

What lies at the interface of regenerative medicine and developmental biology?

Donald E. Ingber^{1,*} and Michael Levin²

At a recent Keystone Symposium on 'Developmental Biology and Tissue Engineering', new findings in areas ranging from stem cell differentiation, embryonic pattern formation and organ regeneration to engineered cell microenvironments, synthetic biomaterials and artificial tissue fabrication were described. Although these new advances were exciting, this symposium clarified that biologists and engineers often view the challenge of tissue formation from different, and sometimes conflicting, perspectives. These dichotomies raise questions regarding the definition of regenerative medicine, but offer the promise of exciting new interdisciplinary approaches to tissue and organ regeneration, if effective alliances can be established.

Introduction

Developmental biologists strive to understand how the cells of our tissues and organs come to be specified and placed in their correct positions. Tissue engineers seek to create artificial materials to repair tissues when they are lost due to injury or disease. Both strive to identify crucial cues that trigger these processes, and to control the cells that execute these programs. That tissue engineering might gain from developmental biology, and vice versa, seems obvious; however, investigators in each of these fields generally attend their own meetings and publish in their own journals. But the recent Keystone Symposium on 'Developmental Biology and Tissue Engineering' (organized by Gordana V. Vunjak-Novakovic, Randall T. Moon and David Kaplan) in Snowbird, Utah, suggests that this paradigm is shifting. Here, we describe key themes from this symposium, and consider the crucial challenges that must be overcome to establish the key principles of regenerative medicine and translate them into powerful therapeutic strategies.

What is regenerative medicine?

Regenerative medicine is a burgeoning new field that promises to improve health and quality of life by repairing or regenerating cells, tissues or organs. This must be accomplished under a diverse set of circumstances, including acute injury, surgical resection, inflammation, pathological remodeling, ageing and progressive degeneration. Some biologists view the goal as the discovery of master switches and stem cells that drive embryonic organ formation, or the inductive organizers that induce a blastema to regenerate a limb, and to use this knowledge to reform damaged organs in humans. Bob Nerem (Georgia Institute of Technology, GA, USA) opened the symposium noting that many, including NIH,

consider tissue engineering to be the replacement of tissues by fabricating substitutes *ex vivo* for implantation. However, this might be an incorrect assumption. Fred Schoen (Brigham and Women's Hospital and Harvard Medical School, MA, USA) described how artificial heart valves composed of resorbable synthetic polymer scaffolds containing cultured bone marrow-derived cells produce functional valves even though the implanted cells are likely to be replaced during remodeling *in vivo*. Apparently, the mechanical microenvironment of the leaflet induces ingrowth and differentiation of the tissue, and results in regeneration of normal valve architecture (Mendelson and Schoen, 2006). Arnold Caplan (Case Western Reserve University, OH, USA) showed results from human clinical trials, as reported by Osiris Therapeutics, in which adult mesenchymal stem cells (MSCs) improved health in patients with myocardial infarct, graft-versus-host disease and Crohn's disease after intravenous injection, not by differentiating into various cell types, but by suppressing immune responses and permitting natural healing. Meanwhile, David Mooney (Harvard University, MA, USA) described injectable polymer systems that control the spatiotemporal dynamics of morphogens and *in situ* programming of stem cells at injury sites (Hill et al., 2006). So rigid definitions of regenerative medicine are not constructive while the principles that define the field are still being delineated.

Won't stem cells solve the problem?

Stem cells are at the center of expectations of regenerative medicine, and many exciting results relating to stem cell therapies were presented at the Snowbird meeting. Robert Lanza (Advanced Cell Technologies, MA, USA) described how somatic cell nuclear transplantation produces immune-compatible cells that can repopulate the bone marrow without requiring myelosuppression, as well as how human embryonic stem cell (hESC) lines can be generated without destroying embryos (Klimanskaya et al., 2006). This is accomplished by extracting a single cell from eight-cell stage embryos, or by creating haploid embryos through parthenogenesis.

Alan Colman (Singapore Institute of Medical Biology and ESC International, Singapore) focused on identifying the crucial growth factors and cytokines necessary to induce hESCs to differentiate into cardiomyocytes. By using cell-free and serum-free culture conditions, combined with genetic selection, they have been able to obtain a 99.9%-pure cardiomyocyte population. However, when GFP-labeled hESCs are injected into ischemic hearts of NOD/SCID mice, most of the implanted cells die. Clinical trials involving injection of stem cells into the heart have been similarly ineffective at maintaining viable cells at the injection site in humans (Hofmann et al., 2005). Nevertheless, the field is still young, and it seems likely that cellular therapies using stem cells will be effective for certain conditions, especially those in which only dysfunctional cells need to be replaced and tissue structure remains intact (e.g. diabetes, Parkinson's disease).

Aren't tissue engineers already building tissues and putting them into people?

If the press seems to be overselling stem cells now, one only needs to go back a few years to find that they had done the same for tissue engineering. Yet only a few engineered tissues (e.g. Apligraf and Integra artificial skin products) are now in clinical use. At Snowbird, Shulamit Levenberg (Technion University, Haifa, Israel), who presented work on co-culturing vascular endothelial cells with other

¹Vascular Biology Program, Departments of Pathology and Surgery, Children's Hospital and Harvard Medical School, Boston, MA 02115, USA. ²Center for Regenerative and Developmental Biology, Forsyth Institute, and Developmental Biology Department, Harvard School of Dental Medicine, Boston, MA 02115, USA.

*Author for correspondence (e-mail: donald.ingber@childrens.harvard.edu)

cell types as an approach to improve the vascularity and functionality of engineered tissues, noted that many developmental biologists ask tissue engineers: "It's so complex, how are you going to try to mimic it?". Farshid Guilak (Duke University, NC, USA) reaffirmed the difficulty of this challenge and explained that some of the frustration in the field might have been based on oversimplified assumptions, such as what works in animals will also work in patients, and that cost is no object. He also described how existing polymer scaffold-cell composites often do not have the appropriate material properties to bear physiological mechanical loads. Guilak is approaching this challenge in a new way by applying a three-dimensional (3D) weaving technology to create porous fabrics that exhibit different moduli (stiffness and elasticity) in different directions and that are composed of interwoven fibers of poly(ϵ -caprolactone) (Moutos et al., 2007); these scaffolds can exhibit mechanical properties similar to those of native articular cartilage.

However, the challenge of integrating this or any other type of artificial scaffold into a diseased tissue filled with inflammatory cytokines remains a tough one. Fred Schoen emphasized that clinical testing and validation of engineered materials will be complicated by the heterogeneity of tissue responses among patients, as well as differences in response in old versus young. Although exciting and effective in certain applications, tissue engineering approaches, like stem cell therapies, need to be greatly improved to match the promises made in the press.

What can we learn from developmental biology?

We should be able to develop new, more powerful regenerative therapeutic strategies if we could decipher the control mechanisms responsible for normal developmental patterning in the embryo and the adult. This promise is seen in powerful regenerative model systems such as Planaria, which perfectly regenerate after the excision of nearly every part of their body, and stop as soon as the pre-existing structures are rebuilt (Sanchez Alvarado, 2003). Human liver can similarly regenerate its original functional mass after removal of more than 50% of the organ, and it shuts off this program when its normal size is restored. However, demonstrating that this type of regenerative capacity can be reactivated in other human organs remains a fundamental challenge in the regenerative medicine field.

Randall Moon (HHMI, University of Washington School of Medicine, WA, USA) showed that Wnt signaling through β -catenin is required for epithelial and mesenchymal cell proliferation, and cell fate specification, during tail regeneration in zebrafish. Conversely, a gain-of-function Wnt8 accelerates the regeneration process without altering final tail size (Stoick-Cooper et al., 2007). Moon also reported that β -catenin-mediated gene expression increases near sites of injury in mouse liver and fish heart, and showed that Wnt5a enhances bone marrow engraftment of hematopoietic progenitor cells when they are injected intravenously in mice (Trowbridge et al., 2006). This is an example of where the dissection of a developmental signaling mechanism has led to tangible results that might have a significant impact on tissue engineering and stem cell therapies. However, the question of morphogen specificity remains: how can a molecule, such as Wnt, that produces diverse effects in many tissues at different times and in different spatial contexts be used as part of a therapeutic strategy in humans where specificity and lack of toxicity are crucial? Also, as Wnts can contribute to tumor formation (Kikuchi, 2003), how can this potential complication be prevented?

Another major approach pursued by developmental biologists centers on defining inductive factors that guide stem cells to differentiate into various specialized cell types. Didier Stainier (University of California San Francisco, CA, USA) described how Wnt2b, bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and retinoic acid (RA) are required for endoderm to differentiate into liver, whereas FGF and RA promote exocrine pancreas formation, and hedgehog signaling and RA stimulate pancreatic β -cell differentiation (Ober et al., 2006). Thomas Reh (University of Washington, WA, USA) showed that NOTCH maintains hESC progenitors in a self-renewing state, and that neural differentiation can be synchronized by inhibiting NOTCH. Moreover, in other experiments with human ES cells, he reported that insulin-like growth factor, DKK1 and noggin direct the cells to generate human retinal progenitors, which can be induced to differentiate into retinal photoreceptors by simultaneously blocking NOTCH and adding RA (Lamba et al., 2006).

But generating the right kinds of cell types is not the same as regenerating tissues and organs. The correct cues must also be provided to position these cells appropriately, to induce them to deposit extracellular matrices (ECMs), and to organize these elements spatially across several levels of scale as components of larger tissue and organ structures (Fig. 1). For example, in mouse *Ds* (disorganization) mutants, normal cell and tissue types are produced, but their overall placement with respect to each other is disturbed at the organ/appendage level (Robin and Nadeau, 2001). So induction of cell differentiation alone is not sufficient to meet the regenerative medicine challenge (Fig. 1). Interestingly, the spatial organization of the microenvironment itself can dictate the final output of multicellular and multimolecular developmental programs. For example, Stainier found that although Smoothed (Smo) is required for pancreatic β -cell differentiation in zebrafish, cells from *smo* knockouts differentiate into β cells when placed in wild-type embryos. In this case, spatial context overrides gene expression. Reh also showed that when GFP-labeled hESCs are injected into the vitreous of newborn mice, the cells invade all of the retinal layers and differentiate into appropriate neural cell types (Lamba et al., 2006). Thus, stem cells can sense local environmental cues, place themselves in relatively normal positions, and differentiate appropriately based on their location.

But one of the goals of regenerative medicine is to produce stem cells that can recreate normal tissue architectural arrangements when these spatial relationships are completely lost (e.g. owing to injury or amputation). This is a considerable challenge, however, as in the absence of the correct morphogenetic cues, implanted stem cells can give rise to teratomas – tumors that exemplify tissue differentiation and growth in the absence of higher-order pattern controls (Wakitani et al., 2003). Thus, we need to learn more about normal tissue construction, and not only how to chemically induce stem cells to form various specialized cell types (Fig. 1).

Classic epithelium-mesenchyme recombination experiments demonstrated that whereas the epithelium specifies the function of its cells (cytodifferentiation), the mesenchyme often governs the 3D form of the epithelium (histodifferentiation) and the overall morphology of the organ (Sakakura et al., 1976). The mesenchyme influences pattern formation by producing morphogens, and by secreting ECM components and matrix-modifying enzymes. At Snowbird, Donald Ingber (Children's Hospital and Harvard Medical School, MA, USA) extended this view by describing how cytoskeletal contractile forces that cells exert on ECM scaffolds and on each other contribute to morphogenetic control during mouse embryonic lung development. When he altered physical interactions

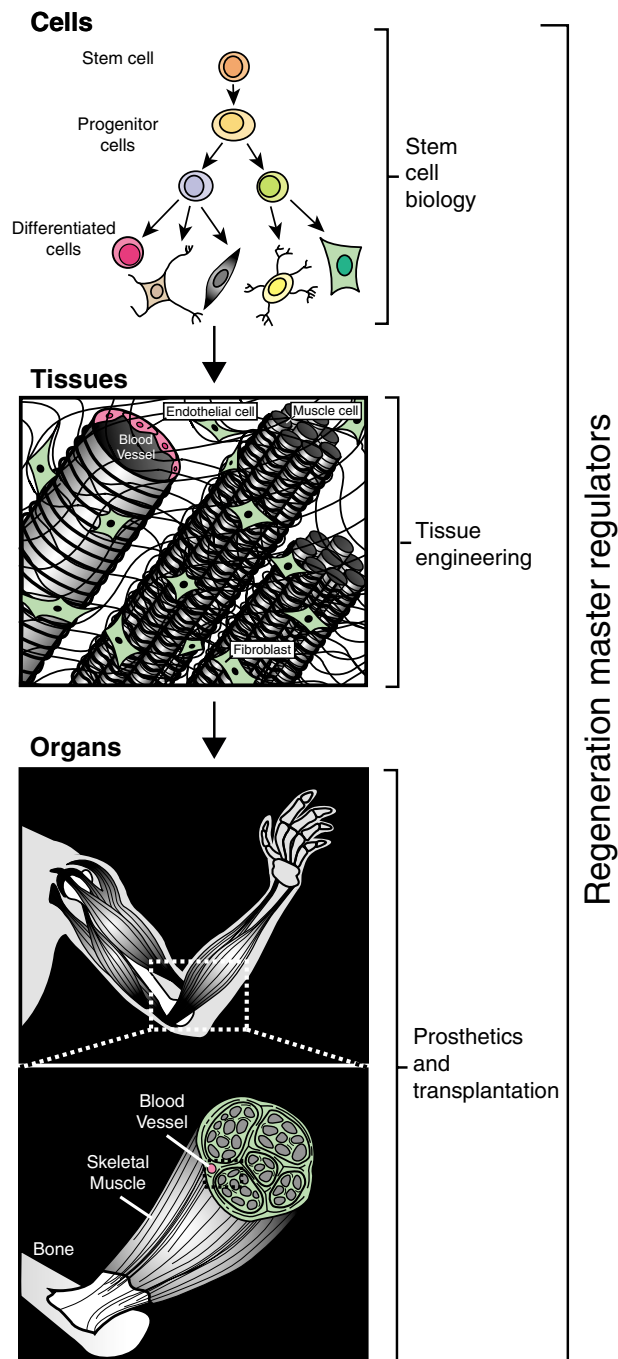


Fig. 1. Current and future approaches to tissue and organ regeneration. At present, the replacement of an organ, such as a whole limb, is carried out through surgical transplantation or implantation of prosthetic devices (bottom panel). Therapeutic stem cell strategies (top panel) present an alternative approach to identifying the controls that drive these cells to form the various specialized cell types that comprise an organ. Tissue engineering (middle panel) focuses mostly on the cell-to-tissue transition by combining cells with scaffolds that mimic the ECM to promote multicellular assembly and provide mechanical functions. Future approaches are likely to integrate stem cells into tissue-engineered products, or use implantable engineered materials to guide stem cell behavior in vivo. Another developmental biology-based approach, which could promote the entire cell-to-organ regeneration cascade, requires identification of 'master regulators' that are analogous to those that produce whole-limb and appendage regeneration in lower organisms.

between cells and ECM by modulating cytoskeletal tension generation by manipulating Rho GTPase or Rho-associated kinase (ROCK), he was able to selectively speed up or slow down epithelial budding morphogenesis and angiogenesis (Moore et al., 2005). Other groups have found that physical forces and cell distortion regulate axis formation (Farge, 2003), tissue remodeling during gastrulation (Belousov et al., 1990) and whole organ size in animals (van Rooij et al., 2007).

Jeff Axelrod (Stanford University, CA, USA) and Suzanne Eaton (Max Planck Institute, Dresden, Germany) explored the importance of cell-cell adhesions and mechanical forces during the establishment of epithelial planar cell polarity (PCP) in *Drosophila*. PCP requires the coordination of local and long-range signaling pathways to orient cells consistently in an epithelial sheet with respect to the polarity of the whole tissue. Axelrod showed that in a domino-like cascade, the interaction between Fz and Vang is mediated by the atypical cadherin Flamingo (Starry night – Flybase) that is present on both sides of the cell-cell adhesion complex and transmits a directional signal between adjacent cells. Eaton examined the mechanism of PCP in the *Drosophila* wing epithelium. She finds that wing cells become hexagonally packed just before initiating bristle formation in a time frame that exactly correlates with polarization of PCP proteins. By using physical modeling approaches, she showed that different cell packing and cell shape configurations are governed by the level of mechanical tension at the junctional region, and demonstrated the presence of tension directly using laser ablation. Thus, cell packing geometry in *Drosophila* wings is governed by a balance of mechanical forces, much like what Ingber observed during the control of mouse lung development.

Michael Levin (Forsyth Institute and Harvard School of Dental Medicine, MA, USA) demonstrated that another physical cue – bioelectricity – drives regeneration in lower organisms (Adams et al., 2007; Levin, 2007). He found that induction of spinal cord and muscle regeneration in the *Xenopus* tail requires the expression of a cell-surface H^+ pump in the wound epithelium that changes transmembrane potential in the regeneration bud, and creates a long-range electric field that appears to promote nerve ingrowth. Moreover, misexpression of a heterologous (yeast) H^+ pump is sufficient to induce the whole regeneration cascade in the *Xenopus* tail at non-regenerative stages. Most intriguing was the finding that ectopic expression of a K^+ channel in *Xenopus* can induce the formation of complex eyes that contain both expressor and non-expressor cells. Together, these findings provide another example of how a physical cue, such as an electric potential, can be as important as chemicals and genes are for developmental control. They also clarify that organ regeneration is a unique developmental program (distinct from wound repair) that may be reinitiated by a relatively simple signal (e.g. the activity of specific ion transporters), which reboots adult cells and reprograms their development, if presented in a receptive tissue microenvironment.

Does combining engineering and biological approaches advance the field?

Numerous presentations described examples of how developmental biologists and engineers are beginning to work together and incorporate each other's tools and approaches. These experiments often fundamentally change the way in which we view the problem. Ingber, Christopher Chen (University of Pennsylvania, PA, USA) and Dennis Discher (University of Pennsylvania) all presented results using engineered substrates that show that mechanical interactions between cells and ECM (and related changes of cell

shape) control cell fate switching in vitro. Ingber described how endothelial cells, liver cells and smooth muscle cells can be switched between growth, differentiation and apoptosis in the presence of a constant amount of soluble growth factors by varying cell spreading (Singhvi et al., 1994; Chen et al., 1997). This was accomplished by culturing cells on micrometer-sized ECM islands created with a microfabrication technique that holds one cell on each island. Chen used a similar approach to show that when larger ECM islands are created that support the adhesion of multicellular monolayers, growth patterns are not homogeneous; rather, increased DNA synthesis is observed in regions where tensional forces are concentrated owing to the geometry of the island (McBeath et al., 2004). He also discovered that cell shape distortion governs stem cell lineage switching (McBeath et al., 2004). hMSCs differentiate into bone cells with high efficiency when cultured on large ECM islands that promote spreading, whereas the same MSCs in the same medium switch on adipose cell differentiation when plated on small islands. Discher fabricated ECM-coated polyacrylamide gels of different stiffnesses to match the elasticity of soft tissues that include brain, muscle and the bone matrix called 'osteoid'. The hMSCs sense these differences in elasticity and respond by differentiating into neurons, muscle cells and osteoblasts, respectively (Engler et al., 2006). As in Chen's studies with shape distortion, ECM mechanics proved as potent as induction cocktails. Matthias Chiquet (Friedrich Miescher Institute, Basel, Switzerland) showed that altering the mechanical forces balanced across integrin receptors induces expression of the tenascin-C-encoding gene in embryonic fibroblasts (Sarasa-Renedo et al., 2006). Interestingly, this mechanical signaling pathway is mediated by integrin-linked kinase, as well as Rho and ROCK, which are also crucial for establishment of localized growth differentials in vitro (Nelson et al., 2005) and in vivo (Moore et al., 2005).

These results provide the first examples of quantitative design criteria that tissue engineers might use to help design artificial tissue scaffolds. In fact, Jeremy Mao (Columbia University, NY, USA), David Kaplan (Tufts University, MA, USA), Gordana Vunjak-Novakovic (Columbia University), and others are already incorporating mechanical loading in their tissue engineering design strategies. Equally important, however, these results suggest that developmental biologists might want to focus more on the role of mechanical forces and material elasticity in their studies on 'stem cell niches' that are responsible for controlling stem cell behavior in situ.

Biologists tend to consider one cytokine (or gene) at a time, and to determine whether it is present (on) or absent (off) when analyzing its role in cell and tissue regulation. Mooney described how the use of synthetic polymer-based drug delivery systems to deliver two angiogenic factors – VEGFA and PDGFBB – with different dynamics (VEGFA fast; PDGFBB slow) in a defined spatial gradient in the same tissue produces much more robust vascular development than either alone, or when both are added simultaneously (Hao et al., 2007). Moreover, he showed that controlling the spatiotemporal dynamics of growth factor delivery can help regenerate the vasculature and save whole limbs in a mouse leg ischemia model. These findings are likely to resonate with developmental biologists because they know that tissue formation is regulated by multiple soluble factors that exhibit varying concentrations over time and space. But it is often difficult, if not impossible, to measure or control these parameters; so this type of engineering approach might open entirely new avenues of research in embryology.

Peter Zandstra (University of Toronto, Canada) described related studies using microfabricated substrates and automated microfluidic systems in which he found that mouse progenitor cells and ESCs

differ in their ability to sense signal strength and spatial cues, and that they exhibit different time windows of sensitivity to the same factors. Zandstra's computational models also revealed that ESCs exhibit robust autocrine signaling that has a buffering effect on differentiation, that they exhibit feed-forward regulatory loops, and that loss of signal responsiveness is an early reversible step in ESC commitment prior to differentiation (Davey and Zandstra, 2006). Furthermore, when he cultured ESCs on microfabricated ECM islands of different sizes, he observed that endogenous signaling gradients can be regulated in a spatially controlled manner to control ESC fate. These engineering approaches might prove extremely useful for ESC production for regenerative medicine applications, as well as for developmental biologists interested in stem cell niche function.

A common theme that emerged in this meeting was the need to understand how robust behaviors emerge from collective interactions. Anand Asthagiri (California Institute of Technology, CA, USA) created quantitative integrative models of vulva cell type specification in *C. elegans*. He deduced a phase diagram of multicellular patterns in a parameter-unbiased approach, and identified parameters that optimally shift a wild-type phenotype into a mutant. This revealed that the phenotypic capacity of the molecular circuit he studied is constrained (i.e. not all possible variations can be explored), and that this approach enables control over large-scale patterns (e.g. transitioning from reflectional to translational symmetry in the linear order of vulval cell types) (Giurumescu et al., 2006). These types of systems-based approaches may be augmented by automated text-mining/curating systems, such as the one described by Andrey Rzhetsky (Columbia University) (Rzhetsky et al., 2004).

Cells cannot explore all possible phenotypic states because regulatory interactions within their gene and signaling networks make certain states impossible. Regulatory constraints also exist at the level of cell-cell and tissue-tissue networks that exhibit similar complex interdependent interactions during embryological development. As Ingber discussed, this phenomenon of dynamic network complexity has been analyzed using physics-based mathematical approaches to explore the implications of much larger networks, on the scale of whole genomes (Kauffman, 2004). This work revealed that the complex web of gene interactions funnels down to only a limited number of stable states over time called 'attractors' (in the same way that water droplets falling on a hilltop eventually roll down to a common low point in one valley or another). In this framework, a 'master' gene or inductive signal effectively acts by lowering the hilltops in the attractor landscape (e.g. by simultaneously altering the activation state of multiple downstream network elements). Once the system (gene, cell or tissue network) passes over the lowered peak, it will fall into another stable attractor and hence generate only distinct types of cells, tissues or organs, because the fixed landscape is determined by the architecture and system-wide dynamics of the underlying regulatory networks. Ingber described gene microarray studies that experimentally confirmed the existence of attractors in the gene regulatory network of human HL60 promyelocytic precursor cells that were induced to differentiate into neutrophils by two different factors, one specific (RA) and one highly non-specific (the solvent DMSO) (Huang et al., 2005). The existence of attractors might explain how generalized stimuli, such as cell shape distortion, changes in ECM mechanics, or a short-circuit electric current flowing through breaks in epithelia, can control cell fate switching and produce identical responses to those induced by cytokines. This physics-based view of cellular signaling appears to conflict with

current paradigms in the biology; however, it might greatly simplify approaches to regenerative medicine because it suggests that we may not have to recreate every step in a signaling cascade to produce a stable functional tissue.

Presentations by engineers described many tools and approaches that might also be of value to developmental biologists. Mooney and Tabata described biodegradable polymers that provide the controlled delivery of proteins, plasmid DNA and siRNAs to selected sites (Silva and Mooney, 2007). Levin described techniques to guide cell migration and differentiation using voltage gradients (Adams et al., 2007), and Vunjak-Novakovic showed how cultured cardiomyocytes exhibit regular cardiac beating rhythms in engineered microsystem bioreactors when electrical potentials are applied (Gerecht-Nir et al., 2006). These bioreactors, which culture cells and tissues at high density within micro-channelled scaffolds perfused with culture medium containing oxygen carriers, might also prove useful for embryological studies.

Another area where biologists and engineers could learn from each other is the materials science of living tissues. Gabor Forgacs (University of Missouri, MI, USA) described an automated 3D printer that produces functional organoid-like structures of any shape by depositing liquid-like multicellular spheroids as ink particles drop-by-drop. Buddy Ratner (University of Washington) summarized results showing that biocompatibility can be controlled by selectively engineering the surface properties of materials to control protein orientation. Peter Lelkes (Drexel University, PA, USA) has used FGF proteins and tenascin C to stimulate concomitant epithelial and endothelial branching morphogenesis in an in vitro model of lung assembly (Mondrinos et al., 2006). Kaplan demonstrated the ability to combine the strength and biocompatibility of natural silk with the specific adhesivity of cell-binding sequences from ECM molecules within chimeric proteins that mimic natural hierarchical self-assembly properties (Wong Po Foo et al., 2006).

These various engineering approaches could potentially revolutionize developmental biology. But the Symposium made it clear that tissue engineers can learn a great deal from developmental biologists as well. Caplan highlighted properties of MSCs, such as homing and secretion of trophic or inhibitory factors, that might prove helpful to those desiring to create materials that can recruit endogenous MSCs or modify their function when injected in vivo (Caplan and Dennis, 2006). Charles Murry (University of Washington) is engineering a 3D scaffold-free patch of human cardiac tissue using hESC-derived cardiac myocytes (McDevitt et al., 2003). Levenberg finds that co-culturing similar hESC-derived cardiomyocytes with hESC-derived endothelial cells and embryonic fibroblasts results in increased cardiac cell proliferation and enhanced vascularization (Caspi et al., 2007). Dan Gazit (Hebrew University and Cedars Sinai Medical Center, CA, USA) is genetically engineering MSCs to express osteogenic BMP or brachyury transcription factor to regenerate bone and nucleus pulposus within intervertebral spinal discs (Aslan et al., 2006). He has also developed numerous sophisticated in vivo imaging techniques, including micro-CT analysis and fiber optic-based confocal fluorescence imaging, that might help developmental biologists and tissue engineers alike.

A new area of biology that might impact tissue engineers, as well as biologists, is that of micro-RNAs (miRNAs) in developmental control. Alexander Schier (Harvard University) showed that a specific miRNA (miR430) regulates the maternal-zygotic transition in zebrafish, not by inducing the switch, but by erasing the previous

state (Giraldez et al., 2006). He speculated that tissue engineers might potentially use miRNAs in the future to hold cells in a particular developmental state. Eric Olson (University Texas Southwestern Medical Center, TX, USA) used microarrays to identify miRNAs that increase their expression in response to structural perturbations that produce cardiac muscle hypertrophy and heart dilation in mice (van Rooij et al., 2007). He found that a particular miRNA (miR208) in the intron of the myosin heavy chain gene plays a central role in the control of heart form and function: strikingly, hearts of *miR208*-knockout mice do not undergo hypertrophy or fibrosis in response to thoracic artery banding. Tissue engineers and biologists may find miRNAs to be extremely useful gene-silencing tools.

Why don't more tissue engineers and developmental biologists work together?

When it comes to clinical translation of fundamental research, tissue engineering should be to developmental biology what drug development is to molecular biology. Yet many biologists find it difficult to see how they can gain from engineers who mix cells together with polymers, inject them into animals, and expect to find that they can regenerate lost tissues without understanding basic developmental principles. Tissue engineers cannot always appreciate why biologists are so fascinated by individual molecules or genes, or even by entire signaling pathways, because they concentrate more on the physical properties of the microenvironment. Developmental biologists also work with cell and molecular biologists in basic academic departments, whereas tissue engineers often collaborate with clinical champions in a hospital setting or interact with industry. Thus, it is not surprising that there has been significant tension between tissue engineers and developmental biologists, or at least a lack of camaraderie, in the past. However, the symposium clarified that this clash of perspectives has not restricted some investigators from bridging this gap. Moreover, a positive feed-back loop has been created, as those scientists and engineers who effectively span this interface are now able to act as 'match-makers' between other biologists and engineers who seek to form new alliances.

So what is the best regeneration strategy?

More questions were generated than answered at the symposium. Do we develop optimal culture conditions for growing and differentiating stem cells outside our bodies, or do we fabricate injectable biomaterials that target to injury sites and recruit endogenous stem cells in vivo? Do we build fully functional adult organ replacements, or do we construct microenvironments that mimic embryonic organs, healing wounds or developmentally active inducer tissues? Should we develop cells and biomaterials that enhance existing tissue repair processes, or create reprogramming protocols that can induce adult human tissues to form blastemas and regenerate whole organs, as newts can? Do different kinds of injury (acute amputation, slow degeneration, hypoxic death, crush, etc.) exhibit the same propensity for regeneration, and how might we determine or control this behavior? Much of the discussion centered on exactly how much micromanaging of cellular behavior will be necessary to promote regeneration, regardless of the approach. Must we provide detailed instructions, such as precise time-varying changes of concentration and spatial gradients for every cytokine and morphogen used during normal development, or will we be able to rely on self-organizing properties of cell collectives and endogenous cues provided by the host environment that harness natural attractor-switching mechanisms?

Each investigator must follow their own vision of what form regenerative therapies will take in the future. However, the possibility of identifying ‘master regulators’ – signals that activate numerous downstream events in a coordinated fashion to generate complex structures – is an attractive one (Fig. 1). This approach is plausible given the demonstrated master regulator functions of molecular signaling molecules, such as Wnt and β -catenin, which can induce a new primary axis during embryogenesis (Funayama et al., 1995), or of the *Apc* and *Runx2* genes whose knockout results in supernumerary teeth (Aberg et al., 2004). However, this meeting clarified that there are also biophysical master regulators, such as ion fluxes that alter electrical potential and trigger regeneration of whole organs in *Xenopus* (see above).

The activation of such high-level morphogenetic programs has the benefit of inducing a coherent response that stops when the structure is rebuilt. This is important because little is known about the precise signals that tell organs when to stop growing. However, triggering embryonic cascades is not always desirable. For example, Olson showed that stress-induced heart remodeling triggers activation of embryonic gene programs (expression of fetal forms of myosin heavy chain) that cause pathological changes in adult cardiac myocytes; reactivation of embryonic programs is also one of the hallmarks of cancer. Thus, a key question is whether we can fully reactivate embryonic programs in a regenerative context. If not, it will be necessary to develop more-focused strategies that selectively trigger limited aspects of the regenerative process, or artificial approaches that are ‘inspired’ by normal developmental programs, rather than attempting to recreate them step-by-step.

It is also essential to identify the best target for a regeneration strategy. Should we implant ESCs, control the activities of adult MSCs, stimulate tissue renewal by somatic cells, dedifferentiate mature terminal cells, or a combination of the above? All of these may be of value, and although the current emphasis seems to focus on stem cells, data suggest that exploring the neglected possibility of triggering growth of terminally differentiated somatic cells (e.g. central nervous system neurons) might also pay off (Cone and Cone, 1976; Stillwell et al., 1973). Likewise, biophysical approaches (e.g. using fluorescent voltage- or pH-reporter dyes, measuring micromechanical properties) might make it feasible to identify stem cell niches in vivo and to isolate and grow cells from adult tissues with important self-renewal and differentiation properties. Discovery of unique chemical or biophysical properties of these cells or of the local tissue microenvironment might also lead to novel ways to preferentially target drug payloads or novel self-assembling nanomaterials to these regions, and thereby provide new approaches to modulate stem cell function and developmental programs in situ.

Yet another problem that must be overcome is that developmental signaling can be very context-dependent. The same biochemical (e.g. Wnt) or physical signal (e.g. cell distortion) might be interpreted differently at different times and places, and thus give rise to entirely different developmental responses. The importance of a cell’s provenance through the embryo for its final functional specialization emphasizes the crucial role that spatial and temporal context play in the conversion of a signal into a response in living systems. Appropriately crafting the regenerative microenvironment is likely to require combining biological and engineering strategies, as well as computational modeling approaches.

Conclusion

The emergence of regenerative medicine has created a new and larger tent, within which tissue engineers and developmental biologists find themselves to be only smaller acts. But at present, it

appears unlikely that a stem cell, morphogen or artificial biomaterial will solve the problem of organ regeneration on its own. In the embryo, developmental responses result from the cumulative life experiences of cells as they pass through different spatial contexts, each with its own special regulatory milieu. Tissue engineers could not do what they do without the knowledge of the specific molecular regulators and ECM components that guide these developmental processes, which were uncovered by biologists. Biologists, likewise, are beginning to seek out engineers and physical scientists to address questions that cannot be effectively answered using their existing biological tools, such as how physical forces, electric potentials, spatiotemporal gradients and system-level dynamics influence morphogenetic control. Thus, alliances between researchers in these different fields are already self-organizing. However, complex biological behaviors emerge from collective interactions among numerous components, whether at the molecular, cellular, tissue or organ levels. Thus, to create therapeutics that repair injuries by promoting tissue and organ reconstruction rather than by scarring, we must recreate the correct microenvironment containing the right combination of physical, as well as chemical cues, and ensure that they are acting in the appropriate spatial and temporal context. To meet this challenge, we will need to combine our expertise in biology and engineering, and appropriate tools and approaches from many other disciplines as well. Only then will it be possible to develop effective therapeutics that can reprogram damaged tissues so that they are able to regenerate themselves.

We thank G. Vunjak-Novakovic, R. T. Moon and D. Kaplan for organizing the meeting, P. G. Chao and N. Tandon for sharing their presentation notes, and Kristin Johnson for artwork. We apologize to those whose work could not be discussed because of space constraints. D.E.I. and M.L. gratefully acknowledge support from NIH, NSF, AHA and NHTSA.

References

- Aberg, T., Cavender, A., Gaikwad, J. S., Bronckers, A. L., Wang, X., Waltimo-Siren, J., Thesleff, I. and D’Souza, R. N. (2004). Phenotypic changes in dentition of *Runx2* homozygote-null mutant mice. *J. Histochem. Cytochem.* **52**, 131–139.
- Adams, D. S., Masi, A. and Levin, M. (2007). H^+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce *Xenopus* tail regeneration. *Development* **134**, 1323–1335.
- Aslan, H., Zilberman, Y., Kandel, L., Liebergall, M., Oskouian, R. J., Gazit, D. and Gazit, Z. (2006). Osteogenic differentiation of noncultured immunisolated bone marrow-derived CD105+ cells. *Stem Cells* **24**, 1728–1737.
- Belousov, L. V., Lakirev, A. V., Naumidi, I. I. and Novoselov, V. V. (1990). Effects of relaxation of mechanical tensions upon the early morphogenesis of *Xenopus laevis* embryos. *Int. J. Dev. Biol.* **34**, 409–419.
- Caplan, A. I. and Dennis, J. E. (2006). Mesenchymal stem cells as trophic mediators. *J. Cell Biochem.* **98**, 1076–1084.
- Caspi, O., Lesman, A., Basevitch, Y., Gepstein, A., Arbel, G., Habib, I. H., Gepstein, L. and Levenberg, S. (2007). Tissue engineering of vascularized cardiac muscle from human embryonic stem cells. *Circ. Res.* **100**, 263–272.
- Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. and Ingber, D. E. (1997). Geometric control of cell life and death. *Science* **276**, 1425–1428.
- Cone, C. D. and Cone, C. M. (1976). Induction of mitosis in mature neurons in central nervous system by sustained depolarization. *Science* **192**, 155–158.
- Davey, R. E. and Zandstra, P. W. (2006). Spatial organization of embryonic stem cell responsiveness to autocrine gp130 ligands reveals an autoregulatory stem cell niche. *Stem Cells* **24**, 2538–2548.
- Engler, A. J., Sen, S., Sweeney, H. L. and Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689.
- Farge, E. (2003). Mechanical induction of Twist in the *Drosophila* foregut/stomodaeal primordium. *Curr. Biol.* **13**, 1365–1377.
- Funayama, N., Fagotto, F., McCreia, P. and Gumbiner, B. M. (1995). Embryonic axis induction by the armadillo repeat domain of β -catenin: evidence for intracellular signaling. *J. Cell Biol.* **128**, 959–968.
- Gerecht-Nir, S., Radisic, M., Park, H., Cannizzaro, C., Boublik, J., Langer, R. and Vunjak-Novakovic, G. (2006). Biophysical regulation during cardiac development and application to tissue engineering. *Int. J. Dev. Biol.* **50**, 233–243.
- Giraldez, A. J., Mishima, Y., Rihel, J., Grocock, R. J., Van Dongen, S., Inoue, K., Enright, A. J. and Schier, A. F. (2006). Zebrafish miR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* **312**, 75–79.

- Giurumescu, C. A., Sternberg, P. W. and Asthagiri, A. R.** (2006). Intercellular coupling amplifies fate segregation during *Caenorhabditis elegans* vulval development. *Proc. Natl. Acad. Sci. USA* **103**, 1331-1336.
- Hao, X., Silva, E. A., Mansson-Broberg, A., Grinnemo, K. H., Siddiqui, A. J., Deligren, G., Wardell, E., Brodin, L. A., Mooney, D. J. and Sylven, C.** (2007). Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. *Cardiovasc. Res.* (in press).
- Hill, E., Boontheekul, T. and Mooney, D. J.** (2006). Regulating activation of transplanted cells controls tissue regeneration. *Proc. Natl. Acad. Sci. USA* **103**, 2494-2499.
- Hofmann, M., Wollert, K. C., Meyer, G. P., Menke, A., Arseniev, L., Hertenstein, B., Ganser, A., Knapp, W. H. and Drexler, H.** (2005). Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation* **111**, 2198-2202.
- Huang, S., Eichler, G., Bar-Yam, Y. and Ingber, D. E.** (2005). Cell fates as high-dimensional attractor states of a complex gene regulatory network. *Phys. Rev. Lett.* **94**, 128701.
- Kauffman, S.** (2004). A proposal for using the ensemble approach to understand genetic regulatory networks. *J. Theor. Biol.* **230**, 581-590.
- Kikuchi, A.** (2003). Tumor formation by genetic mutations in the components of the Wnt signaling pathway. *Cancer Sci.* **94**, 225-229.
- Klimanskaya, I., Chung, Y., Becker, S., Lu, S. J. and Lanza, R.** (2006). Human embryonic stem cell lines derived from single blastomeres. *Nature* **444**, 481-485.
- Lamba, D. A., Karl, M. O., Ware, C. B. and Reh, T. A.** (2006). Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **103**, 12769-12774.
- Levin, M.** (2007). Large-scale biophysics: ion flows and regeneration. *Trends Cell Biol.* **17**, 262-271.
- McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. and Chen, C. S.** (2004). Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483-495.
- McDevitt, T. C., Woodhouse, K. A., Hauschka, S. D., Murry, C. E. and Stayton, P. S.** (2003). Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair. *J. Biomed. Mater. Res. A* **66**, 586-595.
- Mendelson, K. and Schoen, F. J.** (2006). Heart valve tissue engineering: concepts, approaches, progress, and challenges. *Ann. Biomed. Eng.* **34**, 1799-1819.
- Mondrinos, M. J., Koutzaki, S., Jiwanmali, E., Li, M., Dechadarevian, J. P., Lelkes, P. I. and Finck, C. M.** (2006). Engineering three-dimensional pulmonary tissue constructs. *Tissue Eng.* **12**, 717-728.
- Moore, K. A., Polte, T., Huang, S., Shi, B., Alsberg, E., Sunday, M. E. and Ingber, D. E.** (2005). Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by Rho and cytoskeletal tension. *Dev. Dyn.* **232**, 268-281.
- Moutos, F. T., Freed, L. E. and Guilak, F.** (2007). A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. *Nat. Mater.* **6**, 162-167.
- Nelson, C. M., Jean, R. P., Tan, J. L., Liu, W. F., Sniadecki, N. J., Spector, A. A. and Chen, C. S.** (2005). Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl. Acad. Sci. USA* **102**, 11594-11599.
- Ober, E. A., Verkade, H., Field, H. A. and Stainier, D. Y.** (2006). Mesodermal Wnt2b signalling positively regulates liver specification. *Nature* **442**, 688-691.
- Robin, N. H. and Nadeau, J. H.** (2001). Disorganization in mice and humans. *Am. J. Med. Genet.* **101**, 334-338.
- Rzhetsky, A., Iossifov, I., Koike, T., Krauthammer, M., Kra, P., Morris, M., Yu, H., Duboue, P. A., Weng, W., Wilbur, W. J. et al.** (2004). GeneWays: a system for extracting, analyzing, visualizing, and integrating molecular pathway data. *J. Biomed. Inform.* **37**, 43-53.
- Sakakura, T., Nishizuka, Y. and Dawe, C. J.** (1976). Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. *Science* **194**, 1439-1441.
- Sanchez Alvarado, A.** (2003). The freshwater planarian *Schmidtea mediterranea*: embryogenesis, stem cells and regeneration. *Curr. Opin. Genet. Dev.* **13**, 438-444.
- Sarasa-Renedo, A., Tunc-Civelek, V. and Chiquet, M.** (2006). Role of RhoA/ROCK-dependent actin contractility in the induction of tenascin-C by cyclic tensile strain. *Exp. Cell Res.* **312**, 1361-1370.
- Silva, E. A. and Mooney, D. J.** (2007). Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. *J. Thromb. Haemost.* **5**, 590-598.
- Singhvi, R., Kumar, A., Lopez, G., Stephanopoulos, G. N., Wang, D. I. C., Whitesides, G. M. and Ingber, D. E.** (1994). Engineering cell shape and function. *Science* **264**, 696-698.
- Stillwell, E. F., Cone, C. M. and Cone, C. D.** (1973). Stimulation of DNA synthesis in CNS neurones by sustained depolarisation. *Nat. New Biol.* **246**, 110-111.
- Stoick-Cooper, C. L., Weidinger, G., Riehle, K. J., Hubbert, C., Major, M. B., Fausto, N. and Moon, R. T.** (2007). Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development* **134**, 479-489.
- Trowbridge, J. J., Xenocostas, A., Moon, R. T. and Bhatia, M.** (2006). Glycogen synthase kinase-3 is an in vivo regulator of hematopoietic stem cell repopulation. *Nat. Med.* **12**, 89-98.
- van Rooij, E., Sutherland, L. B., Qi, X., Richardson, J. A., Hill, J. and Olson, E. N.** (2007). Control of stress-dependent cardiac growth and gene expression by a MicroRNA. *Science* **316**, 575-579.
- Wakitani, S., Takaoka, K., Hattori, T., Miyazawa, N., Iwanaga, T., Takeda, S., Watanabe, T. K. and Tanigami, A.** (2003). Embryonic stem cells injected into the mouse knee joint form teratomas and subsequently destroy the joint. *Rheumatology Oxford* **42**, 162-165.
- Wong Po Foo, C., Patwardhan, S. V., Belton, D. J., Kitchel, B., Anastasiades, D., Huang, J., Naik, R. R., Perry, C. C. and Kaplan, D. L.** (2006). Novel nanocomposites from spider silk-silica fusion (chimeric) proteins. *Proc. Natl. Acad. Sci. USA* **103**, 9428-9433.