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Advanced Material Strategies for Tissue Engineering Scaffolds

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Abstract

Tissue engineering seeks to restore the function of diseased or damaged tissues through the use of cells and biomaterial scaffolds. It is now apparent that the next generation of functional tissue replacements will require advanced material strategies to achieve many of the important requirements for long-term success. Here we provide representative examples of engineered skeletal and myocardial tissue constructs in which scaffolds were explicitly designed to match native tissue mechanical properties as well as to promote cell alignment. We discuss recent progress in microfluidic devices that can potentially serve as tissue engineering scaffolds, since mass transport via microvascular-like structures will be essential in the development of tissue engineered constructs on the length scale of native tissues. Given the rapid evolution of the field of tissue engineering, it is important to consider the use of advanced materials in light of the emerging role of genetics, growth factors, bioreactors, and other technologies.

Keywords

Cartilage repair; heart repair; biomimetic; microfabrication

1. Introduction

Within the context of many tissues and organ systems, including the skeletal and cardiovascular systems, function relies on transmission or generation of mechanical forces and maintenance of blood circulation. Tissues such as articular cartilage and myocardium

possess highly specialized structures and compositions that provide unique mechanical and transport properties. The loss of function of these tissues with injury, disease, or aging accounts for a significant number of clinical disorders at a tremendous social and economic cost.[1,2] Tissue engineering seeks to restore the function of diseased or damaged tissues and organs through the use of cells and biomaterials. However, there are few engineered tissue products available for clinical use. Many grafts fail because physiologic loading leads to the breakdown of repair tissues or because transport limitations lead to death of the implanted cells.

The magnitudes of mechanical stresses that tissues may be subjected to *in vivo* can be quite large, and few engineered tissue constructs possess the properties to withstand such stresses at the time of implantation. Also, the challenge is not as simple as matching a single mechanical parameter, such as modulus or strength; rather, most tissues possess complex viscoelastic, nonlinear, and anisotropic mechanical properties that may vary with age, site, and other factors.[3] The long-term maintenance of cell phenotype in engineered constructs may require the delivery of multiple factors in a sustained and locally prescribed manner. Furthermore, convective transport of oxygen, nutrients, and waste products is required for cell and tissue survival, but most tissue engineered constructs do not possess a microvascular network at the time of implantation. Without microvasculature, oxygen transfer becomes critically inadequate due to its low solubility in aqueous media and limited diffusional penetration of 100–200 μm in native tissues.[4,5]

In this Research News article, we provide representative examples in which advanced material strategies were exploited to improve the cellular and mechanical function of tissue-engineered cartilage and myocardium. In addition, we discuss microfluidic devices of relevance to these and other tissues and organs that serve some mechanical function (e.g., muscle, tendon, ligament, bone, blood vessels, heart valves, bladder etc.) and are targets of “functional tissue engineering” research efforts.[6]

2. Scaffolds for engineering skeletal tissues

Articular cartilage possesses unique mechanical and tribological properties to withstand the demands of repetitive joint loading. Under normal conditions, this tissue exhibits little or no wear, with millions of cycles of loading that may exceed 10 times body weight. Its unique properties have been attributed to the complex structure and composition of its extracellular matrix,[7] which possesses mechanical properties that are anisotropic, nonlinear, inhomogeneous, and viscoelastic. The primary mechanism of viscoelasticity in cartilage arises from frictional interactions between the solid and fluid phases as the interstitial water moves through the porous extracellular matrix due to gradients in pressure.[8] The mechanical properties of the extracellular matrix are nonlinear, exhibiting characteristics such as strain-dependent moduli and permeability[9,10] as well as approximately two orders of magnitude difference in tensile and compressive moduli.[11] In particular, this tension-compression nonlinearity serves an important biomechanical function by enhancing fluid load support in the tissue during physiologic compressive loading.[12] Both the tensile and compressive properties are anisotropic and vary significantly with depth from the cartilage surface.[11] The presence of “fixed” negative charge in the extracellular matrix due to the high concentration of aggrecan and other proteoglycans results in internal swelling pressures that give rise to inhomogeneous residual stresses within normal articular cartilage.[13] Additionally, these material properties, coupled with the complex geometry of the diarthrodial joint, provide for extremely low friction properties that result in minimal tissue wear, even under high physiologic loading conditions.[14]

While the design parameters that will ultimately lead to long-term restoration of cartilage function remain to be determined,[15] novel scaffold designs have attempted to mimic certain aspects of these properties by using composite materials (i.e., combinations of dissimilar materials), such as fiber reinforcement or layered structures.[16] By embedding fibers within bulk matrix materials, composite scaffolds for cartilage tissue engineering have been fabricated with prescribed physical and mechanical properties that cannot be achieved with a single, homogeneous material. These designs have typically involved the use of organized textile structures such as non-woven, knitted, and three dimensional (3-D) woven fabrics (Figure 1) to reinforce a cell-supporting matrix (e.g., agarose, alginate, or fibrin). In this scaffold configuration, the matrix phase serves to support the delivery, proliferation, and differentiation of embedded cells while the fiber phase determines its mechanical characteristics.

For example, Marijnissen et al. seeded non-woven poly(lactic-co-glycolic acid) (PLGA) meshes either directly with bovine chondrocytes, or with chondrocytes suspended in alginate, to test the effects of using a hydrogel as a cell carrier on chondrogenesis.[17] After 8 weeks of subcutaneous implantation in nude mice, the presence of the alginate cell-carrier increased seeding efficiency by retaining and uniformly dispersing cells throughout the pores of the non-woven mesh. It was also noted that this combination of materials yielded a physically robust construct that maintained its initial geometry over time, without a negative effect on extracellular matrix protein synthesis. Similar results have been reported for non-woven poly(glycolic acid) (PGA) meshes used in combination with chondrocyte-laden fibrin gel[18–20] or PGA meshes used with mesenchymal stem cells suspended in type I collagen and alginate gel.[21]

As with hydrogels, the mechanical properties of commonly used foams and sponge-like scaffolds can also be enhanced through fiber reinforcement. Chen et al.[22] demonstrated this concept by developing a scaffold consisting of a web-like collagen microsphere formed upon a knitted PLGA fabric. This study reported that the knitted fabric in this two-material approach provided the scaffold with mechanical integrity, while the collagen microsphere filled the large pores of the fabric, thereby facilitating uniform cell distribution and cartilage-like tissue formation. Similarly, reinforced PLGA foam scaffolds were produced by Mooney and colleagues[23] by embedding short PGA fibers into the bulk polymer prior to foaming. It was later demonstrated that the mechanical properties of these scaffolds could be tailored for potential use in articular cartilage repair by adjusting their relative material composition.[24] The researchers concluded that fiber reinforcement is a controllable design variable that can be manipulated when engineering scaffolds to suit specific load-bearing applications.

Recent work has sought to replicate the complex biomechanical behavior of native articular cartilage by using fibers and hydrogels known to support chondrogenesis.[25] The basis of this work was a microscale 3-D weaving technique that arranges multiple layers of continuous fibers in three orthogonal directions (Figure 1). Composite scaffolds comprised of a 3-D weave of PGA used in combination with fibrin gel were shown to recreate the physical and structural properties of native articular cartilage; specifically, their inhomogeneous, anisotropic, nonlinear, and viscoelastic mechanical properties.[25] Moreover, a 3-D weave of poly(ϵ -caprolactone) (PCL) and Matrigel^R was shown to support the generation of functional, cartilaginous constructs from human adult mesenchymal stem cells in only 3 weeks *in vitro*. [26] A unique advantage of this composite structure is that scaffolds can be designed and built with predetermined control of site-dependent variations in mechanical properties and porosity within a biocompatible matrix.

Articular cartilage possesses a complex, zonal architecture that varies in composition to allow for smooth articulation at the joint surface and rigid attachment at the subchondral bone interface. While this structural inhomogeneity within the tissue is well recognized, its function is not well understood. Nonetheless, many groups have attempted to recreate this hierarchy by layering various biomaterials to form a stratified scaffold that may improve tissue regeneration and implantation. In an attempt to mimic the depth-dependent material properties of native cartilage, Ng et al. employed a layer-by-layer casting method to form a chondrocyte-laden, stacked hydrogel system consisting of 2% agarose as a top layer and 3% agarose as a bottom layer.[27] Immediately after being formed, compression testing revealed a significantly higher Young's modulus in the bottom layer of the construct than in the top layer. A similar layered approach showed that histological characteristics similar to those of native cartilage could be recreated using chondrocytes encapsulated in photopolymerizing poly(ethylene glycol) diacrylate (PEGDA) hydrogel.[28]

Previous work suggests that tissue engineered cartilage constructs pose a significant challenge with regard to variable and incomplete integration potential.[29–33] Since bone-to-bone interfaces are known to integrate better and faster than cartilage-to-bone interfaces, tissue-engineered osteochondral constructs are widely regarded as one of the most promising techniques for repairing full-thickness articular cartilage defects. Thus, numerous attempts have been made to develop cartilage-bone bilayered constructs using multi-material strategies. In one such study, an anatomically-shaped osteochondral construct was developed by casting a layer of chondrocyte-seeded agarose gel on top of devitalized trabecular bone.[34] Other approaches have included: engineered cartilage grown on nonwoven PGA sutured to a subchondral support made of blended PLGA-PEG or Collagraft^R,[35,36] an esterified hyaluronan sponge (HYAFF^R11) for cartilage regeneration attached with fibrin glue to a calcium phosphate ceramic sponge for bone regeneration,[37] differentially applied chondrogenic and osteogenic growth factors on mesenchymal stem cells seeded in stacked silk scaffolds,[38] a 3-D printed scaffold of mixed PLGA/PLA for the upper cartilage region with a PLGA/tricalcium phosphate mix for the lower bone region, [39] a collagen gel for chondrogenesis cultured upon a bone-inducing hydroxyapatite base, [40] and a cartilaginous tissue layer overlying a porous biodegradable calcium polyphosphate substrate [41].

3. Scaffolds for engineering myocardial patches

Never resting, cardiac muscle requires robust microvascular and mechanical properties to contract continuously and efficiently while resisting fatigue over the three hundred million cardiac cycles of an average human lifespan. From a materials perspective, these demands are met via a unique ensemble of cellular and extracellular matrix structures. Cardiac muscle fibers are highly branched, with implications for stability during contraction,[42] and are embedded in a hierarchically structured, 3-D collagen network, comprising distinct endomysial,[43] perimysial,[44] and epimysial levels of organization. It is therefore not surprising that efforts to tissue engineer cardiac muscle have been challenging. Nevertheless, with ~770,000 new myocardial infarctions per year in the USA,[2] the clinical need remains without question. Progress toward a tissue engineered “myocardial patch” has been made on several fronts, including cardiogenic stem cells[45,46] and the design of myocardial-specific scaffold materials.

As reviewed by Eschenhagen and Zimmermann,[47] a variety of scaffolds have been tested, including natural and synthetic polymer-based materials; “scaffold-less” approaches are also under investigation.[48,49] Few scaffolds, however, have been explicitly designed with myocardium in mind. More often, off-the-shelf materials are selected *ad hoc* and tested for heart cell compatibility. Nevertheless, significant advances have thereby been made:

Zimmermann et al.[50] demonstrated improved left ventricular function after myocardial infarction in rodents after implanting heart cells embedded in a mixture of type I collagen gel and Matrigel®. However, as attention turns towards clinical applicability, limitations intrinsic to previous scaffolds are becoming more apparent (e.g., mechanical weakness of collagen gels[51] and foams,[52] random microstructures in foams made of collagen[53] and some synthetic polymers,[54] and random microstructures and/or excessive stiffness of other synthetic polymers[55,56]). Advanced material strategies for myocardial tissue engineering are thus under development, with an emphasis on mimicking native structural, mechanical, and transport properties.

In developing a myocardial-mimetic scaffold, Ott et al.[57] began with the native tissue itself. The researchers rationalized that decellularized hearts could serve as platforms for repopulation with heart cells. Moreover, they hypothesized that the coronary-derived microvascular network could be used to oxygenate and nourish the repopulated cells, potentially overcoming previous transport limitations.[58,59] To test these hypotheses, scaffolds were prepared by decellularizing adult rat hearts with 1% sodium dodecyl sulfate (SDS). Antegrade SDS perfusion largely preserved the myocardial extracellular matrix structure and blood vessels. Upon repopulation with neonatal rat heart cells, spontaneous macroscopic contractility was demonstrated both in isolated hoops of tissue and, to a greater degree, in perfused whole hearts. Upon perfusion and electrical stimulation, nascent pump function was also restored, with repopulated hearts generating ~2.4 mm Hg of pressure (~2% of adult rat heart).

One potential limitation of decellularized heart scaffolds is their relatively high mechanical stiffness, which exceeded that of native heart by 17- and 29-fold in the circumferential and longitudinal directions, respectively.[57] Increases in scaffold stiffness were likely due to extracellular matrix compaction during decellularization, based on an associated ~93% decrease in ventricular thickness. Also possible are alterations in collagen fibril structure due to SDS-mediated denaturation[60] and/or disruption of protein-protein interactions.[61] A second potential limitation concerns cellular repopulation of the scaffolds, which was achieved by relatively inefficient and spatially non-uniform intramural injections. Hence, it remains to be seen whether this approach is ultimately scalable to human hearts. Regardless, Ott et al.[57] have inspired a new appreciation for native extracellular matrix microstructures, and new approaches to rendering these microstructures in synthetic polymers.

Recent work has sought to replicate the complex mechanical properties of native myocardium by microfabricating an accordion-like honeycomb scaffold.[62] From the hexagonal networks of wax built by bees to the intricate trabeculations of bone, honeycomb-like materials abound in nature, presumably evolved to balance mechanical properties to weight.[63] In the recent work,[62] a unique honeycomb motif was developed, with undulated structural elements circumscribing accordion-shaped pores (Figure 2a). Several honeycomb-like structures manifest in native myocardium, including the collagenous endomysial sheaths cradling and interconnecting individual cardiac muscle fibers[43] and the network of undulated perimysial collagen fibers functionally associated with limiting sarcomere extension.[64] Indeed, it was the undulated shape of the perimysial collagen fibers that inspired the accordion-like honeycomb scaffold design, albeit rendered on a length scale of 50–250 μm versus the 1–10 μm length scale of the perimysial collagen.

Functionally, the accordion-like honeycomb scaffold was designed to guide parallel heart cell alignment (as fibroblasts align in diamond-shaped pores[65]), and to impart anisotropic mechanical properties like those of native myocardium. These two milestones were largely demonstrated. Preferential heart cell alignment was observed after 1 week of culture (Figure

2b) and mechanical properties similar to native rat right ventricular myocardium were achieved after optimization of polymer curing time. Importantly, anisotropic mechanical properties were largely retained following 1 week of culture with neonatal rat heart cells, or following 1 week of *in vitro* cyclic fatigue mimicking the dynamic, physiologic epicardial strains. Construct mechanical properties were attributable not only to the unique accordion-like honeycomb microstructure, but also to the material of scaffold construction, poly(glycerol sebacate) (PGS).[66] Developed by Wang et al., PGS is a bioresorbable, thermoset elastomer that degrades primarily by surface hydrolysis over 4–6 weeks *in vivo*. [67,68] As a salt-leached foam,[69] PGS was recently implanted as an acellular, epicardial patch in a preliminary rodent study.[54] *Ex vivo*, particular success was achieved when PGS foams with laser-perforated flow channels were seeded with neonatal rat heart cells and perfused with culture medium supplemented with perfluorocarbons to increase oxygen carrying capacity.[70]

One potential limitation of the PGS foam scaffold for myocardial tissue engineering is its mechanical weakness under tensile loading. A second disadvantage is the effectively isotropic microstructure of PGS foam which, like collagen foam, yields essentially random orientations of cultured heart cells in the absence of exogenous physical stimuli such as cyclic stretch[52] or electrical field stimulation.[71] However, an advantage of PGS foam is its compliance, which allowed for macroscopic contractions in the resulting engineered myocardium.[54] In contrast, heart cells cultured on accordion-like honeycomb scaffolds contracted in synchrony, but in a manner that was largely isometric under the conditions tested.[62] Conceptually, the accordion-like honeycomb scaffold could yield contractile properties similar to those of foam. Consider that it is in part the undulated shape of the perimysial collagen fibers which lend myocardium its requisite compliance.[72] Ongoing studies aim to improve the accordion-like honeycomb scaffold contractile properties, as well as to generate thicker, multi-laminar honeycomb scaffolds.

4. Scaffolds for engineering thick, vascularized tissue

Most tissues of the body, other than cartilage and cornea, are vascularized and as such require an exquisite microvascular network for transport of oxygen, nutrients, and waste products to sustain the viability of their component cells. Likewise, engineering of tissue constructs on the length scale of native tissues will require microvascular-like networks to sustain living cells. Recently, biodegradable microfluidic devices have been designed and constructed with an overall objective of integrating mass transport and drug delivery capability to cultured cells in tissue engineered constructs. Conceptually, microfluidic devices can be fabricated using materials selected for their mechanical and degradative properties and modified by the addition of surface moieties to enhance cell attachment and function. Progress has been reported in culturing vascular [73,74], liver [75–79] and kidney[80] cells.

The design of scaffold microvascular networks is driven by native tissue requirements for oxygen and, depending upon the specific application, consideration of the role of fluid mechanical forces and fluid transport functions. Analysis of the microcirculation in a wide range of animal tissues provided a basis for designing devices,[81] and determined that specific scaling laws apply to the diameter of blood vessels and the length between bifurcations. These principles have been used to design networks that mimic physiologic blood velocities, pressure drops, hematocrit distributions and shear stresses present in native tissues.[82] One of the most important aspects of these designs is proper replication of the shear stress environment within vessels, which is required in order to generate appropriate mechanotransductive signaling for endothelialization (i.e., lining of interior surfaces of these microfluidic channels with endothelial cells).

Early work in microfluidic devices[73] was carried out using poly(dimethyl siloxane) (PDMS), a non-degradable but generally biocompatible substrate. This transparent, elastomeric polymer is useful because of its ease of processing and device assembly, low cost, and high oxygen permeability. However, PDMS also has several drawbacks, including unstable surface chemistry and a tendency to absorb molecular contaminants. Microfluidic devices have also been fabricated using a wide range of biodegradable substrates including PCL,[83], PLGA,[84,85] and silk fibroin [78]. However, drawbacks of these “off-the-shelf” biomaterials are becoming increasingly apparent, including rigid mechanical properties (PLGA), difficulties in microfluidic device assembly (PCL, silk), very slow degradation (PCL, silk), and an associated inflammatory response upon *in vivo* implantation (PLGA). These limitations have spurred the design of new synthetic polymers such as the aforementioned PGS, an elastomeric material that is relatively easy to process and degrades over 4–6 weeks while eliciting only minimal inflammation *in vivo*. [67,68] Endothelialization[74] and hepatocyte carcinoma cell culture[77] within the microchannels of PGS-based microfluidic devices have been demonstrated. Other biodegradable elastomers currently under investigation include poly(ester amide)[86], in which tunable ratios of ester and amide bonds provide great flexibility in degradation rate and mechanical and surface chemical properties, and poly(polyol sebacate),[87] in which the type of polyol monomer and the stoichiometric ratio of sebacic acid determine material properties. Moreover, microfluidic devices have been made of hydrogels, such as calcium alginate,[88] wherein mass transport is provided both by molecular permeation of the bulk hydrogel and convective diffusion through an embedded microchannel network.

Fabrication technologies used to produce microfluidic devices are typically based upon casting or molding from high precision lithographic silicon master molds; recently other methods such as excimer laser microablation[62] have also emerged. Elastomeric materials such as PDMS and PGS can be de-molded directly from rigid silicon master molds, whereas for rigid materials such as PLGA the replica molding process requires an intermediate transfer molding step using a compliant material such as PDMS. Microfluidic devices are typically formed by molding a thin film (on the order of 100–200 μm in total thickness) of the selected material against a silicon master containing a network of ridges comprising the inverse of a bifurcated microchannel network (Figure 3A). Conventional lithographic processes result in rectangular structures on the silicon master, and therefore the polymer microchannels have vertical sidewalls, sharp corners, and sudden transitions at vessel diameter changes and branching points. Such features can be undesirable for microvascular networks for blood transport because they generate non-uniform flow and dead zones that can lead to coagulation. Several reports suggest that lithographic[89] and etching[90] processes can be tuned to provide rounded microchannels to enable a more physiologic flow of blood in these networks.

The goal of 3-D assembly of a microfluidic device is to seal the microfabricated layers together without the use of adhesives or foreign materials. Sealing can be done by adhering a semi-cured film to a cured film, while ensuring that channels and other precision structures are not substantially deformed, or by applying a thin film of liquid (e.g., non-cured) material between solid (e.g., cured) films.[77] Compression molded membranes such as PLGA can be bonded into 3-D devices by melting the layers using precise control over the time-temperature excursions to prevent deformation of microstructures.[85] For *in vitro* testing, device perfusion can be achieved by fluidic connections linking the microchannel networks to an external pump, e.g. by attaching inlet and outlet tubes.[91] Ultimately, for *in vivo* applications, device perfusion can potentially be achieved by integrating these inlet and outlet conduits to the host blood vessels.

In one representative microfluidic device constructed using a stack of laminated sheets of PLGA,[85] each layer was comprised of a planar, bifurcated microchannel network, and the channels within subsequent layers were interconnected using vertical through-holes (Figure 3B). In this microfluidic device the microchannels ranged in diameter from a maximum diameter of approximately 500 μm to a minimum diameter of approximately 20 μm . Perfusion was readily demonstrated by using fluorescent dyes and tracer particles (Figure 3B,C). Other prototype devices have been constructed using up to 35 individual layers of PDMS, each on the order of $100 \times 100 \times 0.2$ mm in size, that were stacked and sealed together to form an integrated device with one inlet and one outlet.[92]

Parameters for microfluidic device perfusion are highly dependent upon structural and mechanical properties, and on the type of cultured cell. For microchannel endothelialization, [73,74] fluid flow rates are initially very low ($< 1 \mu\text{L}/\text{min}$) to enable cell adhesion, and then increased by one or two orders of magnitude ($10\text{--}100 \mu\text{L}/\text{min}$) to approximately match shear rates present in native microvasculature; dynamic, pulsatile flow may also be desired. In contrast, low flow rates are required for the culture of hepatocytes inside the channels of such a device to avoid cell damage caused by hemodynamic shear forces.[76] Ongoing studies of microfluidic devices for tissue engineering include scale-up as well as functionalization of interior surfaces of the microchannels to enable higher flow rates while also maintaining cell attachment and spreading.

Recent work[93] has focused on the design and fabrication of multi-component microfluidic devices in which distinct compartments for fluid flow and for cell culture co-exist on either side of a thin membrane. A representative prototype, in which convective diffusive transport between “intravascular” and “extravascular” compartments occurs through a 10 μm thick, non-degradable porous membrane, is shown in Figure 3D. Ongoing efforts to improve multi-component devices for tissue engineering include the incorporation of a robust biodegradable membrane instead of currently used non-degradable membranes, and the design and microfabrication of fully integrated intravascular and extravascular spaces.

5. Outlook on advanced material strategies for tissue engineering scaffolds

In this section, we integrate the breadth of scaffold design concepts introduced herein with a particular emphasis on bridging the remaining gaps between synthetic and naturally-derived scaffold structures. We envision that future generations of tissue engineering scaffolds will provide tissue-like mechanical properties as well as micro-to-nanoscale structural features capable of modulating tissue regeneration at the cell level. Although scaffolds with relatively large ($\sim 100 \mu\text{m}$) anisotropic pore structures have been demonstrated to guide the bulk alignment of cells and collagen in engineered tissues,[62,65] the induction of smaller (~ 100 nm to $10 \mu\text{m}$) collagenous microstructures mimicking the exquisite architecture of native collagen networks can reasonably be expected to yield more functional engineered tissues.

For example, in native adult articular cartilage, the collagen fibers in the superficial-most zone of the tissue are aligned parallel to the tissue surface, whereas in the deeper zones the collagen fibers are aligned perpendicular to the surface (i.e., in parallel to the direction of compressive loading), as elegantly demonstrated by Rieppo et al.[94](Figure 4). This collagen architecture results in tension-compression nonlinearity of cartilage tissue that varies with depth from the surface. As a second example, in native adult myocardium, the perimysial collagen is aligned in parallel to the aligned cardiac myocytes from the epicardial surface through to the endocardium, in a curved transmural cutting plane defined normal to the local fiber orientation at every point, as elegantly demonstrated by Pope et al.[44](Figure 5). This collagen architecture maintains the spatial registration of heart cells to enable

cardiomyocyte contraction during systole while also protecting the cells from over-extension during diastole, thereby contributing to the robust, elastomeric material properties required for cardiac pump function.[44]

One possible approach to guiding formation of collagen-scale structural features is to apply electrospun layers onto both the top and bottom surfaces of scaffolds with relatively large microfabricated pores. While the small pore sizes of electrospun scaffolds can pose a barrier to cellular in-growth and mass transport, these limitations might be overcome by integration of the aforementioned microfluidic networks. Electrospun nanofibrous meshes of bioresorbable synthetic polymers have been described and applied previously to myocardial tissue engineering.[95–97] In the context of the PGS used to construct accordion-like honeycomb scaffolds and microfluidic devices, electrospinning was recently demonstrated by a core/shell method.[98]

A second possible approach is to induce directional fibrillogenesis from nucleation points within microfabricated scaffolds. It was recently demonstrated[99] that microfluidic networks constructed from collagen-doped alginate could initiate nucleation and directional growth of collagen fibers in perfused collagen solutions. Thus, cell cultivation on microfabricated scaffolds doped with collagen, such as in the form of a surface interpenetrating network, might similarly initiate nucleation and directional growth of cell-secreted collagen fibers. In another possible approach, interpenetrating networks of collagen within microfabricated scaffolds could be achieved by photopolymerization of collagen mixed with the acrylated version of PGS, i.e., poly(glycerol sebacate acrylate)(PGSA).[100] Alternatively, scaffolds could be directly microfabricated from collagen-doped alginate, which has been demonstrated to support heart cell cultivation.[101]

6. Conclusions

The past two decades have witnessed a remarkable progression in the development of tissue engineering scaffolds that can be attributed in large measure to novel advanced materials. Whereas early cartilage tissue engineering scaffolds were too weak to provide weight-bearing function without a prolonged period of tissue culture,[102] and early cardiac tissue engineering scaffolds were too weak to support physiologic cardiac function,[51] scaffold mechanical properties can now be explicitly designed to match native tissues. Whereas early scaffolds were amorphous materials without specific provisions for mass transport,[103,104] we envision that new scaffold materials and structures will be critical for the future success of engineered tissue replacements. In one approach, the design requirements of this next generation of scaffolds will include seamless integration of thin-walled, bifurcating microchannels lined with endothelial cells with extravascular compartments containing other cell types, collagen-like networks, and systems for controlled release of angiogenic or other growth factors to form a pre-defined template for tissue vasculature.

The examples described herein represent only a few of the myriad ways in which disparate fabrication techniques might synergize in the context of advanced scaffold designs for cartilage or myocardial tissue engineering. Similar concepts could be invoked in mimicking the structure of other tissues or organs using synthetic polymeric biomaterials or native tissues as scaffolds. Within the context of tissue engineering and regenerative medicine, there continues to be a clear and present need to develop advanced materials design and processing methods that can better replicate the exquisite architecture and functional properties of native tissues. Importantly, other new and rapidly evolving technologies also continue to significantly impact the role of materials in tissue engineering, and the development of new materials and structures will be influenced by advances in stem cells, RNA/DNA-based technologies, bioreactors, gene therapy, and other emerging technologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Anisotropic three-dimensional woven scaffold for cartilage tissue engineering. (A) Scanning electron micrograph; (B,C) Safranin-O stained histological section of scaffold with cultured bovine calf articular chondrocytes. Cells are round with black nuclei and are embedded in a red-orange extracellular matrix; scaffold is colored lighter pink. Scale bars: 1 mm (A), 500 μm (B), and 50 μm (C). Adapted with permission from [25] Copyright 2007, Nature Publishing Group (A), and from [105] Copyright 2006, Mary Ann Liebert, Inc. (B)

Figure 2. Accordion-like honeycomb scaffold for myocardial tissue engineering. (A) Scanning electron micrograph. (B) Confocal micrograph of the scaffold with cultured rat heart cells. Cells and filamentous actin are colored green, cell nuclei are colored blue, and the scaffold is colored blue. Scale bars 200 μm . Adapted with permission from [62] Copyright 2008, Nature Publishing Group.

Figure 3.

Microfluidic device design and fabrication. (A) Silicon micromachined master used for compression molding. (B,C) Microfluidic devices constructed using a stack of laminated sheets, each layer comprising a planar bifurcated microchannel network and connected to adjacent layers using vertical through-holes (not shown). The fluidic structures are intentionally offset to illustrate the stacking process. Perfusion is demonstrated by fluorescein dye (B) and tracer particles (C). (D) Multi-compartment device comprised of intravascular (*) and extravascular (**) spaces and a porous membrane positioned between these two compartments (arrow). Scale bars: 500 μm (A,B) and 100 μm (C,D). Adapted from [85] Copyright 2004, Wiley-VCH (A,B).

Figure 4. Collagen organization in relation to the articular surface of native cartilage, as shown in a transverse section by polarized light microscopy. Reproduced with permission from [94]. Copyright 2008, Elsevier.

Figure 5.

Perimysial collagen organization in relation to opposing surfaces of the myocardium, as shown in a curved, transmural plane (A). Collagen can be seen forming long, intra-laminar cords (B and E), spanning the cleavage planes separating adjacent layers (D), and fusing into tendonous structures near the edges of the laminae (C). Reproduced with permission from [44]. Copyright 2008, The American Physiological Society.