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Approaches to *in vitro* tissue regeneration with application for human disease modeling and drug development

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Abstract

Reliable *in vitro* human disease models that capture the complexity of *in vivo* tissue behaviors are crucial to gain mechanistic insights into human disease and enable the development of treatments that are effective across broad patient populations. The integration of stem cell technologies, tissue engineering, emerging biomaterials strategies and microfabrication processes, as well as computational and systems biology approaches, is enabling new tools to generate reliable *in vitro* systems to study the molecular basis of human disease and facilitate drug development. In this review, we discuss these recently developed tools and emphasize opportunities and challenges involved in combining these technologies toward regenerative science.

Keywords

Tissue engineering; regenerative medicine; drug discovery; systems biology; humanon-a-chip; organ-on-a-chip

Introduction

It is estimated that on average more than US$1 billion are expended over a span of roughly 12.5 years to deliver a new drug to market [1]. In total 85% of therapies fail in early clinical trials, whereas of those that continue to Phase III (generally the last step before regulatory approval) only 50% are approved [2]. The current drug development process is inefficient and unsustainable, thus requiring state-of-the-art innovations and tools to survive. This

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situation is exacerbated in the case of large numbers of diseases with small and geographically dispersed patient populations, for which it is even more difficult to collect sufficient data for translational research [1,3]. A central challenge in drug development is the divergence between results obtained from animal studies and from human trials. Animal studies often fail to predict actual clinical outcomes, because the doses, formulations and schedules of medication in the clinic differ from those given to the animals and because of differences between species [2]. Thus, there is an immediate need to develop human surrogate models that bridge the gap between conventional rodent studies and human trials, not only to achieve a greater understanding of disease mechanisms and drug discovery efforts but also to evaluate new therapeutic compounds.

Injury to cells and tissues sets in motion a series of events that contain the damage and initiate the healing process by means of regeneration and repair [4]. Inadequate tissue repair following trauma or surgery and misregulated tissue regeneration and repair responses, such as diabetes mellitus, aging, cancer, osteoarthritis and fibrosis, affect millions of patients worldwide each year [5,6]. The molecular mechanisms underlying tissue repair or its failure are not completely understood and current therapeutic options are limited. Thus, tissue regeneration technology has emerged as a useful platform for development of reliable *in vitro* systems, with applications in drug development and disease modeling [7]. However, a major limitation of simplified *in vitro* model systems is that they often fail to recapitulate the appropriate microenvironmental context in terms of biological cues (including chemical and physical effectors) [8]. Current progress in cell biology and stem cell science, in convergence with advances in microfabrication technologies and biomaterials, has created a unique opportunity to generate relevant humanized micro-tissue constructs. These new capabilities can serve as a crucial toolset for probing human physiology and disease states. As such, these systems provide platforms capable of directing, manipulating and analyzing cellular behavior in the context of an *in-vivo*-like microenvironment. Here, we review several major advances in cell biology and fabrication technology that are being utilized in these applications. First we will describe how cells can be genetically engineered to produce various types of cells with distinct behavior. This aspect is the first building block in engineering tissue constructs. Hierarchical structural features of tissues can be achieved at multiple levels by using tools to engineer the cellular microenvironment in either a static or dynamic fashion (Figure 1a). Thus, in the next section we explain how biomaterial properties can alter environmental cues, cellular fate and function, and how advances in micro- and nano-fabrication techniques have enabled the formation of microengineered structures, which mimic natural tissues with a high degree of spatial resolution. To achieve complex tissue features and capture inter- and intra-organ communications, dynamic environments incorporating fluid flow will be needed. Therefore, we will review bioreactor and microfluidic technologies capable of providing these dynamic elements (Figure 1a). In conclusion, we summarize various ways to glean insights from the wealth of data that is extracted from these systems using computational and systems biology approaches.

Engineering cellular phenotype and function

Cell sources

Identifying the ideal source of human cells is the first major step in the development of human surrogate *in vitro* models. Various sources have been explored toward this end, including cell lines, primary cryopreserved cells and freshly isolated cells, as well as differentiated cells derived from stem cells. Although freshly isolated cells remain the gold standard in many situations, there are certain key constraints such as limited availability of donors and the variability associated with genetic and epigenetic background of donor subjects. Cryopreserved human cells are commercially accessible, and can be controlled for lot-to-lot variations. Consequently, studies performed across numerous laboratories can be carried out with cells from the same lot number so as to maintain identical genetic and epigenetic backgrounds. However, for many cell types, access to fresh or cryopreserved human cells is constrained. This situation includes, but is not limited to, various types of brain cells such as astrocytes, oligodendrocytes, cells of the conductive system in the heart, liver sinusoidal endothelial cells, pancreatic cells and retinal cells. By contrast, to recapitulate human disease, we need to be able to capture at least the minimum required complexity of an organ by including multiple cell types and crucial insoluble or soluble microenvironmental cues. Yet, it is extremely difficult to predict or define the minimum requirements of complexity needed *in vitro* to capture *in vivo* behavior accurately. Presumably these requirements vary depending on the applications pursued; however, they remain a challenge especially during testing new drugs or in the case of diseases with a limited knowledge of molecular mechanisms.

Induced pluripotent stem cells

Isolation of human embryonic stem cells by Thompson *et al*. heralded a new age in which a cell population could be used to differentiate a population to various cell types [9]. This presented a powerful new opportunity for a source of cells for tissue engineering, drug development or therapeutic transplantation. Following that pioneering advance, in 2006 Yamanaka and Takahashi introduced the ‘somatic reprogramming’ technique in which the epigenetic state of somatic cells can be reprogrammed into a pluripotent state [10]. This success was followed by the development of various differentiation protocols to generate specific cell populations, and this technique was immediately heralded as a novel tool for use in modeling human diseases. Differentiation of human induced pluripotent stem cells (iPSCs) into several cell types has been achieved including neural progenitors [11], motor neurons [12], retinal cells [13], hematopoietic progenitor cells and blood cells [14,15], endothelial cells [15] and hepatocytes [16], reviewed in [17]. Notably, differentiated iPSCs could rescue the disease phenotype when transplanted into donor mice [14]. Although these advances are very promising, several compelling technical challenges remain [18]. For example, the differentiation process of stem cells is stochastic, which results in significant variability in the efficiency of differentiation. As a consequence, a heterogeneous population of cells is produced at different developmental stages. For instance, induced hepatocytes currently closely resemble fetal hepatocytes; therefore, understanding the required environmental cues, signaling modalities and timescales necessary for true maturation of a cell lineage remains the topic of future efforts. Additionally, transcription factor or surface

marker expression is currently the standard approach to assess the developmental stage of differentiation [17]. However, defined protocols are needed to evaluate the function of these differentiated cells at each step [17]. In parallel, genetic targeting strategies have recently been developed [19] that can significantly improve our ability to create cells with genetically controlled backgrounds in which the disease-causing genetic variation is the sole experimental variable [20]. This technique now targets multiple genetic loci with acceptable efficiency [21] providing opportunities to generate endogenous reporters as markers for differentiation, gene corrections and relevant cellular sources for disease modeling and validation within the drug development pipeline.

Engineering tissue behavior and function

Biomaterials for substrates and matrices

Cells in the body reside in a microenvironment that encounters multiple cues from neighbor cells, extracellular matrix (ECM) and fluid stress. These cues can be categorized as adhesive, mechanical, chemical and topographical cues [22,23]. The ECM is composed of a complex assembly of many proteins and polysaccharides the particular composition of which varies from tissue to tissue. The primary components include insoluble fibrous structural proteins (i.e. elastin, collagens, laminins and fibronectin), proteoglycans and specialized proteins (i.e. growth factors, small integrin-binding glycoproteins). The ECM components continuously modulate physical forces that are sensed by cells as well as soluble cues that are sequestered and released in a temporal and spatial manner. This microenvironment evolves continuously as a consequence of cell–cell interaction or cell–matrix crosstalk. Additionally, ECM provides a basic structural scaffold to maintain robust tissue architecture. Taken together, biomaterials play a central part in emerging strategies within regenerative medicine and tissue engineering, and provide a scaffold to control and direct morphogenesis and cell function. These materials are used to mimic various facets of extracellular environment such as stiffness, release of various growth factors and presentation of extracellular proteins of interest. Material selection for each specific application depends on several variables, including physical properties (e.g. mechanics, degradation, gel formation), mass transport properties (e.g. diffusion) and biological properties (e.g. cell adhesion and signaling) [24]. Biologically derived (e.g. collagen gels, matrigel) and synthetic biomaterials (e.g. synthetic hydrogels) have been developed and used in various studies. Synthetic materials have become more important in recent years because they promote a more controlled and reproducible physicochemical microenvironment. When working with these materials, multiple parameters are considered tunable such as crosslinking density, porosity, presentation mode of adhesion ligands and their chemistry, as well as the degradation rate of the material [23–25]. Additionally, growth factors and soluble cues can be bound by synthetically tethering linker molecules to a substrate to explore various attributes of the ECM environment fully [26].

Many past studies applied these biomaterial systems to cultures of mature cells at a fully differentiated state to maintain their phenotype after isolation or to direct the organization and tissue morphogenesis. Recent studies have attempted to address the role of biomaterials in controlling stem cell fate and function. Classically, genetic and molecular mediators (e.g.

growth factors, transcription factors) were utilized as a preferred approach for control of stem cell fate. However, increasing evidence has revealed that a diverse array of additional environmental factors contribute to the overall regulation of stem cell activity. In fact, tissue regeneration requires resident stem cells to survey the status of the microenvironment (stem cell niche) and respond appropriately to alterations from aging, injury or inflammation.

Using a micropatterning approach, it was found that cell shape (i.e. rounded versus flattened morphologies) controls the lineage commitment of mesenchymal stem cells (MSCs) into an adipogenic or osteoblastic phenotype [27]. In another study, substrate elasticity was shown to regulate skeletal muscle stem cell self-renewal in culture. Muscle stem cells cultured on soft hydrogel substrates that mimic the elasticity of muscle (12 kPa) self-renew *in vitro* and contribute extensively to muscle regeneration [28]. Another study fabricated physically crosslinked RGD-modified alginate hydrogels with a wide range of mechanics (2.5–110 kPa), and reported optimal osteogenic differentiation of encapsulated MSCs for intermediate stiffness values (11–30 kPa) [29]. In an attempt to mimic dynamically changing matrix mechanics, hydrogel mechanics were temporally manipulated *in situ* in two elegant studies [30,31]. Recently, Lutolf and co-workers described a high-throughput hydrogel microwell system that can be used to probe features such as cell density, substrate mechanics and protein incorporation. This system comprised soft hydrogel microwell arrays with modular stiffness (shear moduli of 1–50 kPa) in which individual microwells were functionalized with combinations of proteins spotted by robotic technology [32,33]. Using this device, more than 2000 experiments can be performed on a single glass slide. This system was demonstrated successfully by probing the combinatorial effects of these parameters on human mesenchymal and mouse neural stem cell differentiation [33]. Advances in material fabrication methods, such as the ones discussed here, present a unique opportunity to control the cellular microenvironment to instruct stem cell functions. Other advances include the introduction of 3D environments while presenting multiple cues such as growth factors [23], as well as new experimental and computational approaches to quantify cell behavior in a robust and systematic fashion.

Micro- and nano-fabrication technologies

Controlling the assembly of cells and environmental cues in three dimensions is crucial for engineering functional tissues. To address the need for spatial heterogeneity of cues in microtissue structures, various microfabrication techniques have been used to generate patterns of cells on surfaces. Various techniques including microcontact printing, microfluidic patterning using microchannels and laminar flow patterning have been applied in this respect, reviewed elsewhere in [34,35]. Photolithographic techniques are another highly developed method used for patterning cells. Soft lithography involves the use of a patterned elastomeric stamp composed of polydimethylsiloxane (PDMS) to generate desired micro- or nano-scale patterns. Various micropatterned surfaces have been prepared by using these techniques to investigate the effect of cell–cell interaction or distinct biological cues and their influence on the differentiation and function of human stem cells [36]. Recently, stereolithography techniques were used to enable 3D photopatterning of hydrogels [37,38]. Stereolithography offers advantages such as computer-aided design (CAD) capabilities and multicell and multimaterial fabrication with the possibility to encapsulate cells at the time of

fabrication [39,40]. Additionally, through integration of dielectrophoresis with stereolithography, 3D cellular assembly was controlled in encapsulation of embryonic stem cells as well as embryoid bodies [41]. To achieve greater spatial resolution, a stereolithographic technique using digital light processors (DLP) and projectors, termed projection stereolithography, was also developed [42–44]. Using 3D projection stereolithography, different cell types including adipose-derived stem cells were incorporated successfully within the fabricated hydrogel scaffolds; this method has also been applied to generate complex 3D designer scaffolds [40,42].

Nanolithography techniques (with precision in the range of ten to hundreds of nm) are another facet of emerging tools to create nanotopographical cues that control cellular fate and function [45]. For instance, a recent study used dip pen nanolithography to produced homogenously nanopatterned chemically modified surfaces to initiate a directed cellular response such as MSC differentiation, in a highly reproducible manner without the need for exogenous biological factors or heavily supplemented cell media [46]. In another study, electron-beam lithography was used to create arrays of nanopits with various spacing to control osteogenic differentiation in the absence of osteogenic supplements [47]. These efforts open up crucial avenues for recapitulating the *in vivo* cellular microenvironment and the wide variation in nanotopographical cues between different tissues and various regions within a tissue.

Microfluidic and microreactor technologies

It has long been understood that the supply of oxygen and soluble nutrients becomes a crucial limiting factor in the *in vitro* culture of 3D tissues. Previous studies have shown that cellular spheroids larger than 1 mm in diameter generally contain a hypoxic, necrotic center surrounded by a rim of viable cells [48]. Incorporation of flow in the system can regulate oxygen gradients while providing essential nutrients to facilitate 3D structure. Additionally, mechanical forces are known to be important modulators of cell physiology and can increase the biosynthetic activity of cells in bioartificial matrices, thus potentially improving or accelerating tissue regeneration *in vitro* [49]. However our current knowledge regarding the specifics of mechanical forces or regimes of application (i.e. magnitude, frequency, continuous or intermittent) with respect to cellular function is limited. Although mechanical forces can be generated in real time from the fluid in circulation, stretching or compression modes of materials in contact with cells can also produce important organ-specific mechanical cues. These aspects are important when providing organ-specific biomechanical cues (e.g. in alveolar epithelium or cardiac muscles) [50]. In general, bioreactors are designed to be devices in which biochemical and/or biological events are conducted under closely monitored and tightly controlled environmental factors (e.g. temperature, pH, waste removal or nutrient supply). Microfluidics comprise systems involving fluids with geometries having characteristic length dimensions in the order of tens to hundreds of microns [51], yet the tissue organoid structures that exist within the same systems can encompass a range from single cells to large cell populations. Therefore, both systems when combined with properly selected biomaterials and the requisite microfabrication processes can provide control over the physicochemical environment in cells spanning all dimensions of cell–cell, cell–matrix and cell–fluid interactions.

Traditionally, most microfluidic culture systems were designed to culture cells in 2D monolayers. However 3D ECM and hydrogel fabrication technologies have recently been merged with microfluidics to achieve 3D cellular microenvironments in flow-competent platforms [52,53]. In one study, a 3D hydrogel was used directly within culture chambers of microfluidic systems, enabling spatial control defined by the structure of chambers [52]. A pneumatic actuation strategy was applied to permit fabrication of gels while keeping the channels open throughout the casting process. In another effort, microfluidic channels were incorporated directly in a hydrogel and ECM scaffold to define a vascular architecture [54]. The microfluidic hydrogel fabrication approach enables a more efficient transport of water and soluble factors, improves control over gradients of soluble factors across 2D and/or 3D cell culture and enables perfusion of an engineered tissue scaffold [55]. Similarly, morphogenesis of 3D tissue structures under continuous perfusion was performed to engineer the hepatic lobule as a functional unit of the liver [56,57]. In this system, morphogenesis of seeded cells is guided in part by scaffold surface chemistry, which controls the relative values of cell–cell and cell–substrate adhesion strength, and by the channel geometry. With the emergence of microengineering technologies, 3D vascularized materials were developed as a result of advances in microfabrication techniques, reviewed elsewhere [51,58]. For instance, a recent study used rigid 3D filament networks of carbohydrate glass, as a cytocompatible sacrificial template in engineered tissues containing living cells, to generate cylindrical networks that could be lined with endothelial cells and perfused with blood under high-pressure pulsatile flow [59]. Notably, 3D vascular features were also achieved by modular approaches that consist of stacks or the assembly of multiple 2D produced structures [60,61].

Isolated complex tissue and multiorgans in communication

Using various fabrication technologies different facets of complex tissue behavior have been elucidated. Many of these efforts attempted to engineer minimal functional units of each tissue by implementing features such as tissue-specific interfaces, cellular organization or biomechanical cues including air or fluid flows. A lung-on-a-chip was accomplished by microfabricating a microfluidic system containing two closely opposed microchannels separated by a porous, flexible membrane coated with ECM that contains alveolar epithelial cells and endothelial cells at opposite sides to represent an air–liquid interface [62]. A similar experimental system was applied to mimic the complex structure and physiology of the intestine using human intestinal epithelial (Caco-2) cells [63]. A topographically patterned porous membrane in a microfluidic device was used as an *in vitro* model of renal reabsorptive barriers [64]. In another study, a circular microfluidic compartmentalized co-culture platform was used to explore the central nervous system (CNS) axon–glia interaction and signaling [65]. In a recent chip-based system, *ex vivo* skin and single hair follicular units were cultured in a bioreactor platform to extend the static maintenance period required for substance testing [66]. Most of these studies have addressed an isolated organ model on a single fluidic platform. However, many aspects of human disease or drug toxicity are dependent on intricate interactions between multiple organs inside the body. Examples of such crosstalk include hepatic clearance of a drug acting on a distant target site or the effect of barrier tissues such as the epithelium of the gastrointestinal (GI) tract, the lung epithelium, the skin and the blood–brain barrier to reduce the bioavailability of drugs that

are taken up orally, through inhalation or through application to the skin. In fact, past efforts have explored human-on-a-chip models that can simulate multi-tissue interactions under fluidic flow using cell lines representing each organ [67]. However, current efforts are focused on engineering complex behavior of organs on integrated platform technologies. These integrated fluidic platforms maintain the capability to host multiple organ structures and provide in-depth insights regarding human pathophysiology.

Understanding complex biological systems through data integration and computational modeling

In the above sections we have reviewed advances in experimental cell biology and fabrication technologies that together help offer at least some aspects of biological complexity at molecular, cellular and tissue levels. Most often, however, the resulting platforms to date have not been readily amenable to the kinds of high-throughput data-rich studies that have become more commonplace in simpler experimental methods [68], such as transcriptomic, proteomic, phosphoproteomic and metabolomic measurement techniques. Moreover, these existing platforms have rarely gained the greater, integrative insights that are available by bringing multivariate computational modeling and analyses to bear on these questions. Thus, in this section, we describe computational frameworks that are proving to offer significant benefit in advancing experimental molecular, cell and tissue biology toward a more predictive capability. We highlight the potential of these approaches to assist in the elucidation of disease pathophysiology, facilitate the design of new therapeutic strategies and expedite the drug discovery process.

The emergence of systems analyses

Owing to the intrinsic complexity of biological systems, integration of experimental and computational analyses is required for a more in-depth understanding of cell physiology and behavior. Systems biology approaches emphasize a multivariate understanding that encompasses contributions of many components, enabling more-robust insights that cannot be obtained from reductionist studies focusing on individual entities [69]. It is becoming increasingly appreciated that the most powerful avenue for systems biology extends beyond simply the ‘horizontal integration’ dimension of -omics, requiring concomitantly an ‘operational integration’ dimension of kinetics and dynamics along with a multiscale ‘vertical integration’ dimension [70].

With respect to practical research and investigative procedures, systems biology involves an iterative, strategic interplay between data-driven and hypothesis-driven perspectives. Global observations are matched against model predictions in an iterative manner, leading to the formation of improved models, new predictions and elegant experiments to test them [71]. A systems biology framework can encompass multiple aims to: (i) characterize all molecular components of the system; (ii) identify molecular interactions within the system, including the details associated with how those interactions change following perturbations; (iii) assess the spatiotemporal dynamics of the behavior and interactions of the constituents; and (iv) integrate this information into a quantitative and potentially predictive mathematical model [72]. Collectively, this basic infrastructure facilitates a more in-depth understanding of the

mechanistic formalism associated with observed behaviors, the manner in which the system responds to internal and external perturbations and which, of several competing hypothesis or models, is most consistent with experimental observations thus most likely to represent cell physiology accurately.

Computational models consist of two distinct classifications: knowledge discovery (i.e. data-mining), which extracts hidden patterns from huge quantities of experimental data to form resultant hypotheses; and simulation-based analysis, which tests hypotheses via *in silico* experiments that, in turn, provide predictions to be examined by *in vitro* and *in vivo* studies [69]. These methods are not mutually exclusive; data mining efforts increasingly inform simulation-based analyses. Knowledge discovery is used extensively within bioinformatics, such as the inference of gene regulatory networks from expression profiles [73–75]. To understand, predict and ultimately control the behavior of biological systems, the development of predictive mathematical models – solidly grounded in experimental data – is essential. In contrast to knowledge discovery, simulation-based approaches attempt to predict the dynamics of systems so that the validity of the underlying assumptions is investigated. Models that survive initial validation are used to make predictions tested then by experiments, as well as to explore biological phenomena not amenable to experimental enquiry [69].

Computational models and statistical approaches

Similar to microfluidic platforms and microreactors described in the previous section, there does not exist a ‘one size fits all’ approach that sufficiently models cellular behavior [76]. Interestingly, it is this aspect that promotes discussion between the experimentalist and modeler, resulting in selection of the most appropriate mathematical formalism for each biological question studied. In turn, experiments are designed to attain the maximum level of spatiotemporal resolution.

Computational models that reconstruct a mechanism are categorized as either deterministic or probabilistic. Ordinary differential equations (ODEs) are the most popular deterministic formulism which, when accounting for spatial parameters, are described by partial differential equations (PDEs) or compartmental models. Overall, each equation represents a species’ continuous concentration over a specified timescale, typically denoted by mass-action kinetics. Alternatively, algebraic substitutions such as Michaelis–Menten functions, transfer functions or power laws [77] result in a condensed algorithm with more-complex rates terms. Deterministic models remain the most appropriate framework to capture the behavior of systems where species are abundant and reaction events occur frequently; however, many cellular processes consist of molecular interactions that are intrinsically random as a consequence of sufficiently low concentrations of molecules, or very slow kinetics. To model these cellular processes, stochastic simulation algorithms or Monte Carlo methods can be used. The latter approaches cope with different reaction timescales by modeling molecules individually, where reaction events are calculated based on probability. In recent years, hybrid approaches have evolved to minimize computational efforts by partitioning fast kinetics within a continuous framework, whereas discrete stochastic algorithms simulate slow reactions [78,79]. The fundamental assumptions underlying

deterministic and stochastic algorithms, model design, verification, calibration (i.e. model regression, training) and validation are beyond the scope of this review. Insightful perspectives and reviews focused on applications in cell biology, systems biomedicine and drug discovery are referenced elsewhere [76,77,80–82].

In contrast to modeling approaches based on prior knowledge of molecular mechanisms, inference models are constructed from the actual data in the absence of any underlying assumptions. Defined as data-driven models, a spectrum of inference techniques can be employed to gain insights concerning relationships, interactions and influences among multivariate biological components (Benedict and Lauffenburger 2013). Data-driven techniques enable the integration of data obtained from entirely different metrics assessed at diverse physiological scales. Although more abstract than deterministic models, data-driven modeling approaches such as clustering, principle component analysis (PCA), partial least squares (PLS; e.g. PLSR and PLSDA), decision trees and Bayesian inference networks extract groups of molecular activities that are statistically associated with a given cell phenotype or behavior. New hypotheses, derived directly from the mathematical analyses, can be formulated and tested experimentally in an iterative manner.

During the past decade, data-driven models have emerged as standard tools for systems-level research in signaling networks, as reviewed by Janes and Yaffe [83]. For example, a cuesignal-response paradigm has been used in combination with multivariate analysis techniques (e.g. PCA, PLS) to evaluate liver hepatotoxicity [84] and drug toxicity [85], pathogenesis of inflammatory bowel disease [86] and applications for adoptive T cell therapy of cancer [87]. In these designs, statistical methods glean signal–response relationships from heterogeneous multivariate signaling data. To illustrate a cue-signal-response compendium, Cosgrove *et al*. [85] evaluated idiosyncratic drug hepatotoxicity by administering drugs to primary cells from multiple donors across a landscape of inflammatory contexts including bacterial analog, lipopolysaccharide (LPS) and select cytokines. Seventeen phosphoproteins were analyzed over time to capture the dynamic state of the intracellular signaling network governing cell death as the phenotypic metric. This framework reproduced clinical drug hepatotoxicity signatures *in vitro* and demonstrated novel regulatory schemes contributing to hepatic cytotoxicity. Figure 2 illustrates the cue-signal-response compendium and alternative analyses, which hold great promise in all areas of regenerative medicine beyond signaling events to cell phenotypes, cell–cell interactions and *in vivo* tissue function.

Contemporary experimental techniques offer biological insights at the molecular level; yet, the collective knowledge associated with regulatory circuitry yields networks that are irreducibly complex. An informative review by Hecker *et al*. [88] describes multiple network inference models including one of the simplest architectures referred to as information theory models [89], in addition to discrete dynamic Boolean networks [90] and Bayesian networks that represent complex, stochastic and nonlinear relationships among multiple interacting molecules [91]. Within the realm of cellular reprogramming, tissue engineering and disease progression, network analyses have assessed the core transcriptional regulatory circuitry of stem cell differentiation [92,93], cell–matrix adhesion networks (e.g. integrin adhesome) [94] and cancer progression and regulation, respectively [95,96].

The role of systems analyses in drug discovery

The systems biology approach, with its combination of computational, experimental and observational enquiry, has extended from model systems to human physiology and now to areas of pharmacology and human health. Systems biology exemplifies an integrative perspective that is essential in drug discovery, thus enabling informed decisions (i.e. target selection, isolated or combinatorial therapy, selection of the most appropriate model and experimental system, a comprehensive understanding of the target and its regulatory role in various pathways). Dynamic models promote drug discovery efforts [82,97], as well as quantitative pharmacology and drug development [98,99]. Mechanistic and data-driven models reviewed here are also highly relevant. For example, network analyses have identified new drug targets, drug regimes and mechanisms of action [100–102], whereas alternative statistical techniques are employed routinely to evaluate combinatorial effects of drug screens. Similar to the methodologies reviewed, signature-based approaches are defined by a series of drug-induced molecular and phenotypic measurements resulting in multivariate signatures. Signature-based prediction is a compelling new strategy now used to investigate drug mechanisms, and has recently been applied to combination chemotherapies [103]. In conclusion, it is the multidisciplinary nature of systems biology that has the potential to revolutionize our understanding of human physiology and pathophysiology. We believe that data-driven models and dynamic multiscale models of biological systems will ultimately transform drug discovery efforts toward a more holistic approach that accelerates innovation and improves patient health.

Concluding remarks and future perspectives

The endeavors described above use interdisciplinary work coming from a broad range of backgrounds, predominantly molecular and cell biology and physiology along with traditional engineering disciplines such as chemical and mechanical engineering. At the same time, the new discipline of biological engineering is arising as an intimately seamless fusion of molecular and cellular life science with the most germane concepts and methods for analysis and synthesis inherent in engineering (http://web.mit.edu/be/about/). Biological engineering is precisely aimed at helping make biology-based technologies more gainfully predictive in all realms of application, and prominent among these should be *in vitro* tissue regeneration toward the goal of more-effective modeling of human disease and enhanced prospects in drug discovery and development.

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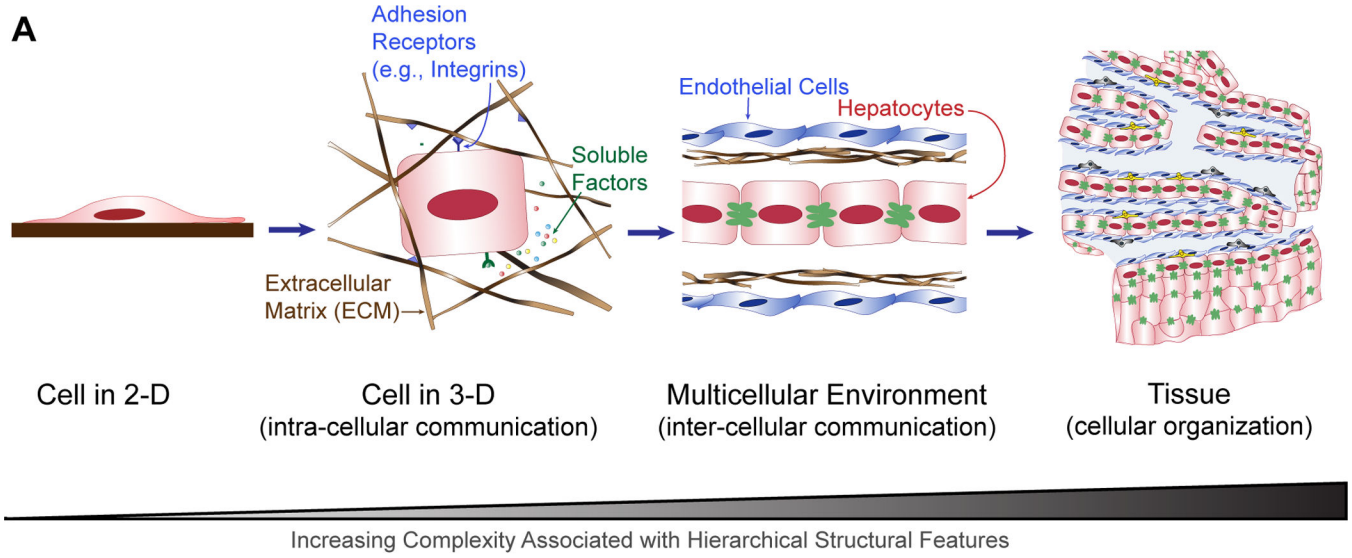
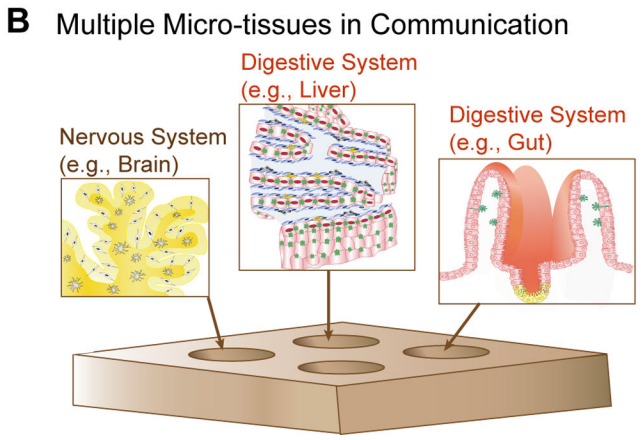
Highlights

- Reliable *in vitro* models for human disease are needed
- Human cell-based models including iPS cell-based models are emerging
- Biomimetic matrices and substrates are crucial to recapitulate microenvironment
- Microfluidics and microfabrication technologies enable dynamic organ models
- Systems biology and multidisciplinary approaches are crucial to drug discovery





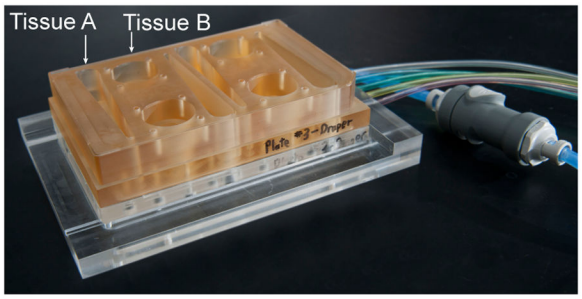


Figure 1.

(a) Schematic diagram showing the hierarchical structural features of a tissue (e.g. the liver). Cells seeded in conventional 2D systems are flat, and thus do not fully capture the complexity of *in vivo* tissue behavior. Using biomaterial strategies and micro- or nano-fabrication techniques, it is possible to introduce various cues such as the extracellular matrix (ECM) and adhesion ligands that dictate release and sequestration of soluble cues such as growth factors. At the next level, using multiple cell types (e.g. endothelial cells and hepatocytes) can impact the fate and function of each cell type within the system while establishing intercellular communications. Using various patterning techniques, it is possible to control positioning of cells and to achieve improved homotypic and heterotypic cellular organization. At the tissue level, the combinatorial effects of ECM and environmental cues, as well as multicellular interactions in the 3D architecture, direct complex tissue behavior. **(b)** Bioreactors and microfluidic devices enable perfusion of 3D tissue structures and provide spatial and temporal control over soluble factors. Different micro-tissues can be cultured and integrated into a single device, and can be engineered with organ-specific biological cues connected by microfluidic systems. Integration of these features enables investigation of interorgan communications through cytokines and additional soluble factors

to capture the complex, dynamic behaviors of human organs. This same system can be used during drug development processes to introduce various types of drugs through different routes (e.g. oral, intravenous, topical or inhalation) and evaluate drug–tissue crosstalk. **(c)** A fabricated platform to study communication between two micro-tissues using a microfluidic system.

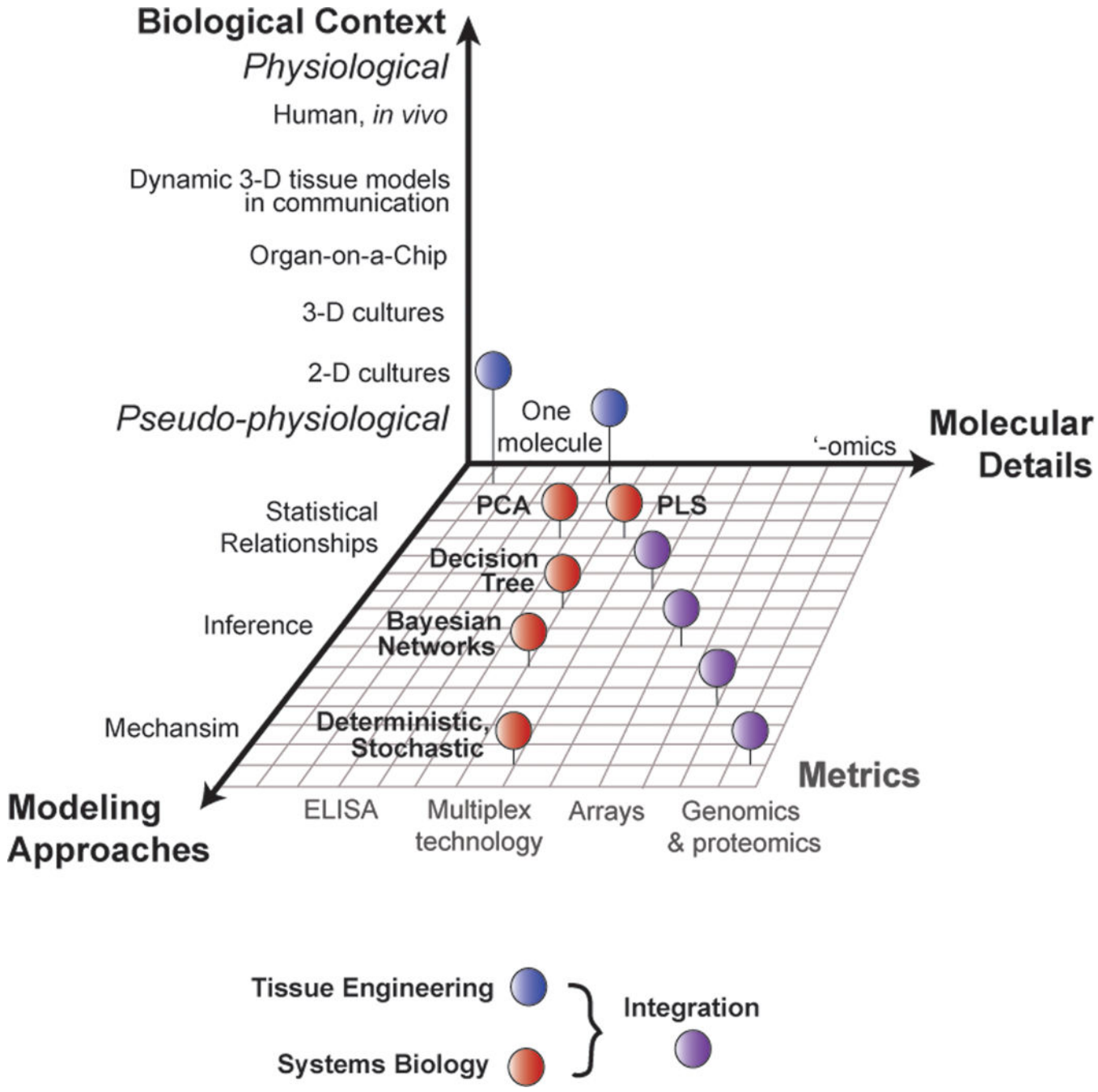


Figure 2.

Data integration, beyond intracellular signaling events to cell phenotype, cell–cell interactions and *in vivo* tissue function, is essential to advance experimental biology toward a more predictive capability. Tissue engineering (blue circles) and systems biology approaches (red circles) progress toward a research paradigm that integrates both disciplines in parallel (purple circles), adapted, with permission, from [68]. Systems-level computational efforts encompass physiochemical models (i.e. deterministic and stochastic) as well as data-driven approaches [e.g. principle component analysis (PCA), partial least

squares (PLS), Decision Tree and Bayesian networks]. Within regenerative medicine research, the implementation of a systems biology framework is illustrated with respect to modeling details that span mechanistic models to statistical relationships and inference models. Collectively, these efforts incorporate biological contexts that extend from prototypical cell lines or primary cells (e.g. 2D cultures) through monocultures or heterotypic cell cultures (e.g. 3D cultures) to engineered and native tissue to organ complexity (e.g. organ-on-a-chip) and communication between tissue models (e.g. human-on-a-chip), while incorporating molecular detail assessed by metrics at multiple scales (gray font).