with no immunosuppression (Macchiarini et al, 2008; Bader & Macchiarini, 2010). Dr. Birchall indicated that three patients have successfully undergone this procedure, though two of which died due to progression of the underlying disease process, despite technically successful grafts and excellent airway function. The growing body of literature using this approach includes results of a two-year follow-up study of the first successful tissue engineered tracheal replacement in a child (Elliott et al, 2012).

Dr. Birchall indicated that integrating leading edge TE technologies with standard medical procedures to create new organs, particularly hollow organs, could address a critical need in the field of tissue transplantation. There are many drawbacks to the current method of allogeneic organ replacement, including a shortage of organs suitable for transplantation, complicated ethical considerations, and the requirement of longterm immunosuppression. Transplantation of complex functional organs such as heart, lung, liver, (Orlando et al, 2011) and kidney (Song et al, 2013) using decellularized and reseeded natural scaffolds have already been successfully generated in rodent models. Although many challenges remain in generating complex functional organs by this approach, combining TE and stem cell approaches is advancing the generation of hollow organs.

The 3D architecture of decellularized ECM does not need to be preserved for the product to be useful clinically. Whereas Dr. Birchall's approach preserves the shape and size of the donor tissue, Dr. Karen Christman processes heart tissue into a novel scaffolding material. Dr Christman has developed an injectable, decellularized ECM derived from porcine myocardium as a scaffold for cardiac tissue engineering. These injectable myocardial matrices (IMMs) undergo self-assembly after transplantation into a rat model of myocardial infarction, are well tolerated, and trigger a relatively low immune response (Singelyn et al, 2009; Singelyn & Christman, 2010). Endothelial cells and smooth muscle cells migrated towards the matrix and arterial formation increased. Furthermore, ckit+ stem cells (putative cardiac stem cells) were found inside the material. In the study, the matrix was injected through a clinically used catheter with no affect on the matrix's mechanical properties using this delivery method, suggesting the material could be used in a minimally invasive procedure (Singelyn et al, 2009).

Although there are numerous examples of promising therapies based on natural ECM-based scaffolds, there are some issues with natural ECM-derived scaffolds that must be

considered when using such materials. The ECM is inherently heterogeneous and comprised of large, complex fibrous proteins and proteoglycans with numerous isoforms, splice variants and glycoforms that can make the molecules difficult to purify (Little et al, 2008; 2011). They contain a variety of signaling motifs that are not well understood, making lot-to-lot variability a challenge. Finally, they can be limited in supply and are prone to contamination by pathogens and immunogens, making them unfavorable targets for therapeutic development (Little et al, 2008; 2011).

Some researchers are addressing the challenges endemic to using natural ECM scaffolding by developing synthetic platforms with the desired physical and biochemical properties of ECM. Dr. Christopher Breuer presented promising data using a composite vascular graft being developed for the treatment of congenital heart diseases in children (Mirensky & Breuer, 2008). The graft consists of a biodegradable polyglycolic acid scaffold which can be seeded with autologous endothelial cells or bone marrow mononuclear cells (Hibino et al, 2011; Roh et al, 2008). Using a preclinical mouse model, Dr. Breuer showed that seeding a graft with autologous bone marrow cells led to a notable improvement in its performance (Hibino et al, 2011). However, he also observed that the seeded cells do not form the bulk of the regenerated tissue. Instead, the grafts become populated with endogenous cells, indicating that transplantation of engineered tissue constructs seems to augment the body's own repair mechanisms. Dr. Breuer is in the process of testing these seeded grafts in human patients.

Synthetic scaffolds are an active area of exploration in the field. Dr. Birchall, for instance, indicated that artificial scaffolds are being developed to replace the decellularized cadaveric scaffolds for engineered tracheal use, although current experience indicates that it is more difficult to induce appropriate cell growth on these scaffolds than on natural scaffolds. This observation seems to be dependent upon the tissue of interest; however, since Dr. Tracy Grikscheit's laboratory is using a synthetic scaffold to develop a bioengineered gut. In Grikscheit's therapeutic model of intestinal resection, multicellular clusters of epithelium and mesenchyme from a donor animal were cultured on a biodegradable polymer scaffold made from bio poly-L-lactic acid or polyglycolic acid (Sala et al*,* 2009; 2011) to yield an engineered intestine that was surgically implanted into a host. Dr. Grikscheit found that transplantation of implants that included intestinal crypts regenerated normal-looking small intestine in both rodents and pigs, as assessed by histology and immunohistochemistry. The transplanted tissue differentiated into multiple cell types including muscle, nerve and epithelial cells, probably because the stem cell niche was preserved in the intestinal crypt transplants. Interestingly, both donor and host cells contributed to regeneration, suggesting that these multicellular organs stimulate both exogenous cell replacement and endogenous repair mechanisms (Sala et al, 2009; 2011).

In another example of a new synthetic products being tested in animal models, Dr. Robert Sah highlighted the value of 3D tissue engineering using synthetic scaffolds to replace articular cartilage, a tissue with complex physical and functional requirements. Articular cartilage is the smooth white tissue that covers the end of bones and joints and is essential for allowing bones to glide over each other with little friction. Since articular

cartilage bears significant weight over an individual's lifetime and has a high incidence of injury, degeneration or damage of this tissue is common. Slow and often at times poor mechanisms of self-repair result in significant long-term effects on human mobility and quality of life (Williams et al, 2010). Depending on the nature of the cartilage damage, different repair strategies might be required. Currently available treatments include microfracture surgery (a technique in which tiny fractures in the underlying bone are made to form a blood clot with cells that help replace the damaged articular cartilage), other surface treatments that may be used to repair superficial cartilage damage, or cell-based tissue or osteochondral implants to replace more severely damaged tissue (Williams et al, 2010). Given the nature of the tissue, bioengineered devices for cartilage repair must use a material that 1) can fill the defect and bear significant weight load, 2) has adhesive properties to facilitate integration with the surrounding environment and 3) has anti-adhesive properties to facilitate the maintenance of a low-friction surface (Sah, 2004).

Dr. Sah discussed his laboratory's efforts to design and test bioengineered osteografts to replace damaged articular cartilage (Han et al, 2010; 2012). These osteografts are composed of chondrocytes in a dissolvable 3D scaffold made of agarose, collagen and proteoglycans (Han et al, 2010; 2012). His lab has worked extensively to determine the optimal proportion of cells to scaffold components, which is critical for promoting both the desired physical properties of the graft and integration into the surrounding tissue. This ratio can also affect the durability of the graft. Although the first products developed consisted of a cell-matrix mix, Dr. Sah's approach has evolved to implanting cells and matrix as a compacted product using a method called Tissue Engineering by Assembly which appears to be a promising approach for replacing tissues with complex physical and biological characteristics such as articular cartilage.

> **<u>Recommendation #1</u>: Support research using scaffolds pre-seeded with cells for transplantation as well as cell-free scaffold approaches.** If tissue engineering is to progress, both cell-seeded and cell-free scaffolds approaches must be studied and developed further. These approaches must utilize scaffolding that integrates into the transplant site. Furthermore, the scaffolding must help create a microenvironment that is conducive for the delivered cells to thrive and/or be able to recruit the appropriate cells to the scaffold, depending on the therapeutic approach.

B. Hydrogels for Cell Delivery and Tissue Repair

Hydrogels have become increasingly attractive substrates for tissue engineering. These materials consist mostly of water with a small amount of natural or synthetic material, so they allow for good diffusion of oxygen and metabolites. They can be chemically modified to carry growth factors or other chemical moieties important for cell performance, and they can be molded into different shapes to optimize tissue performance. Hydrogels can be used either as scaffolding for 3D implants or as injectable carriers for improving cell transplantation and tissue repair. During injection, they can help protect cells from mechanical stress, localize them to the transplantation

site, and direct their organization and differentiation *in vivo*. Two entire sessions of the CIRM workshop were dedicated to describing different uses for hydrogels in tissue engineering, reflective of the tremendous growth and therapeutic potential in this area of research. Table 2 summarizes the hydrogel approaches presented at the workshop.

Table 2. Summary of applications of hydrogels presented at CIRM workshop.

Hydrogels	Intended Application
Synthetic peptide-based hydrogel	Improve cell therapy approaches by maintaining cell viability during delivery and retaining cells at the transplantation site
Blended natural materials-based hydrogel	Improve cell therapy to treat retinal disease
Decellularized heart ECM-derived hydrogel	Inject into the heart post-MI to promote endogenous cardiac repair
ECM peptide-derived hydrogel	Inject into the heart post-MI to promote endogenous cardiac repair
Growth factor-releasing hydrogel	Improve recovery from spinal cord injury
Growth factor-encoding AAV released from natural material-based hydrogel	Improve cartilage injury repair

Dr. Sarah Heilshorn, whose laboratory at Stanford University designs novel biomaterials for cell transplantation via surgical implantation or direct injection, presented studies using bioengineered hydrogels to improve survival and retention of injected stem cells. Her group has developed protein-based hydrogels that self-assemble and are fully resorbable. Dr. Heilshorn highlighted the flexibility and advantages of hydrogels for a variety of applications (Aguado et al, 2012; Wong Po Foo et al, 2009). Due to their high water content, hydrogels can easily accommodate a payload such as cells, drugs, or growth factors. Hydrogels also have mechanical properties similar to native tissue, which greatly impacts cell behavior. In the protein-based hydrogel developed by the

Heilshorn laboratory, the mechanical properties can be controlled by modifications to the peptide sequence of the hydrogel components, which may be important for adapting the product for use with different cell types (Wong Po Foo et al, 2009).

Dr. Randall Lee from UCSF presented studies injecting hydrogels with ECM-derived peptides to promote angiogenesis and recruit myofibroblasts for myocardial regeneration. His research found that injection of algysil-based hydrogel improved ventricular geometry and diastolic/systolic function in rat and dog models of dilated myopathy (Lee et al, 2012). Preliminary results from first-in-human studies show decreased ventricular wall stress as well as reduced ventricular ectopy, indicating a promising safety profile as well as the potential efficacy of the approach (Lee et al, 2012).

Dr. Molly Shoichet's group is developing injectable hydrogels to improve regenerative therapies to treat vision loss and nervous system degeneration (Ballios et al, 2010; Pakulska et al, 2012). Degeneration of retinal photoreceptors is a devastating cause of vision loss in disorders such as age-related macular degeneration, retinitis pigmentosa and diabetic retinopathy. Dr. Shoichet showed data evaluating the effects of a blend of hyaluronan and methylcellulose (HA-MC) on the viability of transplanted cells for the ultimate treatment of vision loss. HA-MC combines the shear thinning properties (the ability for a fluid to increase its ability to flow as more force is applied) of hyaluronan with methylcellulose that gels upon change in temperature. Addition of HA-MC to cells during transplantation decreased cell aggregation and improved survival of retinal stem cells and their progeny transplanted into the subretinal space (Ballios et al, 2010; Pakulska et al, 2012). Dr. Shoichet's group is using this model to identify molecular factors, such as CD44, that may provide a mechanistic understanding of cell survival and integration in this model. Injectable scaffolds thus can help stabilize and distribute cells during transplantation, may be essential for protecting therapeutic cells from shearing forces during transplantation, and may promote cell survival and function.

Hydrogels can also be used to deliver tissue explants that have been constructed in culture on a hydrogel-based scaffold. Dr. Milica Radisic's group is comparing the performance of engineered patches using a combination of cells and either hydrogels or biomaterial scaffolds for healing cardiac infarct scars (Tandon et al, 2009; Radisic, 2004, 2008). Dr. Radisic showed that hydrogel-based bioengineered patches seeded with iPSC-derived cardiomyocytes beat synchronously and are capable of propagating electrical signals (Tandon et al, 2009; Radisic, 2004, 2008). The group is experimenting with providing gradients of growth factors to prevent necrosis in the center of large cell patches to enable scaling up and translation of the technology to more clinically relevant studies (Odedra et al, 2011).

Dr. Constance Chu has been experimenting with methods to deliver TGFβ1 to promote cartilage repair during microfracture, which is the current standard of care procedure to treat cartilage defects. In microfracture, small puncture wounds are made in the bone face of the affected knee to induce bleeding from the bone marrow into the joint and leads to the formation of a clot, which serves as a scaffold for chondrogenesis from bone marrow mesenchymal stem cells. Although this surgical procedure is the treatment-of-choice to repair superficial cartilage damage, the results are mixed, as some patients have lower levels of successful chondrocyte differentiation. TGFβ1 is well known for its chondrogenesis-stimulating properties (Pagnotto et al, 2007), so her approach is aimed at boosting chondrocyte differentiation by increasing the concentration of this factor at the site of repair. Dr. Chu originally tested a delivery method of TGFβ1 using smart polymers based on hydrogels made from polyethylene glycol (PEG) modified with genipin, but these were unable to sustain local delivery to the damage site. An alternative gene therapy approach delivering TGFβ1-encodingadeno-associated virus has proved more viable. When combined with a fibrin scaffold, this approach offers some important advantages over cell-based approaches for cartilage repair, namely the direct transduction of host repair cells while limiting the

disadvantages of indiscriminate transduction and the requirement of expensive ex vivo manipulations (Lee et al, 2011).

> **<u>Recommendation #2</u>**: **Support the development of hydrogels that mimic natural tissue properties.** Hydrogels are being tested in a wide range of regenerative medicine applications and represent an important funding focus for CIRM. Hydrogels present an opportunity for therapeutic approaches where traditional, more rigid scaffold may not be appropriate. The physician, scientist, or engineer devising the therapy must distinguish the important considerations of a particular disease state and determine whether a hydrogel would give the best chance for an effective therapy.

C. Synthetic Niches to Enhance & Direct Stem Cell Fate

The ultimate goal of the tissue engineering field lies with *in vivo* applications, but TE-based approaches could have profound impacts on stem cell studies *in vitro*. A supportive 3D architecture contributes significantly to the development and proper function of differentiated cells. Both rigid and hydrogel scaffolds are particularly important when considering how to scale-up the production of differentiated cells or 3D tissues. An active area of research in bioengineering involves developing 3D scaffolds that support *in vitro* studies regarding stem cell growth and differentiation.

A common strategy for *in vitro* studies is the incorporation of growth factors and morphogens embedded within the extracellular environment. This can occur via interaction of specific domains of the trophic factor with ECM molecules (e.g., heparin-binding domains that link to collagen and fibronectin) or by the direct anchoring of growth factors to cell membranes. The immobilization of these trophic cues can be important for creating environmental niches by increasing the local concentration of a protein or by establishing temporal or spatial gradients of trophic factors (Eshghi & Schaffer, 2008). The ECM has an important and well-accepted role in establishing the extracellular milieu. The elucidation and recapitulation of the molecular mechanisms underlying growth factor gradient development, as well as how the ECM and the extracellular environment work individually and together to support the proper growth and function of cells and tissues, was an area explored during the workshop.

As with *in vivo* TE approaches, synthetic scaffolds are valuable in cell culture models. A number of cell culture studies are examining the properties of protein versus polysaccharide-based biomaterialsand synthetic scaffolds on growth factor release kinetics (Willerth & Sakiyama-Elbert, 2008). These different biomaterials, when seeded with stem cells, can promote the differentiation of a variety of cell types *in vitro.* Dr. Shelly Sakiyama-Elbert has shown that fibrin-based hydrogel scaffolds formulated with the appropriate growth factors support differentiation of embryonic stem cell (ES)-derived neural stem cells into neurons and glial cells (Willerth et al, 2008; McCreedy & Sakiyama-Elbert, 2012). Dr. Sakiyama-Elbert has shown that growth factors can be incorporated into the scaffolds and are released in a controlled manner, resulting in cell

differentiation over a period of days to weeks without having to add any factors to the cell culture media itself (Willerth et al, 2008). This is a critical advance when considering the implantation of scaffolds into a native environment, where they would be expected to support cell differentiation over a protracted repair period *in vivo*. Furthermore, a scaffold that can be formulated for slow and controlled diffusion-mediated release more closely mimics how endogenous growth factors are presented within the ECM during development. Dr. Sakiyama-Elbert showed data on successful implantation of these scaffolds *in vivo* following spinal cord injury, demonstrating proof of concept for the possible clinical application of these observations (Johnson et al, 2010a; 2010b).

ECM-based hydrogel scaffolds are comprised of polymer backbones that can be functionalized with adhesion motifs, and can be used to simulate the mechanisms by which the ECM governs cell fate decisions (Keung et al, 2010a, 2010b). Dr. David Schaffer's lab designs ECM-like scaffolds that allow for precise control over the material architecture and the presentation of bioactive ligands. Recent studies in his lab were devoted to screening bacterial peptide display libraries to identify and characterize bioactive peptides that support neural stem cell culture (Little et al, 2011). Furthermore, his group found that lipid bilayers functionalized with peptide sequences with known adhesive qualities (e.g. various RGD peptides) display differential efficacy in promoting the differentiation of neural stem cells (Ananthanarayanan et al, 2010). His group is also characterizing components from ECM and on cell membranes that support the growth and differentiation of stem cells. Studies such as these could yield fully synthetic and defined culture systems that recapitulate the stem cell microenvironment or induce tissues to differentiate, while allowing for control of the purity and reproducibility of the scaffold material.

The ability to direct the differentiation of stem cells in an efficient and reproducible manner is essential for the success of regenerative therapies. One method to affect differentiation is by modulating the geometry of the cellular microenvironment (Karp et al, 2007; Mohr et al, 2006; Keung et al, 2010a). In culture, 100 μm-square microwells are able to maintain the integrity of human embryonic stem cell colonies for weeks, without inducing spontaneous differentiation, and yield embryoid bodies (EBs), or 3D aggregates of embryonic stem cells, of uniform size that exhibit lower differentiation variability compared with those generated in typical suspension cultures (Mohr et al, 2006; Karp et al, 2007). Recent studies have also found an important relationship between mechanical stimulation and changes in gene expression that influence the differentiation state of stem cells (Engler et al, 2006; Hwang et al, 2007).

The most common differentiation method in use today, spontaneous self-assembly of ESCs to form EBs, does not allow for precise spatial and temporal control of the 3D microenvironment and tends to yield a heterogeneous mixture of stem cells and progenitor or differentiating cell populations (Bratt-Leal et al, 2009; Kinney et al, 2011). Although cellular heterogeneity might be essential for producing complex tissue, there is great value to increasing our ability to direct cellular differentiation. At the workshop, Dr. Todd McDevitt gave an overview of these issues while presenting work from his own lab, which focuses on understanding the importance of the 3D microenvironment in cellular

differentiation. The goal of this research is to increase our ability to direct stem cell differentiation along specific paths and simultaneously improve the scalability of cell production to the large capacity that will be needed for clinical applications. Dr. McDevitt's lab uses biodegradable microparticles to deliver morphogenic factors directly within EB microenvironments in a spatially and temporally controlled manner. His lab delivered biodegradable microspheres, made from the widely utilized polymer PLGA, coated with retinoic acid into EBs and found that the microspheres promoted the formation of spheroids that phenotypically and morphologically resembled mouse embryos at the early primitive streak stage, where gastrulation and germ layer formation occur (Carpenedo et al, 2009). This discovery could be adapted to deliver any number of critical morphogens for directed differentiation with the precision and control required for clinical applications.

Dr. Kyriacos Athanasiou highlighted the importance of considering mechanical factors in the development of tissue engineered products for cartilage repair. Since biomechanical factors have profound effects on cell differentiation in the tissue microenvironment, it is likely that matching key factors, such as the matrix stiffness, to the therapeutic target tissue may help direct the differentiation of cells to desired lineages and optimize their function *in vivo* (Keung et al, 2010a,b). During development, biomechanical stimuli—such as hydrostatic pressure or compression—critically promote articular cartilage development by enhancing the re-differentiation of chondrocytes and promoting the chondroinduction of other cell types (Responte et al, 2012). Not surprisingly, static culture-based approaches are typically inferior and produce constructs that lack the full functionality of cartilage shaped by both growth and mechanical factors. To address these issues, Dr. Athanasiou described his laboratory's efforts to develop scaffold-free, self-assembled 3D cartilage constructs generated through a combination of cell culture and application of growth factors and mechanical stimuli (Hu & Athanasiou, 2006; Elder & Athanasiou, 2008). This approach begins with chondrocytes seeded at high density in agarose molds, which facilitates endogenous intercellular adhesion and chondrocyte self-assembly. The cells eventually become embedded in a scaffold derived from chondrocyte-secreted ECM molecules. When this nascent cartilage is exposed to hydrostatic pressure, and in combination with added TGFβ1, the resulting construct exhibits compressive and tensile properties that resemble native cartilage that is capable of holding a suture (Elder & Athanasiou, 2008). His group has also shown that skin cells can be coaxed into producing cartilage using a similar combination of approaches (Deng et al, 2007).

Using the 3D physical microenvironment as a tool to control cell differentiation is an alternative to the conventional approach of diffusion-based chemical modification of the cellular environment (i.e., by adding growth factors directly into the tissue culture media), and offers significantly improved spatial control and reproducibility (Willerth & Sakiyama-Elbert, 2008). Furthermore, there is increasing evidence supporting the importance of the physical microenvironment in stem cell transplantation, not only for providing soluble trophic support but also in delivering specific biophysical cues—such as tissue stiffness, shear stress, repetitive stretch (during pulsatile blood flow), and compressive impacts—that strongly influence cell behavior (Willerth & Sakiyama-Elbert,

2008; Keung et al, 2010a, b). A current focus in the field is to further understand the role of a 3D culture environment and biomechanical factors in tissue formation and function. The results of these studies may impact stem cell biology but will also lead to new paradigms that can be applied to developing regenerative approaches *in vivo*.

> **<u>Recommendation #3</u>: Develop biomaterials-based methods to model stem cell niches in vivo to enhance and direct stem cell expansion in vitro.** Biomaterials-based techniques and tools present a major opportunity for scientists and engineers to elucidate the biology of the stem cell niche *in vivo* using *in vitro* means. Understanding the stem cell niche better will ultimately help aid in the development of more efficacious stem cell expansion in vitro, and *ex vivo*, as well as developing more efficient means to modulate stem cell fate.

CHAPTER 3: BIOLOGICAL FACTORS AND ANALYTICAL TOOLS THAT IMPACT TISSUE ENGINEERED PRODUCTS

Once a tissue engineered product has been conceptualized and developed, the product must be tested for safety and efficacy before it can be advanced to the clinic. This requires animal models, tools for tracking engraftment and survival, and consideration of the immunological profile of the product. Participants in CIRM's workshop on tissue engineering discussed the approaches and tools needed to evaluate the scientific merit of a TE product before therapeutic development could be warranted.

A. Analytical Tools: *in vivo* Cell Tracking and Monitoring Biological Activity

Many of the standard preclinical assessment tests used in other fields might be applicable to cell therapy, including assessment of the cardiac, neural, liver, and reproductive toxicity profiles of each product. However, there are evaluations that are more specific to TE products, and some of the tools to conduct these evaluations remain to be developed. For instance, there are currently no good methods for real-time, non-destructive, high-content assessment of the health and stability of an engineered tissue either *in vitro* or *in vivo*. Dr. Athanasiou indicated that a need in the field and an active area of research is the development of new analytical techniques to monitor successful engraftment and function of engineered tissues. Techniques are also needed to evaluate host responses to the implant including inflammation, apoptosis, cell trafficking and gene expression. These tools might incorporate intelligent nanosensors, which can non-invasively sense particular chemical signals indicative of their respective cellular events, into engineered tissues to monitor tissue behavior. More research is required to characterize the optimal approach for implantation of these devices into the joint environment. In addition, Dr. Sah highlighted the need for better quantitative tools for testing TE products, such as polarized light microscopy to determine fiber/tissue orientation and 3D imaging for evaluating repair processes at the joint level *in vivo*.

> **Recommendation #4: Develop better quantitative tools for testing tissue-engineered products.** The development of these tools will enable characterization of materials in a TE product and assessing cell quality. In preparing for clinical trials, for instance, scale-up of current good manufacturing processes (cGMP) must incorporate the use of appropriate assays to ensure that the resulting cell product has maintained its biological activity.

B. Preclinical Models for Testing Tissue Engineered Products

Another area of development in TE is determining the appropriate combination of animal, cell culture, and human models in which to test a new product. Experience in engineered tissues indicates that TE products, irrespective of the presence of cells within the scaffold before implantation, often fail *in vivo* because of a lack of functional integration with native tissue. Integration of engineered and host tissues is a challenging area for translational of TE products, as the human response cannot always be adequately modeled in animals. Workshop participants indicated that it is essential to invest resources into developing preclinical animal models that may better predict the

behavior of a product in human patients. In turn, robust and predictive preclinical models will positively impact the development of TE-based approaches by making the process more efficient. However, it was noted that in some cases first-in-man studies were essential to test the safety and efficacy of TE products.

Dr. Chu raised the issue of appropriate model systems specifically in the context of tissue engineered cartilage therapies, describing the advantages and disadvantages of a number of animal systems that are commonly used. Small animal (e.g. mouse, rat and rabbit) models offer certain significant advantages, namely, the ease of access to experimental material and, in the case of mice, the availability of genetically modified strains, which can facilitate mechanistic in vivo studies. However, the small joint size, thin cartilage and greater propensity for intrinsic healing limit the translational value of some small animal models (Chu et al, 2010). In addition, the history of predicting safety and efficacy in small animal models has not been very strong, particularly since rats, rabbits and mice are inherently more resistant to infection than humans. Studying immune processes in animal models such as the rat or rabbit, for instance, may not yield sufficient insight to guide best practices in humans (Frey-Vasconcells et al, 2012).

Larger animals, while carrying greater logistical, financial and ethical considerations, can be of great value for evaluating cartilage therapies. Dr. Chu described the value of testing these approaches in equine models, which, similar to humans, can suffer from osteoarthritis, develop cartilage-based injuries, and exhibit low intrinsic capacity for repair (Chu et al, 2010). Importantly, equine models permit researchers to study cartilage defects that approximate the size, depth and complexity of those observed in humans, noting that the tissue closely resembles its human counterpart. When used judiciously, larger animal models will be of great translational value for testing these such tissue engineered products to the clinic.

Unless more appropriate models are developed, workshop participants agreed that researchers must take great care when using animals to model human reactions to devices with respect to infection. Dr. Birchall brought an interesting perspective to this discussion by highlighting differences in the regulatory path of tissue engineered products between Europe and the U.S. In Europe, regulations requiring extensive preclinical testing in animals are being relaxed and first-in-human models are becoming increasingly common. In cases of urgent medical need, physicians can approach local ethical committees to request approval of experimental therapies to treat individual patients. This means that Europe is attracting some of the most experimental TE researchers and physicians, and making new therapies available to patients relatively quickly. Many researchers at the workshop agreed that this approach might be more useful for evaluating the safety and efficacy of TE products. Since this approach is not currently feasible in the United States, participants highlighted the value of collaborations with European scientists and continued advocacy with the FDA.

C. Tissue Engineering and the Immune Response

A universal consideration for TE products and their translation to the clinic is the immune system's foreign body response (FBR), which is triggered soon after implantation at any blood-biomaterial interface (Anderson et al, 2008; Higgins et al, 2009). The FBR is the end-stage result of a series of immunological and vascular reactions by the body to foreign objects and may ultimately result in the degradation of the implanted device. Dr. David Grainger gave an overview of the FBR and highlighted its importance to tissue engineered products and regenerative medicine.

In short, the FBR culminates with a "privileged microenvironment" that exists for the lifetime of any implanted product. Rather than protecting the implant, the reactivity of immune cells and the bioactive agents that they release in this space can create enormous problems for the function of the implant. Infection can take hold and immune cells can secrete inflammatory mediators, cytokines, reactive oxygen species and metabolic acid that can lead to degradation of the implant biomaterial. Growth and angiogenic factors secreted by macrophages can also influence ECM remodeling, which constitutes another factor that can influence biomaterial performance (Anderson et al, 2008; Higgins et al, 2009).

An important message from Dr. Grainger's presentation and the associated discussion was that many, but not all, implants trigger a foreign body response, leading to fibrotic capsule formation and/or the toxic byproducts of phagocytosis accumulating at the implant site. Therefore, a major outstanding question is how to consider and minimize this response when designing tissue engineered products. One approach is to include small amounts of antioxidants, an approach that has been taken with medical devices containing additional polymers such as polyethylene (Anderson et al, 2008). Biodegradable materials appear to be more successful at limiting the FBR than permanent implants, leading Dr. Grainger to suggest that resorbable materials should be used wherever possible. When resorbable materials cannot be used, Dr. Grainger felt it would be essential to develop implants that foil the FBR to the extent possible.

Infection is important outside of the context of a reaction to the biomaterial. The human body has many trillions of cells but ten times more commensal bacteria. Given that no environment, either at the device-manufacturing level or in the operating room, is truly sterile, the likelihood of infection is high and steps should be taken to minimize the risk of implant contamination wherever possible. An approach that was discussed at the workshop was to coat the surface of devices with "probiotic" bacteria that could out-compete the colonization of more damaging strains, thereby protecting the device.

A final immunological consideration in evaluating a TE product containing cells is rejection. Immunosuppression is a challenging insult for patients, but it is currently the only available tool to avoid rejection of allogeneic cells. CIRM hosted a previous workshop on this topic, which resulted in the release of a specific Request for Applications for Stem Cell Transplantation Immunology projects. Immune rejection was, therefore, not discussed in depth at this workshop which was focused on tissue engineering. The report from that and other CIRM workshops, as well as commissioned

reports, are available at http://www.cirm.ca.gov/our-funding/publications-cirm-meetings-and-workshop.

> **Recommendation #5: Develop materials that mitigate immune response and enhance engraftment.** Better understanding the immune response to tissue engineered products presents an area of opportunity for CIRM and for the field as a whole. In some sense, each of the previous technical recommendations plays a role in the elucidation and expansion of this area of study. Whether a cell-seeded or cell-free rigid polymeric or hydrogel scaffold is chosen, the patient's immune response should be considered. Furthermore, better knowledge of the niche, characterization tools, and *in vivo* and *in vitro* model systems may aid in understanding the human immune response.

Table 2 below summarizes approaches currently under development for engineered tissues/organs and engineered functions that were presented at the workshop.

Table 2. Current Tissue Engineering Projects Under Development			
		Approach	**Advantage(s)**
Engineered Tissue or Organ	**Articular Cartilage**	Dissolvable 3D scaffold made of agarose, collagen and proteoglycans seeded with chondrocytes	Can recreate the complex biological and mechanical environment of the native tissue
		Fibrin+PEG+genipin hydrogel with TGFβ1 adeno-associated virus	Direct transduction of host repair cells while limiting indiscriminate transduction
		Scaffold-free, self-assembled 3D cartilage constructs generated through cell culture + application of growth factors + mechanical stimuli	Allows recapitulation of the *in vivo* microenvironment for articular cartilage formation
	Blood Vessel	Biodegradable polyglycolic acid (PGA) scaffold seeded with autologous endothelial cells or bone marrow mononuclear cells	Autologous approach high degree of integration with host tissue
	Cardiac Tissue	Decellularized porcine heart ECM matrix processed into an injectable form	Minimally invasive delivery with low immunogenicity
		Injectable alginate-based hydrogels with ECM-derived peptides	Minimally invasive delivery
		Hydrogels using poly-(glycerol sebacate), collagen, fibrin or biodegradable polymers or combinations	Scale-up of process is achievable
	Intestine	Biodegradable scaffold with poly-L-lactic acid and polyglycolic acid cells?	Microenvironment is preserved; promotes integration of donor cells with host tissue
	Retinal Photoreceptors	Shear thinning hydrogel blend of hyaluronan and methylcellulose	Protects therapeutic cell suspension from shear stresses and improve cell survival
	Trachea	Decellularized scaffold with patient's cells	No immunosuppression
Intended Biological Function	**Cell Delivery / Cell Retention**	Customized, resorbable two component peptide-based hydrogel for stem cell survival and retention	Highly adaptable platform technology
		Fibrin-based hydrogel with incorporation of growth factors for differentiation of hESC-derived neural stem cells into glial cells or neurons	Controlled release of growth factors from the scaffold, thus eliminating the need to add growth factors to culture media
	Differentiation & Growth	Biodegradable poly-(lactic-co-glycolic acid) microparticles coated with retinoic acid to deliver morphogenic factors for spatial/temporal control of embryoid body microenvironment	Highly adaptable for controlled delivery of various critical morphogens for directed differentiation in clinical applications
		ECM-like hydrogels with bioactive ligands for the growth & differentiation of neural stem cells	Immobilization of trophic or growth factors creates temporal and/or spatial gradients which help re-create the native microenvironment
	Immune Modulation	Resorbable biomaterials and/or integration of antioxidants for tissue engineered implants	Mitigates foreign body response to implant

CHAPTER 4. CONSIDERATIONS FOR TRANSLATION OF TISSUE ENGINEERED PRODUCTS

> **Recommendation #6: Help educate researchers about the translational considerations of cell-scaffold combination products.** The interdisciplinary nature of TE products has the potential to complicate the process of filing for FDA approval, as TE products sometimes straddle the traditional divide between biologics and devices. Additionally, practical considerations influence the translation of TE products to the patient bedside. For instance, many TE-based medical products must be implanted surgically so, in addition to being effective and easy to implant in the patient, they must be designed for easy storage, manipulation, and sometimes even assembly in the operating room. Helping researchers understand some of these considerations early in the development process will streamline the translational process.

Developing a tissue engineered product is a long and costly process. Some of the necessary steps to successfully commercialize a development candidate include: extensive basic research into human biology and materials science, knowledge of the possible molecular and cellular mechanisms-of-action, preclinical testing in animal models, and first-in-human clinical trials. In addition to these considerations, stem cell/TE-based products face additional challenges along their development path. The ability to efficiently differentiate stem cells into some desired cell types is limited, which presents challenges both to basic research and product manufacturing. Promising new TE products combine biological material with cell-free scaffolds or matrices, but design of these products depends on the combined expertise of cell physiology, matrix chemistry, and scaffold physics, which are disciplines that traditionally do not interact with one another.

The characteristics of TE products are forcing developers to look for new models of product development and commercialization and to chart new paths through the regulatory process. In this chapter we summarize the experiences of scientists and venture capital investors (VCs) who have been involved in the development of therapeutic TE products as case studies of some of the specific challenges faced in commercializing these products.

A. Challenges involving interdisciplinary collaborations

Developing and commercializing a TE product requires a wide range of experts. Basic scientists who study tissue growth and stem cell differentiation and the engineers who develop new biomaterials and devices lay much of the foundational work, and collaborations between the two fields are and will continue to be critically important.

> **Recommendation #7: Promote interaction between the stem cell and biomaterials communities.**

> Participants discussed the importance of fostering collaboration between these disciplines to help develop innovative tissue engineered products that are aligned with biological systems applications and clinical needs. The biomaterials community has unique tools, such as three-dimensional degradable scaffolds and sustained-release systems, which can be useful in addressing complex biological challenges. Stem cell biologists, meanwhile, have the biological resources and model systems to which biomaterials can be applied. It is critically important to continue to promote interaction between these two fields to encourage collaboration.

With basic researchers conducting early phase discovery science, at the other end of the spectrum are the clinicians and surgeons who apply new therapies and surgical approaches to patients. In the middle ground, applied scientists and engineers attempt to translate basic research discoveries into a product and ultimately, large-scale production. In addition to scientists, clinicians and engineers, the commercial success of a TE product requires a firm grasp of FDA regulations, sound business development, a good understanding of healthcare costs and economics, and the successful navigation of the private- and public-funding environment. This complexity leads to great potential for dead-ends, such as promising products that fail to gain FDA approval or engineered tissues that surgeons find too difficult or impractical to use on their patients. It also drives VCs to invest in products at later stages of development. Meeting participants stressed the need to promote interactions between the people involved at each step of the development process, so that they become better informed of the potential challenges and can better anticipate and mitigate issues along the way.

Many speakers emphasized that surgeons should be involved as early as possible in the product development process. A new TE product will only be successfully applied to patients, and therefore commercially viable, if surgeons are willing and able to develop the skills to implant them. While TE cartilage, for instance, may be successful in laboratory and animal models, it must be a product that surgeons can easily and readily use in the operating room. It is also important for researchers and developers involved in the early steps of product development to be better aware of regulatory complexities down the road. For instance, Matrigel is commonly used as a surface coating or as a hydrogel to grow stem cells in the lab, but because of the source from which it is derived and resulting batch-to-batch variability it is not likely to be approved for use in clinical products by the FDA. These examples highlight the need to consider the entire development process when designing a TE product and establishing laboratory model systems.

Communicating with stakeholders early in the development process was a recurring theme of the workshop, within which early and frequent communication with the FDA was strongly encouraged. Such ongoing dialogue increases the likelihood of approval by anticipating and proactively addressing comments and suggestions made by the agency. In addition to FDA interaction, communicating with private funders and patient advocates can lead to more favorable funding prospects or faster market adoption of a

product. Improving communication with non-scientists or regulatory personnel was highlighted as integral to the success of a TE product.

B. Manufacturing and Scalability Challenges

Once a TE product candidate has been identified and tested in vivo, methods to produce it in a reliable, scalable fashion need to be developed. Scaling up the production of a TE therapy can pose many challenges, especially if the product includes live cells or complex structures. While simplicity in product design can be more desirable from a manufacturing perspective, discussion at the workshop noted the fact that simple products are not optimal for all biological applications.

i. Material scale-up: One category in the TE product landscape is cell-free synthetic scaffolds. Once implanted into a patient or animal model, the material can support the recovery of ailing tissues or regeneration of an organ from endogenous stem cells. Cell-free scaffolds are relatively straightforward and amenable in principle to large-scale production, and standard engineering principles can often be applied to produce them at scale. Scaffolds need to interact with cells or tissues in the body, and biomaterials for tissue engineering applications are increasingly being designed in conjugation with growth factors or other proteins or small molecules that help bind to cells or recruit endogenous repair mechanisms, adding a level of complexity to their scale-up.

Scaffolds derived from biologic tissue can be more challenging to produce efficiently, and they can suffer from quality control issues and lot-to-lot variability. Scaffolds such as decellularized trachea have limited ability to be made in a high-throughput manner and thus will be difficult to translate to a large number of patients.

Finally, even when simple scaffolds or hydrogels have a promising biological effect they might not be the optimal therapeutic product. In the infarcted heart, for instance, hydrogel injection supports heart tissue repair but not growth of a new organ. In many cases, a combination product or a patient-specific tailored product must be considered as the long-term therapeutic approach, even if in the short-term the TE product is a simple biomaterial.

ii. Cell production scale-up: A second TE product category involves cells, either undifferentiated or differentiated into a desirable cell type, which can be delivered alone or in combination with scaffolds to repair or regenerate damaged tissue. Commercialization of cell-based therapeutics requires the ability to produce large amounts of the desired cell type in the desired configuration in a reproducible fashion. In addition to the scientific hurdles that must be overcome to develop the appropriate differentiation protocol, cell production and product delivery have a number of challenges, including:

1. Space requirements: it takes a lot of space to grow cells in liquid medium or three-dimensional culture system and must be able to comply with regulatory

requirements, such as current good manufacturing practices (cGMP) and/or good tissue practices (cGTP).

2. Purity: if the desired product is a specific cell type differentiated from stem or progenitor cells in culture, methodologies are required to ensure a consistent composition of the final product, with a defined purity of the desired cell type and an understanding of the identity of the additional cells in the population.
3. Stability: some differentiated cell types, such as chondrocytes, de-differentiate over multiple passages in culture. Equally challenging is the fact that certain types of undifferentiated cells can be difficult to maintain in their pluripotent state in culture. The instability of cell phenotypes in culture makes it difficult to maintain batches of a desired cell type in culture for a long time, which can lead to high production costs.
4. Reproducibility and quality control: Scale-up using cGMP must incorporate the use of appropriate assays to ensure that the resulting cell product has maintained its biological activity. Given the problems cited above and the FDA requirements for cell-based therapeutics, the quality of each batch must be verified. For instance, Cytograft runs a series of tests on its vascular graft product, which requires the use of a significant portion of its workforce.
5. Delivery: Product designers must consider the mode of delivery of the TE product, and work closely with the clinicians or surgeons that will be transplanting the cell product to maintain acceptable delivery characteristics through scale-up.

Finally, as discussed in Chapter 2 there is increasing interest in developing autologous cell-based products derived from a patient's own cells. Surgeons have had positive results conducting one-off experimental therapies using personalized medicine approaches, which present unique scalability challenges. In these cases, the procedure must be tailored to an individual patient and to the particular therapeutic application, meaning that scale-up opportunities are more limited.

iii. Complex TE product manufacturing: More complex tissue-engineered products, such as 3D tissues, organoids, or engineered stem cell niches hold great promise for but may be very difficult to produce in an automated way in large quantities. For complex TE products, scale-up might be applicable to only a portion of the product, or will require developing procedures that allow products to be made at the clinical site. Complex products may also require training of doctors on the implantation and/or utilization of the TE product.

As an example, Dr. Birchall discussed the challenges that will be faced in scaling up the tracheal replacement therapy he is using. He indicated that stem cells required to seed the scaffold in this case are unique to the patient. However, the manufacturing of the requisite scaffold, whether that entails decellularizing and processing a donor cadaver trachea or manufacturing one using synthetic materials, is amenable to scale-up to be able to treat a larger number of patients. In addition, any method to facilitate the culture of stem cells and their attachment to the scaffold would probably have general applicability and could be scaled-up.

C. Regulatory Challenges

Obtaining regulatory approval is, of course, necessary to conduct a first-in-human clinical study of a TE product. Regulatory approval is challenging for any therapeutic candidates, but tissue engineered products pose unique challenges. For some TE products one of the early steps in the regulatory process is the FDA's definition of the product as either a "biologic" or "device" (consult the FDA definitions for Devices http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051512.htm and Biologics http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm113522.htm). A product with live cells will typically falls in the biologic category, whereas synthetic, cell-free inserts are often regulated as devices, but all cases depend upon the proposed mechanism of action. The regulatory path can be unclear for hydrogels or scaffolds derived from biological materials such as decellularized tissue or extracellular matrix components. The intended use of the product and primary mode of action are important factors in defining the regulatory path. An example presented at the meeting was that of an algisyl-based hydrogel used to improve ventricular geometry and function in patients with dilated cardiomyopathy (Lee et al, 2012). The hydrogel was considered a device in that case based on the proposed mechanism of action of improving heart mechanics without actively promoting regeneration. Conversely, for other applications a hydrogel or scaffold derived from biological sources may be considered a biologic depending upon the mechanism of action. Predicting the primary regulatory jurisdiction is more difficult with products that combine device and biologic components, known as combination products. These are cases in which discussions with the FDA early in the development process can be useful to understand the regulatory expectations and inform preclinical study design.

The FDA regulates not only the final therapeutic product and its applications but also the manufacturing process, which can impact product safety. Manufacturing challenges have led many TE product developers to opt for simpler products in which the composition and manufacturing are well defined and reproducible, such as by selecting synthetic rather than biologically-derived gels or scaffolds. By contrast, complex products such as organoids, biomimetic stem cell niches, and cell-seeded scaffolds can be more challenging from a regulatory perspective because of the many steps required to achieve the final product. Reproducibility of the manufacturing process and the ability to fully characterize the end product will be safety concerns that should be considered.

A number of workshop participants shared their respective experiences interacting the FDA. Dr. Breuer reported a positive experience in receiving FDA approval for his IND for a complex product, which is a vascular graft composed of a biodegradable synthetic scaffold seeded with patient-derived autologous endothelial cells (Hibino et al, 2011; Roh et al, 2008). Dr. Breuer attributed a large part of his success to early discussions with FDA representatives early in his project through which he was able to discuss his approach and gain feedback on what data might be requested by regulatory reviewers. He indicated that the FDA was a thoughtful partner and that their primary focus on patient safety was aligned with his concerns as a physician. He was able to alleviate many safety concerns associated with cell-based therapies by using autologous cells. Finally, since he was targeting pediatric congenital heart diseases, the FDA was more

willing to accept efficacy parameters that were more vague than might be the case for other indications, although other workshop participants noted that the agency is often willing to be more flexible about efficacy than safety. A similarly positive experience with the FDA was reported for Carticel, an autologous cell-therapy product that was approved by the FDA in 1997 to repair articular cartilage injuries in the knee of adults who have not responded to prior treatments. This product faced regulatory hurdles as the first cell therapy, but these were overcome and the product was approved.

> **<u>Recommendation #8:</u> Facilitate dialogue with the FDA regarding regulation of tissue engineered products.**
> Many participants indicated that their early interactions with FDA representatives had not been predictive of their experience during the official regulatory review period, in contrast to some of the positive experiences shared. There was a strong feeling that education is needed both to inform researchers and developers of the FDA's mission and regulatory guidelines as well as to raise awareness at the FDA of the special needs and challenges facing the TE community. Participants commented that the TE products or approaches approved by the FDA to date target diseases with no or few therapeutic options. FDA approval of TE products in areas where alternative therapeutic options exist was perceived as slow or even unachievable, and some workshop participants were frustrated by their inability to deliver therapies to patients in a timely and cost-feasible manner CIRM was identified as an important broker between the FDA and the TE product developers.

D. Structural and Financial Challenges: Bridging the Valley of Death

Workshop participants discussed the fact that many TE products fail to be developed due to the so-called Valley of Death which affects many other biotechnology discoveries. The "Valley of Death" is a metaphor for the gap that exists between basic biomedical breakthroughs made in the laboratory and therapeutic leads that enter clinical trials (Coller & Califf, 2009; Finkbeiner, 2010). Many discoveries are not successfully translated to the clinic and/or marketplace. In some cases, this is because the technology is flawed or because of competing technologies which make the approach financially unattractive. The inability to bridge this gap has enormous potential costs for patients who do then not have access to potentially life-saving new therapeutic options (Butler, 2008).

There are multiple reasons for the widening Valley of Death in therapeutic development (Coller & Califf, 2009; Finkbeiner, 2010; Lysaght et al, 2008). Academic intellectual property is secured based on early-stage research and typically does not include preclinical toxicology studies or human data. Most academic researchers lack the funding, regulatory know-how, appropriate expertise, and incentives to advance their breakthroughs beyond the laboratory scale. This issue has become more pronounced as research funding has become scarce. Many agencies have responded to budgetary constraints with a reduced willingness to fund risky translational projects. Dr. Jennifer

Elisseeff pointed out that there are few options for funding the manufacturing of TE products, for instance. As a consequence, many academic research projects remain stuck at an early stage of therapeutic development.

In the past, the significant financial risks involved in developing, testing, receiving regulatory approval, and marketing a therapeutic candidate were shouldered by the pharmaceutical/biotech industry, who licensed discoveries from scientists and brought the product to market. Industry was willing to take these risks because therapeutic breakthroughs led to generous financial rewards. However, industry has become more risk-averse, and TE products are at a particular disadvantage for many reasons. First, TE products have a limited track record in the medical industry, and the financial rewards involved in developing new products are not as certain as they are for developing a second or third generation of an existing pharmaceutical. Second, since each TE product is unique the regulatory pathway may not be clear and are therefore considered risky. Third, the manufacturing and scale-up of TE products can be complicated and potentially expensive. Finally, the business model is less clear for TE products and ability to secure insurance reimbursement is less certain than for small molecule pharmaceuticals. These realities present barriers to entry of TE products from academia selected for further therapeutic development by industry. The field has responded by focusing on more developed products that are achievable and commercially viable in the short term, and by shifting the burden of risk management to development-stage firms or even to academia (Lysaght et al, 2008).

E. Case Studies in the Commercialization of Tissue Engineered Products

A final set of challenges in working with any therapeutic product involves a number of non-scientific factors that impact commercial success and should be considered in product development. These encompass marketing inefficiencies, the ability of a product to reach its intended customer, insurance reimbursement and other economic considerations, and many other intangibles. Table 4 summarizes the case studies of tissue engineered product development presented by industry participants.

Workshop participants described several cases in which a promising TE product was approved by the FDA but did not lead to a commercial success. Dr. Gail Naughton recounted the story of Advanced Tissue Sciences (ATS), a company she co-founded in 1987. ATS developed two skin substitutes to facilitate wound closure. Both products, eventually commercialized under the names Transcyte and Dermagraft, consist of a resorbable scaffolding material populated by human neonatal foreskin fibroblasts. The FDA approved Transcyte in 1996 as a temporary wound covering in burn patients awaiting an autograft; the similar product Dermagraft was approved to treat diabetic foot ulcers in 2001.

Table 4. Industry Case Studies of Product Development			
Company	**Product**	**Approved Indication**	**Product Description**
Advanced Tissue Sciences	Transcyte	Burn wounds	Resorbable scaffold seeded with human neonatal foreskin fibroblasts
	Dermagraft	Diabetic foot ulcers	Human fibroblasts, an extracellular matrix, and a bioabsorbable polyglactin mesh scaffold
Cytograft	LifeLine	Not yet approved (under development for hemodialysis access)	Tissue-engineered blood vessels made of sheaths of patient-derived fibroblasts lined with autologous endothelial cells
Genzyme	Carticel	Symptomatic cartilage defects of the femoral condyle in patients who have failed a prior surgical procedure	Autologous cultured chondrocytes
	Matrix-assisted Autologous Chondrocyte Implant (MACI)	Articular cartilage defects of femoral condyle	Membrane-bound form of the Carticel product with a simplified surgical implantation procedure

In spite of positive clinical data for these two products, the company generated far less than the anticipated revenue. Unable to recoup its development costs, ATS eventually filed for Chapter 11 bankruptcy protection in 2003. Transcyte and Dermagraft were acquired by other companies and eventually commercialized by Advanced BioHealing (ABHB) in 2007. ABHB, recently acquired by Shire, became the first company to profit from the sale of these products, more than 20 years after their development.

Dr. Naughton attributed ATS' financial woes to several factors. 1) The product had trouble gaining regulatory approval, both in the U.S. and in Europe, which delayed the time to market. 2) The company still had not established insurance reimbursement five years after its first Dermagraft shipment. 3) The manufacturing process was not robust, since one in three lots failed to meet product release specifications, therefore increasing production costs. 4) Investor excitement allowed ATS to raise a significant amount of funding which it used to build a manufacturing facility based on an anticipated $300 million market. However, the facility incurred large overhead costs that could not be paid for by the modest initial profits. These and other financial pressures led ATS to file for Chapter 11 bankruptcy protection in October 2002 (Pangarkar et al, 2010).

If ATS presented a case study of overly ambitious projections stymied by regulatory and insurance delays, Carticel provided an example of the importance of stakeholder buy-in. Stephen Duguay presented the scientific and business history of Carticel, an autologous chondrocyte product first developed in Sweden and approved by the FDA in 1997 for

the treatment of symptomatic lesions of the femoral condyle and patella in patients that have failed a prior surgical procedure. Carticel has been a success in several regards: it has been used to treat more than 16,000 patients. It was the first cell therapy approved by the FDA. Although it was originally approved by the FDA as a device prior to current regulations for cell-based therapies, it has demonstrated the feasibility of commercially producing an autologous cell-based therapeutic product. But while Carticel earned value for its U.S. manufacturer Genzyme, its profitability remains questionable. Dr. Duguay attributed the limited profits to Carticel's poor market penetration which had multiple causes: 1) it was approved as a second line of treatment; 2) its initial cost was higher than that of other treatments (though its better durability would eventually offset this cost); and 3) transplant requires a challenging surgical procedure, including harvesting periosteum and performing fine suturing to create a delivery pouch which has limited the adoption of the product by surgeons.

To overcome the obstacles experienced by Carticel, Genzyme acquired a second generation of autologous cartilage implant, called Matrix-assisted Autologous Chondrocyte Implant (MACI). MACI is in essence a membrane-bound form of the Carticel product with a simplified and shortened surgical implantation procedure. MACI is currently being tested in a clinical trial designed to show its superiority to the current standard of care in an effort to obtain approval as a first-line treatment. The expectation is that surgeons will adopt MACI more readily than Carticel because of its simpler use and should result in larger sales.

Although increasing sales is one path to improving commercial success, reducing overhead and streamlining development can also help. Dr. Todd McAllister described Cytograft's strategy to develop and commercialize LifeLine, a line of tissue-engineered blood vessels made of sheaths of patient-derived fibroblasts lined with autologous endothelial cells. The product was successful in Phase I and II trials designed to test its use as hemodialysis access and is currently undergoing Phase III trials. Dr. McAllister emphasized that Cytograft was able to keep operational costs through Phase I and II trials under $20 million, in part by conducting its clinical trials outside of the U.S. The discussion highlighted the importance of reducing overhead costs and flexible product design options.

Greg Bonfiglio offered statistics for the general field of biotechnology; namely, an average time to market of 10-15 years, average development and production costs of $1 billion, failure rate of 90% by the clinical-trial stage, and a less than 30% chance that an approved drug will generate enough revenue to recoup its own development costs. Such statistics explain why venture capitalists (VCs) are increasingly unlikely to invest in tissue engineered and other biotechnology products prior to Phase III clinical trials, when the product has passed enough functional and regulatory hurdles to have a reasonable chance of success. These figures highlighted the importance of containing development costs, improving design, and maintaining flexible commercialization strategies and timelines.
While the specifics might differ between products, presenters agreed that new models of development and commercialization are needed, particularly in light of scarce funding

opportunities. Several possible strategies to increase the success rate of new TE products were offered, including 1) keeping a focused product line, 2) positioning the product as a first-line treatment to increase market penetration and improve the likelihood of insurance reimbursement, 3) avoiding large infrastructure and personnel costs, 4) keeping product development through early stage clinical trials in academia, 5) acquiring seed funding from people with a personal stake in the product's success, 6) outsourcing some items of production to the pharmaceutical industry, and 7) lightening the regulatory burden by using already approved products or procedures.

These translational realities highlight the critical role of public funding and academic research in inventing a new generation of breakthrough technologies, and de-risking them sufficiently that they are either profitable for their developers or attractive to more traditional financing sources. Academia has traditionally been an incubator for transformative research, and academic scientists are very willing to take creative risks (Finkbeiner, 2010). Because of their mission to promote the public welfare and not simply provide a financial return on investment, public agencies can invest in high-risk projects that have the potential to deliver novel treatments, even if only a fraction of these products will be commercially successful. The task for CIRM in supporting tissue engineering research and development is to identify the projects with the greatest potential to revolutionize patient care. In this way, a public agency can contribute to our knowledge of wellness and disease, create jobs for a highly skilled local workforce, and potentially be responsible for bringing a commercially successful therapy to market.

CONCLUSION

The choice of cells and materials for tissue engineering is often done without the human clinical endpoint in mind. Translating discoveries in the laboratory into a tissue-engineered product, however, faces not only technical challenges with the tissue construct itself but business challenges including scale-up and manufacturing issues, navigating FDA approval, addressing the appropriate market needs, and raising funds needed to bring the product to market. Workshop participants opined that translation of discoveries from the bench to the bedside would be greatly eased if these non-scientific challenges were identified and addressed up front. They commented that CIRM was ideally situated to shepherd research in these directions.

CHAPTER 5: OUTCOMES: ADVANCING TISSUE ENGINEERING APPROACHES TO THE CLINIC

Pioneering clinicians and scientists are helping bring tissue-engineered products to the bedside to aid patients, and stem cells are beginning to play a central role in this effort. However, participants in CIRM's workshop of Tissue Engineering admitted that stem cell-derived products face unique and difficult challenges at all aspects of development. They indicated that there is great value in developing a stronger multidisciplinary community that can collaboratively address some of these challenges, and that CIRM could play an important role in nurturing this community by sponsoring funding initiatives and other supportive activities that offset the high-risk nature of this novel field. The areas in which workshop participants felt CIRM could have an impact included:

1. **Support basic research using stem cells for tissue engineering.** TE-directed research will focus on areas such as the differentiation of stem cells, their aggregation into 3D structures or tissues *in vivo* or *in vitro*, the immune response and foreign body response, and the development of adequate analytical tools test the safety and efficacy of TE products in either preclinical animal models (where adequate) or first-in-man studies.

2. **Support basic research in stem cell/biomaterial interactions.** CIRM should increase its effort to fund basic research that will elucidate the interaction of stem cells with synthetic and biologically derived materials, by targeting groups that combine strong bioengineering and stem cell experience.

3. **Promote collaborations between stakeholders in TE therapies, particularly basic scientists, surgeons, and industry.** Workshop participants indicated that TE products must be developed with the clinical goal in mind, so early communication between all collaborators is essential to increasing a product's potential. In its more translational funding initiatives, CIRM should aim to improve therapeutic success of TE products by insisting upon collaborative projects that have concrete avenues for early and regular communication between varied stakeholders.

4. **Contribute to regulatory transparency for TE products.** The FDA is in the position of needing to regulate TE products that are heterogeneous in their composition and intended uses. The challenge can make the regulatory process treacherous for both regulators and product developers. As a dual advocate for patients and stem cell scientists, CIRM has an interest in making regulatory processes as transparent and effective as possible. CIRM is in a position to present developments in tissue engineering to the FDA, and conversely make researchers be aware of regulatory expectations to avoid pitfalls in their development process.

5. **Highlighting non-scientific factors that impact commercial success**. Many TE products are intended for a clinical market. Again, as a function of its interaction with patients and stem cell scientists, CIRM has an interest in ensuring that researchers consider non-scientific factors that will contribute to their products' future success. In

designing its grant applications and educational outreach activities for therapeutic TE products, participants urged CIRM to prioritize clinical need, develop measures to both evaluate the market and provide insurance coverage for the product being developed, and ensure that products will be well accepted among medical practitioners who will be the end-users of the technology. Considering these factors will help these products be successful in the marketplace.

REFERENCES

Aguado B, Mulyasasmita MS, Su J, Lampe KJ, Heilshorn SC. (2012) Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. *Tissue Eng. Part A* **18(7-8):** 806-815.

Ananthanarayanan B, Little L, Schaffer DV, Healy KE, Tirrell M. (2010) Neural stem cell adhesion and proliferation on phospholipid bilayers functionalized with RGD peptides. *Biomaterials* **31:** 8706-8715.

Anderson JM, Rodriguez A, Chang DT. (2008) Foreign body reaction to biomaterials. *Seminar Immunol.* **20:** 86-100.

Atala A. (2012) Regenerative medicine strategies. *J. Ped. Surg*. **47:** 17-28.

Bader A, Macchiarini P. (2010) Moving towards *in situ* tracheal regeneration: the bionic tissue engineered transplantation approach. *J. Cell. Mol .Med*. **14(7):** 1877-1889.

Badylak SF, Weiss DJ, Caplan A, Macchiarini P. (2012) Engineered whole organs and complex tissues*. Lancet* **379:** 943-952.

Ballios BG, Cooke MJ, van der Kooy D, Shoichet MS. (2010) A hydrogel-based stem cell delivery system to treat retinal degenerative diseases. *Biomaterials* **31(9):** 2555-2564.

Bratt-Leal AM, Carpenedo RL, McDevitt TC. (2009) Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation. *Biotech. Prog.* **25:** 43-51.

Butler D. (2008) Crossing the valley of death. *Nature* **453:** 840-842.

Carpenedo RL, Bratt-Leal AM, Marklein RA, Seaman SA, Bowen NJ, McDonald JF, McDevitt TC. (2009) Homogeneous and organized differentiation within embryoid bodies induced by microsphere-mediated delivery of small molecules. *Biomaterials* **30:** 2507-2515.

Chu CR, Szczodry M, Bruno S. (2010) Animal models for cartilage regeneration and repair. *Tissue Eng. Part B* **16(1):** 105-115.

Coller BS, Califf RM. (2009) Traversing the valley of death: A guide to assessing prospects for translational success. *Science Translat. Med.* **1(10):** 1-5.

Deng Y, Hu, JC, Athanasiou KA. (2007) Isolation and chondroinduction of a dermis-isolated, aggrecan-sensitive subpopulation with high chondrogenic potential. *Arthritis Rheum.* **56(1):** 168-176.

Elder BD, Athanasiou KA. (2008) Synergistic and additive effects of hydrostatic pressure and growth factors on tissue formation. *PLoS One* **3(6):** e2341.

Elliott MJ, De Coppi P, Speggiorin S, Roebuck D, Butler CR, Samuel E, Crowley C, McLaren C, Fierens A, Vondrys D, Cochrane L, Jephson C, Janes S, Beaumont NJ, Cogan T, Bader A, Seifalian AM, Hsuan JJ, Lowdell MW, Birchall MA. (2012) *Lancet.* 380(9846):994-1000.

Engler AJ, Sen S, Sweeney HL, Discher DE. (2006) Matrix elasticity directs stem cell lineage specification. *Cell* **126:** 677-689.

Eshghi S, Schaffer DV. (2008) Engineering microenvironments to control stem cell fate and function. *Stembook [Internet.]* Cambridge (MA), Harvard Stem Cell Institute.

Finkbeiner S. (2010) Bridging the valley of death of therapeutics for neurodegeneration. *Nat. Med.* **16(10):** 1227-1232.

Frey-Vasconcells J, Whittlesey KJ, Baum E, Feigal EG. (2012) Translation of stem cell research: points to consider in designing preclinical animal studies. *Stem Cells Translat. Med.* **1:** 1-6.

Han E, Ge C, Chen AC, Schumacher BL, Sah RL. (2012) Compaction enhances extracellular matrix content and mechanical properties of tissue-engineered cartilaginous constructs. *Tissue Eng. Part A* **18(11-12):** 1151-1160.

Han E, Wilensky LM, Schumacher BL, Chen AC, Masuda K, Sah RL. (2010) Tissue engineering by molecular disassembly and reassembly: Biomimetic retention of mechanically functional aggrecan in hydrogel. *Tissue Eng. Part C* **16(6):** 1471-1479.

Hibino N, Yi T, Duncan DR, Rathore A, Dean E, Naito J, Dardik A, Kyriakides T, Madri J, Pober JS, Shinoka T, Breuer CK. (2011) A critical role for macrophages in neovessel formation and the development of stenosis in tissue-engineered vascular grafts. *FASEB J.* **25:** 4253-4263.

Higgins DM, Basaraba RJ, Hohnbaum AC, Lee EJ, Grainger DW, Gonzalez-Juarrero M. (2009) Localized immunosuppressive environment in the foreign body response to implanted biomaterials. *Am. J. Pathol*. **175:** 161-170.

Hu JC, Athanasiou KA. (2006) The effects of intermittent hydrostatic pressure on self-assembled articular cartilage constructs. *Tissue Eng.* **12(5):** 1337-1344.

Hwang NS, Varghese S, Elisseeff J. (2008) Controlled differentiation of stem cells. *Adv. Drug Deliv. Rev*. **60:** 199-214.

Johnson PJ, Tatara A, Shiu A, Sakiyama-Elbert SE. (2010a) Controlled release of neurotrophin-3 and platelet-derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. *Cell Transplant.* **19(1):** 89-101.

Johnson PJ, Parker SR, Sakiyama-Elbert SE. (2010b) Fibrin-based tissue engineering scaffolds enhance neural fiber sprouting and delay the accumulation of reactive astrocytes at the lesion in a subacute model of spinal cord injury. *J. Biomed. Mater. Res. A* **92(1):** 152-63.

Karp JM, Yeh J, Eng G, Fukuda J, Blumling J, Suh KY, Cheng J, Mahdavi A, Borenstein J, Langer R, Khademhosseini A. (2007) Controlling size, shape, and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. *Lab on a Chip* **7:** 786-794.

Keung AJ, Healy KE, Kumar S, Schaffer DV. (2010a) Biophysics and dynamics of natural and engineered stem cell microenvironments. *WIREs Syst. Biol. Med.* **2:** 49-64.

Keung AJ, Kumar S, Schaffer DV. (2010b) Presentation counts: Microenvironmental regulation of stem cells by biophysical and material cues. *Annu. Rev. Cell Dev. Biol.* **26:** 533-556.

Kinney MA, Sargent CY, McDevitt TC. (2011) The multiparametric effects of hydrodynamic environments on stem cell culture. *Tissue Eng. Part B* **17(4):** 249-262.

Lee HH, Haleem AM, Yao V, Li J, Xiao X, Chu CR. (2011) Release of bioactive adeno-associated virus from fibrin scaffolds: Effects of fibrin glue concentrations. *Tissue Eng. Part A* **17(15-16):** 1969-1978.

Lee RJ, Hinson A, Helgerson S. (2012) Polymer-based restoration of left ventricular mechanics. *Cell. Transplant.* In press.

Little L, Dane K, Daugherty P, Healy K, Schaffer D. (2011) Exploiting bacterial peptide display technology to engineer biomaterials for neural stem cell culture*. Biomaterials* **32:** 1484-1494.

Little L, Healy KE, Schaffer D. (2008) Engineering biomaterials for synthetic neural stem cell microenvironments. *Chem. Rev.* **108:** 1787-1796.

Lysaght MJ, Jaklenec A, Deweerd E. (2008) Great expectations: Private sector activity in tissue engineering, regenerative medicine and stem cell therapeutics. *Tissue Eng. Part A* **14(2):** 305-315.

Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Dodson A, Martorell J, Bellini S, Parnigotto PP, Dickinson SC, Hollander AP, Mantero S, Conconi MT, Birchall MA. (2008) Clinical transplantation of a tissue-engineered airway. *Lancet* **372:** 2023-2030.

Multi-Agency Tissue Engineering Science Interagency Working Group. (2007) Advancing Tissue Science and Engineering: A Foundation for the Future/A Multi-agency Strategic Plan (Executive Summary). *Tissue Eng.* **13(12):** 2825-2827.

McCreedy D, Sakiyama-Elbert SE. (2012) Combination therapies in the CNS: Engineering the environment. *Neurosci. Lett.* In press.

Mirensky TL, Breuer CK. (2008) The development of tissue-engineered grafts for reconstructive cardiothoracic surgical applications. *Pediatr. Res.* **63(5):** 559-568.

Mohr JC, de Pablo JJ, Palecek SP. (2006) 3-D microwell culture of human embryonic stem cells. *Biomaterials* **27:** 6032-6042.

Odedra D, Chiu LL, Shoichet M, Radisic M. (2011) Endothelial cells guided by immobilized gradients of vascular endothelial growth factor on porous collagen scaffolds. *Acta Biomater.* **7(8):** 3025-3035.

Orlando G, Wood KJ, Stratta RJ, Yoo JJ, Atala A, Soker S. (2011) Regenerative medicine and organ transplantation: Past, present and future. *Transplantation* **91:** 1310-1317.

Pagnotto MR, Wang Z, Karpie JC, Ferretti M, Xiao X, Chu CR. (2007) Adeno-associated viral gene transfer of transforming growth factor-b1 to human mesenchymal stem cells improves cartilage repair. *Gene Ther.* **14:** 804-813.

Pakulska MM, Ballios BG, Shoichet MS. (2012) Injectable hydrogels for central nervous system therapy. *Biomed. Mater.* **7(2):** 024101.

Pangarkar N, Pharoah M, Nigam A, Hutmacher DW, Champ S (2010). Advanced Tissue Sciences Inc.: learning from the past, a case study for regenerative medicine. *Regen Med.* 5(5): 823-35.

Radisic M, Marsano A, Maidhof R, Wang Y, Vunjak-Novakovic G. (2008) Cardiac tissue engineering using perfusion bioreactor systems. *Nat. Protoc.* **3(4):** 719-738.

Radisic M, Park H, Shing H, Consi T, Schoen FJ, Langer R, Freed LE, Vunjak-Novakovic G. (2004) Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. *Proc. Natl. Acad. Sci. USA* **101(52):** 18129-18134.

Responte DJ, Lee JK, Hu JC, Athanasiou KA. (2012) Biomechanics-driven chondrogenesis: from embryo to adult. *FASEB J.* **26:** 1-11.

Roh JD, Nelson GN, Brennan MP, Mirensky TL, Yi T, Hazlett T, Tellides G, Sinusas AJ, Pober JS, Saltzman WM, Kyriakides TR, Breuer CK. (2008) Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. *Biomaterials* **29(10):** 1454-1463.

Sah RL. (2004) Interface and bulk regions in the repair, regeneration and replacement of articular cartilage. *J. Musculoskel. Neuron Interact.* **4(4):** 393-395.

Sala FG, Kunisaki SM, Ochoa ER, Vacanti J, Grikscheit TC. (2009) Engineered small intestine and stomach form autologous tissue in a preclinical large animal model. *J. Surg. Res.* **156:** 205-212.

Sala FG, Matthews JA, Speer AL, Torashima Y, Barthel ER, Grikscheit TC. (2011) A multicellular approach forms a significant amount of tissue-engineered small intestine in the mouse. *Tissue Eng. Part A* **17:** 1841-1850.

Singelyn JM, Christman KL. (2010) Injectable materials for the treatment of myocardial infarction and heart failure: The promise of decellularlized matrices. *J. Cardiovasc. Trans. Res.* **3:** 478-486.

Singelyn JM, DeQuach, JA, Seif-Naraghi SB, Littlefield, RB, Schup-Magoffin P, Christman KL. (2009) Naturally derived myocardial matrix as an injectable scaffold for cardiac engineering. *Biomaterials* **30:** 5409-5416.

Song JJ, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP, Ott HC. (2013) Regeneration and experimental orthotopic transplantation of a bioengineered kidney. *Nature Medicine* **19**:646-651.

Terraciano V, Hwang N, Moroni L, Park HB, Zhang Z, Mizrahi J, Seliktar D, Elisseeff J. (2007) Differential response of adult and embryonic mesenchymal progenitor cells to mechanical compression in hydrogels. *Stem Cells* **25:** 2730-2738.

Tandon N, Cannizzaro C, Chao PH, Maidhof R, Marsano A, Au HT, Radisic M, Vunjak-Novakovic G. (2009) Electrical stimulation systems for cardiac tissue engineering. *Nat. Protoc.* **4(2):** 155-173.

Willerth SM, Rader A, Sakiyama-Elbert SE. (2008) The effect of controlled growth factor delivery on embryonic stem cell differentiation inside fibrin scaffolds. *Stem Cell Res.* **1:** 205-218.

Willerth SM, Sakiyama-Elbert SE. (2008) Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery. *Stembook [Internet.]* Cambridge (MA), Harvard Stem Cell Institute.

Williams GM, Chan EF, Temple-Wong MM, Bae WC, Masuda K, Bugbee WD, Sah RL. (2010) Shape, loading, and motion in the bioengineering design, fabrication and testing of personalized synovial joints. *J. Biomechanics* **43:** 156-165.

Wong Po Foo CTS, Lee JS, Mulyasasmita W, Parisi-Amon A, Heilshorn SC. (2009) Two-component protein-engineered physical hydrogels for cell encapsulation. *Proc. Natl. Acad. Sci. USA* **106(52):** 22067-22072.

Engineering Strategies, Opportunities, and Challenges for Tissue Repair and Regeneration

January 12-13, 2012

Location: Wyndham Parc 55 Hotel (55 Cyril Magnin Street, San Francisco, CA 94102). All sessions will be located in the Market Street Meeting Room (3rd Floor) unless indicated otherwise.

Day One: Thursday, January 12, 2012	
7:00 am	**Breakfast available**
8:00 am	**President's Welcome** **Alan Trounson, PhD**; CIRM President
8:15 am	**CIRM's Translational Portfolio** **Patricia Olson, PhD;** Executive Director of Scientific Activities
Three–dimensional Cell Scaffolding for Tissue Repair	
Chair	**Robert Sah, MD, ScD; UC San Diego**
8:30 am	**Robert Sah, MD, ScD; UC San Diego** *Scaffold-directed cartilage engineering*
8:55 am	**Martin Birchall, MD; UC Davis** *Building to Breathe: Clinical applications of scaffolds and stem cells in airway surgery*
9:15 am	**Tracy Grikscheit, MD; Children's Hospital Los Angeles** *Tissue engineered small intestine relies on the stem cell niche*
9:35 am	**Karen Christman, PhD; UC San Diego** *Cardiac repair using decellularized matrix*
9:55 am	**Questions for all 4 speakers**
10:25 am	**Coffee break**
Immunological Considerations for Tissue Engineered Products	
10:45 am	**David Grainger, PhD, University of Utah** *Host foreign body and infection response to scaffolding materials*
11:15 am	**Questions**

Panel Discussion I – Challenges and bottlenecks in tissue engineering **Moderator: Molly Shoichet (University of Toronto)**	

11:30 am	Panel members:
	Stephen Duguay (Genzyme)
	Christine Kelley (NIBIB/NIH)
	Tracy Grikscheit (Children's Hospital Los Angeles)
	
	Discussion topics may include:
	• Issues related to sourcing of materials and/or cells
	• Are adequate analytical tools and characterization methods available?
	• Scaffold fabrication methods that enable translation
	  ○ Process consistency
	  ○ Scale-up considerations
	• Understanding regulatory expectations

12:15 pm	**Lunch (Powell meeting room)**
1:30 pm	**Keynote Address:** **Anthony Atala, MD; Wake Forest University** *Tissue Engineering and Cell Therapy – Current Concepts and Changing Trends*
Hydrogels for Cell Delivery & Tissue Repair I	
Chair	**Milica Radisic, PhD; University of Toronto**
2:20 pm	**Sarah Heilshorn, PhD; Stanford University** *Hydrogels to improve stem cell delivery*
2:45 pm	**Molly Shoichet, PhD; University of Toronto** *Injectable hydrogels for cell therapy of retinal disease*
3:05 pm	**Coffee Break**
3:25 pm	**Randall Lee, MD, PhD; UC San Francisco** *Hydrogels to regenerate the myocardium*
3:45 pm	**Milica Radisic, PhD; University of Toronto** *Collagen patches for cardiac repair*
4:05 pm	**Questions for all 4 speakers**
Translation/Commercial Consideration of Tissue Engineered Products I	
4:25 pm	**Greg Bonfiglio, JD; Proteus Ventures** *Venture capital perspective on translation of tissue engineered products*
4:45 pm	**Questions**

Panel Discussion II – Needs and limitations of *in vitro* and *in vivo* models for tissue engineered products **Moderator: David Grainger (University of Utah)**	
4:55 pm	Panelists: Anthony Atala (Wake Forest University) Chris Breuer (Yale University) Randall Lee (UC San Francisco) Discussion topics may include: • What criteria validate a relevant preclinical model for tissue engineered devices? ◦ What are the critical issues (pro/con) presented by small animal models? • Are there general criteria for monitoring and validating improved tissue integration in TE strategies that are conserved across various tissue types in vivo in preclinical models?
5:40 pm	**Adjourn Day 1**
6:30 pm	**Dinner (Kuleto's; 221 Powell Street, San Francisco, CA 94102)**
Day 2: Friday, January 13, 2012	
7:30 am	**Breakfast available**
Hydrogels for Cell Delivery & Tissue Repair II	
8:15 am	**Constance Chu, MD; University of Pittsburgh** *Innovative delivery systems for cartilage repair*
8:40 am	**Shelly Sakiyama-Elbert, PhD; Washington University** *Growth factor delivery from fibrin scaffolds to direct ES derived neural progenitor survival and differentiation for spinal cord injury*
9:05 am	**Questions for both speakers**
9:25 am	**Coffee Break**
Novel Stem Cell Culture Methods	
Chair	**David Schaffer, PhD; UC Berkeley**
9:45 am	**David Schaffer, PhD; UC Berkeley** *Engineering strategies to emulate the stem cell niche*
10:05 am	**Todd McDevitt, PhD; Georgia Institute of Technology** *Directing differentiation with microenvironmental cues*
10:25 am	**Kyriacos Athanasiou, PhD; UC Davis** *Self-assembling engineered cartilage*

10:45 am	**Questions for all 3 speakers**
Translation/Commercial Consideration of Tissue Engineered Products II	
Chair	**Greg Bonfiglio, JD; Proteus Venture Partners**
11:15 am	**Gail Naughton, PhD; Histogen Inc.** *Bringing engineered skin from lab bench to market*
11:45 pm	**Lunch (Powell meeting room)**
12:45 pm	**Jennifer Elisseeff, PhD; Johns Hopkins University** *Technology spinouts from academia*
1:05 pm	**Stephen Duguay, PhD; Genzyme** *Commercialization of cartilage repair products*
1:25 pm	**Chris Breuer, MD; Yale University** *Bench to bedside in academia*
1:45 pm	**Todd McAllister, PhD; Cytograft Tissue Engineering** *Commercialization of tissue engineered blood vessels*
2:05 pm	**Questions for all 5 speakers**
Summary & Next Steps Panel Discussion	
2:30 pm	**(Moderator) – Alan Trounson, CIRM President** **Christine Kelley, PhD; NIBIB/NIH** **Mark Furth, PhD; Wake Forest University** **Todd McDevitt, PhD; Georgia Institute of Technology**
3:30 pm	**ADJOURN**