STEM CELLS®

REGENERATIVE MEDICINE

Organ Engineering: Design, Technology, and Integration

GAURAV KAUSHIK[1,2,*], JEROEN LEIJTEN[1,2,3,*] AND ALI KHADEMHOSSEINI[1,2,4,5,6,#]

[1]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA; [2]Department of Medicine, Biomaterials Innovation Research Center, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA; [3]Department of Developmental BioEngineering, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands; [4]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA; [5]Department of Bioindustrial Technologies, College of Animal Bioscience and Technology, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea; [6]Department of Physics, King Abdulaziz University, Jeddah 21569, Saudi Arabia

Correspondence: Ali Khademhosseini, Harvard Medical School, 65 Landsdowne St, Cambridge Massachusetts 02139, United States, T: 617-768-8395, alik@rics.bwh.harvard.edu; *Shared first authors, author sequence randomly determined; [#]Correspondence: alik@bwh.harvard.edu

Received September 27, 2015; accepted for publication September 06, 2016; available online without subscription through the open access option.

1066-5099/2016/$30.00/0

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/stem.2502

Key words. Organ Engineering • Tissue Engineering • 3D Printing • Microfluidics • Developmental Biology

ABSTRACT

Engineering complex tissues and whole organs has the potential to dramatically impact translational medicine in several avenues. Organ engineering is a discipline that integrates biological knowledge of embryological development, anatomy, physiology, and cellular interactions with enabling technologies including biocompatible biomaterials and biofabrication platforms such as 3D bioprinting. When engineering complex tissues and organs, core design principles must be taken into account, such as the structure-function relationship, biochemical signaling, mechanics, gradients, and spatial constraints. Technological advances in biomaterials, biofabrication, and biomedical imaging allow for *in vitro* control of these factors to recreate *in vivo* phenomena. Finally, organ engineering emerges as an integration of biological design and technical rigor. An overall workflow for organ engineering and guiding technology to advance biology as well as a perspective on necessary future iterations in the field is discussed. STEM CELLS *2016; 00:000–000*

SIGNIFICANCE STATEMENT

Organ engineering offers tremendous promise for regenerative medicine on multiple fronts, including transplants for patients and improved preclinincal diagnostic modeling. This review encompasses integrative approaches in engineering in an accessible manner. In addition to its core subject, improved culture systems are discussed which could benefit biologists across fields, not just stem cell biology and regenerative medicine.

INTRODUCTION

Organ development is the intricate process of coordinated differentiation, morphogenesis, and maturation of diverse populations of cells into complex tissues [1]. Organ development is orchestrated by intricate physicochemical and spatiotemporal cues including growth factors such as Wnts, bone morphogenetic proteins, and fibroblast growth factors [2-4]. Gaining the ability to engineer complex tissues and whole organs has tremendous implications for advancing our understanding of developmental processes and empowering translational and precision medicine. For example, model organs have the promise of delivering next-generation

drug testing platforms. This is greatly desired as the number of new FDA approved drugs has steadily declined, while drug development cost has progressively risen to $2.6 billion [5]. This is largely caused by the low likelihood of FDA approval; only 10,4 % of all drugs that enter a phase I clinical trial will gain market approval, as determined by Hay [6]. Generating platforms that yield greater efficiency, accuracy, fewer false positives will lower development costs and thus increase drug affordability [7-9]. It is expected that recapitulating the complexity of organogenesis *in vitro* could provide such a platform, but achieving such complexity in a facile manner is proving to be a significant engineering challenge. However, great advances have been made through the combination of technological rigor and biological design (Figure 1). In this review, we will outline key design principles and successful approaches for engineering complex tissues and organ systems to maximize biological insight. Core physiological principles that underlie development and disease progression will be discussed. We also offer a perspective on future iterations and interdisciplinary collaborations that can accelerate our understanding of organ systems and the creation of complex functional tissue and organs.

Design Principles

Biomimicry of Organ Structure-Function

The structure of an organ is inherent to its function. For example, bone's trabecular structure determines its load bearing capacity, kidney's intricate anatomical structure is essential for its filtering and resorbing function, and the high density vascular structure within the pancreas enables a rapid glucose/insulin response rate of islets of Langerhans, which prevents the occurrence of hypoglycemia and hyperglycemia. Altering an organ's tissue structure therefore changes its ability to function, and structural remodeling is often associated with pathological organ function [10, 11]. For example, cardiac muscle is a complex tissue as it is organized in aligned, layered sheets, and alterations in cell morphology or misalignment is associated with impaired force production [12] and arrhythmia [13]. Despite cells' ability to self-assemble into organized micro-organoids, they are incapable of spontaneously self-assembling into macro-sized organs that contain fully anatomically correct complex tissue structures *in vitro* [14-16]. Instead, they must be carefully guided with structural, mechanical, and biochemical cues.

When designing organs, it is crucial to have a firm grasp of the organ's basic tissue structures. With regards to engineering complexity, tissues and organs can be organized into three basic groups in ascending order: two-dimensional, hollow, and three-dimensional/solid tissues [17, 18]. Two-dimensional tissues have the fewest engineering hurdles. Simple 2D deposition techniques, such as sputter-coating or simple printing of cells and materials, can be leveraged to replace damaged tissue [19-21]. For example, collagen-glycosaminoglycan scaffolds improved the healing of burn wounds. Moreover, the addition of mesenchymal stem cells into these scaffolds can further improve healing, keratinization, and vascularization [22]. Less facile are hollow tubes, such as large vessels and vasculature. Hollow organs can be thought of as two-dimensional tissues that are folded into three dimensions. Hollow organ disorders, such as bladder cancer and vascular calcification, are numerous and the ability to engineer these in the lab for implantation will be a huge boon to patients. For example, Atala successfully treated patients in need of cystoplasty with an engineered bladder composed of omentum wrapped collagen-polyglycolic acid bladder-shaped scaffold laden with autologous cells [23]. The most complex organ type is composed of solid tissues, which incorporate hollow tissues (microvasculature), several types of tissue-specific adult cell types, and stem cells. Solid organ engineering represents the final frontier, in which previous iterations and approaches must be integrated to meticulously recapitulate the native tissue's structures/functions.

Multiscale Biological Imaging

A primary requirement for accurate recreation of a structured organ is clean, detailed, and multiscale digital imaging data. A great deal of historical histological data exist, which could be mined to create macroscale digital printing data e.g. archives of histological, MRI, and CT images. However, such data has often limited resolution e.g. CT data is often limited to a few hundred micrometer resolution, and may only provide planar structural information about an organ e.g. single histological sections. In order to print whole, functional organs, integration of imaging modalities across length scales may be required. An elegant example of a multiscale and multi-image modality dataset is represented by the Cancer Digital Slide Archive in which histological and immunohistochemical data are paired with MRI data and clinical data [24].

Recent advances in bioimaging may allow for the collection of data to create blue prints for high resolution print designs. For example, CLARITY is a technique that transforms tissues into transparent, porous hydrogels-tissue hybrids of which the lipid content is removed, while maintaining intact tissue structures [25, 26]. This is achieved via the incorporation of a swellable polymer that covalently links with the tissue's proteins and nucleic acid, while the lipids are actively removed using charged ionic SDS-micelles via electrophoretic tissue clearing. This technique can visualize it's the tissue's structure and molecular composition with a resolution as high as 70 nanometer [27]. Moreover, this technique allows for precise, repeatable immunolabeling of individual molecules with sectioning enabling the mapping of numerous proteins within a single tissue volume [28]. Although this technique is primarily used for studying brain structures, it is uniquely suited to provide comprehensive insights into the spatial compo-

sition of a wide range of tissues. Such data might prove valuable for the production of engineered native-like tissues and organs. In addition, contrast-enhanced nanotomography (nanoCT), magnetic resonance imaging (MRI), or histological data can provide large-scale structural information whereas molecular imaging can provide information about how proteins and cells are distributed through a tissue or organ. Such information could be used to design multiphasic structures with both structural and biochemical heterogeneity. Open source workflows for converting and integrating multiple imaging modalities into usable printing data are needed.

Passive and Active Forces

Numerous factors are able to influence cell behavior and organ function [29]. For the sake of brevity, we will here discuss broad concepts in cell biology that can be controlled in engineered systems.

A major advance in our understanding of the development of bioengineered organs was the discovery of mechanotransduction, the concept that mechanical forces regulate cell fate and function through the entire lifespan of an organ [30, 31]. The ability of cells to sense, respond to, and impart forces is crucial to cellular function and has been linked to behaviors ranging from stem cell differentiation [32] to aging [33-35]. Mechanotransduction thus influences tissue behavior, and by extension organ function, by stimulating cells through cell-based forces. Mechanical forces can be sensed in several ways, including protein-mediated signaling, cellular deformations, and membrane tension [36-38].

Passive mechanical stimuli can affect how cells conform and respond to their environment. Stem cell lineage commitment is partially determined by the stiffness of the surrounding microenvironment [39]. For example, Engler has reported that mesenchymal stem cells seeded on hydrogels with a stiffness of ~0.5 kPa acquired a neurogenic phenotype, while ~10 kPa or ~30 kPa induced a myogenic or osteogenic phenotype, respectively [39]. In addition, Guvendiren reported that temporally stiffening a methacrylated hyaluronic acid hydrogel containing mesenchymal stem cells cultured for 2 weeks with a 1:1 mix of osteogenic and adipogenic medium from 1 kPa to 10 kPa shifted stem cell differentiation from adipogenic to osteogenic [40]. This shift became progressively weaker when the hydrogels were stiffened at later time points indicating that substrate stiffness is important throughout the differentiation process. The stiffness of mature tissue is also a factor in how organs and tissues function [41]. For example, Weisbrod concluded based on a 5 month follow up study of diet-induced obese mice that arterial stiffening is not simply a consequence of hypertension, but in fact, also a causing factor of the development of hypertension and organ dysfunction as arterial stiffening preceded systolic hypertension [42]. Other physicochemical properties, including the distribution of adhesion ligands [43] and the hydrophobicity/hydrophilicity of the environment [44, 45] have also been shown to affect how cells function. Cells can sense mechanical cues, amongst others, via integrin-mediated signal transduction and focal adhesions, a molecular complex that is able to deform its environment, dynamically remodel in response to external loads, and trigger signaling cascades with a wide array of biological ramifications [31, 41, 46-48]. Interestingly, data of Huebsch has suggested that mesenchymal stem cells might sense substrate stiffness differently in 2D than in 3D, via integrin alphaV and integrin alpha5, respectively [49]. In addition, actomyosin contractibility is essential to mechanosensing as blocking this process using chemicals such as Cytochalasin D, Y-27632, or Blebbistatin prevents stem cells from appropriately responding to stiffness induced stimuli [39, 40, 50]. It should be noted that focal adhesion-based signaling is extremely sensitive not only to stiffness but also the exact content of the extracellular matrix [51, 52], suggesting a balance between stiffness and biochemistry.

Active or imparted mechanical loads directly influence cell physiology from internal cell signaling to cell morphology. A wide range of cells are known to be affected by shear force and tugging forces from their neighbors. During embryonic morphogenesis cells can impart forces on their neighbors via cell-adhesion molecules [36, 53]. For example, Farge reported that the formation of the anterior gut formation was partially orchestrated via the mechanical forces of morphogenetic movement, which controlled the expression of important developmental genes. In this study, transient 10% uniaxial lateral deformation of early Drosophila embryos induced ectopic expression of Twist around the entire dorsal-ventral axis, which was triggered by mechanically induced nuclear translocation of Armadillo (Drosophila's homologue for β-catenin) [54]. The polarity of these molecules and the subsequent forces could thus dictate the eventual shape of the organism. In mammals, the presence of excessive shear forces on vascular endothelial cells is associated with progression of vessel disorders such as hypertension [55]. Shear [56] and hydrodynamic [57] forces are able to dynamically remodel cells by altering their volume or aspect ratio, which are key parameters that affect the molecular signaling gradients within cells [58].

When cells and tissues are removed from the body, they begin to degenerate and malfunction. A critical reason for this is the lack of proper mechanical feedback, which is a continuously ongoing process in most tissue systems *in vivo*. It is of particular importance to note that overstimulation can induce maladaptive remodeling, as in the case of hypertension-associated vascular stiffening with age [59]. In addition to function following form, form also follows function. For example, it is known that hypertension can induce arterial stiffening through increased extracellular matrix production, vascular thickness, and structural stiffness. However, arterial stiffening can also induce hypertension through increased structural stiffening [60]. Thus, balancing the

design of an engineered tissue or organ with the presented mechanical environment is required for maintaining proper tissue and organ function and preventing maladaptive remodeling.

Spatiotemporal Biochemical Signaling

Biochemical cues such as medium composition, and in particular growth factor supplementation, are the current gold standard for inducing stem cell differentiation e.g. TGB1 is standardly used to induce cartilage formation, while BMP2 is standardly used to induce bone formation. *In vivo*, organisms deploy protein gradients to affect the motion of cells, a process known as chemotaxis. In this way, cells may be guided to their eventual location in the body [61]. The neo-angiogenesis factor VEGF is a classic example of a concentrated growth factor that induces localized changes in physiology [62]. Growth factors embedded in the extracellular matrix can stimulate cell adhesion and proliferation [63, 64]. These cues are highly variable in both space and time. A canonical example of this effect is time-dependent Wnt signaling in the heart. At different stages of cardiac tissue development, Wnt is required and then must be silenced for proper lineage specification [65]. Recapitulating biphasic, time-dependent signals have yielded extraordinarily results when differentiating cells *in vitro*. Cyclical temporal signaling must also be appreciated e.g. circadian clock genes can impact on stem cell differentiation and proliferation [66]. Mice models have demonstrated that Period Circadian Clock 3 (Per3) and Nocturnin (Noc) play key roles in adipogenesis by controlling peroxisome proliferator-activated receptor γ expression and activity [67, 68], and *in vitro* experiments have shown that disruption of brain and muscle Arnt-like 1 (Bma1) increased adipogenesis and decreased myogenesis potentially via WNT signaling *in vitro* [69, 70]. Yet, most culture systems do not incorporate the concept of *in vitro* rhythm.

Spatial Constraints

Cells remodel and conform to the physical environment in which they exist. A parameter as simple as cell seeding density can impact cellular metabolism, migration, proliferation, and differentiation [71]. In recent years, various approaches have allowed for control of cell spreading in 2D. Micropatterning and 2D printing allow for deposition of cell-adhesive materials to which cells conform [72]. Soft photolithography approaches allow for materials with roughness or curvature, such as channels or pits, in which cells align [73]. Such approaches have enabled insightful studies of how single cell morphology impact function but also allowed for careful engineering of 2D cell assemblies or microtissues [48]. Bioprinting technologies, discussed below, now allow for designing three dimensional structures where cell alignment can be controlled.

Decellularized organs can also be thought of as an enforced spatial constraint. In this technique, animal organs are treated to remove all soluble matter, leaving behind a “ghost-white” scaffold composed of insoluble extracellular matrix [74]. When cells are reseeded into these scaffolds, a modest amount of organ function is restored [75, 76]. Such approaches ignore time-dependent changes in organ development but capture a critical spatial component.

Technological Advances

The recognition of aforementioned design principles and challenges in engineering biology has engendered the creation of new technologies that can advance our understanding of cell behavior and allow for controlled engineering of tissues (Figure 2) [77-81]. In this section, we will cover existing technologies, how they have advanced, and project how future iterations might enable the engineering of complex tissues and whole organs.

Biomaterials and 2D Microfabrication

Stem cells interact with their external environment and alter their structure and function in response [30, 52]. This knowledge has fueled the development of biomaterials that are highly tunable, with the ability to alter their mechanical and chemical properties, including porosity, stiffness, cell-attachment sites, and hydrophobicity/hydrophilicity [30]. The biomaterial toolkit continues to grow rapidly and provides solutions for nearly all biological tissues or engineering challenges. Regardless of these breakthroughs, nearly all attempts at clinical translation have been attempted with relatively simple materials e.g. distinct formulations of collagen or alginate containing a possible growth factor. As such, the true commercial value of advanced materials with higher levels of control, and complexity, remains to be proven.

Bioprinting has emerged as the premier method for fabricating three dimensional, macroscale designs, and has the promise of reproducible, reliable assembly of biological structures. Early bioprinting approaches can be traced back to microfabrication-based two-dimensional matrix/protein deposition [82, 83]. In such applications, selective adhesive domains can be arranged in specific geometries or “shapes” on a surface. When cells are plated, they autonomously adhere to these shapes and take on its form [48]. These studies have enabled biologists to understand how geometric symmetry relates to function. In particular, 2D printing led to the insight that patterns that force cells to break their geometrical symmetry can dramatically affect their behavior [84]. For example, Downing reported that patterned surfaces could improve cellular reprogramming efficiencies via mechanomodulation of the cells’ epigenetic state. Specifically, microgrooved surfaces induced a cytoskeletal reorganization that correlated with decreased histone deacetylase activity and increased WD repeat domain 5 (WDR5) expression, which resulted in increased histone H3 acetylation and methylation and increased mesenchymal-to-epithelial transition in adult fibroblasts [85].

Scaffolding and Biofabrication

3D printing relies on a methodology known as additive manufacturing [86]. In additive manufacturing, digital data of a 3D structure is converted into an actual object. In contrast to methods that involve sacrificial molds or solvents, 3D printing can create complex structures from the bottom-up.

There are three major approaches for 3D bioprinting currently: inkjet printing, extrusion printing, and stereolithography [17]. Much like how ink in a cartridge is used to deliver droplets to paper to create documents, inkjet bioprinters can deliver biomaterials and cells in controlled volumes. Inkjet printers have controllable yet finite resolution (i.e. droplet size), high printing speed, and relatively low material cost. However, droplets are created from a reservoir through either thermal or mechanical means, which can perturb cells during the printing process. Regardless, inkjet-based bioprinters can operate with a dispensing frequency of 1–10,000 Hz with a spatial resolution that ranges approximately from 50 μm to 1 mm. Dispensing speeds can be even further improved via piezoelectric inkjet printing, which can operate at speeds of approximately 15-25 kHz. However, instant heat exposure and shear stress could induce cell damage. Another drawback is that the "ink" must be a fluid in order for droplets to form. This limits the concentration of cells, thereby limiting cell density. Additionally, in order to form actual tissue, deposited droplets must be cross-linked via potentially-cytotoxic factors (i.e. pH, photoinitiators, or ultraviolet light). As such, inkjet printing is best for 2D tissues with relatively low cell density and complexity, such as cartilage or tendon.

For extrusion printing, cells and/or materials are extruded through a small print head in a line-by-line fashion [87]. Extrusion printing is an extremely efficient and low cost method for printing cell encapsulating constructs or structures into which cells can be seeded. However, printing resolution is limited by the print head's diameter, which in turn is limited by the viscosity of the biomaterial. Depending on the printer's design, it can be challenging to print with viscous materials due to the required pressures. Shear-thinning biomaterials with strain rate [88] or temperature-dependent [87] properties have emerged as a solution for this challenge. Shear-thinning materials flow like a liquid once pressure is applied, but become solid once that pressure is removed. Examples of shear thinning biomaterials are β-Hairpin peptide-based hydrogels, gelatin and silicate nanoplatelets mixtures, adamantane modified hyaluronic acid and β-cyclodextrin modified HA mixtures, and oppositely charged gelatin nanospheres [88-90]. Extrusion printing of shear-thinning biomaterials in tandem with microfluidics can enable continuous printing of multiple materials and contribute to development of biofabrication platforms that allow for the facile engineering of chemically and structurally complex tissues.

In stereolithography, a photopolymerizable material is printed layer-by-layer. As in extrusion printing, digital data is used to construct the object. Specifically, digital data is used in in stereolithographic bioprinting to instruct where the light should be focused to locally polymerize the biomaterial. A related platform is digital micromirror device (DMD)-based printing, in which an array of digitally-controlled mirrors reflect light to create a 2D projection on a surface. By changing the 2D projection over the height of a biomaterial, a 3D object of desired shape can be made.

A plethora of ultraviolet or blue-light curable biomaterials make this a convenient platform for many labs interested in using their "2D" materials for 3D bioprinting [91]. Some drawbacks exist, however. The use of light poses a risk of toxicity for cells, so printing time and light source parameters must be optimized to ensure cells are not damaged during the printing process. As in extrusion printing, complex scaffolds can be made entirely from biomaterials with cells being seeded after. In such ways, cells could be encouraged to adhere to particular geometries by varying factors such as curvature, porosity, roughness, or surface-to-volume ratio. The ability to vary spatial properties over multiple length scales is a crucial engineering requirement for organs as structure is meticulously tied to function.

Biomaterials for 3D printing often require photoinitiators that cross-link monomers upon activation by ultraviolet or blue light. However, materials and particles that react to visible [92] or near-infrared [93] light for controlled cross-linking or release of biomolecules have also been developed and been used to cage chemical cues. "Dual-wavelength" bioprinters may be able to print biological scaffolds with controlled distribution of chemical signals or adhesive domains. This would offer a huge advance for the field since organs are distinguished by spatial heterogeneity with regards to cellular and molecular composition.

Integrated Engineering Approaches

Organ development is orchestrated by dynamic gradients and combinations of growth factors. Organs are relentlessly perfused to replenish and control oxygen, pH, nutrients, and temperature levels throughout their lifetime. In contrast, standard cell culture is performed in petri dishes under static conditions. Furthermore, the concentration of cues can be varied in time and deliver gradients of chemical factors to improve the biomimicry *in vitro*. It is of note that no single factor can induce the spontaneous self-assembly of a complex tissue or an organ from a pool of stem cells. A concert of tunable factors that can be deployed in a controlled manner following their expression patterns during natural development will likely yield the most promising results. Organ development also requires long-term culture and post-processing or maturation of naive tissues after cell seeding.

Fortunately, engineering advances have enabled us to recreate various aspects of organ development *in vitro* (Figure 3). In the case of physical cues, materials with tunable properties, e.g. stiffness and ligand density

have allowed us to understand how these factors dictate stem cell fate and guided their fate in well-controlled *in vitro* environments. In recent years, the engineering of complex tissues and organs has been expanded by three integrated engineering approaches: 1) microfluidics-based organ systems or "organs-on-a-chip", 2) three-dimensional organoids, and 3) engineering complex tissues for whole organ replacement.

Organs-on-a-Chip

Drug development cost are rising progressively as drug candidates fail in clinical trials despite promising results from traditional preclinical model systems. The current drug development cost has reached a record high of $2.6 billion per FDA approved drug [5]. Moreover, the development cost per approved drug has on average doubled every nine year since 1950 [94]. Two main reasons for the increased drug costs are 1) the limited predictability of *in vitro* results for *in vivo* outcomes and 2) the small size of patient cohorts in phase I and phase II clinical trials, which cannot sufficiently inform on probable clinical efficiency [94]. Together this has led to a large failure rate of highly expensive phase III trials. Improved *in vitro* models that better predict drug efficacy and safety in humans are thus sorely needed. In response to this need, and assisted by the advent of induced pluripotent stem cells, the field of miniaturized organ systems or "organs-on-a-chip" has emerged. Advanced organs-on-a-chip models can provide complex physical and biochemical cues to human-derived cells to form biomimetic research platforms [95]. The integration of these engineering efforts allow organs-on-a-chip systems to surpass traditional "cells-in-a-dish" models with regards to how well they can recapitulate *in vivo* phenomena [96]. Furthermore, individual organ systems can be connected in an integrated circuit to form a theorized "human-on-a-chip". Systemic signals can dramatically influence an organism's health as organ function is strongly affected by inter-organ communication [97, 98]. For example, muscle performance can alter metabolism in distal tissues [99] and even influence aging [35]. With regards to drug testing, individual drugs may perform well on their intended organ, but once metabolized by another organ it can result in toxic byproducts, unexpected side-effects, or attenuated clinical effects [8, 100, 101]. By studying individual and isolated organs, we risk forfeiting vital information that emerges from integrated organ systems. Thus integration of physical, chemical, and biological variables and continued iteration of systemic events will enable more sophisticated drug screens in not only healthy systems, but also those that mimic specific inherited or acquired disease etiologies [7-9, 77, 102].

3D Organoids

Spatial constraints can be used to control cell assembly in two dimensions in the lab. However, cells lack firm, non-permissive physical constraints in the body. Yet, organs are able to undergo controlled growth to appropriate length scales in three dimensions. 3D organoids have been developed to recapture this phenomenon in the lab. These organoids typically are co-cultures of pluripotent and adult cells that act in concert with matrix and biochemical factors. Rather than adhere to a preconstructed scaffold with firm, defined physical cues, organoids undergo autonomous self-assembly [103, 104], a process in which growth and remodeling occurs spontaneously and in an externally-uncontrolled fashion [105]. In recent years, organoids have shown tremendous prowess as advanced culture models that provide insight in the development and pathology of miniature organ-like structures [106]. Long-term culture is made possible through the use of microbioreactors, extracellular matrices, or scaffolding to give cells an initial platform in which to form tissue while maintaining a stem cell population for continuous tissue renewal. Organoid cultures can be easily integrated with complementary organ-on-chip models via microfluidics.

Outlook

Organs are highly complex structures that pose a tremendous engineering challenge. Fortunately, rapid technological and methodological developments have advanced our capabilities to capture and recapitulate crucial aspects of organ structure and function. Organ-on-chip models can create two-dimensional models in which cell function can be monitored over time in response to precisely controlled physicochemical cues. Organoid models enable our understanding of morphogenesis and developmental disorders. Moreover, nascent organ replacement models are paving the way for lab-grown, transplantable organs derived from a patient's own cells. Recent advances in creating 3D organ-on-chip models are expected to further aid this revolution [107].

Each individual organ poses its own engineering challenge. The spatial constraints, biochemical cues, role of physical forces, individual cell types, and growth program varies between each tissue type. Early organ models, while they have a large measure of effectiveness over traditional culture techniques, cannot fully replicate *in vivo* biology. Specifically, single organ models do not include interaction between different tissues and organs and thus largely exclude the dynamic effects of an organism's metabolic, immune, and hormonal status. For example, adipose tissue can influence skeletal muscles, cardiovascular tissues, and the pancreas' β-cells, through endocrine organ crosstalk e.g. via adiponectin, leptin, DPP4, and vistatin [108]. This suggests that not all the requirements for proper organ formation have become known. In addition, different stem cell sources e.g. adult stem cells, embryonic stem cells, and induced pluripotent stem cells most likely require distinct design parameters to effectively induce their differentiation and tissue formation. Therefore, engineering expertise must be coupled with the ever progressing biological knowledge to drive the biodesign of engineered tissues and organs.

An excellent case study in organ design is a heart that combines both solid organ engineering (ventricular wall) with hollow organ engineering (four chambers). Engineering cardiac tissue can be achieved via either bottom-up, starting from organized sheets of myocytes [109], or from the top-down, starting with decellularized matrix and reintroducing the appropriate cells in the right places [74]. Regarding the bottom-up approach, the need for myocyte alignment is a must; misaligned myocardium underlies heart failure and dysfunction [73]. However, alignment sheets of myocytes are organized in a particular way in the heart wall to ensure proper contraction [110]. At this stage, two-dimensional models of heart organ function exist, but the ability to bottom-up engineer a tissue with a complex such as a heart's organized muscle has remained largely out of reach. For example, despite our capability to 3D print the macroshape of a trabeculated heart, these printed constructs do not yet possess proper alignment of cells and extracellular matrix [111]. In contrast, top-down approaches such as using decellularized donor hearts as scaffolds provides an excellent template of organized matrix in which patient-specific cells can integrate. However, expanding and placing cells in an efficient and aligned manner in the scaffold has remained a challenge. The tissue development rates of top-down approaches might also differ from those we have evolved to form native tissues under embryological conditions; the degree of self-assembly that is required or the remodeling that the engineered organ will undergo after cells are properly seeded are still largely unknown. Consequently, the final engineered organ may be dissimilar from its native counterpart in both a structural and a functional manner.

Combining top-down and bottom-up approaches could lead to improved designs; a combination of intelligent engineering and spatiotemporal control of cell placement is needed along with an appreciation for the changes that occur in an organ during development. In the future, novel 3D bioprinting technologies may be able to print heterogeneous and complex tissues with diverse populations of cells, matrix, and growth factors from high-resolution, multiscale imaging data. As additional data informs our understanding of organogenesis, these insights can be folded back into engineering platforms, which can in turn generate more nuanced experiments in developmental biology.

Acknowledgments

The authors acknowledge funding from the National Science Foundation (EFRI-1240443), IMMODGEL (602694), and the National Institutes of Health (EB012597, AR057837, DE021468, HL099073, AI105024, AR063745, 5T32EB016652-02). Dr. Leijten acknowledges financial support from Innovative Research Incentives Scheme Veni #14328 of the Netherlands Organization for Scientific Research (NWO).

Author Contributions

G.K. and J.L. designed the review, GK and JL drew the figures, G.K. and J.L. wrote the review's first draft, and G.K., J.L. and A.K. reviewed and approved the work.

References

1 Costantini F, Kopan R. Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development. **Developmental cell**. 2010;18:698-712.

2 Nelson CM. Geometric control of tissue morphogenesis. **Biochimica et biophysica acta**. 2009;1793:903-910.

3 Marvin MJ, Di Rocco G, Gardiner A et al. Inhibition of Wnt activity induces heart formation from posterior mesoderm. **Genes & development**. 2001;15:316-327.

4 Leijten J, Georgi N, Moreira Teixeira L et al. Metabolic programming of mesenchymal stromal cells by oxygen tension directs chondrogenic cell fate. **Proceedings of the National Academy of Sciences of the United States of America**. 2014;84:30-44.

5 Avorn J. The $2.6 billion pill--methodologic and policy considerations. **The New England journal of medicine**. 2015;372:1877-1879.

6 Hay M, Thomas DW, Craighead JL et al. Clinical development success rates for investigational drugs. **Nature biotechnology**. 2014;32:40-51.

7 Selimovic S, Dokmeci MR, Khademhosseini A. Organs-on-a-chip for drug discovery. **Current opinion in pharmacology**. 2013;13:829-833.

8 Polini A, Prodanov L, Bhise NS et al. Organs-on-a-chip: a new tool for drug discovery. **Expert opinion on drug discovery**. 2014;9:335-352.

9 Kang L, Chung BG, Langer R et al. Microfluidics for drug discovery and development: from target selection to product lifecycle management. **Drug discovery today**. 2008;13:1-13.

10 Sullivan KE, Black LD. The role of cardiac fibroblasts in extracellular matrix-mediated signaling during normal and pathological cardiac development. **Journal of biomechanical engineering**. 2013;135:71001.

11 Brandi ML. Microarchitecture, the key to bone quality. **Rheumatology**. 2009;48 Suppl 4:iv3-8.

12 Viswanathan MC, Kaushik G, Engler AJ et al. A Drosophila melanogaster model of diastolic dysfunction and cardiomyopathy based on impaired troponin-T function. **Circulation research**. 2014;114:e6-17.

13 Mezzano V, Sheikh F. Cell-cell junction remodeling in the heart: possible role in cardiac conduction system function and arrhythmias? **Life sciences**. 2012;90:313-321.

14 Unger RE, Sartoris A, Peters K et al. Tissue-like self-assembly in cocultures of endothelial cells and osteoblasts and the formation of microcapillary-like structures on three-dimensional porous biomaterials. **Biomaterials**. 2007;28:3965-3976.

15 Zhang S. Fabrication of novel biomaterials through molecular self-assembly. **Nature biotechnology**. 2003;21:1171-1178.

16 Leijten J, Khademhosseini A. From Nano to Macro: Multiscale Materials for Improved Stem Cell Culturing and Analysis. **Cell stem cell**. 2016;18:20-24.

17 Murphy SV, Atala A. 3D bioprinting of tissues and organs. **Nature biotechnology**. 2014;32:773-785.

18 Leijten J, Chai YC, Papantoniou I et al. Cell based advanced therapeutic medicinal products for bone repair: Keep it simple? **Advanced drug delivery reviews**. 2015;84:30-44.

19 Koch L, Deiwick A, Schlie S et al. Skin tissue generation by laser cell printing. **Biotechnology and bioengineering**. 2012;109:1855-1863.

20 Rimann M, Bono E, Annaheim H et al. Standardized 3D Bioprinting of Soft Tissue Models with Human Primary Cells. **Journal of laboratory automation**. 2015.

21 Algzlan H, Varada S. Three-dimensional printing of the skin. **JAMA dermatology**. 2015;151:207.

22 Liu P, Deng Z, Han S et al. Tissue-engineered skin containing mesenchymal

stem cells improves burn wounds. **Artificial organs**. 2008;32:925-931.

23 Atala A, Bauer SB, Soker S et al. Tissue-engineered autologous bladders for patients needing cystoplasty. **Lancet**. 2006;367:1241-1246.

24 Gutman DA, Cobb J, Somanna D et al. Cancer Digital Slide Archive: an informatics resource to support integrated in silico analysis of TCGA pathology data. **Journal of the American Medical Informatics Association : JAMIA**. 2013;20:1091-1098.

25 Epp JR, Niibori Y, Liz Hsiang HL et al. Optimization of CLARITY for Clearing Whole-Brain and Other Intact Organs(1,2,3). **eNeuro**. 2015;2.

26 Chung K, Wallace J, Kim SY et al. Structural and molecular interrogation of intact biological systems. **Nature**. 2013;497:332-337.

27 Chen F, Tillberg PW, Boyden ES. Optical imaging. Expansion microscopy. **Science**. 2015;347:543-548.

28 Tomer R, Ye L, Hsueh B et al. Advanced CLARITY for rapid and high-resolution imaging of intact tissues. **Nature protocols**. 2014;9:1682-1697.

29 Crowder SW, Leonardo V, Whittaker T et al. Material Cues as Potent Regulators of Epigenetics and Stem Cell Function. **Cell stem cell**. 2016;18:39-52.

30 Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. **Nature materials**. 2014;13:547-557.

31 Spanjaard E, de Rooij J. Mechanotransduction: vinculin provides stability when tension rises. **Current biology : CB**. 2013;23:R159-161.

32 Holle AW, Tang X, Vijayraghavan D et al. In situ mechanotransduction via vinculin regulates stem cell differentiation. **Stem cells**. 2013;31:2467-2477.

33 Kaushik G, Fuhrmann A, Cammarato A et al. In situ mechanical analysis of myofibrillar perturbation and aging on soft, bilayered Drosophila myocardium. **Biophysical journal**. 2011;101:2629-2637.

34 Kaushik G, Zambon AC, Fuhrmann A et al. Measuring passive myocardial stiffness in Drosophila melanogaster to investigate diastolic dysfunction. **Journal of cellular and molecular medicine**. 2012;16:1656-1662.

35 Kaushik G, Spenlehauer A, Sessions AO et al. Vinculin network-mediated cytoskeletal remodeling regulates contractile function in the aging heart. **Science translational medicine**. 2015;7:292ra299.

36 Huveneers S, de Rooij J. Mechanosensitive systems at the cadherin-F-actin interface. **Journal of cell science**. 2013;126:403-413.

37 Orr AW, Helmke BP, Blackman BR et al. Mechanisms of mechanotransduction. **Developmental cell**. 2006;10:11-20.

38 Holle AW, Engler AJ. More than a feeling: discovering, understanding, and influencing mechanosensing pathways. **Current opinion in biotechnology**. 2011;22:648-654.

39 Engler AJ, Sen S, Sweeney HL et al. Matrix elasticity directs stem cell lineage specification. **Cell**. 2006;126:677-689.

40 Guvendiren M, Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. **Nature communications**. 2012;3:792.

41 Young JL, Kretchmer K, Ondeck MG et al. Mechanosensitive kinases regulate stiffness-induced cardiomyocyte maturation. **Scientific reports**. 2014;4:6425.

42 Weisbrod RM, Shiang T, Al Sayah L et al. Arterial stiffening precedes systolic hypertension in diet-induced obesity. **Hypertension**. 2013;62:1105-1110.

43 Viswanathan P, Ondeck MG, Chirasatitsin S et al. 3D surface topology guides stem cell adhesion and differentiation. **Biomaterials**. 2015;52:140-147.

44 Phadke A, Zhang C, Arman B et al. Rapid self-healing hydrogels. **Proceedings of the National Academy of Sciences of the United States of America**. 2012;109:4383-4388.

45 Phadke A, Zhang C, Hwang Y et al. Templated mineralization of synthetic hydrogels for bone-like composite materials: role of matrix hydrophobicity. **Biomacromolecules**. 2010;11:2060-2068.

46 Fuhrmann A, Engler AJ. The cytoskeleton regulates cell attachment strength. **Biophysical journal**. 2015;109:57-65.

47 Jahed Z, Shams H, Mehrbod M et al. Mechanotransduction pathways linking the extracellular matrix to the nucleus. **International review of cell and molecular biology**. 2014;310:171-220.

48 Oakes PW, Banerjee S, Marchetti MC et al. Geometry regulates traction stresses in adherent cells. **Biophysical journal**. 2014;107:825-833.

49 Huebsch N, Arany PR, Mao AS et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. **Nature materials**. 2010;9:518-526.

50 Inoue Y, Tsuda S, Nakagawa K et al. Modeling myosin-dependent rearrangement and force generation in an actomyosin network. **Journal of theoretical biology**. 2011;281:65-73.

51 Taylor-Weiner H, Ravi N, Engler AJ. Traction forces mediated by integrin signaling are necessary for definitive endoderm specification. **Journal of cell science**. 2015;128:1961-1968.

52 Wen JH, Vincent LG, Fuhrmann A et al. Interplay of matrix stiffness and protein tethering in stem cell differentiation. **Nature materials**. 2014;13:979-987.

53 Duband JL, Thiery JP. Spatio-temporal distribution of the adherens junction-associated molecules vinculin and talin in the early avian embryo. **Cell differentiation and development : the official journal of the International Society of Developmental Biologists**. 1990;30:55-76.

54 Farge E. Mechanical induction of Twist in the Drosophila foregut/stomodeal primordium. **Current biology : CB**. 2003;13:1365-1377.

55 Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. **JAMA : the journal of the American Medical Association**. 1999;282:2035-2042.

56 Fuhrmann A, Engler AJ. Acute shear stress direction dictates adherent cell remodeling and verifies shear profile of spinning disk assays. **Physical biology**. 2015;12:016011.

57 Sinha B, Koster D, Ruez R et al. Cells respond to mechanical stress by rapid disassembly of caveolae. **Cell**. 2011;144:402-413.

58 Rangamani P, Lipshtat A, Azeloglu EU et al. Decoding information in cell shape. **Cell**. 2013;154:1356-1369.

59 Qiu H, Zhu Y, Sun Z et al. Short communication: vascular smooth muscle cell stiffness as a mechanism for increased aortic stiffness with aging. **Circulation research**. 2010;107:615-619.

60 Humphrey JD, Harrison DG, Figueroa CA et al. Central Artery Stiffness in Hypertension and Aging: A Problem With Cause and Consequence. **Circulation research**. 2016;118:379-381.

61 Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. **Cell**. 1996;84:359-369.

62 Barleon B, Sozzani S, Zhou D et al. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. **Blood**. 1996;87:3336-3343.

63 Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. **Current opinion in cell biology**. 2012;24:645-651.

64 Moreira Teixeira LS, Leijten JC, Wennink JW et al. The effect of platelet lysate supplementation of a dextran-based hydrogel on cartilage formation. **Biomaterials**. 2012;33:3651-3661.

65 Lian X, Hsiao C, Wilson G et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. **Proceedings of the National Academy of Sciences of the United States of America**. 2012;109:E1848-1857.

66 Zlotorynski E. Circadian rhythms: Translating the clock. **Nature reviews Molecular cell biology**. 2015;16:390.

67 Costa MJ, So AY, Kaasik K et al. Circadian rhythm gene period 3 is an inhibitor of the adipocyte cell fate. **The Journal of biological chemistry**. 2011;286:9063-9070.

68 Kawai M, Green CB, Lecka-Czernik B et al. A circadian-regulated gene, Nocturnin, promotes adipogenesis by stimulating PPAR-gamma nuclear translocation. **Proceedings of the National Academy of Sciences of the United States of America**. 2010;107:10508-10513.

69 Guo B, Chatterjee S, Li L et al. The clock gene, brain and muscle Arnt-like 1, regulates adipogenesis via Wnt signaling pathway. **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**. 2012;26:3453-3463.

70 Chatterjee S, Nam D, Guo B et al. Brain and muscle Arnt-like 1 is a key regulator of myogenesis. **Journal of cell science**. 2013;126:2213-2224.

71 Erickson IE, Kestle SR, Zellars KH et al. High mesenchymal stem cell seeding densities in hyaluronic acid hydrogels produce engineered cartilage with native tissue properties. **Acta biomaterialia**. 2012;8:3027-3034.

72 Parker KK, Tan J, Chen CS et al. Myofibrillar architecture in engineered cardiac myocytes. **Circulation research**. 2008;103:340-342.

73 Pfeiffer ER, Wright AT, Edwards AG et al. Caveolae in ventricular myocytes are required for stretch-dependent conduction slowing.

Journal of molecular and cellular cardiology. 2014;76:265-274.

7**4** Ott HC, Matthiesen TS, Goh SK et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. **Nature medicine**. 2008;14:213-221.

7**5** Bao J, Shi Y, Sun H et al. Construction of a portal implantable functional tissue-engineered liver using perfusion-decellularized matrix and hepatocytes in rats. **Cell transplantation**. 2011;20:753-766.

7**6** Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. **Annual review of biomedical engineering**. 2011;13:27-53.

7**7** Bae H, Chu H, Edalat F et al. Development of functional biomaterials with micro- and nanoscale technologies for tissue engineering and drug delivery applications. **Journal of tissue engineering and regenerative medicine**. 2014;8:1-14.

7**8** Camci-Unal G, Alemdar N, Annabi N et al. Oxygen Releasing Biomaterials for Tissue Engineering. **Polymer international**. 2013;62:843-848.

7**9** Khademhosseini A, Peppas NA. Micro- and nanoengineering of biomaterials for healthcare applications. **Advanced healthcare materials**. 2013;2:10-12.

8**0** Cha C, Liechty WB, Khademhosseini A et al. Designing biomaterials to direct stem cell fate. **ACS nano**. 2012;6:9353-9358.

8**1** Zorlutuna P, Annabi N, Camci-Unal G et al. Microfabricated biomaterials for engineering 3D tissues. **Advanced materials**. 2012;24:1782-1804.

8**2** Thery M. Micropatterning as a tool to decipher cell morphogenesis and functions. **Journal of cell science**. 2010;123:4201-4213.

8**3** Jackman RJ, Wilbur JL, Whitesides GM. Fabrication of submicrometer features on curved substrates by microcontact printing. **Science**. 1995;269:664-666.

8**4** McCain ML, Parker KK. Mechanotransduction: the role of mechanical stress, myocyte shape, and cytoskeletal architecture on cardiac function. **Pflugers Archiv : European journal of physiology**. 2011;462:89-104.

8**5** Downing TL, Soto J, Morez C et al. Biophysical regulation of epigenetic state and cell reprogramming. **Nature materials**. 2013;12:1154-1162.

8**6** Tumbleston JR, Shirvanyants D, Ermoshkin N et al. Additive manufacturing. Continuous liquid interface production of 3D objects. **Science**. 2015;347:1349-1352.

8**7** Kolesky DB, Truby RL, Gladman AS et al. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. **Advanced materials**. 2014;26:3124-3130.

8**8** Gaharwar AK, Avery RK, Assmann A et al. Shear-thinning nanocomposite hydrogels for the treatment of hemorrhage. **ACS nano**. 2014;8:9833-9842.

8**9** Yan C, Altunbas A, Yucel T et al. Injectable solid hydrogel: mechanism of shear-thinning and immediate recovery of injectable beta-hairpin peptide hydrogels. **Soft matter**. 2010;6:5143-5156.

9**0** Rodell CB, Kaminski AL, Burdick JA. Rational design of network properties in guest-host assembled and shear-thinning hyaluronic acid hydrogels. **Biomacromolecules**. 2013;14:4125-4134.

9**1** Peppas NA, Hilt JZ, Khademhosseini A et al. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. **Advanced materials**. 2006;18:1345-1360.

9**2** Mosiewicz KA, Kolb L, van der Vlies AJ et al. In situ cell manipulation through enzymatic hydrogel photopatterning. **Nature materials**. 2013;12:1072-1078.

9**3** Yan B, Boyer JC, Habault D et al. Near infrared light triggered release of biomacromolecules from hydrogels loaded with upconversion nanoparticles. **Journal of the American Chemical Society**. 2012;134:16558-16561.

9**4** Scannell JW, Blanckley A, Boldon H et al. Diagnosing the decline in pharmaceutical R&D efficiency. **Nature reviews Drug discovery**. 2012;11:191-200.

9**5** Bhatia SN, Ingber DE. Microfluidic organs-on-chips. **Nature biotechnology**. 2014;32:760-772.

9**6** Huh D, Matthews BD, Mammoto A et al. Reconstituting organ-level lung functions on a chip. **Science**. 2010;328:1662-1668.

9**7** Grueter CE, van Rooij E, Johnson BA et al. A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. **Cell**. 2012;149:671-683.

9**8** Ulgherait M, Rana A, Rera M et al. AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. **Cell reports**. 2014;8:1767-1780.

9**9** Bland ML, Lee RJ, Magallanes JM et al. AMPK supports growth in Drosophila by regulating muscle activity and nutrient uptake in the gut. **Developmental biology**. 2010;344:293-303.

1**00.Park** BK, Boobis A, Clarke S et al. Managing the challenge of chemically reactive metabolites in drug development. **Nature reviews Drug discovery**. 2011;10:292-306.

1**01.Tacar** O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. **The Journal of pharmacy and pharmacology**. 2013;65:157-170.

1**02.Khademhosseini** A, Bettinger C, Karp JM et al. Interplay of biomaterials and microscale technologies for advancing biomedical applications. **Journal of biomaterials science Polymer edition**. 2006;17:1221-1240.

1**03.Sasai** Y. Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. **Cell stem cell**. 2013;12:520-530.

1**04.Moreira** Teixeira LS, Leijten JC, Sobral J et al. High throughput generated micro-aggregates of chondrocytes stimulate cartilage formation in vitro and in vivo. **European cells & materials**. 2012;23:387-399.

1**05.Khademhosseini** A, Eng G, Yeh J et al. Microfluidic patterning for fabrication of contractile cardiac organoids. **Biomedical microdevices**. 2007;9:149-157.

1**06.Lancaster** MA, Renner M, Martin CA et al. Cerebral organoids model human brain development and microcephaly. **Nature**. 2013;501:373-379.

1**07.Madden** L, Juhas M, Kraus WE et al. Bioengineered human myobundles mimic clinical responses of skeletal muscle to drugs. **eLife**. 2015;4:e04885.

1**08.Romacho** T, Elsen M, Rohrborn D et al. Adipose tissue and its role in organ crosstalk. **Acta physiologica**. 2014;210:733-753.

1**09.Wang** G, McCain ML, Yang L et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. **Nature medicine**. 2014.

1**10.McCain** ML, Sheehy SP, Grosberg A et al. Recapitulating maladaptive, multiscale remodeling of failing myocardium on a chip. **Proceedings of the National Academy of Sciences of the United States of America**. 2013;110:9770-9775.

1**11.Hinton** J, Jallerat Q, Palchesko Q et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. **Science Advances**. 2015;1:10.

1**12.Shin** SR, Aghaei-Ghareh-Bolagh B, Dang TT et al. Cell-laden microengineered and mechanically tunable hybrid hydrogels of gelatin and graphene oxide. **Advanced materials**. 2013;25:6385-6391.

1**13.Young** JL, Engler AJ. Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro. **Biomaterials**. 2011;32:1002-1009.

1**14.Wang** H, Liu K, Chen KJ et al. A rapid pathway toward a superb gene delivery system: programming structural and functional diversity into a supramolecular nanoparticle library. **ACS nano**. 2010;4:6235-6243.

1**15.Hardin** JO, Ober TJ, Valentine AD et al. Microfluidic Printheads for Multimaterial 3D Printing of Viscoelastic Inks. **Advanced materials**. 2015;27:3279-3284.

1**16.Zhu** W, Li J, Leong YJ et al. 3D-Printed Artificial Microfish. **Advanced materials**. 2015.

1**17.Huh** D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. **Trends in cell biology**. 2011;21:745-754.

1**18.Antonica** F, Kasprzyk DF, Opitz R et al. Generation of functional thyroid from embryonic stem cells. **Nature**. 2012;491:66-71.

Figure 1. Organ Engineering Workflow. The principle workflow for organ engineering can be divided into three major components: discovery, design, and technology. In this process, the discovery of new biological phenomena (e.g. mechanotransduction, genome editing) informs the design of technologies, which furthers biological discovery. Framing organ engineering from this workflow, equal value is placed on biology and technology.

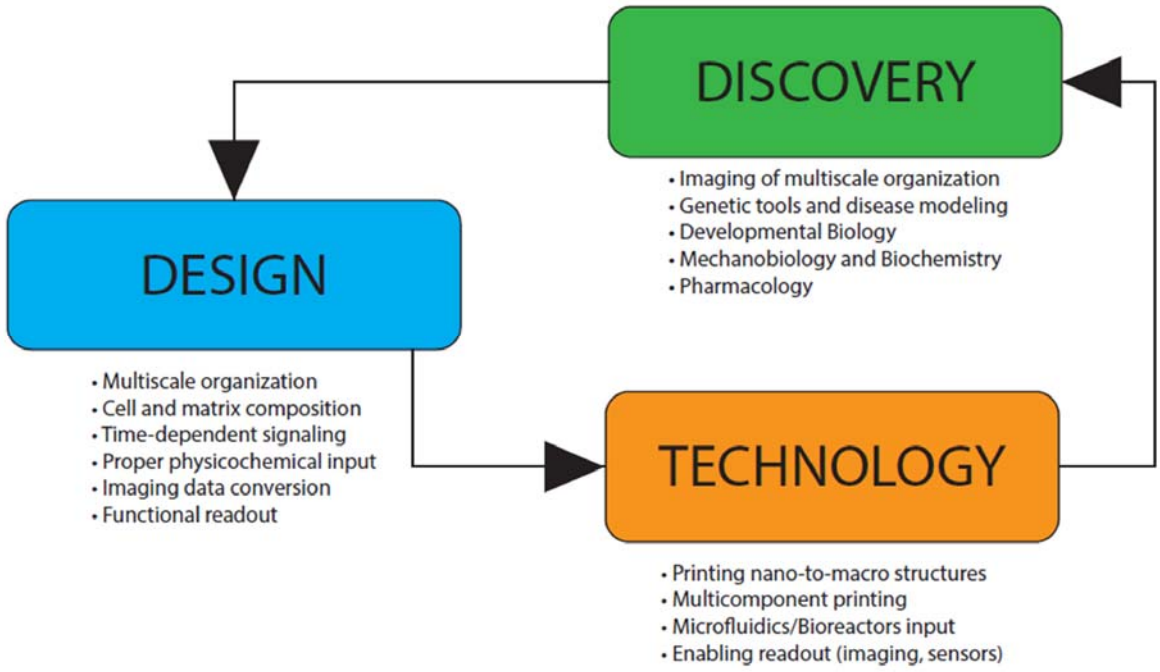

Figure 2. Technologies for Organ Engineering. Advanced and emerging technologies in the fields of biomaterials, microfluidics/bioreactors, and biofabrication now allow for more precise control over cell and tissue structure and function. (A) Biomaterials can be hybridized with synthetic materials to increase mechanical stability to enable printing large, stable structures [112] (left scale bar, 500 µm, middle and right scale bars, 100 µm) or (B) can have time-dependent stiffening to mimic development and improve cell maturation *in vitro* [113]. (Scale bars, 25 µm) (C) Microbioreactors and microfluidics can provide physicochemical cues to sustain biological tissues over time and allow for autonomous self-assembly [106]. (D) Advanced microfluidics can allow for spatial patterning [114] or (E) continuous printing of multiple materials [115]. (Upper scale bar, 5 mm; lower scale bar, 200 µm). Biofabrication techniques such as (F) extrusion [87] (scale bar, 300 µm) or (G) stereolithography-based [116] printing can build up complex tissue architectures from the ground-up. (Scale bar, 100 µm). Images are reprinted with permission.

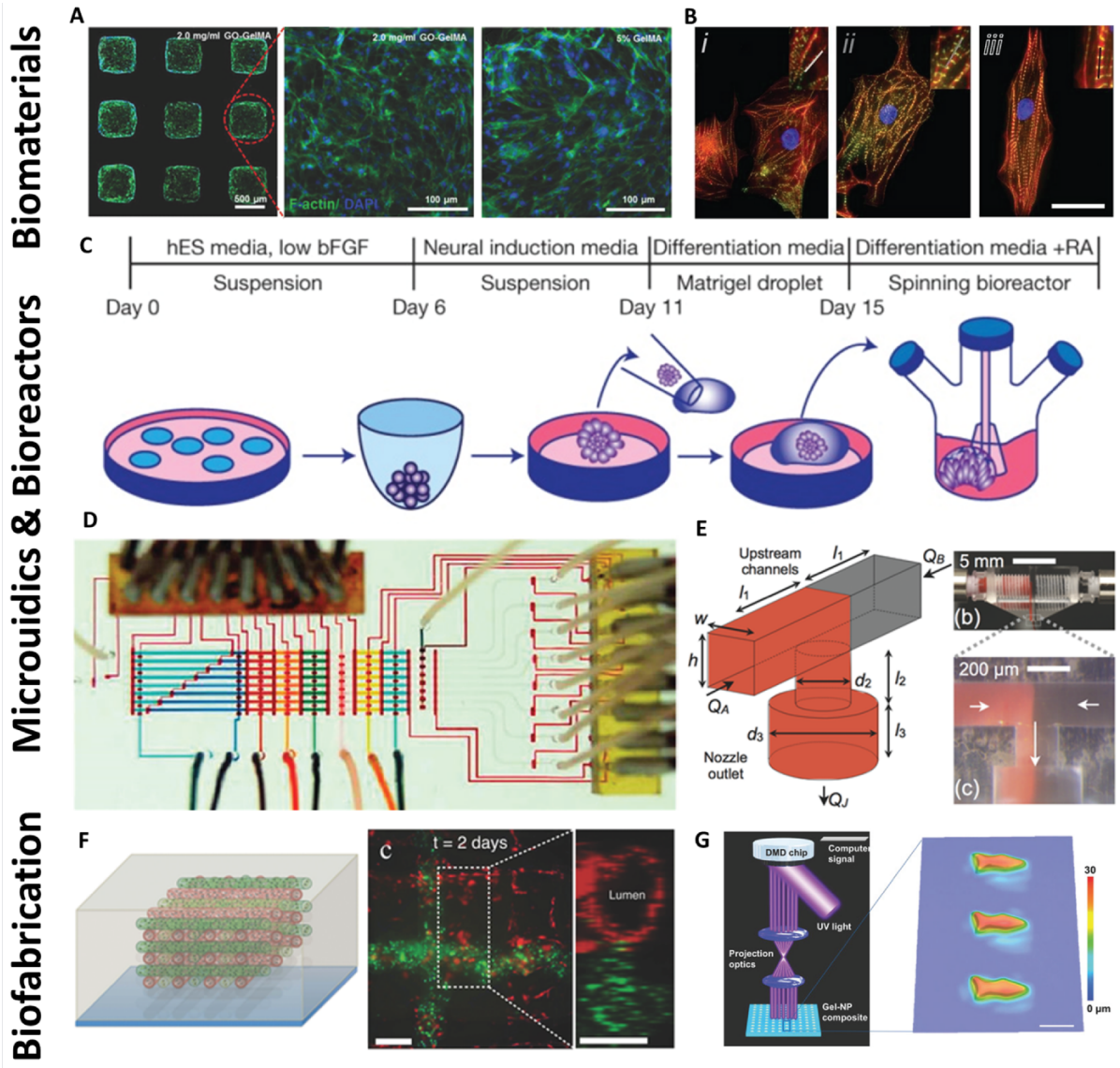

Figure 3. Integrated Engineering Approaches for Organ Engineering. Complex tissue and organ models have developed on two frontiers: organ-on-chip devices and 3D organoids. (A) organs-on-a-chip devices integrate microfluidics, biomaterials, and other microfabrication approaches to create two dimensional representations that recapitulate function or phenomena of the original organ, as in the case of the lung-on-chip model [96]. (B) A "human-on-chip", in which several organ types are placed in a directed, organized circuit has also been proposed [117]. Organoids are mixed populations of cells that undergo autonomous self-assembly in a bioreactor given appropriate physicochemical cues. Organoids have been able to recapitulate (C) neural [106] (scale bar, 200 µm) and (D) thyroid tissues [118]. (Scale bars, 20 µm). Images are reprinted with permission.

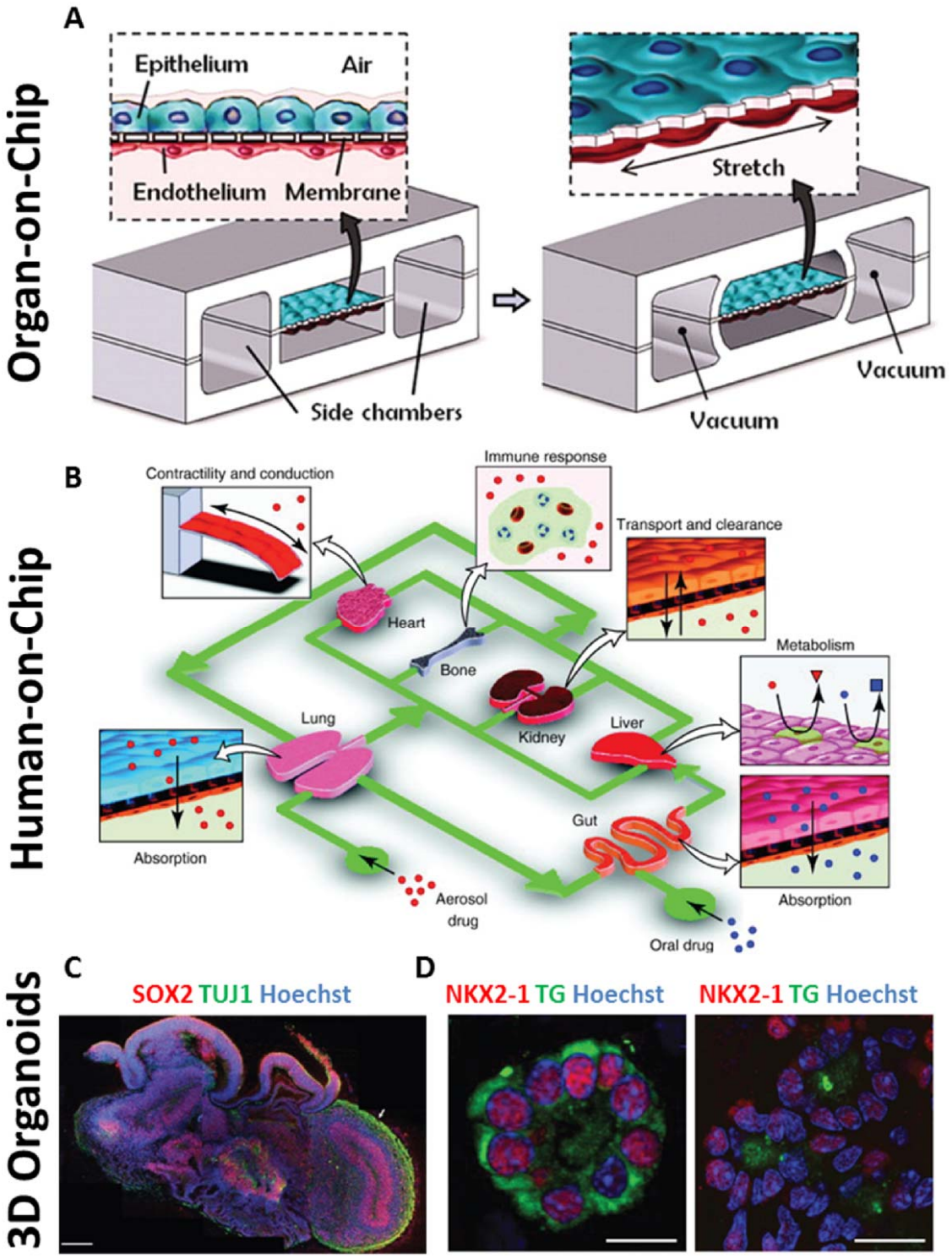

Table of Content Abstract

Organ engineering offers tremendous promise for regenerative medicine on multiple fronts, including transplants for patients and improved preclinincal diagnostic modeling. This review encompasses integrative approaches in engineering in an accessible manner. In addition to its core subject, improved culture systems are discussed which could benefit biologists across fields, not just stem cell biology and regenerative medicine.