Systems Biology Characterization of Engineered Tissues

Padmavathy Rajagopalan,[1,2,5] Simon Kasif,[3] and T.M. Murali[4,5]

[1]Department of Chemical Engineering, Virginia Tech, Blacksburg, Virginia 24060; email: padmar@vt.edu

[2]School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, Virginia 24060

[3]Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215

[4]Department of Computer Science, Virginia Tech, Blacksburg, Virginia 24060

[5]ICTAS Center for Systems Biology of Engineered Tissues, Virginia Tech, Blacksburg, Virginia 24060

Annu. Rev. Biomed. Eng. 2013. 15:55–70

The *Annual Review of Biomedical Engineering* is online at bioeng.annualreviews.org

This article's doi: 10.1146/annurev-bioeng-071811-150120

Keywords

tissue and organ mimics, biomaterials, 'omics data sets, molecular interaction networks, data integration algorithms

Abstract

Tissue engineering and molecular systems biology are inherently interdisciplinary fields that have been developed independently so far. In this review, we first provide a brief introduction to tissue engineering and to molecular systems biology. Next, we highlight some prominent applications of systems biology techniques in tissue engineering. Finally, we outline research directions that can successfully blend these two fields. Through these examples, we propose that experimental and computational advances in molecular systems biology can lead to predictive models of bioengineered tissues that enhance our understanding of bioengineered systems. In turn, the unique challenges posed by tissue engineering will usher in new experimental techniques and computational advances in systems biology.

Contents

1. INTRODUCTION

In vitro tissue mimics can serve as reliable models of in vivo structures that can be systematically probed with a wide range of cues. Because they are engineered, experimentation on such models is considerably less complex than that needed to probe tissues and organisms in vivo. Currently, tissue engineers draw heavily from a range of disciplines in order to successfully design tissue or organ mimics. These fields include cell and molecular biology, biomedical engineering, materials science, and chemical and mechanical engineering.

Concomitant with the rapid developments in tissue engineering, the genomic revolution of the past two decades and rapid advances in high-throughput experimental techniques are enabling measurements of mRNA, protein, and metabolite levels and the detection of molecular interactions on a massive scale. These advances are transforming molecular biology from a reductionist, hypothesis-driven experimental field into an increasingly data-driven science focused on understanding the functioning of the living cell at a systems level. The grand challenges that constitute the field of molecular systems biology include understanding the complex interactions between diverse and large bodies of molecules at various levels and inferring the intricate pathways that govern each biological process.

So far, the inherently interdisciplinary fields of tissue engineering and systems biology have been developed independently. Through this review, we aim to demonstrate that there is a natural synergy between the two areas that has not yet been fully explored. After providing a brief introduction to tissue engineering and to molecular systems biology, we highlight some prominent applications of systems biology techniques in tissue engineering. Finally, we describe how these fields can be merged using computational science as a driving force (**Figure 1**).

2. TISSUE ENGINEERING OVERVIEW

Age and disease often result in the deterioration of human tissues and organs. Although changes in lifestyle and administration of drugs can delay or, in some cases, prevent tissue degradation, organ failure is an inevitable outcome for a large section of an aging population. Surgical transplantation can provide relief in some cases. However, the scarcity of viable donors, the difficulty in preventing an immune response, and rising medical costs can make such treatment options unattainable. An alternative is tissue engineering, a field of research that attempts to create replacements for living tissues and organs (1, 2). The goal of tissue engineering is to create organs and tissues that recapitulate several components of native tissue or to design cellular systems that closely mimic structures found in vivo (3–5). Researchers typically use natural or nonimmunogenic synthetic

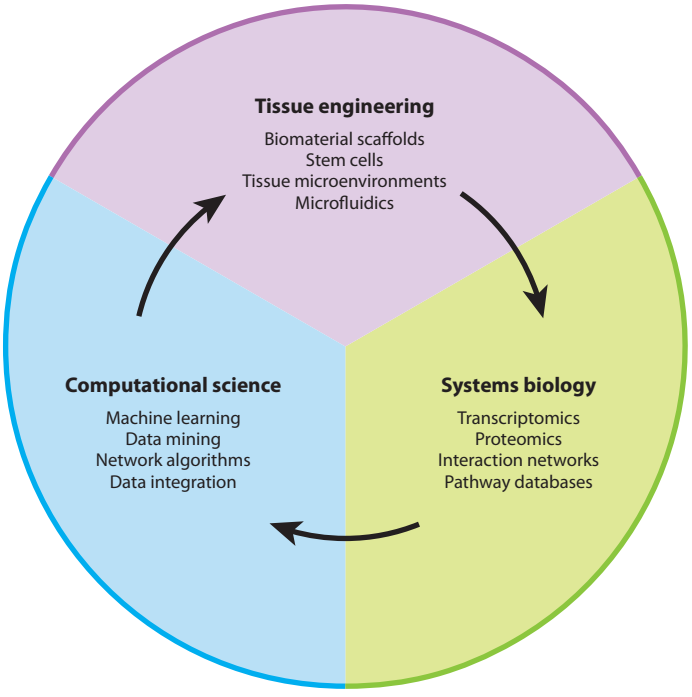

Figure 1

Illustration of how tissue engineering, systems biology, and computational science can influence, and be mutually beneficial to, one another.

materials and cells derived from native tissue. To date, research in this field has resulted in a wide array of engineered tissues. Examples are vascular grafts (6), urinary bladder (7), skin (8), kidney (9), cornea (10), bone (11), cartilage (11), liver (12), muscle (13), and nerve (14, 15).

Tissue engineers must match the physical, chemical, biological, and structural details of each tissue (16–23). Because tissue architecture is a complex combination of proteins, proteoglycans, basement membranes, multiple cell types, and different oxygen requirements, the design space can be daunting. Furthermore, it is critical that components in engineered tissues be nonimmunogenic in vivo, which poses additional challenges in materials synthesis and tissue assembly (15, 22, 24–26). Typically, engineers first work to obtain the optimal chemical composition and match the mechanical properties of the tissue and then try to optimize its shape and structure. Today, researchers finely tune the surface topography (23, 27), include chemical and mechanical gradients (23), and vary the dynamic conditions a tissue experiences in vivo (28). Once an engineered tissue has been assembled, the next challenge is to measure, understand, analyze, and then modify intra- and intercellular signals within the engineered construct. Information obtained from the complex modes by which cells communicate often leads to changes of the assembly, modification, and subsequent use of engineered tissues. Finding an optimal solution that combines all design parameters makes this experimentally reliant research field extremely labor and resource intensive.

In the past, studies focused primarily on the design of two-dimensional (2D) tissues (29–31). Such approaches included a biomaterial in contact with either a single cell type (monolayer) or multiple cell types (2D cocultures). The choice of the biomaterial and its properties played a

critical role in maintaining cellular phenotype and physiological functions. In such systems, either primary or genetically modified cells taken from the native tissue were cultured as monolayers or in the presence of other tissue-specific cells.

Tissue engineers have benefited from advances in stem cell research that have opened up numerous possibilities in their ability to control tissue composition and function (32–35). In order to obtain multiple cell types, pluripotent, embryonic, or mesenchymal stem cells (MSCs) (32–37) are used. Although stem cells can radically change research strategies for tissue engineering, they require careful manipulation with respect to their rates of proliferation, presentation of chemical signals (e.g., growth factors), and tuning of chemical and mechanical properties of the biomaterial scaffold. Today, researchers are placing more emphasis on the size and spatial specifications of embryoid bodies, the incorporation of different extracellular matrix (ECM) proteins, and the presentation of morphogens and growth factors (38–41). With new opportunities, stem cells also bring forth challenges in eliciting and maintaining cellular function and preventing the formation of cancerous tissues over longer time periods (42).

The field of tissue engineering has made rapid strides in past decades. From simple cell-biomaterial systems, the field has evolved toward smart materials, stem cells, and sophisticated analytical methods. Despite tremendous progress, this rapidly evolving research field is often based on empirical evidence and intensive experimentation. For these reasons, it is difficult to fully explore the design space or to test a wide range of conditions and treatments. These shortcomings could result in experimentalists missing important insights into signaling molecules, metabolites, and pathways. Therefore, tissue engineers could benefit from a systems approach that combines computational predictions followed by experimental validations.

3. SYSTEMS BIOLOGY OVERVIEW

Cells, tissues, organs, and organisms are systems of components whose interactions have been defined, refined, and optimized over hundreds of millions of years of evolution. Molecular systems biology is a field that works toward a comprehensive and unified understanding of cells by integrating experimental and computational approaches in order to answer the following key questions: What are the basic structures and properties of the biological networks in a living cell? How does a cell function over time under various conditions? How does a cell maintain its robustness and stability? How can we modify or construct biological systems to achieve desired properties? The explosive progress of genome sequencing projects and the massive amounts of data that high-throughput experiments in DNA microarrays, proteomics, and metabolomics yield drive advances in this field. Sophisticated computational ideas process these data sources in an effort to systematically analyze and unravel the complex biological phenomena that take place in a cell. Experimental and computational approaches used in systems biology fall along a continuum between high-level, or top-down, and low-level, or bottom-up, analyses (43). Top-down approaches reveal and analyze large-scale, high-throughput data sets for interactions or correlations that are relevant to a specific cell behavior. They provide a more global and less biased view of regulatory networks and can often uncover connections that may have escaped the attention of more focused methods. In contrast, bottom-up approaches include detailed kinetic models of cellular systems, enabling quantitative predictions about the dynamical properties of regulatory networks. Typically, these models are relatively small and handcrafted. Although we recognize the importance of both approaches to systems biology, we focus our attention on top-down methods in this review.

3.1. Systems Biology Data Sets

A fundamental tenet of systems biology is that studying the cell as a whole will lead to discoveries that were not possible through studying individual components (44, 45). This paradigm necessitates measuring the state of a cell along multiple modalities and dimensions. In this section, we describe the diverse types of data sets used by systems biologists.

3.1.1. 'Omics technologies. The Encyclopedia of DNA Elements (ENCODE) Project has recently published and analyzed a large number of data sets that discover and define the functional elements encoded in the human genome (46). The elements include genes, transcripts, and regions that regulate transcription, as well as the chromatin states and DNA methylation patterns of these regions. Efforts are underway to apply the same analysis pipeline to the mouse genome (47). These data promise to usher in a new chapter in our understanding of mammalian genomes. Among more mature 'omics approaches, transcriptome analysis has been most reproducibly performed on a genomewide scale using DNA microarrays, and next generation sequencing has rapidly emerged as a reliable method to measure gene-expression levels (48).

Protein levels in cells or tissues can be determined by mass spectrometry or by protein arrays. Multiple reaction monitoring enables mass spectrometric techniques to be targeted at specific peptides (49–51). By contrast, unbiased and global methods (52, 53) are more likely to detect more abundant proteins. Commercially available arrays are useful in the identification of multiple classes of proteins, such as those involved in cell signaling, including cytokines and kinases. However, because antibodies can cross-react, arrays often result in several false positives. An additional complication in mammalian systems is that protein expression is cell-type specific (54). In contrast to mRNA, proteins are difficult to amplify, raising additional challenges in this field.

3.1.2. Protein-protein interactions. Direct physical interactions between proteins are often necessary for the proteins to carry out their biological functions. Such protein-protein interactions (PPIs) play important roles in virtually every biological process. A number of methods, such as yeast two-hybrid screening, coimmunoprecipitation, and tandem-affinity precipitation followed by mass spectrometry to identify proteins, have been developed to detect these interactions. Systematic, unbiased, and high-throughput experimental strategies have incorporated these techniques to discover interactions at the scale of whole genomes or proteomes (55). Databases storing these interactions have proliferated (56). Despite numerous systematic efforts to chart the landscape of PPIs in mammalian cells, our knowledge of these interactions remains sparse (57), with human PPIs being the most investigated and research on other mammals lagging behind.

3.1.3. Regulatory interactions. Genes and proteins regulate each other to coordinate cellular processes. Such interactions include transcription factors (TFs) binding to regulatory sequences on DNA to govern the expression of target genes and kinases, or phosphatases conferring post-translation modifications such as (de)phosphorylation on their substrates. Chromatin immunoprecipitation (ChIP) is widely used to detect TF-DNA interactions (58). Such experiments must be performed for each TF and in each cell type of interest, as a TF's targets can vary dramatically from cell to cell (59). By and large, regulatory interactions are much less comprehensively known than PPIs.

3.1.4. Biochemical and signaling pathways. Biochemical and signaling pathway databases are another important source of information about gene and protein function. Here, we use the term

pathway to refer to a network of physical and regulatory interactions between genes and gene products that together perform a specific biological function. This definition is to be contrasted with the notion of a pathway simply as a set of genes that perform some discrete function. Numerous databases contain biochemical and signaling pathways manually curated from the literature (60–64). Among mammalian organisms, *Homo sapiens* is best represented in these databases. Many sources also store mappings to interaction pathways in other organisms based on sequence and structural homology.

3.1.5. Interactome prediction. To complement these experimentally derived interaction data sets, numerous computational approaches can predict physical (65, 66), regulatory (67), or functional interactions (68) or simultaneously predict all these types (69). Here, a functional interaction or association is a specific interaction between two genes that jointly contribute to the same biological process. Apart from incorporating experimental interaction data, these approaches use multiple modalities of orthogonal information including sequence orthology, similar transcriptional response across a variety of conditions (coexpression), text-mining, and gene families that share above-random similarities in their evolutionary histories. They include schemes to score each predicted interaction against a common reference of well-curated gold standard interactions. Reverse-engineering regulatory interactions among genes from large compendia of gene-expression profiles (70, 71) constitute another class of powerful approaches for discovering networks from high-throughput data.

3.1.6. Functional annotations. To take optimal advantage of systems biology data sets, information about the functions of genes and other cellular components needs to be described by a well-structured set of standardized terms, amenable to computational analysis. The Gene Ontology (GO) (72) provides a widely used, standardized, and organism-independent set of terms to describe biological processes, molecular functions, and cellular components. Each of these three ontologies consists of a hierarchical arrangement of terms with precise definitions and interrelationships. Specifically, the hierarchy is a directed acyclic graph (DAG) that connects less-specific parent terms to their more-specific child terms. By construction, genes annotated to a term are also annotated to the term's parents. The GO also includes a set of evidence codes that characterize the reasoning behind a particular annotation. As the GO is organism independent, genes with similar functions in different organisms can be annotated to the same term even when there is little sequence similarity among the genes. The GO Consortium oversees the development and refinement of the ontologies, and individual genome databases are responsible for assigning annotations of genes to appropriate GO terms.

3.2. Systems Biology Algorithms

In this section, we describe state-of-the-art algorithms that integrate genomic, proteomics, metabolomics, and pathway databases to obtain meaningful information at the cellular and tissue levels. A number of approaches have been developed to integrate diverse types of 'omics data sets (45, 71, 73, 74) to discover gene modules, infer gene networks, and predict functional links and gene functions. We focus on methods that have been applied primarily to mammals, rather than to single-celled or to other less complex organisms.

3.2.1. Functional modules. Specific cellular functions are carried out by sets of genes and proteins and interactions among them. A functional module may be defined as a set of molecules that interact to produce a discrete biological function. For example, protein synthesis is a discrete

function carried out by the ribosome and its associated proteins. A signal transduction cascade transmits a signal initiated by the binding of a specific chemical to a receptor protein into the nucleus. Biochemical pathways exist that synthesize specific amino acids. Over the past many decades, hypothesis-driven experiments have yielded numerous instances of such modules. In their seminal paper, Hartwell et al. (75) issued a call for shifting the focus of cell biology from molecules to modules. Since then, a vast number of approaches have been developed to dissect complex molecular networks into modules or communities (76–80). Other methods have computed gene modules by integrating gene-expression data across multiple cellular conditions (77, 81–83). Such gene modules can reveal similarities and differences between multiple cellular conditions (84, 85), for instance by characterizing gene-expression profiles in specific (sets of) tumors as a combination of activated and deactivated modules (84).

3.2.2. Response networks. An intricate network of molecular interactions governs a cell's response to its environment. These interactions dynamically change in response to a myriad of cues. Therefore, discovering the set of molecular interactions that are active in a given cellular context is a fundamental question in systems biology (74). A number of techniques compute the response network of interactions perturbed in a given condition by integrating gene-expression data with interaction networks (86–91). Recently, attention has turned to combining transcriptional and proteomic measurements within the context of interaction networks (92, 93). Lack of correlation between mRNA and protein levels can complicate such analyses (94, 95).

3.2.3. Prediction and analysis of signaling pathways. Another line of research exploits interaction networks as a scaffold to find subnetworks that can suggest connections between causes (e.g., genes that are knocked out or located near single nucleotide polymorphisms) and effects (e.g., differentially expressed genes) (96, 97) or to discover hidden components of signaling networks from transcriptomic and proteomic data sets (98, 99). These methods have been used to recover known signaling pathways, for example, those that start at a membrane protein and end at a transcription factor in baker's yeast (100). Methods have been developed to encode expert knowledge about a well-understood pathway into a logical model (101) and propose experiments to clarify regulatory relationships that are downstream of the pathway (102).

3.2.4. Gene function prediction. Diverse large-scale functional and structural genomic data sets create the potential to compute quantified, testable predictions of the functions of poorly characterized genes. Predicting gene function based on sequence similarity (103) offers limited benefits, as this method provides clear and unambiguous annotations for only about 30–50% of genes in an organism (104); the remaining genes have unknown, tentative, or marginal annotations. Annotations may also be incorrect, as they are transmitted from one gene to another via weak chains of inference (105), a problem that can be propagated throughout public databases (105, 106), contaminating future functional predictions.

The functional association or linkage networks (FLNs) described above provide a useful basis for predicting gene function (107–109). For instance, two genes may have similar functions if their protein products interact or if they have very similar patterns of gene expression. Because FLNs by themselves do not predict GO terms for genes of unknown function, many methods have emerged to explicitly propagate functional evidence across FLNs in order to predict the functions of uncharacterized genes (107, 110–112). These methods can exploit both local and long-distance connections in the FLN in a controlled manner in order to provide predictions with quantifiable estimates of reliability.

4. APPLICATIONS OF SYSTEMS BIOLOGY IN TISSUE ENGINEERING

Engineered tissues are assembled on biomaterial scaffolds that can support adhesion, locomotion, and timely colonization of cells. In a recent study, Wu et al. (113) used a multilevel approach to control cell speed and persistence in order to optimize the mean free path of motile multipotent stromal cells. The authors measured cell motility properties and the phosphorylation levels of several activities of important signaling molecules on 19 polymeric substrates. By training decision trees on these measurements, they identified the appropriate combinations of growth factors and ECM proteins that resulted in both high speed and directional persistence. This combination of experimental and computational methods enables systematic identification of a small group of molecules and specific substrate conditions that yield optimal cellular responses.

Once an engineered construct has been implanted, its long-term success depends on its ability to integrate with tissue in vivo. Toward this goal, Sun et al. (114) used a combinatorial approach to deliver cytokines from hydrogels to obtain a desired bone density upon the implantation of a tissue-engineered bone graft (114). The authors modeled the dynamics of the balance between bone regeneration and resorption by a mixture of ordinary (for intracellular signaling pathways) and stochastic (for intercellular signaling pathways and cell populations) differential equations. Their simulations suggested combinations of cytokines, for example, Wnt/BMP2, and optimal dose ratios of these combinations that would provide the most benefit during bone remodeling.

Stem cell research is a vast, expanding field. A comprehensive overview of this research is beyond the scope of this review. From the viewpoint of tissue engineering, stem cells present tremendous potential in the design of implants, for which the source of cells can often play a decisive role in a positive outcome. We highlight DNA microarray–based analyses that provide the tissue engineering community with important information on stem cell sourcing. Transcriptional data on embryonic-, mesenchymal-, and umbilical cord–derived cells are leading to an improved understanding of the similarities and differences bewteen these different types of stem cells. Microarray studies have been conducted on mouse embryonic versus adult stem cells (34). Comparisons have also been made between human embryonic stem cells and differentiated cells (115, 116). Ivanova et al. (115) observed that several gene products and regulatory pathways were conserved between murine embryonic and hematopoietic stem cells. Tanaka and coworkers (116) found that fewer embryonic stem cell genes and more tropoblast-related genes were found in materials derived from embryos at different stages, although only the embryonic stem cells contained the *Esg-1* gene associated with pluripotency. Sperger et al. (117) compared the transcriptional profiles between human pluripotent and germ cell teratomas. Using hierarchical clustering, the authors identified similarities between different human embryonic stem cells and embryonal carcinoma. One of the many outcomes of this study is the ability to test the candidate genes that can potentially transform stem cells into their cancerous counterparts.

In some cases, due to the unavailability of embryonic stem cells, researchers have relied on MSCs. MSCs can also be sourced from different tissues. For example, MSCs are most commonly obtained from adult bone marrow, but other sources such as fetal amniotic fluid, term pregnancy amniotic membrane, and term pregnancy umbilical cord blood are also utilized. Recent studies have analyzed detailed gene-expression profiles of MSCs derived from different sources (118, 119). These studies have discovered that a core set of gene-expression profiles was maintained in all four cell types. When the gene expressions of MSCs were compared with those of fetal organs, the authors discovered similarities in regulation of ECM proteins, in Wnt signaling, and in TGF-β (transforming growth factor-beta) receptor signaling. They also discovered variations between MSCs obtained from each of the four tissues. Their findings could prove to be a powerful resource to tissue engineers embarking on the use of MSCs for implants and therapeutic applications.

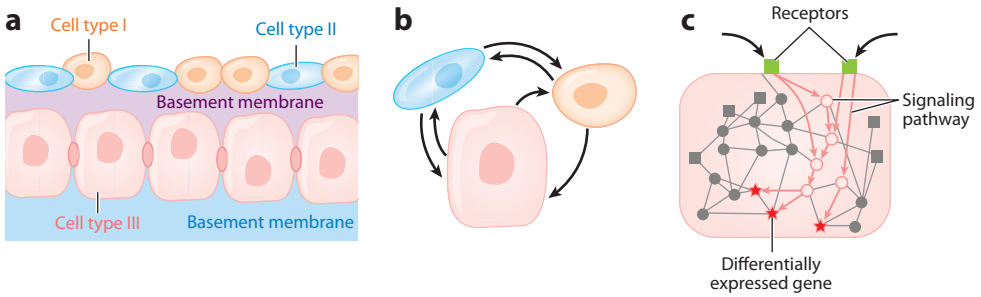

Figure 2

(*a*) Illustration of an engineered tissue with three cell types. (*b*) The three cell types exchange signals to maintain their phenotype. (*c*) Systems biology experiments and computational analysis help to decipher the exchanged signals and the internal cellular responses.

5. DEFINING A NEW SYNTHESIS BETWEEN TISSUE ENGINEERING AND SYSTEMS BIOLOGY

In this section, we describe several examples of how tissue engineering and systems biology can inspire each other to solve the next generation of challenges in both fields. As described in earlier sections, biomedical engineers and scientists currently design in vitro tissue mimics by combining and integrating available knowledge on cellular behavior, tissue organization, and experimental capabilities. So far, predictive models have not played a major role in tissue engineering. More specifically, current approaches to tissue engineering have not yet effectively tapped into the information about cellular systems that is rapidly accumulating with the advent of systems biology. Conversely, systems biology approaches have so far primarily been used to study model organisms and human diseases such as cancer. They have not been utilized to tackle the challenging question of how different cell types could be organized into complex engineered tissues. Adding the tools of systems biology to the repertoire of biomedical engineers and scientists can revolutionize the development of in vitro tissue mimics and accelerate translation of these engineered tissues into applications for human health.

Tissue mimics that incorporate multiple types of cells (120) offer a promising and controlled environment for studying intercellular signaling. For instance, liver models often include at least one cell type in addition to hepatocytes (120, 121). Analysis of transcriptional measurements in hepatocytes in the models may point to cellular pathways that are perturbed in response to signals from the other cell type(s). Algorithms for predicting signaling pathways can then be applied to prioritize proteins for further delineation of intercellular communication networks (**Figure 2**). In this scenario, tissue engineering provides an ideal system (the in vitro liver model) to study a fundamental question in cell biology (signaling between cells in a tissue) and spurs developments in novel algorithms for pathway prediction. In turn, these algorithms help to prioritize proteins that can be further studied using new experimental techniques in systems biology. The resulting data can lead to algorithmic improvements, to incorporation into refined computational models of intercellular signaling, and ultimately to better design of the tissue engineered systems themselves.

Microfluidics offers another avenue for the integration of tissue engineering with systems biology. Microfluidic devices can mimic flows that perfuse tissues and organs in vivo (122–125). Moreover, they can be engineered to generate well-defined and controllable flows. The low flow rates they facilitate permit the direct analysis of media samples using mass spectrometric techniques (126, 127). Culturing tissue mimics inside microfluidic systems coupled to mass spectrometers opens up the possibility of real-time measurements of the extracellular protein and metabolite

pool. These data can be combined with measurements of intercellular molecules. Analysis of these dense temporal data sets may require the development of new systems biology algorithms. Applying these approaches to these data can produce phenomenological and predictive models of how the cell receives external signals and processes them internally to produce its responses. These approaches can also be used to study organs connected by microfluidics and cultured on a chip (128–130), leading to improved systemic models of how organs network and interact with each other in the body.

The topography of biomaterials on which cells are cultured can play an important role in eliciting specific cellular responses (131). Unadkat et al. (132) developed an approach to construct a library of nonbiased, random combinations of simple surface topographies. The authors showed that novel surface topographies could cause human mesenchymal stromal cells to proliferate or undergo osteogenic differentiation. They were also able to correlate parameters of the algorithms used to generate the topographies to cellular responses. In the future, such methods can be integrated with systems biology ideas to unearth the intra- and intercellular molecular machinery that transduces changes in biomaterial properties into cellular responses.

Although systems biology approaches have been widely used to investigate cancer, they have not been utilized from a tissue engineering perspective. In the past several years, there has been a major effort to develop tissue-engineered tumor microenvironments (133–137). Engineered tumor models that mimic the three-dimensional (3D) environment found in vivo are increasingly being used for elucidating cancer cell behavior because the underlying cellular and molecular mechanisms have been shown to be different in 2D and 3D cultures (137). However, given the inherent complexity of the signaling pathways within each type of cancer and the wide range of cancers, the computational approaches could serve to unearth regulatory pathways of interest for further experimental analysis. For example, large-scale data sets on the expression of genes, the levels and activities of proteins, and the synthesis of intermediate metabolites can enable the identification of key signaling molecules whose dysregulation may lead to cancer (138). Incorporating these approaches in the study of tissue-engineered tumor microenvironments may lead to the identification of new drug targets, predict the side effects of drugs, and bring down the costs and time associated with experimental aspects of this area of research (139).

The research directions and opportunities presented by the natural synergy between tissue engineering and systems biology are virtually unlimited. Scientific explorations at the synthesis of these fields will likely unearth new combinations of experimental and computational approaches to design the next generation of implants. In the future, systems biology approaches will underlie predictive computational models of engineered tissues and drive their novel experimental analysis. Simultaneously, the demands of tissue engineering will inspire new experimental methodologies in systems biology and novel analysis frameworks in computational science. Eventually, seamlessly intertwined computational and experimental models will drive future advances in tissue engineering and in systems biology.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize in advance to all scientists whose research we could not cite due to space limitations. We gratefully acknowledge support from NIH/NIDDK grant 1R21DK077802, NSF grants CAREER 0955873, CBET-0933225, DBI-1062380, and DMR-090750, and EPA grant

EPA-RD-83499801-0. We also acknowledge support from the Institute of Critical Technology and Applied Science Center for Systems Biology of Engineered Tissues at Virginia Tech.

LITERATURE CITED

1. Levenberg S, Langer R. 2004. Advances in tissue engineering. *Curr. Top. Dev. Biol.* 61:113–34
2. Lenas P, Moos M Jr, Luyten FP. 2009. Developmental engineering: a new paradigm for the design and manufacturing of cell-based products. Part II: from genes to networks: tissue engineering from the viewpoint of systems biology and network science. *Tissue Eng. Part B Rev.* 15:395–422
3. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. 2006. Microscale technologies for tissue engineering and biology. *Proc. Natl. Acad. Sci USA* 103:2480–87
4. Dvir T, Timko BP, Kohane DS, Langer R. 2011. Nanotechnological strategies for engineering complex tissues. *Nat. Nanotechnol.* 6:13–22
5. Vunjak-Novakovic G, Kaplan DL. 2006. Tissue engineering: the next generation. *Tissue Eng.* 12:3261–63
6. L'Heureux N, Dusserre N, Marini A, Garrido S, de la Fuente L, McAllister T. 2007. Technology insight: the evolution of tissue-engineered vascular grafts—from research to clinical practice. *Nat. Clin. Pract. Cardiovasc. Med.* 4:389–95
7. Oberpenning F, Meng J, Yoo JJ, Atala A. 1999. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat. Biotechnol.* 17:149–55
8. MacNeil S. 2007. Progress and opportunities for tissue-engineered skin. *Nature* 445:874–80
9. Hammerman MR. 2003. Tissue engineering the kidney. *Kidney Int.* 63:1195–204
10. Shah A, Brugnano J, Sun S, Vase A, Orwin E. 2008. The development of a tissue-engineered cornea: biomaterials and culture methods. *Pediatr. Res.* 63:535–44
11. Bianco P, Robey PG. 2001. Stem cells in tissue engineering. *Nature* 414:118–21
12. Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, et al. 2010. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat. Med.* 16:814–20
13. Kim BS, Nikolovski J, Bonadio J, Mooney DJ. 1999. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nat. Biotechnol.* 17:979–83
14. Horner PJ, Gage FH. 2000. Regenerating the damaged central nervous system. *Nature* 407:963–70
15. Mikos AG, Herring SW, Ochareon P, Elisseeff J, Lu HH, et al. 2006. Engineering complex tissues. *Tissue Eng.* 12:3307–39
16. Badylak S, Nerem R. 2010. Progress in tissue engineering and regenerative medicine. *Proc. Natl. Acad. Sci. USA* 107:3285–86
17. Kelm J. 2004. Microscale tissue engineering using gravity-enforced cell assembly. *Trends Biotechnol.* 22:195–202
18. Liu C, Xia Z, Czernuszka J. 2007. Design and development of three-dimensional scaffolds for tissue engineering. *Chem. Eng. Res. Des.* 85:1051–64
19. Martino MM, Tortelli F, Mochizuki M, Traub S, Ben-David D, et al. 2011. Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. *Sci. Transl. Med.* 3:100ra89
20. Petersen T, Calle E, Zhao L, Lee E, Gui L, et al. 2010. Tissue-engineered lungs for in vivo implantation. *Science* 329:538–41
21. Mayer J, Karamuk E, Akaike T, Wintermantel E. 2000. Matrices for tissue engineering-scaffold structure for a bioartificial liver support system. *J. Control. Release* 64:81–90
22. Kaplan DL, Moon RT, Vunjak-Novakovic G. 2005. It takes a village to grow a tissue. *Nat. Biotechnol.* 23:1237–39
23. Griffith LG, Swartz MA. 2006. Capturing complex 3D tissue physiology in vitro. *Nat. Rev. Mol. Cell Biol.* 7:211–24
24. Bryan N, Ashwin H, Smart N, Bayon Y, Scarborough N, Hunt JA. 2012. The innate oxygen dependant immune pathway as a sensitive parameter to predict the performance of biological graft materials. *Biomaterials* 33:6380–92

25. Methe H, Edelman ER. 2006. Tissue engineering of endothelial cells and the immune response. *Transpl. Proc.* 38:3293–99
26. Sefton MV, Babensee JE, Woodhouse KA. 2008. Innate and adaptive immune responses in tissue engineering. *Semin. Immunol.* 20:83–85
27. Kubo K, Tsukimura N, Iwasa F, Ueno T, Saruwatari L, et al. 2009. Cellular behavior on TiO_2 nanonodular structures in a micro-to-nanoscale hierarchy model. *Biomaterials* 30:5319–29
28. Freed LE, Langer R, Martin I, Pellis NR, Vunjak-Novakovic G. 1997. Tissue engineering of cartilage in space. *Proc. Natl. Acad. Sci. USA* 94:13885–90
29. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1999. Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. *FASEB J.* 13:1883–900
30. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1998. Probing heterotypic cell interactions: hepatocyte function in microfabricated co-cultures. *J. Biomater. Sci. Polym. Ed.* 9:1137–60
31. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1998. Microfabrication of hepatocyte/fibroblast cocultures: role of homotypic cell interactions. *Biotechnol. Prog.* 14:378–87
32. Orford KW, Scadden DT. 2008. Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. *Nat. Rev. Genet.* 9:115–28
33. Purton LE, Scadden DT. 2008. The hematopoietic stem cell niche. In *StemBook* [Internet], ed. Stem Cell Res. Community. Cambridge, MA: Harvard Stem Cell Inst. **http://www.ncbi.nlm.nih.gov/books/NBK27051/**
34. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. 2002. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* 298:597–600
35. Osafune K, Caron L, Borowiak M, Martinez RJ, Fitz-Gerald CS, et al. 2008. Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat. Biotechnol.* 26:313–15
36. Robinton DA, Daley GQ. 2012. The promise of induced pluripotent stem cells in research and therapy. *Nature* 481:295–305
37. Passier R, van Laake LW, Mummery CL. 2008. Stem-cell-based therapy and lessons from the heart. *Nature* 453:322–29
38. Bratt-Leal AM, Carpenedo RL, McDevitt TC. 2009. Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation. *Biotechnol. Prog.* 25:43–51
39. Carpenedo RL, Bratt-Leal AM, Marklein RA, Seaman SA, Bowen NJ, et al. 2009. Homogeneous and organized differentiation within embryoid bodies induced by microsphere-mediated delivery of small molecules. *Biomaterials* 30:2507–15
40. Carpenedo RL, Seaman SA, McDevitt TC. 2010. Microsphere size effects on embryoid body incorporation and embryonic stem cell differentiation. *J. Biomed. Mater. Res. Part A* 94:466–75
41. Newman KD, McBurney MW. 2004. Poly(D,L lactic-co-glycolic acid) microspheres as biodegradable microcarriers for pluripotent stem cells. *Biomaterials* 25:5763–71
42. Reya T, Morrison SJ, Clarke MF, Weissman IL. 2001. Stem cells, cancer, and cancer stem cells. *Nature* 414:105–11
43. Ideker T, Lauffenburger D. 2003. Building with a scaffold: emerging strategies for high- to low-level cellular modeling. *Trends Biotechnol.* 21:252–62
44. Kitano H. 2002. Systems biology: a brief overview. *Science* 295:1662–64
45. Joyce AR, Palsson BØ. 2006. The model organism as a system: integrating 'omics' data sets. *Nat. Rev. Mol. Cell Biol.* 7:198–210
46. Skipper M, Dhand R, Campbell P. 2012. Presenting ENCODE. *Nature* 489:45
47. Stamatoyannopoulos JA, Snyder M, Hardison R, Ren B, Gingeras T, et al. 2012. An encyclopedia of mouse DNA elements (Mouse ENCODE). *Genome Biol.* 13:418
48. Mane SP, Evans C, Cooper KL, Crasta OR, Folkerts O, et al. 2009. Transcriptome sequencing of the Microarray Quality Control (MAQC) RNA reference samples using next generation sequencing. *BMC Genomics* 10:264
49. Picotti P, Rinner O, Stallmach R, Dautel F, Farrah T, et al. 2010. High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nat. Methods* 7:43–46
50. Hewel JA, Liu J, Onishi K, Fong V, Chandran S, et al. 2010. Synthetic peptide arrays for pathway-level protein monitoring by liquid chromatography-tandem mass spectrometry. *Mol. Cell Proteomics* 9:2460–73

51. Kiyonami R, Schoen A, Prakash A, Peterman S, Zabrouskov V, et al. 2011. Increased selectivity, analytical precision, and throughput in targeted proteomics. *Mol. Cell Proteomics* 10:M110.002931
52. Duncan MW, Aebersold R, Caprioli RM. The pros and cons of peptide-centric proteomics. *Nat. Biotechnol.* 28:659–64
53. Hackett M. 2008. Science, marketing and wishful thinking in quantitative proteomics. *Proteomics* 8:4618–23
54. Gouw JW, Krijgsveld J, Heck AJ. 2010. Quantitative proteomics by metabolic labeling of model organisms. *Mol. Cell Proteomics* 9:11–24
55. Vidal M, Cusick ME, Barabasi AL. 2011. Interactome networks and human disease. *Cell* 144:986–98
56. Cerami EG, Gross BE, Demir E, Rodchenkov I, Babur O, et al. 2011. Pathway Commons, a web resource for biological pathway data. *Nucleic Acids Res.* 39:D685–90
57. Stumpf MP, Thorne T, de Silva E, Stewart R, An HJ, et al. 2008. Estimating the size of the human interactome. *Proc. Natl. Acad. Sci. USA* 105:6959–64
58. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, et al. 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 122:947–56
59. Neph S, Stergachis AB, Reynolds A, Sandstrom R, Borenstein E, Stamatoyannopoulos JA. 2012. Circuitry and dynamics of human transcription factor regulatory networks. *Cell* 150:1274–86
60. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, et al. 2008. KEGG for linking genomes to life and the environment. *Nucleic Acids Res.* 36:D480–84
61. Kandasamy K, Mohan SS, Raju R, Keerthikumar S, Kumar GS, et al. 2010. NetPath: a public resource of curated signal transduction pathways. *Genome Biol.* 11:R3
62. Schaefer CF, Anthony K, Krupa S, Buchoff J, Day M, et al. 2009. PID: the Pathway Interaction Database. *Nucleic Acids Res.* 37:D674–79
63. Joshi-Tope G, Gillespie M, Vastrik I, D'Eustachio P, Schmidt E, et al. 2005. Reactome: a knowledgebase of biological pathways. *Nucleic Acids Res.* 33:D428–32
64. Kelder T, van Iersel MP, Hanspers K, Kutmon M, Conklin BR, et al. 2012. WikiPathways: building research communities on biological pathways. *Nucleic Acids Res.* 40:D1301–7
65. Dyer MD, Murali TM, Sobral BW. 2007. Computational prediction of host-pathogen protein–protein interactions. *Bioinformatics* 23:i159–66
66. Huttenhower C, Haley EM, Hibbs MA, Dumeaux V, Barrett DR, et al. 2009. Exploring the human genome with functional maps. *Genome Res.* 19:1093–106
67. Gardner TS, Faith JJ. 2005. Reverse-engineering transcription control networks. *Phys. Life Rev.* 2:65–88
68. Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, et al. 2009. STRING 8—a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.* 37:D412–16
69. Park CY, Hess DC, Huttenhower C, Troyanskaya OG. 2010. Simultaneous genome-wide inference of physical, genetic, regulatory, and functional pathway components. *PLoS Comput. Biol.* 6:e1001009
70. Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A. 2005. Reverse engineering of regulatory networks in human B cells. *Nat. Genet.* 37:382–90
71. Markowetz F, Spang R. 2007. Inferring cellular networks—a review. *BMC Bioinformatics* 8:S5
72. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, et al. 2000. Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25:25–29
73. Sharan R, Ideker T. 2006. Modeling cellular machinery through biological network comparison. *Nat. Biotechnol.* 24:427–33
74. Ideker T, Sharan R. 2008. Protein networks in disease. *Genome Res.* 18:644–52
75. Hartwell LH, Hopfield JJ, Leibler S, Murray AW. 1999. From molecular to modular cell biology. *Nature* 402:C47–52
76. Girvan M, Newman ME. 2002. Community structure in social and biological networks. *Proc. Natl. Acad. Sci. USA* 99:7821–26
77. Hu H, Yan X, Huang Y, Han J, Zhou XJ. 2005. Mining coherent dense subgraphs across massive biological networks for functional discovery. *Bioinformatics* 21(Suppl. 1):i213–21
78. Bader GD, Hogue CW. 2003. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4:2

79. Jiang P, Singh M. 2010. SPICi: a fast clustering algorithm for large biological networks. *Bioinformatics* 26:1105–11
80. Newman MEJ. 2006. Modularity and community structure in networks. *Proc. Natl. Acad. Sci. USA* 103:8577–82
81. Bergmann S, Ihmels J, Barkai N. 2003. Similarities and differences in genome-wide expression data of six organisms. *PLoS Biol.* 2:E9
82. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Barrette TR, Ghosh D, Chinnaiyan AM. 2005. Mining for regulatory programs in the cancer transcriptome. *Nat. Genet.* 37:579–83
83. Stuart JM, Segal E, Koller D, Kim SK. 2003. A gene-coexpression network for global discovery of conserved genetic modules. *Science* 302:249–55
84. Segal E, Friedman N, Koller D, Regev A. 2004. A module map showing conditional activity of expression modules in cancer. *Nat. Genet.* 36:1090–98
85. Murali TM, Rivera CG. 2008. Network legos: building blocks of cellular wiring diagrams. *J. Comput. Biol.* 15:829–44
86. Ideker T, Ozier O, Schwikowski B, Siegel AF. 2002. Discovering regulatory and signalling circuits in molecular interaction networks. *Bioinformatics* 18(Suppl. 1):S233–40
87. Liu M, Liberzon A, Kong SW, Lai WR, Park PJ, et al. 2007. Network-based analysis of affected biological processes in type 2 diabetes models. *PLoS Genet.* 3:e96
88. Ulitsky I, Shamir R. 2007. Identification of functional modules using network topology and high-throughput data. *BMC Syst. Biol.* 1:8
89. Murali TM, Rivera CG. 2008. Network legos: building blocks of cellular wiring diagrams. *J. Comput. Biol.* 15:829–44
90. Dittrich MT, Klau GW, Rosenwald A, Dandekar T, Muller T. 2008. Identifying functional modules in protein-protein interaction networks: an integrated exact approach. *Bioinformatics* 24:i223–31
91. Parkkinen JA, Kaski S. 2010. Searching for functional gene modules with interaction component models. *BMC Syst. Biol.* 4:4
92. Nibbe RK, Koyutürk M, Chance MR. 2010. An integrative -omics approach to identify functional sub-networks in human colorectal cancer. *PLoS Comput. Biol.* 6:e1000639
93. Lu R, Markowetz F, Unwin RD, Leek JT, Airoldi EM, et al. 2009. Systems-level dynamic analyses of fate change in murine embryonic stem cells. *Nature* 462:358–62
94. Taniguchi Y, Choi PJ, Li G-W, Chen H, Babu M, et al. Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* 329:533–38
95. Wang H, Wang Q, Pape UJ, Shen B, Huang J, et al. 2010. Systematic investigation of global coordination among mRNA and protein in cellular society. *BMC Genomics* 11:364
96. Suthram S, Beyer A, Karp RM, Eldar Y, Ideker T. 2008. eQED: an efficient method for interpreting eQTL associations using protein networks. *Mol. Syst. Biol.* 4:162
97. Yosef N, Ungar L, Zalckvar E, Kimchi A, Kupiec M, et al. 2009. Toward accurate reconstruction of functional protein networks. *Mol. Syst. Biol.* 5:248
98. Bailly-Bechet M, Borgs C, Braunstein A, Chayes J, Dagkessamanskaia A, et al. 2011. Finding undetected protein associations in cell signaling by belief propagation. *Proc. Natl. Acad. Sci. USA* 108:882–87
99. Huang SS, Fraenkel E. 2009. Integrating proteomic, transcriptional, and interactome data reveals hidden components of signaling and regulatory networks. *Sci. Signal.* 2:ra40
100. Steffen M, Petti A, Aach J, D'Haeseleer P, Church G. 2002. Automated modelling of signal transduction networks. *BMC Bioinformatics* 3:34
101. Karlebach G, Shamir R. 2008. Modelling and analysis of gene regulatory networks. *Nat. Rev. Mol. Cell Biol.* 9:770–80
102. Szczurek E, Gat-Viks I, Tiuryn J, Vingron M. 2009. Elucidating regulatory mechanisms downstream of a signaling pathway using informative experiments. *Mol. Syst. Biol.* 5:287
103. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–10
104. Enright AJ, Kunin V, Ouzounis CA. 2003. Protein families and TRIBES in genome sequence space. *Nucleic Acids Res.* 31:4632–38

105. Boguski MS. 1999. Biosequence exegesis. *Science* 286:453–55
106. Brenner SE. 1999. Errors in genome annotation. *Trends Genet.* 15:132–33
107. Karaoz U, Murali TM, Letovsky S, Zheng Y, Ding C, et al. 2004. Whole genome annotation using evidence integration in functional linkage networks. *Proc. Natl. Acad. Sci. USA* 101:2888–93
108. Lee I, Date SV, Adai AT, Marcotte EM. 2004. A probabilistic functional network of yeast genes. *Science* 306:1555–58
109. Myers CL, Robson D, Wible A, Hibbs MA, Chiriac C, et al. 2005. Discovery of biological networks from diverse functional genomic data. *Genome Biol.* 6:R114
110. Letovsky S, Kasif S. 2003. Predicting protein function from protein/protein interaction data: a probabilistic approach. *Bioinformatics* 19(Suppl. 1):i197–204
111. Mostafavi S, Ray D, Warde-Farley D, Grouios C, Morris Q. 2008. GeneMANIA: a real-time multiple association network integration algorithm for predicting gene function. *Genome Biol.* 9(Suppl. 1):S4
112. Peña-Castillo L, Tasan M, Myers CL, Lee H, Joshi T, et al. 2008. A critical assessment of *Mus musculus* gene function prediction using integrated genomic evidence. *Genome Biol.* 9(Suppl. 1):S2
113. Wu S, Wells A, Griffith LG, Lauffenburger DA. 2011. Controlling multipotent stromal cell migration by integrating "coarse-graining" materials and "fine-tuning" small molecules via decision tree signal-response modeling. *Biomaterials* 32:7524–31
114. Sun X, Su J, Bao J, Peng T, Zhang L, et al. 2012. Cytokine combination therapy prediction for bone remodeling in tissue engineering based on the intracellular signaling pathway. *Biomaterials* 33:8625–76
115. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. 2002. A stem cell molecular signature. *Science* 298:601–4
116. Tanaka TS, Kunath T, Kimber WL, Jaradat SA, Stagg CA, et al. 2002. Gene expression profiling of embryo-derived stem cells reveals candidate genes associated with pluripotency and lineage specificity. *Genome Res.* 12:1921–28
117. Sperger JM, Chen X, Draper JS, Antosiewicz JE, Chon CH, et al. 2003. Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. *Proc. Natl. Acad. Sci. USA* 100:13350–55
118. Wang T-H, Lee Y-S, Hwang S-M. 2011. Transcriptome analysis of common gene expression in human mesenchymal stem cells derived from four different origins. *Methods Mol. Biol.* 698:405–17
119. Tsai M-S, Hwang S-M, Chen K-D, Lee Y-S, Hsu L-W, et al. 2007. Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow. *Stem Cells* 25:2511–23
120. Kim Y, Rajagopalan P. 2011. 3D hepatic cultures simultaneously maintain primary hepatocyte and liver sinusoidal endothelial cell phenotypes. *PLoS One* 5:e15456
121. March S, Hui EE, Underhill GH, Khetani S, Bhatia SN. 2009. Microenvironmental regulation of the sinusoidal endothelial cell phenotype *in vitro*. *Hepatology* 50:920–28
122. Sia SK, Whitesides GM. 2003. Microfluidic devices fabricated in Poly(dimethylsiloxane) for biological studies. *Electrophoresis* 24:3563–76
123. Chen J, Li J, Sun Y. 2012. Microfluidic approaches for cancer cell detection, characterization, and separation. *Lab Chip* 12:1753–67
124. Prabhakarpandian B, Shen M-C, Pant K, Kiani MF. 2011. Microfluidic devices for modeling cell-cell and particle-cell interactions in the microvasculature. *Microvasc. Res.* 82:210–20
125. Inamdar NK, Borenstein JT. 2011. Microfluidic cell culture models for tissue engineering. *Curr. Opin. Biotechnol.* 22:681–89
126. Urban PL, Jefimovs K, Amantonico A, Fagerer SR, Schmid T, et al. 2010. High-density micro-arrays for mass spectrometry. *Lab Chip* 10:3206–9
127. Amantonico A, Urban PL, Zenobi R. 2010. Analytical techniques for single-cell metabolomics: state of the art and trends. *Anal. Bioanal. Chem.* 398:2493–504
128. Kim HJ, Huh D, Hamilton G, Ingber DE. 2012. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* 12:2165–74
129. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. 2010. Reconstituting organ-level lung functions on a chip. *Science* 328:1662–68

130. Huh D, Hamilton GA, Ingber DE. 2011. From 3D cell culture to organs-on-chips. *Trends Cell Biol.* 21:745–54
131. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, et al. 2010. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat. Mater.* 9:518–26
132. Unadkat HV, Hulsman M, Cornelissen K, Papenburg BJ, Truckenmuller RK, et al. 2011. An algorithm-based topographical biomaterials library to instruct cell fate. *Proc. Natl. Acad. Sci. USA* 108:16565–70
133. Verbridge SS, Chandler EM, Fischbach C. 2010. Tissue-engineered three-dimensional tumor models to study tumor angiogenesis. *Tissue Eng. Part A* 16:2147–52
134. Verbridge SS, Choi NW, Zheng Y, Brooks DJ, Stroock AD, Fischbach C. 2010. Oxygen-controlled three-dimensional cultures to analyze tumor angiogenesis. *Tissue Eng. Part A* 16:2133–41
135. Pathi SP, Kowalczewski C, Tadipatri R, Fischbach C. 2010. A novel 3-D mineralized tumor model to study breast cancer bone metastasis. *PLoS One* 5:e8849
136. Fischbach C, Chen R, Matsumoto T, Schmelzle T, Brugge JS, et al. 2007. Engineering tumors with 3D scaffolds. *Nat. Methods* 4:855–60
137. Fischbach C, Kong HJ, Hsiong SX, Evangelista MB, Yuen W, Mooney DJ. 2009. Cancer cell angiogenic capability is regulated by 3D culture and integrin engagement. *Proc. Natl. Acad. Sci USA* 106:399–404
138. Aebersold R, Auffray C, Baney E, Barillot E, Brazma A, et al. 2009. Report on EU-USA workshop: how systems biology can advance cancer research (27 October 2008). *Mol. Oncol.* 3:9–17
139. Bown J, Andrews PS, Deeni Y, Goltsov A, Idowu M, et al. 2012. Engineering simulations for cancer systems biology. *Curr. Drug Targets* 13:1560–74

Annual Review of Biomedical Engineering
Volume 15, 2013

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Biomedical Engineering* articles may be found at http://bioeng.annualreviews.org/