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Toward the Directed Self-Assembly of Engineered Tissues

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Abstract

Using scaffold-based approaches, tissue engineers have made bold steps toward creating replacement tissues in the laboratory. However, many of these engineered constructs do not fully match the functional properties of their native counterparts. This is partially due to our limited quantitative understanding of the growth and remodeling processes that alter the architecture of engineered tissues, both in culture and after implantation. Here, we consider the mechanisms by which physical cues direct this adaptive response. In particular, we highlight recent experimental and computational studies that characterize quantitatively the mechanics of growth and remodeling in tissue constructs. These advances, from fields as diverse as soft tissue biomechanics and developmental biology, can be used to design new tissue engineering approaches that direct the self-assembly of engineered tissues.

INTRODUCTION

ECM: extracellular matrix

Owing to the dire shortage of organs available for transplantation, as well as the complications associated with the rejection of donor organs, tissue engineers seek to construct replacement tissues in the laboratory for patients in the clinic (1). However, the challenges inherent in such a task are complex and multidisciplinary; teams of investigators typically involve engineers, clinicians, materials scientists, and biologists.

Early progress in tissue engineering was rooted in advances from materials science—in particular, the design of synthetic polymeric scaffolds that could be used to mimic the meshwork of extracellular matrix (ECM) proteins in the body (2). As a result, the term tissue engineering largely came to refer to the process of designing and constructing new scaffolds, seeding them with cells expanded in culture, and implanting them in vivo at sites of tissue damage. This approach enjoyed success with engineered skin, cartilage, and other predominately avascular connective tissues (2, 3), but the construction of more complex organ architectures has proven elusive (3). Progress over the past two decades has been promising but far slower than originally anticipated (4). With all due respect to seminal advances initially made in the field, conventional approaches to engineer tissues have become decidedly phenomenological and incremental in nature. Such approaches typically involve subtle modifications to the biochemical properties of the scaffold and, often, the use of different cell sources or different combinations of growth factors. In most of these cases, the geometry of the scaffold is then physically molded to mimic native tissue architecture or facilitate native tissue growth.

As an alternative approach, can we harness insights from developmental biology and use them to devise new strategies for tissue engineering? Remarkable progress has been made in determining how the physical and chemical properties of the microenvironment regulate the growth and remodeling of developing tissues (5). Often, these discoveries are driven by a combination of theoretical and experimental approaches (6, 7). Still, while an established theoretical framework exists for the growth and remodeling of mature soft tissues (8), only recently have these ideas been applied to either developing or engineered tissues. Can the physical cues that control developmental processes be repurposed in the laboratory to direct the self-assembly of new organ rudiments? This is essentially a bottom-up approach to tissue engineering (9). Can this somehow be combined with top-down, scaffold-based approaches?

Here, we discuss the current state of scaffold-based tissue engineering strategies and consider a case in which physical cues have been shown to pattern the growth and remodeling of a tissue-equivalent: engineered, small-caliber blood vessels. We then discuss recent work from the developmental biology of tissues, which suggests that similar mechanical factors govern the formation of tissues both in the embryo and in culture. In particular, we focus on the role of the physical microenvironment and suggest alternative tissue engineering approaches that are motivated by discoveries from fields as diverse as experimental embryology and soft tissue biomechanics.

EARLY SCAFFOLD-BASED APPROACHES TO TISSUE ENGINEERING

Traditional tissue engineering strategies have generally followed one of two paradigms. Polymeric scaffolds are either (*a*) implanted at sites of tissue damage to facilitate tissue ingrowth in vivo or (*b*) cultured ex vivo in a bioreactor to generate engineered tissue equivalents (10). Owing to their biocompatibility, flexible processing methods, and readily tunable material properties, hydrogels have proven ideal candidates for polymeric scaffolds (11–13). Hydrogels can mimic the fibrous architecture of the ECM and can be engineered (by chemical modification to the polymeric network) to promote cell adhesion (14) or allow for the controlled release of growth factors

(15). These hydrogel scaffolds are then typically seeded with a population of cells expanded in culture. Still, alternatively, both cell-free and scaffold-free approaches have also been devised (16). Depending on the application, the seeded cells can be derived from autologous or allogeneic sources (3). Recent studies have employed adult- and embryonic-derived stem cells (17–19) or, more recently, induced pluripotent stem cells (20–22).

However, there are often issues with the quality of donor cells; for instance, it might be untenable to harvest autologous populations of cells from donor tissues compromised by disease or age. In addition, transport issues have made it difficult to construct implantable scaffolds larger than a few hundred micrometers thick (16). In addition, for stem cell–based approaches, it is still unclear what signals drive these cells toward a particular fate. Stem cell differentiation is often incomplete (i.e., not all cells differentiate into the desired cell type) (23), and undifferentiated stem cells have demonstrated a propensity to become tumorigenic (24). Still, despite these challenges, several commercially available engineered tissues are available for clinical use, although largely for avascular tissues like skin, cartilage, and trachea (3, 25–29). For more complex tissues, cells seeded within scaffolds do not self-assemble in a way that mimics the native tissue architecture.

SCAFFOLDS: THE NEXT GENERATION

To address this issue, researchers have developed two approaches to more closely recapitulate the in vivo geometry of native tissues: whole-organ tissue engineering and organ printing. During whole-organ tissue engineering, researchers use detergents to completely decellularize intact organs; the freshly denuded ECM is then used as a biomimetic scaffold that can be seeded with donor cells (27). In this way, the complex geometry of an engineered organ can be determined a priori. This technique has been successful in constructing complex tissues that contain numerous cell types, such as heart (30), lung (31), and kidney (32) (**Figure 1*a***). However, this work has largely been demonstrated in small animals like mice, and it is unclear how these techniques would scale up to larger organisms, where there are likely to be significant transport issues. Moreover, although the initial geometry of the scaffold matches that of native tissues, it is not clear how host cells remodel and alter the original donor architecture after implantation. Despite these challenges, tissue-engineered whole tracheae have been successfully implanted in humans by using autologous cell sources (respiratory epithelial cells and chondrocytes) (26).

Three-dimensional biomimetic scaffolds have also been created using inkjet-like printing technology, which deposits hydrogel onto a surface. In this technique, scaffolds are printed layer by layer using a computerized reconstruction of the target tissue geometry (33). Alternatively, 3D plastic molds of a desired geometry can be created using rapid prototyping. The molds are injected with a suspension of cells and hydrogel, which is then polymerized to create a tissue-engineered construct (34). Similar to whole-organ tissue engineering, this approach attempts to construct an implant geometry that mimics the complex architecture of native tissue. Three-dimensional organ printing has been successful primarily with avascular tissue types, such as engineered cartilage. For instance, chondrocytes embedded in a mixture of fibrin and collagen gel have been printed onto electrospun polycaprolactone fibers. These layers were then stacked to create a composite cartilaginous construct (35). Alternatively, 3D printers have been used to create tissue-engineered ears based on scanned patient geometry (**Figure 1*b***). In this case, a 3D plastic mold was filled with chondrocytes suspended within a type-I collagen gel (34).

Despite closely matching the original tissue architecture, most of these sophisticated scaffold-based constructs still fail to functionally match the complex mechanical behavior of native tissues. In tissue-engineered whole trachea, for instance, there have been issues with scaffold collapse (27). Although it is well established that native tissues remodel under different physiological conditions

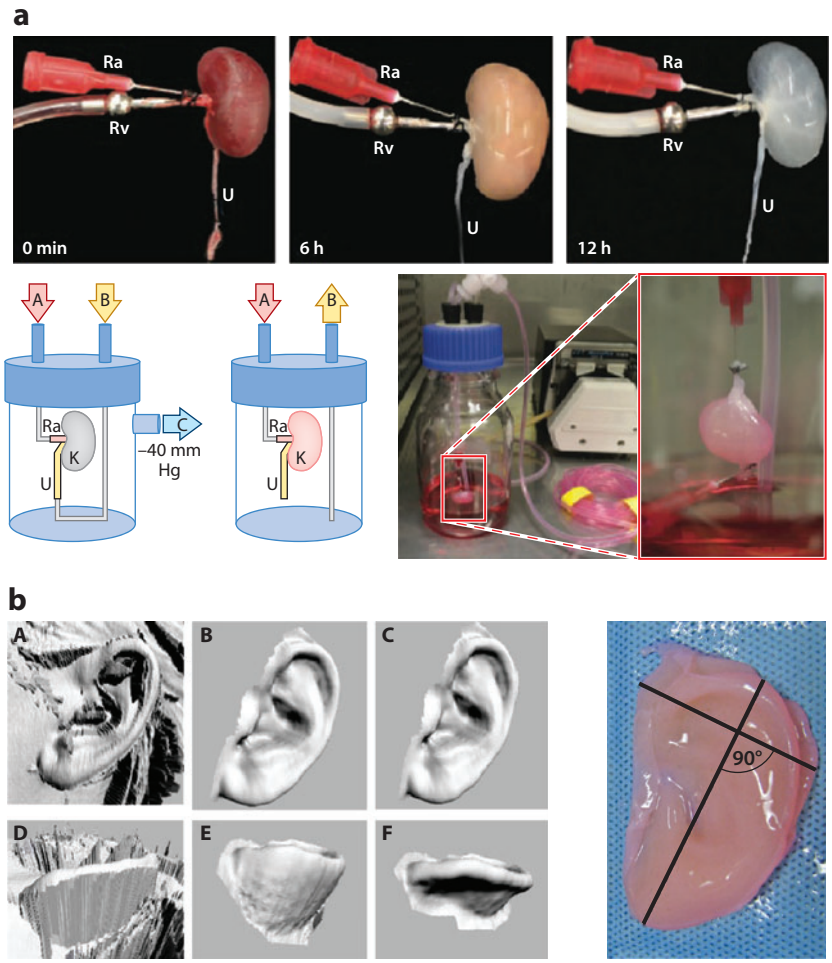

Figure 1

Biomimetic scaffolds: whole-organ tissue engineering and three-dimensional organ printing. (*a*) Whole mouse kidneys decellularized with detergents and then reseeded with both endothelial and epithelial cell populations. The engineered kidneys are cultured in bioreactor before implantation. Adapted from Song et al. (32) with permission from Macmillan Publishers Ltd. (*b*) Three-dimensional organ printing of a human ear, based on scanned patient geometry, using a chondrocyte-seeded type-I collagen gel. Adapted from Reiffel et al. (34). Abbreviations: K, kidney; Ra, renal artery; Rv, renal vein; U, ureter.

(36), how the architecture of engineered tissues changes during culture or after implantation remains unclear.

THE GROWTH AND REMODELING OF ENGINEERED TISSUES—A CASE STUDY: BLOOD VESSELS

Several bioreactors incorporate mechanical loading, which dramatically improves the functional properties of engineered constructs, but the motivation for including these physical cues is largely phenomenological. A quantitative understanding of the remodeling processes that alter

the structure of engineered tissues is only beginning to take shape. Here, because the growth and remodeling of native blood vessels have been studied extensively (37), we consider tissue-engineered blood vessels as a case study and discuss work that has shown how biophysical cues can direct the growth and remodeling of engineered tissues. We then highlight recent advances, which use theoretical models of these processes to guide the construction of engineered vessels.

SMC: smooth muscle cell

Mimicking the Laminar Architecture of Native Vessels

Weinberg & Bell (38) pioneered the development of engineered blood vessels in 1986. Cells suspended in 3D type-I collagen gels had been shown to exert tractions on the surrounding ECM, contracting the network down into a tissue-like structure (39, 40). This approach was first used to create engineered skin equivalents, which were fabricated by seeding collagen gels with dermal fibroblasts that had been expanded previously in culture (41). Building on this work, Weinberg & Bell (38) used a similar approach to create vascular constructs that mimicked the laminar architecture of native blood vessels (**Figure 2*a***) (see sidebar, The Laminar Architecture of Blood Vessels). An annular mold was filled with a suspension of smooth muscle cells (SMCs) and type-I collagen. After one week of culture, the cells had contracted the gel down around the central mandrel to create a tubular construct (**Figure 2*b***). A second layer was then cast around the first using fibroblasts instead of SMCs. The entire construct was then removed from the mold and perfused with a suspension of endothelial cells, which seeded themselves along the luminal surface. The engineered vessels thus included equivalent layers for each of the three compartments of native blood vessels—the intima, media, and adventitia. (Interestingly, Weinberg & Bell did not identify tissue engineering applications as the primary motivation for their work. Rather, they proposed that these constructs could serve as 3D culture platforms to study vascular biology.)

Although these early constructs matched certain gross aspects of vascular morphology, they failed to match the mechanical properties of native blood vessels. The layered constructs were extremely compliant and burst at very low internal pressures (<10 mm Hg). Even constructs reinforced with a Dacron sleeve failed at the subphysiological internal pressures of 40–70 mm Hg. In addition, these engineered vessels included only minimal amounts of elastin, a major structural ECM that endows vessels with much of their elasticity (42). Also, the SMCs seeded in the media-equivalent layer were oriented somewhat longitudinally, in stark contrast to the near-circumferential alignment of both SMCs and collagen fibers in native blood vessels (43).

THE LAMINAR ARCHITECTURE OF BLOOD VESSELS

In general, the blood vessel wall is constructed of three primary layers: the intima, media, and adventitia (37, 42, 146) (**Figure 2*a***). The intimal layer lines the central lumen and consists of endothelial cells overlying a thin basement membrane. These cells regulate transport across the vessel wall and, though involved in fluid shear sensing, contribute minimally to the overall mechanical properties of the wall. The media is separated from the intima by a thin elastic lamina consisting mostly of elastin, a highly distensible structural protein that endows blood vessels with their mechanical elasticity (42). The medial layer contains smooth muscle cells (SMCs), which contract to modulate the vessel diameter. It consists largely of an interconnected lamellar network of elastin in which circumferentially oriented SMCs and collagen fibers are embedded. Lastly, the outermost adventitial layer is separated from the media by another thin elastic lamina and consists of myofibroblasts within a dense network of collagen fibers. In general, the adventitia is thought to serve as a protective sheath, which prevents overdistension of the blood vessel in response to acute pressure increases (37).

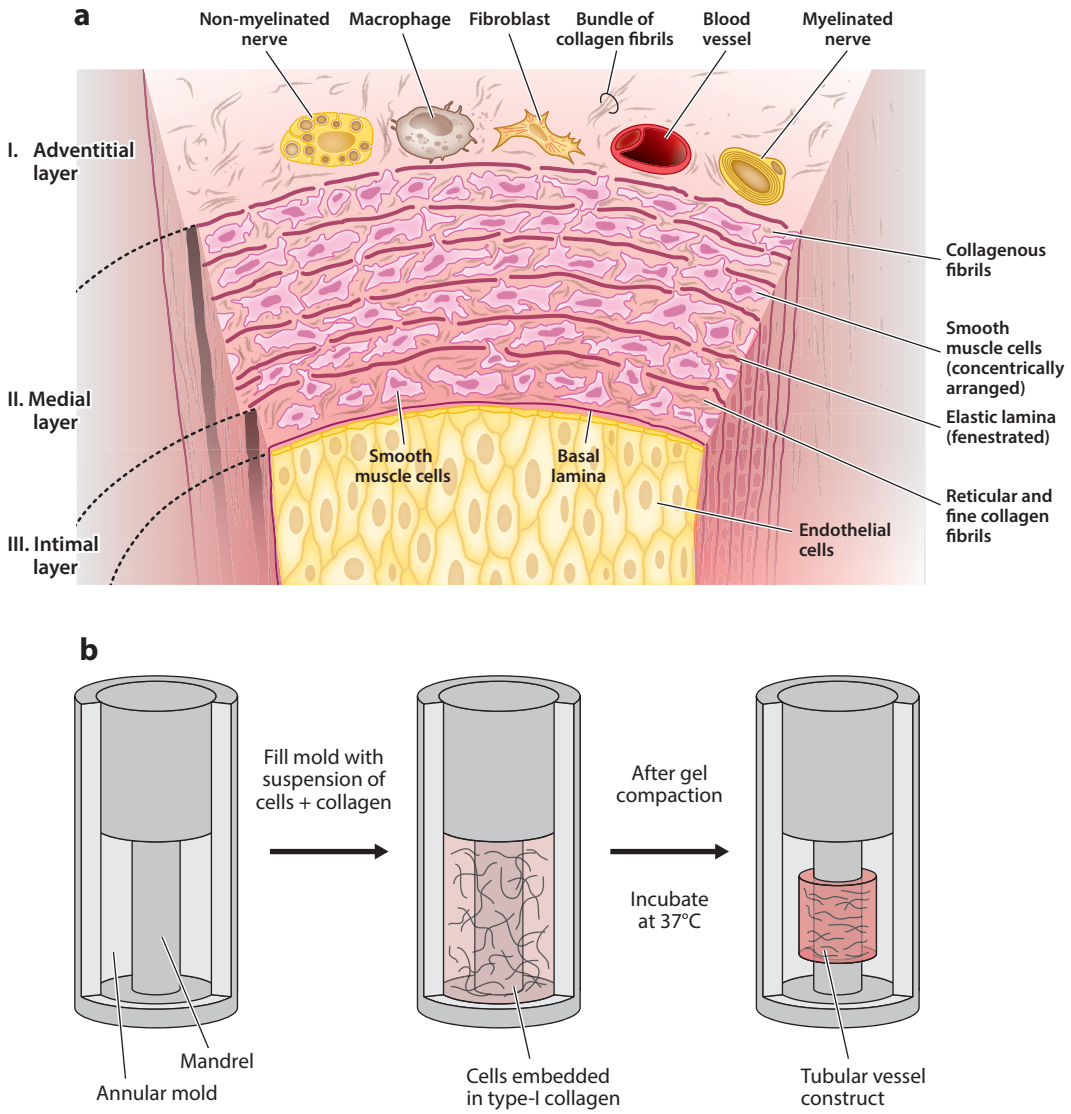

Figure 2

Mimicking the laminar architecture of native blood vessels. (*a*) Schematic of the anatomical structure of an elastic artery. Adapted from Rhodin (146) with permission from John Wiley & Sons, Inc. (*b*) Schematic of biopolymer-based approach used to construct tubular vessel constructs. Based on experimental setup reported by Barocas et al. (47).

Subsequent studies sought to improve construct performance by more closely matching the architecture of native vessels, with hopes of thereby improving their mechanical properties. In 1993, L'Heureux and colleagues (44) used a similar approach to engineer vessels, but found serendipitously that SMCs in the media-equivalent layer aligned circumferentially if adhesion to the underlying mandrel was disrupted (**Figure 3*a***). Simply varying the mechanical boundary conditions along the inner surface of the tissue was sufficient to produce preferential cellular alignment. Alternatively, Tranquillo and colleagues (45) generated circumferential SMCs in media equivalents

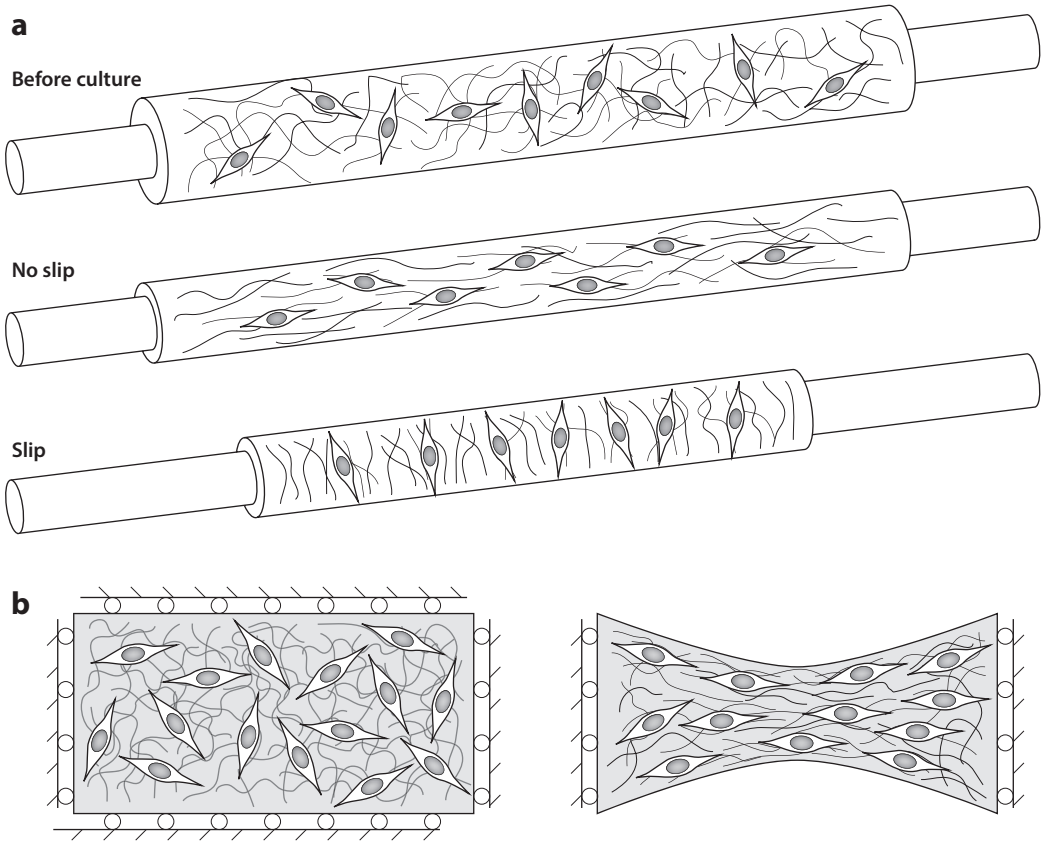

Figure 3

Physical cues dictate patterned alignment in engineered tissues. Varying the mechanical constraints of smooth muscle cell–seeded collagen gels in culture using both (*a*) cylindrical and (*b*) planar geometries. In both cases, physical cues direct cell and extracellular matrix alignment in engineered tissue equivalents. Based on results reported by Barocas et al. (47), Costa et al. (54), and Wagenseil et al. (94).

by exploiting the orientation-inducing effects of a strong magnetic field. Contact guidance along the magnetically oriented collagen fibers then promoted circumferential SMC alignment (46).

The physical effects of this patterned circumferential alignment were later evaluated by mechanical testing using constructs generated by both magnetic prealignment and mandrel compaction. Barocas and colleagues (47) showed that circumferential alignment of SMCs and collagen produced constructs that were considerably stiffer in the circumferential direction than were their isotropic counterparts. In addition, using a combination of theory and experiment, Barocas et al. demonstrated that (free-slip) mandrel compaction was more effective than magnetic prealignment in generating anisotropic media equivalents. (Free compaction of magnetically aligned gels produced near-isotropic tissue constructs.) Using an anisotropic biphasic theory to model the mechanics of tissue equivalents (48, 49), Barocas et al. (47) predicted that SMCs were under higher circumferential stress during mandrel compaction and thereby exerted higher traction forces, which aligned the surrounding network. Dynamic mechanical feedback between the SMCs, the collagen network, and the applied mechanical constraints directed the self-assembly of circumferentially

aligned SMCs and collagen fibers. A similar mechanical feedback likely also removed the prepatterned magnetic alignment in freely compacted gels—a situation in which elevated circumferential stresses were absent. Additional studies using fibroblast-populated collagen gels have also concluded that elevated mechanical tension contributes to cell and collagen fiber orientation during gel compaction (50–53). Other investigators, however, have suggested that the mechanical effects of free edges primarily dictate cell and network alignment (54). Still, both cases suggest that mechanical or geometrical constraints can be used as physical cues to pattern tissue anisotropy.

PGA: polyglycolic acid

MMP: matrix metalloproteinase

Using Physical Cues to Direct the Remodeling of Engineered Vessels

Although patterned circumferential alignment dramatically improved the mechanical properties of engineered vessels, these constructs still failed to match the functional properties of intact veins and arteries. In a clever set of experiments, Girton and colleagues (55) hypothesized that glycation—the typically deleterious mechanism of nonenzymatic tissue stiffening that occurs in conditions like diabetes—could be exploited to increase the strength of engineered vessels. After 10 weeks of culture, uniaxial tensile tests revealed dramatic increases in the stiffness and strength of glycated constructs (56). Tensile strength, for instance, was approximately 50% that of native, intact vessels—a dramatic improvement from earlier engineered vessels. Importantly, the authors (56) demonstrated that patterned fiber alignment enhanced the effects of glycation in these tissues, presumably by increasing the likelihood of crossbridge formation between neighboring fibers.

Alternatively, other groups worked to improve the mechanical performance of vascular constructs by subjecting them to dynamic mechanical loading, an attempt to qualitatively recapitulate the boundary conditions arising from pulsatile blood flow in vivo. In 1999, Niklason and colleagues (57) used an inflatable silicone tube in lieu of a central mandrel to apply cyclic circumferential strains to engineered vessels in culture. In these initial experiments, the SMCs were seeded in a tubular scaffold of polyglycolic acid (PGA), which degraded over time as the cells laid down a network of ECM. Although PGA initially provided mechanical integrity to the construct, residual polymeric fragments were shown to compromise its overall mechanical properties (58). Thus, building on the pioneering work of Kanda and colleagues (59, 60), Seliktar et al. (61) cultured SMC-seeded collagen gels around inflatable silicone tubes. The authors reported elevated circumferential SMC alignment and a 240% increase in construct strength after eight days of culture under pulsatile loading. This response is at least partially mediated by the activity of matrix metalloproteinase-2 (MMP2), as cyclic mechanical strain has been shown to control the production and activity of MMP2 in SMC-populated collagen gels (62). In addition, dynamic stretching has been shown to induce a contractile SMC phenotype (63), as opposed to the synthetic behavior typically observed with SMCs in prolonged primary culture (64). Dynamic mechanical loading might thus lead to greater cell-generated traction forces, thereby increasing the circumferential alignment of cells and collagen in these engineered constructs. The mechanosensory mechanisms that modulate this phenotypic switching are incompletely understood, but a combination of dynamic physical and biochemical factors clearly directs the patterned self-assembly of tissue equivalents (65, 66).

Given their sheer abundance, how might these different physical and biochemical cues be optimized to tune the functional properties of engineered blood vessels? Isenberg & Tranquillo (67) applied cyclic mechanical strains to glycated media equivalents and found that a wide range of parameters—including strain magnitude, strain rate, and relaxation time—influenced the measured modulus and ultimate tensile strength, some in nonintuitive ways. The authors

thus acknowledged that confounding physical factors can complicate our understanding of the remodeling response for a given set of loading conditions.

In general, the behavior of such complex systems often eludes simple intuition, and mathematical models have proven to be useful tools for both interpreting experimental results and suggesting new experiments. Here, in the context of engineered tissues, can they be used to quantitatively guide current experimental efforts to direct the self-assembly of tissue equivalents?

Quantitative Models for the Growth and Remodeling of Engineered Vessels

Since the mid-twentieth century, continuum mechanical approaches have been used to model the biomechanics of soft tissues (68). Typically, these structures undergo large deformations and exhibit nonlinear material properties, so finite elasticity has proven to be a convenient framework for characterizing their mechanical behavior (37, 69). Unlike traditional engineering materials, biological tissues grow and remodel in response to environmental stimuli; that is, their mechanical behavior has both active and passive components. To model this unique capability, engineers have used nonlinear elasticity to construct mathematical frameworks for the growth and remodeling of biological tissues (see sidebar, Theories for Biological Growth and Remodeling) (**Figure 4**).

These growth and remodeling theories have been used extensively to model the mechanics of native blood vessels, especially arteries (37, 70). Typically, the mechanical response of these tissues is assumed to depend on the three primary constituents of the vessel wall: SMCs, collagen, and elastin (71) (**Figure 4*b***). However, most studies have indicated that tissue-engineered blood vessels contain only minimal amounts of elastin (72) and exhibit only limited vasocontractility in the presence of pharmacologic agents (57). Thus, in a first attempt to model the biaxial mechanical behavior of engineered vascular constructs, Dahl and colleagues (73) assumed that the mechanical response was primarily dictated by collagen. They measured the distribution and alignment of collagen fibers in engineered vascular constructs cultured around inflatable silicone tubes (74) and used a microstructurally motivated model to account explicitly for different families of collagen

THEORIES FOR BIOLOGICAL GROWTH AND REMODELING

It has long been acknowledged that biological tissues alter their structure in response to environmental stimuli, such as disease, aging, or physical loading. Using nonlinear elasticity, researchers have developed two theoretical frameworks to model the growth and remodeling of soft tissues: kinematic growth and constrained mixture theory (8). Skalak and colleagues (147) pioneered a theory for kinematic growth, noting that growth is mechanically analogous to thermal expansion (148). These ideas were extended in the theory of finite volumetric growth introduced by Rodriguez et al. (78). Briefly, the overall deformation gradient tensor, F, was decomposed into a growth tensor, G, and an elastic deformation gradient tensor, F^*, by $F = F^* \cdot G$ (**Figure 3*a***) (113). Growth altered the zero-stress state of the tissue, and stress depended only on F^*. Geometrically incompatible growth generated residual stress (149).

Alternatively, Humphrey & Rajagopal (77) introduced a constrained mixture theory for growth and remodeling. Tissues were modeled explicitly as composite materials consisting of several microstructural components (**Figure 3*b***). Each component possessed a unique natural configuration and turnover rate. A theory of mixtures was then used to compute the stress state for each component, and all were assumed to deform together. Residual stress was produced by differences in component turnover rates and natural configurations.

These two theories are not mutually exclusive and have been used in combination to model the mechanics of aortic growth and remodeling (80).

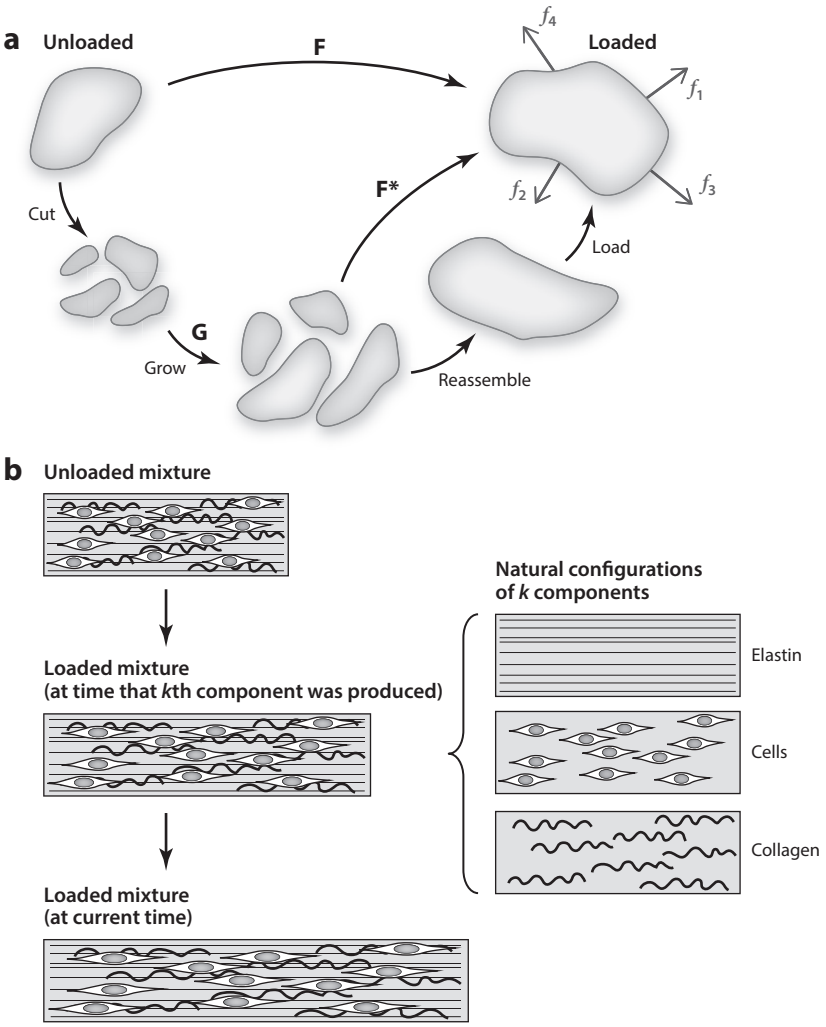

Figure 4

Theoretical models for the growth and remodeling of soft tissues. (*a*) Schematic for kinematic theory of volumetric growth. The overall deformation is decomposed into components representing both growth and elastic deformation. This is expressed mathematically by $\mathbf{F} = \mathbf{F}^* \cdot \mathbf{G}$, where **F** represents the overall deformation gradient tensor, $\mathbf{F}^*$ represents the elastic deformation gradient tensor, and **G** is the growth tensor. Adapted from Taber (113). (*b*) Schematic for constrained mixture theory for biological growth and remodeling. Tissues are treated as composite materials, consisting of *k* components. (Here, the three primary structural constituents of blood vessels are shown: smooth muscle cells, elastin, and collagen). Each component has a unique natural configuration and turnover rate. Based on Humphrey & Rajagopal (77).

fibers (75). By fitting model parameters to data from biaxial mechanical tests, the authors suggested that, although most of the collagen fibers were aligned circumferentially, the few remaining fibers that had aligned either axially or helically contributed more to the overall mechanical behavior of the tissue (i.e., these fibers were stiffer). Intriguingly, the model further suggested that the strain history of the vascular construct during culture strongly affected its mechanical response (73).

Still, this initial study did not address how physical cues might direct the growth and remodeling of the various vessel constituents during culture. Recently, though, Niklason, Humphrey, and colleagues (76) developed a combined experimental and theoretical approach to do exactly this. Using a bioreactor equipped with an imaging window, the authors used two-photon microscopy to image dynamically the SMCs and collagen fibers within cultured vascular constructs. Fourier analysis was then used to compute temporal patterns of collagen fiber alignment, and a constrained mixture model for the growth and remodeling of the engineered tissue (77) was used to predict the evolution of vessel wall constituents, as well as their behavior during biaxial mechanical tests. This integrative approach is extremely promising and, to the best of our knowledge, represents the most comprehensive platform yet developed to investigate the directed self-assembly of engineered blood vessels.

There is compelling evidence that local mechanical stresses modulate the growth and remodeling of intact blood vessels (78–80). It would be interesting to determine if a similar feedback mechanism regulates the formation of engineered vascular constructs. To test this hypothesis, Raykin and colleagues (81) have proposed an axisymmetric, thick-walled model for mechanically mediated growth in engineered vessels. Using a kinematic theory for both growth and plasticity (**Figure 4*a***), the authors tested their model against mechanical data from vascular constructs cultured under dynamic loading (61). Importantly, this framework was able to recapitulate some of the mechanically induced changes in construct properties, such as increases in both the tensile modulus and yield stress, suggesting that mechanical feedback may drive the self-assembly of these engineered tissues (81).

In native blood vessels, growth and remodeling often generate a state of residual stress (82), which can be characterized by measuring the opening angle created by cutting a vessel ring radially (83). Under normal physiological loads, these residual stresses tend to create a more uniform distribution of mechanical stress across the vessel wall, which is a more efficient way for the tissue to bear mechanical loads (82). Intriguingly, preliminary opening angle experiments with engineered vessels have shown that these tissues are also in a state of residual stress (84). Although it is unclear how residual stress varies in both space and time, these stresses are sure to influence the physical microenvironment, and thus vessel growth and remodeling. These promising studies emphasize the need to measure physical forces during the formation of engineered tissues. Engineered tissue equivalents thus offer a unique experimental platform to quantify how cells and ECM bear mechanical loads.

MECHANICS OF ENGINEERED TISSUES

Biological tissues clearly alter their structure in response to mechanical loads (85). Mounting evidence has suggested that cells respond directly to local mechanical cues (e.g., stress, strain, and strain rate) (86), which modulate tissue growth and remodeling (87). At a macroscopic level, most soft biological tissues exhibit nonlinear, viscoelastic material properties (36), but because they are composed of various microstructural components, such as cells and ECM, it is unclear how tissue-level mechanical loads are distributed between each of these different constituents. In cell culture, where the physical microenvironment can be controlled precisely by using micromechanical probes, cells have been shown to respond actively to applied mechanical forces (88, 89). But what forces do cells experience in the more physiological, but less well-characterized, context of engineered tissues?

Because of their relative simplicity compared with native tissues, engineered tissue equivalents have been used to quantify the relative mechanical forces carried by cells and ECM during tissue-level deformations. Zahalak, Elson, and colleagues (90) performed uniaxial mechanical tests with

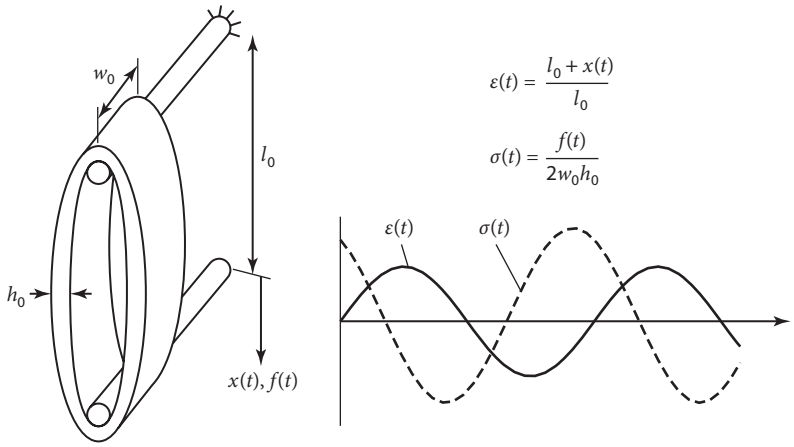

Figure 5

Quantifying the mechanical properties of engineered tissue equivalents. Uniaxial tensile tests can be used to determine the viscoelastic material response of cell-seeded collagen rings. Tissue stress (σ) and strain (ε) are computed as dynamic functions of time.

rings of fibroblast-populated collagen matrices (**Figure 5**) and developed a constitutive model for bioartificial tissues that accounted explicitly for the distinct contributions of both cells and ECM to the overall mechanical behavior (91). Mechanical testing was conducted before and after treatment with cytochalasin D, which disrupts actin filaments, to remove the cellular component of the overall tissue force. Then, by assuming additive stress decomposition, the authors suggested that, during both fast and slow loading, the cells and ECM contributed comparably to the total tissue stiffness. They reported a force of approximately 750 nN for an individual fibroblast. Additional uniaxial mechanical tests were used to further characterize the viscoelastic properties of these fibroblast-populated collagen gels (92, 93) (**Figure 5**). Biaxial mechanical tests, which more closely replicate the multiaxial loading environment experienced by many soft tissues in vivo, were conducted using similar tissue equivalents and demonstrated that cell orientation influences mechanical anisotropy (94, 95).

Other recent studies have shown that cell deformations within tissue equivalents do not match the deformations measured macroscopically during uniaxial mechanical testing—that is, the deformations are nonaffine (96, 97). Similarly, Sander and colleagues (98) have reported heterogeneous, nonaffine deformations in cell-compacted collagen gels, which result from hierarchical interactions within the entangled network of collagen fibers. Importantly, their multiscale simulations predicted compressive forces in certain populations of fibers during equibiaxial tensile tests, where the macroscopic (tissue-level) stresses were purely tensile. These results suggest that microstructural considerations must be taken into account when studying the mechanics of engineered tissues (99), as the physical cues experienced by cells embedded within the network, which direct growth and remodeling, are sure to be influenced by microstructural fiber kinematics.

Still, single isolated cells dispersed within a 3D matrix behave differently than cells grouped together as cohorts—especially cells that reside within an epithelium. Recently, microlithography-based techniques have been used to engineer 3D organotypic epithelial tissues in type-I collagen gels (100). Briefly, elastomeric stamps with lithographically patterned features were used to create arrays of microwells in 3D collagen matrices. The wells were then seeded with epithelial cells, which coalesced to form organotypic tissues of defined geometry. Using this technique, Nelson

et al. (101) showed that tissue geometry specifies branch locations in mammary epithelial tubules. In addition, 3D traction force microscopy has been used to quantify some of the mechanical forces exerted by these micropatterned tissues (102). During tissue culture, cell-generated contractile forces were coordinated across the epithelium, and patterns of mechanical stress were shown to regulate sites of mammary branching (103).

These mechanical studies have helped identify some of the physical mechanisms that drive the growth and remodeling of engineered tissues. Recent experiments with living embryos have suggested that similar physical cues direct tissue formation during development, a process termed morphogenesis.

MECHANICAL CONTROL OF TISSUE ASSEMBLY IN THE EMBRYO

Stem cell–based approaches to tissue engineering have largely focused on creating novel biomaterial scaffolds, which modulate the physical and biochemical signals that instruct stem cell differentiation (23). For instance, because matrix elasticity has been shown to direct the differentiation of mesenchymal stem cells (104), scaffold mechanical properties can be tuned to engineer different tissue types of interest (105). In the embryo, however, this dense signaling milieu occurs within the physical context of tissues that are constantly changing shape: epithelia that fold, stretch, and grow (106). These deformations also have implications for signaling, as far-flung tissues are brought into contact via morphogenetic movements, thereby facilitating inductive interactions between cell populations (107). Tissue assembly in the embryo thus requires the coordination of a mechanical process (the physical shaping of tissues) with a signaling one (the differentiation of organ-specific cell types). However, because physical forces also modulate cell behavior and signaling, this sets up a kind of mechanical feedback that underlies morphogenesis.

Scientists have long been interested in the physical forces that shape the embryo (108, 109). In fact, before the advent of molecular techniques, embryos were largely manipulated physically through cut-and-paste experiments that involved tissue explantation and grafting (110). Thus, up until the mid-twentieth century, much of the language used to describe morphogenesis was decidedly mechanical in nature (110–112). Gathering quantitative mechanical data in embryos is an extremely challenging task (113, 114), but recently investigators have begun using micromechanical probes (115) and mathematical modeling (7) to investigate mechanical feedback during embryogenesis.

In the developing chicken embryo, Taber and colleagues (116–118) have used a combination of theory and experiment to determine the biophysical mechanisms that regulate cardiac c-looping, an important early step in heart development wherein the initially straight heart tube bends and twists to the right side of the embryo. By removing a layer of tissue overlying the heart, the authors altered the external forces driving cardiac torsion and disrupted the looping process (117). However, the altered mechanical loads triggered a contractile response along the right side of the heart tube, which worked to restore the c-looped configuration. A nonlinear finite element model of the looping heart tube, which combined a kinematic theory for growth with a stress-mediated growth law, was able to recapitulate the dynamics of this process, suggesting that mechanical feedback regulates early cardiac development (118). In zebrafish embryos, the fluid forces associated with blood flow have also been shown to regulate cardiac morphogenesis (119), indicating another instance of mechanical feedback between function and form in the developing heart. Along similar lines, Wagenseil (120) constructed a constrained mixture model to simulate growth and remodeling in the developing mouse aorta. (As discussed previously, this framework was also used to characterize the remodeling response of both native and engineered blood vessels.) The model was able to predict the measured mechanical properties of the developing aorta, but

only if the natural configurations for each constituent were free to evolve in time—a reasonable assumption in the highly dynamic environment of developing tissues.

Mechanical feedback has also been implicated during airway branching in the developing lung (121). Initially, the airway lumen is filled with fluid. Both fetal breathing movements and peristaltic smooth-muscle contractions move fluid through the branched network of epithelial tubes (122), locally modulating both luminal pressure and fluid shear stress. Disruptions in fluid flow caused by paralyzed peristalsis (123) or inhibited fetal breathing movements (124) can lead to hypoplastic branching, indicating a role for mechanical feedback in branching morphogenesis of the lung (121). We next consider how these mechanisms of mechanical feedback in the embryo might be harnessed to direct the self-assembly of tissues engineered in the lab.

DIRECTED SELF-ASSEMBLY OF ENGINEERED TISSUES

The physical microenvironment has been shown to regulate cell proliferation (125), differentiation (104, 126, 127), epithelial-mesenchymal transition (128, 129), and apoptosis (130) in 2D culture. How can our understanding of this tissue remodeling be used to direct the construction of the engineered tissues?

In a classic set of experiments, intermixed cell populations were shown to spontaneously form segregated aggregates in culture, with one phase of cells enveloping the other (131). This physical sorting mechanism is thought to be driven by a combination of differential adhesion and differential contractility (132, 133) and has been shown to underlie germ-layer organization in the developing zebrafish embryo (134). By exploiting this phenomenon, a bioengineered tooth germ has been constructed in the laboratory (135). Nakao and colleagues (135) suspended isolated developing tooth epithelial and mesenchymal cells in 3D collagen gels. The suspensions spontaneously formed aggregates, with the epithelial cells enveloping the mesenchyme, thus recapitulating the epithelial-mesenchymal interactions in vivo. When implanted into alveolar bone, the aggregate formed a functional tooth replacement (136). Subsequent work by Mammoto and colleagues (137) has suggested that mechanical feedback between these two layers drives tooth specification. Physical compaction of the mesenchymal cells suppresses RhoA expression and was shown to be sufficient for odontogenic differentiation. Because many other developing organs arise as a consequence of epithelial-mesenchymal interactions (138), it would be interesting to determine whether similar biophysical mechanisms can be co-opted to direct the self-assembly of other engineered organ rudiments. To aid in this process, Khademhosseini and colleagues (139) have developed a method to assemble cell-laden, lock-and-key-shaped hydrogels in predetermined geometrical configurations, which would circumvent the need to rely on physical sorting mechanisms to set up the epithelial-mesenchymal interactions in culture.

Other recent work has demonstrated a capacity for other organ rudiments to self-assemble in 3D culture (140). Remarkably, Eiraku and colleagues (141) recapitulated 3D optic-cup morphogenesis in cultured stem cell aggregates. Biophysical analysis of the self-assembly suggested that the authors were able to reproduce some of the physical forces that drive this process in the embryo (142). In addition, Sato and colleagues (143, 144) demonstrated the spontaneous formation of intestinal crypts from stem cell aggregates embedded in 3D gels of reconstituted basement membrane protein. In the developing embryo, villus formation has been shown to occur via a physical instability, which arises as a consequence of mechanical constraints between adjacent tissue layers (145). It is unclear if a similar physical mechanism underlies crypt formation in 3D culture. It would be interesting to determine if theories for the growth and remodeling of soft tissues can be used to predict the directed self-assembly of these engineered tissue rudiments.

CONCLUSIONS

Tissue morphogenesis is a complex process that spans multiple time- and length-scales. Recent studies investigating tissue assembly—both in the embryo and in culture—have made important advances, but we are still unable to construct replacement tissues that mimic the functional properties of their native counterparts. There is a clear need for new quantitative models that can be used to guide current experimental approaches to tissue engineering. However, this progress will require collaboration between theorists and experimentalists, working in concert to determine the physical and biochemical cues that direct tissue self-assembly.

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141. This paper demonstrates that cultured stem cell aggregates can spontaneously recapitulate optic cup morphogenesis in 3D culture.