

Synthesis and microfabrication of biomaterials for soft-tissue engineering*

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Abstract: Biomaterials synthesis and scaffold fabrication will play an increasingly important role in the design of systems for regenerative medicine and tissue engineering. These rapidly growing fields are converging as scaffold design must begin to incorporate multidisciplinary aspects in order to effectively organize cell-seeded constructs into functional tissue. This review article examines the use of synthetic biomaterials and fabrication strategies across length scales with the ultimate goal of guiding cell function and directing tissue formation. This discussion is parsed into three subsections: (1) biomaterials synthesis, including elastomers and gels; (2) synthetic micro- and nanostructures for engineering the cell–biomaterial interface; and (3) complex biomaterials systems design for controlling aspects of the cellular microenvironment.

Keywords: biomaterials; microfabrication; nanostructures; polymers; tissue engineering.

INTRODUCTION

The promise of tissue engineering

The field of tissue engineering, more broadly termed regenerative medicine, is an interdisciplinary effort that combines aspects of medicine, engineering, biology, and chemistry to reduce mortality and morbidity associated with the loss of healthy organs. The traditional strategy to creating engineered tissues begins with the incorporation of cells with a scaffold fabricated from resorbable materials [1]. Matrices constructed out of natural or synthetic materials act as immunoisolation barriers and provide structural support to allow cell proliferation. The ultimate goal is for the cells to integrate with the host, remodel the scaffold, and ultimately form functional tissue. The promise of tissue engineering has been elevated along with recent advancements in cell biology, genetics, and biotechnology. Human embryonic stem cells (hESCs), induced pluripotent stem cells [2], patient-specific cells lines, gene therapy, and small interfering RNA (siRNA) are recent examples of technologies that could potentially empower and assist in realizing the full potential of tissue engineering [3]. Advances in basic science have also led to nascent discoveries of the geno- and phenotypic effects of the cellular microenvironment on tissue development and organ formation. The combination of powerful new biological tools and expanding knowledge of basic science suggests that the ability to direct cell fate through controlling cell–biomaterial interactions is becoming increasingly important in the field of tissue engineering.

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The traditional role of polymeric biomaterials

Polymeric biomaterials synthesis and development has played a key role in the advancement of biomedical technologies, including drug delivery and tissue engineering. Initial developments in organic biomaterials focused on satisfying several criteria, including: (1) nontoxic monomers; (2) application-specific polymer chemistry; and (3) optimal biocompatibility. The starting point for biomaterials development often relied upon employing traditional engineering materials for applications that interfaced directly with biological systems. For example, polyethylene, polytetrafluoroethylene, and silicone-based polymers have been used in medical implants for a wide variety of applications. In general, these polymers are designed to be biologically inert to minimize negative consequences of biomaterial–tissue interactions [4]. More recent strategies have embraced the notion of inevitable biomaterial–tissue interactions by designing biomaterials to integrate favorably with host tissue as opposed to minimizing the potentially deleterious effects of such interactions. This is an important step as the perceived potential role of biomaterials and implants transformed from devices that were essentially passive into active systems that could dictate specific biological responses. Biomaterials can also be synthesized and fabricated into implants that control the behavior of surrounding tissue, which has a significant potential for biomedical applications, including tissue engineering scaffolds.

Aspects of engineering biomaterials for regenerative medicine

Synthetic biomaterials design has traditionally focused on enhancing chemical and physical aspects, including biodegradation properties, surface chemistry, biocompatibility, etc., oftentimes drawing upon natural materials for inspiration. However, materials design, in the traditional sense, is only one aspect of designing biomaterials systems to interface with biological systems for the purposes of tissue engineering. Other less obtrusive aspects of biomaterials properties have been found to exhibit a profound influence on cell behavior, including such examples as mechanical properties and topographical features. Furthermore, biomaterials must also be developed with downstream post-processing in mind. For example, biomaterials may eventually be fashioned into systems with micro- and nanoscale structures or complex functionality such as controlled release of growth factors or electronic capabilities.

In general, the hierarchical engineering of biomaterials for tissue engineering mimics the same span of complexity of extracellular matrix (ECM) and tissue structures found in native organs. ECM molecules are known to influence cell function in a multifaceted manner. Specific peptide sequences are known to modulate adhesion and migration while structural ECM components are known to affect contractility and migration [5]. Complex arrangements of ECM proteins and homo- and heterotypic cell interactions provide cues for proper tissue function and simultaneously provide a framework to guide efforts in developing tissue engineering scaffolds. In general, the maturing paradigm is to utilize unique strategies of biomaterials fabrication and assembly to form structural subunits of tissue. These subunits can then be assembled into larger structures in order to create functional organs [6]. This short review is designed to highlight some new strategies for guiding cell function using biomaterials in the context of regenerative medicine applications. This objective will be pursued by examining select examples that provide a broad scope of recent themes that interface biomaterials synthesis and microfabrication techniques in tissue engineering. Specific focus will be dedicated to strategies for controlling cellular processes, both simple and complex, and how these can be implemented into tissue engineering systems. Three specific areas will be discussed in the context of the theme of controlling cell function and microenvironment: (1) synthetic biomaterials design, (2) micro- and nanostructured interfaces, and (3) microsystems and devices.

MATERIALS: THE DESIGN OF SYNTHETIC POLYMERIC BIOMATERIALS

ECM proteins as inspiration for synthetic biomaterials

Native ECM proteins serve a multitude of functions *in vivo*. ECM proteins such as fibronectin, vitronectin, laminins (A, B1, B2), collagens (I, II, IV), and thrombospondin [7] provide mechanical structure to support and protect attached cells. The rigidity of these ECM proteins provides a suitable substrate with appropriate stiffness to counteract contractile forces imposed on the surroundings by cells such as contractile fibroblasts and myofibroblasts. The Young's modulus of ECM proteins can vary between 0.3–0.6 kPa for native elastin [8] and 34 MPa for hydrated collagen [9]. The contractility forces imposed upon the ECM is both a function of the matrix as well as the cell type. ECM proteins also play an instrumental role in controlling cell function via other physicochemical mechanisms such as specific cell binding domain sequences. For example, fibronectin, vitronectin, collagen I, and thrombospondin all contain amino acid sequences of arginine–glycine–aspartic acid (RGD) [7], which is known to support the adhesion of many cells. From this and other examples, it is known that the ECM proteins present chemical moieties that are responsible for governing a variety of functions, including adhesion, migration, and extension of cellular protrusions such as neurites, lamellipodia, and filopodia [5]. The physical and chemical properties of native ECM proteins serve as a useful starting point for the design of biomimetic synthetic biomaterials.

Considerations in synthetic biomaterials design for tissue engineering applications

The primary function of first-generation synthetic biomaterials was simply to provide a structural support for cells to coalesce into functional tissue. However, just as the ECM is known to dramatically affect cell function, biomaterials used as tissue engineering scaffolds also affect cell function. Biomaterials utilized as tissue engineering scaffolds are typically designed with a variety of properties in mind, including chemical, physical, and mechanical. The choice of monomeric units and their connectivity is the primary basis for controlling relevant properties in synthetic polymers. Polyesters including poly(α -hydroxy esters) such as poly(D,L-lactide) (PLA), poly(glycolide) (PGA), and their copolymers (PLGA) have been used extensively as tissue engineering biomaterials because of hydrolytically and enzymatically cleavable ester linkages and monomers that can be readily metabolized [10]. However, these linear thermoplastic materials suffer other disadvantages, which suggests the ubiquitous use of these materials in tissue engineering applications is based upon their prior history of widespread use in clinical applications as opposed to their desirable properties. For example, most thermoplastic polyesters used for tissue engineering applications are unable to replicate the chemical, physical, or mechanical properties of native ECM molecules. Surface-modification strategies that include proteinaceous coatings or covalent tethering of biologically active molecules are logical strategies to improve biocompatibility as well as control functions such as adhesion or differentiation [11]. These molecules can also be incorporated throughout the bulk material to ensure presentation of signaling molecules throughout the lifespan of the degrading material. In addition to non-natural surface chemistry, other disadvantages of PLGA copolymers include nonlinear degradation profiles and bulk degradation kinetics [12]. While these disadvantages ultimately limit the utility of thermoplastic polymers, they also serve as a basis with which to motivate future generations of synthetic biomaterials, including biodegradable elastomers, which have a strong potential for use in soft-tissue engineering.

Synthetic biodegradable elastomers

Synthetic biodegradable elastomers are a class of biomaterials that exhibit a wide range of significant advantages to other previously studied biomaterials. The synthetic nature of these materials enables rapid, facile, and scalable synthesis of polymers with a potentially wide range of properties. The control of monomer feed ratios, synthesis conditions, and curing methods enables a wide parameter space

of resulting polymer properties [13,14]. Elastomers with Young's moduli on the order of 1 MPa or less can be routinely synthesized and have been shown to be useful for a variety of soft-tissue engineering applications [13]. Initial work focused on synthesizing elastomeric networks using naturally occurring monomers that were thermally cross-linked to form polyester linkages [13]. This polymer, termed poly(glycerol-*co*-sebacate) (PGS), utilizes sebacic acid as the diacid and glycerol as the polyol. These polymers exhibit a unique set of elastomeric properties with Young's moduli on the order of 1 MPa and maximum elongation at a break of more than 200 %. PGS exhibits excellent biocompatibility and degrades in a nearly linear manner both *in vitro* and *in vivo* [12]. In general, monomers with either carboxylic acids or polyols undergo thermal polycondensation to form cross-linked polyester networks by ensuring that at least one of these monomers has a chemical functionality of more than two and the other has a chemical functionality of at least two. Additional formulations based on cross-linked polyester networks were also studied to further expand the range of properties [15], including polymers based on citric acid. In one study, xylitol, a naturally occurring polyol, is utilized as a monomer in a system that can form a tough elastomer if it is condensed with sebacic acid or a hydrogel if it is condensed with the polyfunctional citric acid [14]. Other studies have demonstrated tunable properties of thermally cured elastomers containing naturally occurring polyols by adjusting the feed ratios of monomers and processing conditions [16]. While the properties of polyester elastomers can be tuned to some degree, the potential use of biodegradable elastomers in a wide range of medical applications suggests that a wider range of properties would be desirable. Using naturally occurring collagen as design inspiration, elastomeric poly(ester amides) were synthesized by polycondensation of a polyol and a polyfunctional monomer containing primary amines with a diacid to form poly(1,3-diamino-2-hydroxy propane-*co*-polyol sebacate) (APS, Fig. 1). Tuning the relative presence of ester and amide bonds serves a more sensitive manner with which to control the physical properties, including degradation half-life, which could be tuned from 8 to 100 weeks *in vivo* [17] with only slight alterations in mechanical properties. Furthermore, the enhanced biocompatibility of these elastomers enabled *in vitro* culture of primary hepatocytes on APS substrates without the need for surface modification [18,19].

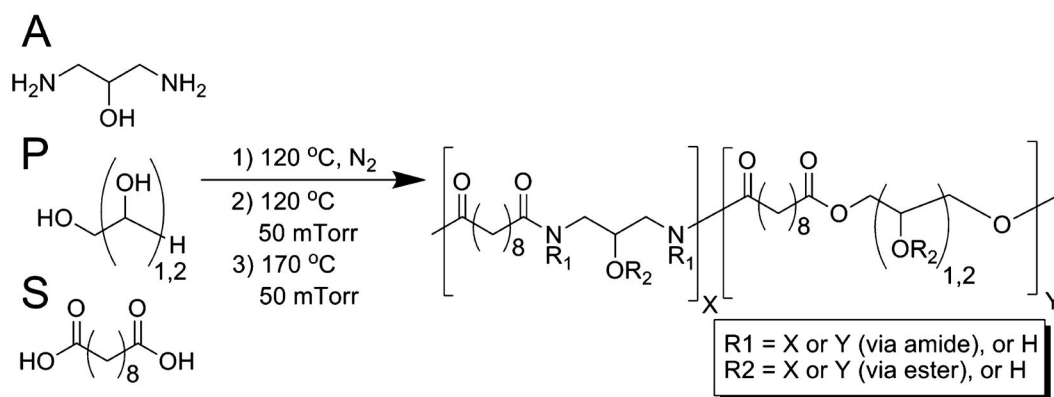


Fig. 1 Synthesis scheme of biomimetic APS polymers. The general synthetic scheme of APS polymers incorporated the following monomers through condensation reaction: (1) a multifunctional amine group (A), which was chosen to be 1,3-diamino-2-hydroxypropane in this example; (2) a polyol (P); and (3) a diacid (S). Glycerol or D,L-threitol were used as polyols while sebacic acid was chosen as the diacid. The first phase of polymerization results in a pre-polymer that can be molded into a variety of geometries. The pre-polymer can then be cured into the final cross-linked form. Reprinted with permission from: C. J. Bettinger, J. P. Bruggeman, J. T. Borenstein, R. Langer. *Biomaterials* **29**, 2315 (2008), copyright © 2008 Elsevier.

Thermally cross-linked elastomers have demonstrated enormous potential in many soft-tissue engineering applications. However, the aggressive conditions required for polymerization have thus far prohibited the use of these polymers for such tissue engineering strategies that encapsulate growth factors or cells directly into the network. Photocrosslinkable biomaterials offer significant advantages compared to their thermally cross-linked counterparts, including the aforementioned class of biodegradable elastomers, such as (1) rapid, mild cross-linking conditions, (2) the potential to pattern films through photolithography, and (3) the ability to precisely control cross-linking through chemistry, an additional mode with which to finely tune physical, mechanical, and chemical properties of polymer networks. Most synthetic routes to obtaining photocrosslinkable biomaterials begin with a polymeric precursor that is chemically modified through the addition of photoactive groups such as acrylates or methacrylates [20]. Poly(ethylene glycol) (PEG) is a simple, water-soluble, synthetic polymer that has been used as a photocrosslinkable polymer for numerous biomedical applications. PEG has been used extensively as a biomaterial because it can be easily modified with photoreactive acrylates as well as bioactive molecules. PEG has been used in a significant number of soft-tissue engineering applications, including the encapsulation of mesenchymal stem cells [21]. Poly(α -hydroxy esters) have also been targeted for use in photocrosslinkable systems, usually serving as blocks in combination with other synthetic polymers. Ring-opening polymerizations of PLA and poly(ϵ -caprolactone) (PCL) monomers have been used to produce photocrosslinkable networks. For example, PCL diols have been prepared with adipic acid and 4-hydroxycinnamic acid to create biodegradable photocrosslinkable elastomers [22]. PCL has also been used in synthesizing block and star copolymer networks, including PCL-b-PEG-b-PCL and star-PCL-b-PLA, respectively [23,24]. Other types of synthetic photocrosslinkable polymers have been explored for tissue engineering, including acrylated forms of poly(vinyl alcohol) [25] and PGS [26].

Natural polymers have also been modified with various reactive side groups to create synthetic analogs that can be cross-linked by photoinitiation. Collagen, an extremely versatile and robust natural polymer, has been modified with methacrylic acid via lysine and hydrolysine residues to form photocrosslinkable gels [27]. Biodegradable polysaccharides have also been synthetically modified, including hyaluronic acid [28], dextran [29], and chitosan [30]. Synthetic versions of these natural polymers have been used for various drug delivery and soft-tissue engineering applications. One general unifying theme in developing elastomeric systems is the enhanced ability to finely tune the properties of these networks. The degree of cross-linking within the network can be controlled at many points along the route toward realization of the polymer in its final form. Modifications of monomer composition, synthesis conditions, and processing parameters can be used to finely tune critical properties, including degradation rate, compressive modulus, porosity, swelling, and the like [16]. Furthermore, there are numerous strategies in which modifying these network properties can be accomplished relatively independent of one another, including the use of copolymers, stoichiometric control of reactions, and incorporation of additional compounds within the network. Future development in synthetic soft materials for tissue engineering will continue to focus on expanding the parameter space of properties to facilitate tissue-specific biodegradable polymeric systems.

Synthetic biomaterials with integrated cell signaling modalities

Synthetic biomaterials can also serve as a unique opportunity to incorporate molecules that provide structural support as well as signaling modalities to direct cell function. The polymerization of diglycidyl esters with primary amines to form linear esters is an efficient means of incorporating biomolecules into the polymer backbone of synthetic biomaterials [31]. Films formed from polymers synthesized by this method (termed PCD) have been shown to enhance differentiation of PC12 neuroblastoma cells relative to films treated with laminin. A similar synthetic route was also employed in the synthesis of acetylcholine-containing polyesters, which also supported neurite growth from dorsal root ganglion explants [32] (Fig. 2). These examples suggest that incorporating biologically active

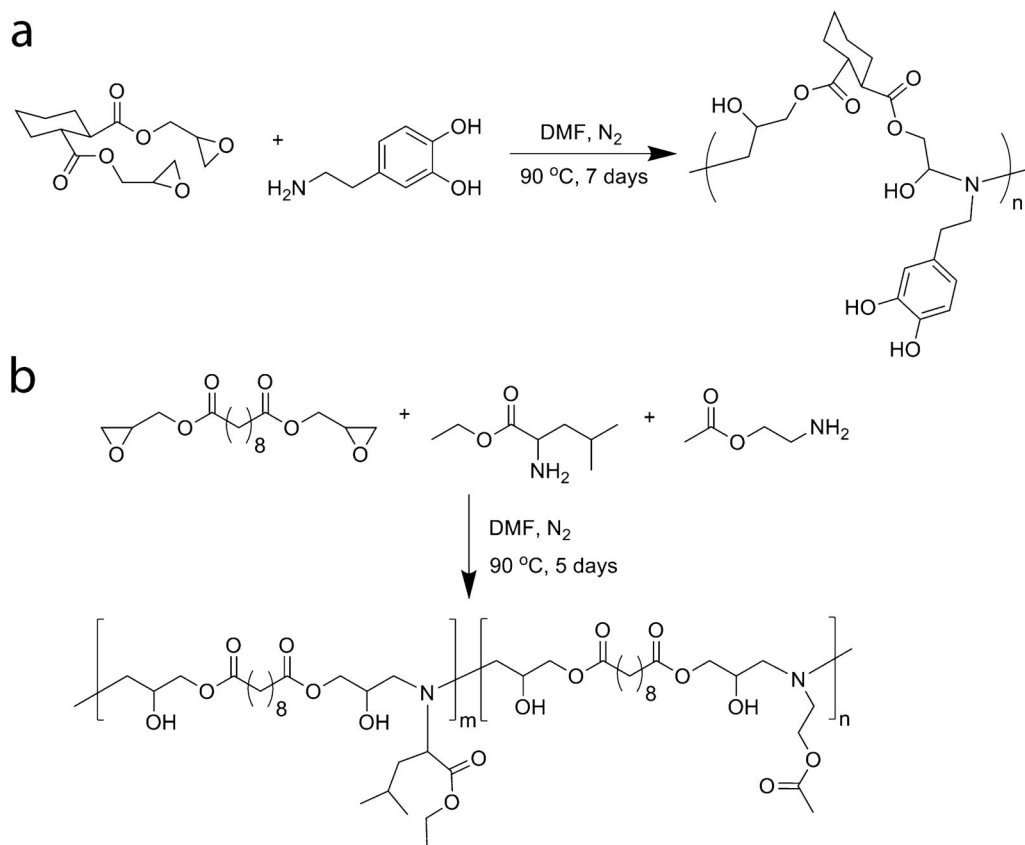


Fig. 2 Neuroinductive biomaterials containing neurotransmitters. (a) PCD is synthesized by using dopamine as a monomer. (b) This synthesis strategy can also be applied to a large number of diglycidyl esters and biomolecules containing primary amines, including acetylcholine. Synthetic biopolymers incorporating neurotransmitters as monomers have demonstrated enhanced biocompatibility with respect to neural tissue both *in vitro* and *in vivo*. (a) Adapted from: J. Gao, Y. M. Kim, H. Coe, B. Zern, B. Sheppard, Y. Wang. *Proc. Natl. Acad. Sci. USA* **103**, 16681 (2007), copyright © 2007 National Academy of Sciences; (b) adapted from C. Gumerá, Y. Wang. *Adv Mater.* **19**, 4404 (2007), copyright © 2007 Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

molecules as a core monomeric component of the polymer can lead to expanded functionality of tissue engineering biomaterials without the additional steps of surface modifications or coatings. Synthetic biomaterials can also be used to construct hydrogel networks for use in creating custom 3D micro-environments for cell culture. Chemically modified hyaluronic acid hydrogels (Fig. 3) have been demonstrated to be useful in hESC culture [33]. Controlling matrix properties can serve as a method to influence cell adhesion and spreading in 3D cell-seeded networks [34]. For example, the incorporation of covalent barriers in cell-seeded networks restricts spreading and directly influences the morphology of cells.

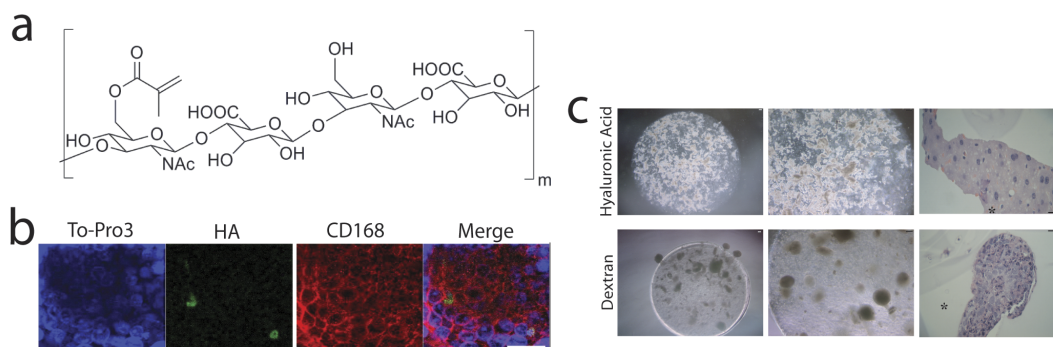


Fig. 3 Photocrosslinkable hyaluronic acid for encapsulation of hESCs. (a) Hyaluronic acid (HA) modified with methacrylate groups can be photocross-linked into hydrogels for tissue engineering strategies such as cell encapsulation. Photocrosslinkable HA has been used to create hydrogel networks to control the self-renewal and differentiation of hESCs by interactions with hyaluronic receptors expressed by hESCs. (b) Specifically, hESCs express CD44 and CD168, two types of cell membrane HA receptors, and have also been found to internalize soluble HA. (c) hESCs cultured in 3D HA hydrogel networks were found to remain in undifferentiated colonies compared with hESCs cultured in dextran hydrogels, which were found to form embryoid bodies, clusters of differentiated stem cells. Scale bars represent 100 μm . Reprinted from S. Gerecht, J. A. Burdick, L. S. Ferreira, S. A. Townsend, R. Langer, G. Vunjak-Novakovic. *Proc. Natl. Acad. Sci. USA* **104**, 11298 (2007), copyright © 2007 National Academy of Sciences.

STRUCTURES: MICRO- AND NANOFABRICATED BIOMATERIALS INTERFACES

Native cell-nanotopography interactions in micro- and nanostructures in natural biomaterials

The topographical aspect of native ECM proteins is an extremely important structural component that guides cell function. Collagen I, for example, is an ECM protein that contains a repeating structural element due to banding that occurs at a pitch of 66 nm [35]. Although the physical dimensions of individual molecules is 300 nm in length and 1.5 nm in width [36], these individual molecules can form fibrils that extend for tens of microns in length and have diameters between 260 and 410 nm [37]. The basement membrane of the human corneal epithelium also has a submicron feature scale [38]. Cells interact with native topographical structures often through a phenomenon known as contact guidance. Contact guidance is an example of a naturally occurring phenomenon that is characterized by the response of cells to structures on the micron and submicron scale. Contact guidance regulates a variety of cell functions involved in physiological processes. Cell migration through the ECM in vivo is modulated by the guidance of cells via organized ECM proteins [39]. For example, in vivo T cell migration is known to be highly dependent upon cell–biomaterial interactions with native ECM proteins [40]. Contact guidance also plays an important role in the migration of cells, both individual and collective [41]. Contact guidance is also an important component in efficient organelle function, including axonal guidance and growth cone motility [42]. In general, the nanometer-scale dimensions of these ECM molecules combined with the ability of cells to detect the features presupposes that topographical cues provide a flexible framework with which to guide a multitude of cell functions. The remaining treatment of this topic presents some examples of how artificially fabricated micro- and nanostructured synthetic biomaterials interfaces can be engineered to control cellular function by virtue of cell–topography interactions.

Engineering simple cell function using synthetic surface topography

Ubiquitous in vivo cell-topography signaling suggests that synthetic interactions may serve as a potent signaling mechanism for use in regenerative medicine. Recent developments in photolithography and microfabrication have produced 2D substrates with micron- and submicron-scale features; a length scale similar to native topography found in ECM molecules. ECM components such as collagen often contain fibrous structural elements that can be recapitulated in synthetic 2D systems through ridge-groove nanograting features. Similarly, the textured surface of the basement membrane in the corneal epithelium can likewise be recapitulated in synthetic 2D substrates through the fabrication of nanoposts and nanopit features. These two examples reflect the potential for using microfabrication strategies to reconstruct native nanotopography in synthetic systems. Generalized micro- and nanofabrication techniques such as replica molding (RM) and soft lithography have demonstrated the ability to create synthetic 2D substrate with micro- and nanometer-scale features in a variety of soft materials [43–45]. RM can reproduce features down to 30 nm and has been adapted to fabricate nanotopographical structures in a variety of natural and synthetic polymers [46–48]. In addition to ordered topography, there have been applications of substrates with random micro- and nanostructures that are fabricated using processes such as electrospinning [49], chemical etching [50], and phase separation [51,52]. These substrates, which exhibit short-range order, are also known to influence cell function. However, in the interest of brevity, only synthetic substrates with features that exhibit long-range order and sizes between approximately 50 nm and 1 μm , termed “nanotopography” in the case of this article, will be discussed. There are three basic nanotopographic geometries that are prevalent in studies of cell–nanotopography interactions: (1) nanogratings, parallel arrays of alternating ridge-groove structure combinations; (2) nanoposts, orthogonal arrays of raised pillars; and (3) nanopit arrays, orthogonal arrays of depressed pits.

Cells cultured on 2D synthetic nanotopography respond in a wide variety of altered behavior, which depend upon many factors, including the physical properties of the bulk substrate material, cell type, feature length scale, and feature geometry [53]. Although almost all mammalian cells have been shown to respond to nanotopography, many effects of cell–nanotopography interactions are equivocal across phenotype, substrate material, feature size, and geometry. The most obvious effect of nanotopography on cell function is morphology. Many cell types typically respond to nanogratings by simultaneously aligning and elongating in the direction of the grating axis. This response has been observed in various cell types, including fibroblasts, endothelial cells, embryonic stem cells, mesenchymal stem cells, smooth muscle cells, epithelial cells, and Schwann cells [54]. Neurites extending from neuroblastoma cells and dorsal root ganglion cultured with soluble nerve growth factor on nanogratings exhibit enhanced alignment and increased length. The morphological response is evident in cells cultured on substrates with features as small as 100 nm and depths as small as 75 nm [55]. Substrates with nanopost and nanopit features elicit a more subtle effect on cellular morphology, for reasons that are not entirely clear. Many studies have demonstrated the reduction of spreading on nanoposts and nanopits, although the overall effect of these structures on cell area is ambiguous.

The length scale of synthetic nanotopography can be designed to mimic that of ECM proteins [56], including collagen [57]. Therefore, the prediction that nanotopography enhances attachment and adhesion of mammalian cells is reasonable. While nanogratings generally appear to enhance the adhesion in various cell–biomaterial–geometry combinations, nanoposts and nanopits generally reduce initial cell attachment. These effects are also highly dependent upon the depth, height, and geometry of the features. Future studies should be aimed at elucidating the apparent geometrical dependence of differential adhesion. Nanotopography also influences proliferation profiles of various cell types [58–60]. While cells cultured on nanogratings exhibit reduced proliferation rates compared to cells cultured on planar substrates, there is no obvious trend to predict the effect of nanopost or nanopit geometries on proliferation. Furthermore, there are currently no widely accepted hypotheses regarding the mechanism for the effect of cell–nanotopography interactions on cell proliferation.

The effect of nanotopography on migration is primarily observed in cells cultured on substrates with nanograting structures. Many cell types have exhibit biased migration in the direction of the grating axis and increased overall migration velocities, including endothelial cells [59], epithelial cells [61–63], osteoblasts [64], and C6 glioma cells [65]. In general, enhanced migration is a response that is typically coupled with elongated morphology and alignment of the cell body with the nanograting axis. There have been limited studies regarding the migration of cells on nanopit or nanopost geometries compared to nanograting geometries, which makes any attempt at drawing conclusions premature at this time.

Directing cell fate and tissue assembly through cell–topography interactions

The impact of nanotopography on basic cell function in many cell types suggests that nanotopography could also be conceivably utilized as a signaling modality for directing complex cell function. Human mesenchymal stem cells (hMSCs) cultured on nanogratings have the potential to be preferentially differentiated into neuronal lineages as determined by the presence of synaptophysin, *tuj1*, and nestin markers as well as the upregulation of MAP2 [66]. The enhanced differentiation of hMSCs has also been explored using nanopit arrays. Long-term culture of osteoprogenitor cells and hMSCs on poly(methyl methacrylate) (PMMA) nanopit arrays of varying order alters the expression of osteopontin and osteocalcin, two bone-specific ECM proteins [67]. Taken together, these studies demonstrate the potential of nanotopography to control cell fate. Additionally, the complementary findings of hMSCs cultured on nanogratings and ordered–disordered nanopits suggest the potential for selective, controllable differentiation based solely on nanotopography geometry.

Modulating cell–nanotopography interactions also has the potential to influence cell–cell interactions with potential to generate complex multicellular structures. One example of this is the culture of human endothelial progenitor cells (EPCs) on PDMS nanograting [59]. EPCs respond to substrates through alterations in morphology, reduced proliferation, and enhanced migration. Endothelial markers appeared to remain constant across EPCs cultured on both nanograting and planar substrates. However, EPCs cultured on nanograting for up to 6 days formed segregated multicellular bands (Fig. 4) approximately 100 μm in width and hundreds of microns in length. These superstructures contrasted significantly with EPCs cultured on planar substrates, which formed confluent monolayers. The band structures found in EPCs cultured on nanograting formed well-defined and organized capillary tubes in an *in vitro* matrigel assay. Confluent layers of EPCs formed during culture on planar substrates did not form distinct capillary tubes. This preferential formation found in EPCs cultured on nanotopography is hypothesized to occur for several reasons. First, alignment, elongation, and increased migration velocities bias morphology and cell–cell interactions to produce band structures, which served as structural precursors to capillary tubes. Second, reduced proliferation of EPCs cultured on nanograting prevented the formation of confluent monolayers. This reduced cell density could serve to enhance cell mobility during the induction of capillary tube formation with matrigel. Regardless of the possible mechanism, this study demonstrates the expanded potential for substrate nanotopography to create simple tissue structures by virtue of cell–nanotopography interactions alone.

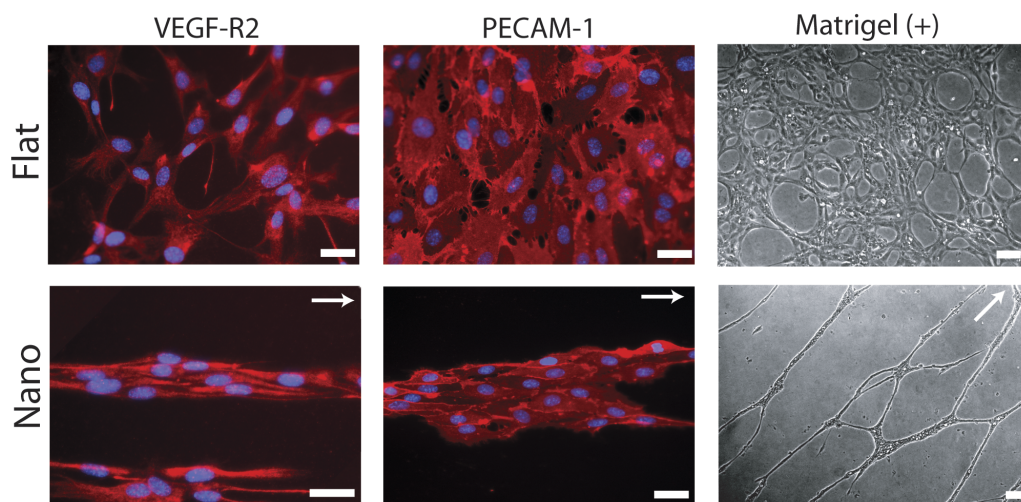


Fig. 4 Nanograting topography promotes organized multicellular structures in endothelial cells. Expression of vascular endothelial growth factor receptor-2 (VEGF-R2) and platelet/endothelial cell adhesion molecule-1 (PECAM-1), two endothelial cell markers, was constant in endothelial cells cultured on both planar and nanograting substrates. However, endothelial cells cultured on nanograting topography were organized into multicellular band structures while those cultured on flat substrates were relatively disorganized. These aligned band structures formed aligned capillary tubes in an in vitro matrigel assay (Matrigel (+)). The grating axis is indicated by the white arrows. Scale bars represent 50 μm . Adapted from: C. J. Bettinger, Z. Zhang, S. Gerecht, J. T. Borenstein, R. Langer. *Adv. Mater.* **20**, 99 (2008), copyright © 2008 Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Potential applications of micro- and nanostructured biomaterials in tissue engineering scaffolds

Cell–nanotopography interactions have the ability to control a variety of cell functions, which have potential utility in tissue engineering applications. The integration of nanotopography as a cue to modulate basic cell function has potential use in scaffold design as well. The influence of nanograting on morphology can be used to form aligned populations of cells, which are important for the structure and function of smooth muscle cells and endothelial cells. Vascular tissue engineering scaffolds are of particular interest because of the correlation with alignment and cell function of multiple cell phenotypes within close proximity to one another, including inner endothelial cells that are aligned parallel to blood vessels and smooth muscle cells that are orthogonal. Recent work has led to the fabrication of a tube-shaped scaffold with multiple nanograting surfaces [68]. Enhanced migration on nanograting also has potential implications for the design of guidance channels for peripheral nerve regeneration. For example, tubular conduits modified with nanograting features could enhance the migration of Schwann cells into the injury site to promote axonal regeneration [69]. These structures could also promote the rapid migration of neurites across nerve gaps that are often observed in peripheral nerve injury.

DEVICES: MICROFABRICATED BIOMATERIALS SYSTEMS FOR TISSUE ENGINEERING

The fabrication of micro- and nanostructures in biomaterials interfaces is an effective method in controlling cell behavior in planar systems, which is suitable for simple tissues. These structures could prove to be useful in modulating cell function in populations of cells or organizing cell structures into tissues that adhere to simple 2D geometries. However, the development of biomaterials systems to accommodate complex 3D structure is a more challenging pursuit. Many native tissues are complex sys-

tems of heterotypic cell populations that interact with biomaterial structures in the presence of precisely coordinated concentrations of soluble factors, all of which are spatially resolved on the micron length scale. Taken together, this complex milieu of signals defines the microenvironmental landscape that ultimately dictates the function of tissues and organs. For example, the liver is an organ that contains dozens of cell types and a pervasive micron-scale vascular network [70]. Tissue engineering systems derived from synthetic cell-seeded biomaterial constructs that are organized into systems with complex dimensionality or functionality may be able to more accurately recapitulate the native microenvironment. This section describes several examples that demonstrate how biomaterials can be fabricated into complex scaffolds, which can direct various aspects of the cell function, and ultimately control the cellular microenvironment for potential use in tissue engineering constructs.

Microfabricated 3D scaffolds

The general field of rapid prototyping (RP), originally developed for rapid manufacturing techniques, forms 3D objects with the help of custom fabrication hardware and computer-generated solid or surface models. Programs such as computer-aided design (CAD), coupled with the ability to fabricate arbitrary and complex 3D structures through the use of RP and solid free-form fabrication (SFF), have allowed the production of designer scaffolds with predefined microarchitecture using many classes of materials, including polymers, composites, and ceramics [71]. Direct ink writing (DIW) of biomaterials has also been studied as a potential scaffold fabrication strategy [72]. DIW of hydrogel-based structures has been shown to produce features on the order of 5 μm , which can be used to control the morphology of seeded cells. Other techniques have focused on the precise deposition of cells, rather than scaffold material. For example, the technique of laser-guided direct cell writing has been used to build up 3D tissue structures with micron-scale precision [73,74]. Controlled cell deposition has also been accomplished by direct deposition of cell solutions using commercially available ink-jet printing technology [75]. In general, the motivation for fabricating tissue engineering constructs using RP, SFF, DIW, direct cell deposition, and related techniques is typically focused on the ability to create scaffolds with predefined and precisely controlled unit cell geometries. Optimizing pore structures is useful in predicting scaffold operating parameters such as void percentage for cell seeding, bulk mechanical properties, and metabolite concentrations. Direct cell deposition also exhibits unique advantages of selectively depositing cells of various phenotypes with micron-scale precision in a spatially controlled manner. This diverse research area is rapidly expanding and has been extensively reviewed elsewhere [71,76]. Hence, this topic will not be the primary focus of this discussion. Instead, examples wherein the application of microfabricated 3D scaffolds is used to control aspects of cell behavior will be presented.

Just as the cell morphology, structure, and function can be controlled by engineering synthetic topography at the cell–biomaterial interface, similar attributes can be engineered through the use of scaffolds with microfabricated 3D structures. Recent work has led to microfabrication of a 3D scaffold for myocardial repair applications. Anisotropy is present in many native tissues and gives rise to unique structure–function relationships that are crucial to maintaining healthy organs. For example, cardiac muscle fibers are bound by collagen sheaths, which ultimately result in directionally dependent mechanical and electrical properties. Tissue engineering strategies for cardiac tissue inherently require a scaffold that is able to appropriately mimic the structural, mechanical, and electrical anisotropies of native tissue. Scaffolds designed for this purpose should ideally (1) match the physiologic mechanical properties, (2) provide low mechanical resistance in order to accommodate large deformations during contraction of cardiac tissue, and (3) provide structural cues to align, orient, and organize cells into physiologically relevant cardiac tissue structures. Three-dimensional porous tissue engineering scaffolds were fabricated using laser ablation of PGS [77]. The resulting scaffolds were honeycomb-shaped cellular solids with physiologically matched properties, including structural features roughly 200 μm in size and stiffness on the order of 2.1 MPa (Fig. 5). Neonatal rat heart cells were seeded on these scaffolds and cultured *in vitro* for up to one week. Anisotropic honeycomb scaffolds aligned seeded heart

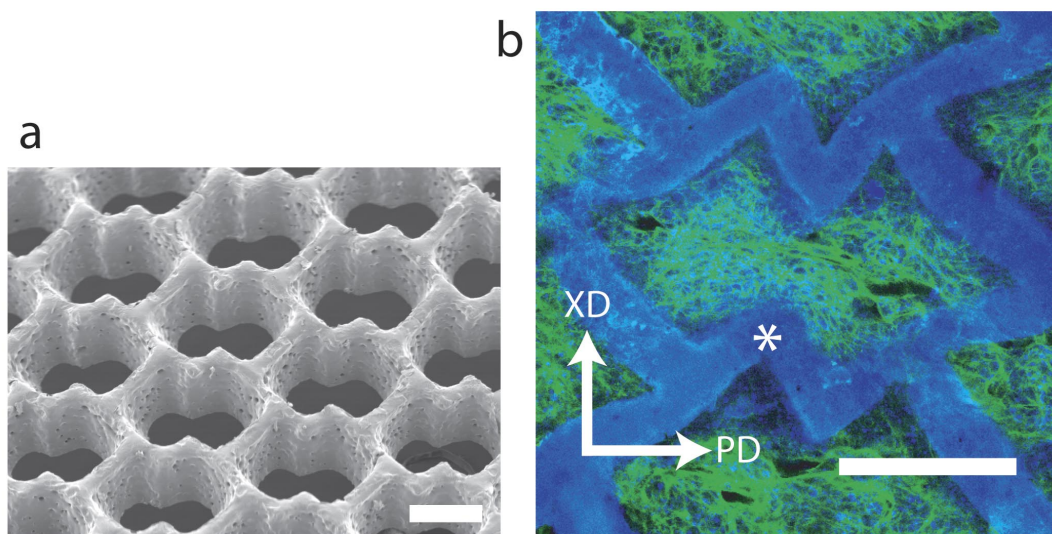


Fig. 5 Microfabricated scaffolds with honeycomb structure provide structural and mechanical cues to guide cardiac regeneration. (a) Micron-scale structures were fabricated in PGS films using an excimer laser to create porous scaffold geometries. (b) These structures (blue) provided were found to guide the alignment and morphology of neonatal heart cells (green). Scaffolds fabricated with honeycomb structures produced tissue-engineered myocardial constructs with anisotropic mechanical and electrical properties, characteristics similar to that of native myocardial tissue. Scale bars represent 200 μm . Reprinted by permission from: *Nat. Mater.* **7**, 1003 (2008), copyright © 2008 Macmillan Publishers, Ltd.

cells and produced a tissue morphology that is similar to native cardiac tissue. Furthermore, tissue constructs formed from these scaffolds exhibited directionally dependent electrophysiological properties as well. This work demonstrates that enhancing tissue engineering with rationally chosen micron-scale features can ultimately yield biomimetic properties that more accurately replicate those of native tissues. Generally speaking, this is an excellent example of integrating structures within a material to create a system that can better control the structure and function of seeded cells.

Biodegradable microfluidic scaffolds

The use of microfluidics as engineering systems has largely focused on miniaturization of molecular analysis and genomics, portable devices for field-deployable biosensors, and interfacing fluid handling with microelectromechanical systems (MEMS) for improved automation [78]. However, the field of tissue engineering can also benefit significantly from advances in microfluidics technology, given that the appropriate biomaterials and biocompatible processes are interfaced with traditional microfabrication methods. One of the main advantages of utilizing microfluidics in tissue engineering applications is the ability to design and fabricate systems that can mimic the complex microarchitecture of native tissues. For example, much work has been performed in creating biomimetic microenvironments for hepatocytes within microfluidic devices [79,80]. These systems, though ultimately limited by their inorganic, nonbiodegradable material, are useful for the study of optimal culture conditions such as fluid shear stress, metabolite concentrations, and heterotypic cell interactions. More recent work has moved beyond traditional microfluidic geometries to fabricate systems that mimic the *in vivo* geometry and mass transport characteristics of a liver sinusoid [81]. Materials used for microfluidic scaffold applications would ideally be resorbable, promote cell attachment, allow surface modification, and be amenable to facile processing. These unmet needs continue to drive active research in synthetic biomaterial synthe-

sis and development as well as natural biomaterials purification and characterization. In addition to materials development, advances in materials processes must also be pursued to allow for efficient microfabrication strategies of novel materials. For example, advanced 3D microfluidic systems using inorganic materials [82] must be translated into biodegradable materials. Developing parallel strategies for novel cell-compatible biomaterials synthesis, processing, and fabrication will result in novel microfluidic scaffolds to further advance scaffold development. One step in this process is the fabrication of biomaterials directly into microfluidic conduits, which can then be perfused with medium and seeded with cells. Initial work in this area examined the possibility of adapting soft-lithography processes to polyesters, including PLGA [83] and PGS [84]. In the latter case, 3D microfluidic constructs were seeded with hepatocytes and perfused in long-term culture conditions. Natural materials have also been explored for potential use in biodegradable microfluidic tissue engineering scaffolds (Fig. 6). For example, replica-molded silk fibroin can be processed into microfluidic scaffolds. Hepatocytes seeded into these devices were maintained in long-term perfusion culture [46]. The integration of vascular networks into cell-seeded scaffolds enables facile *in vitro* perfusion as well as the potential for integration with host vasculature for *in vivo* perfusion. Microfluidic scaffolds have the potential to overcome issues of mass transport limitations of nutrient supply and waste product removal in order to enable the scale-up of cell-seeded constructs.

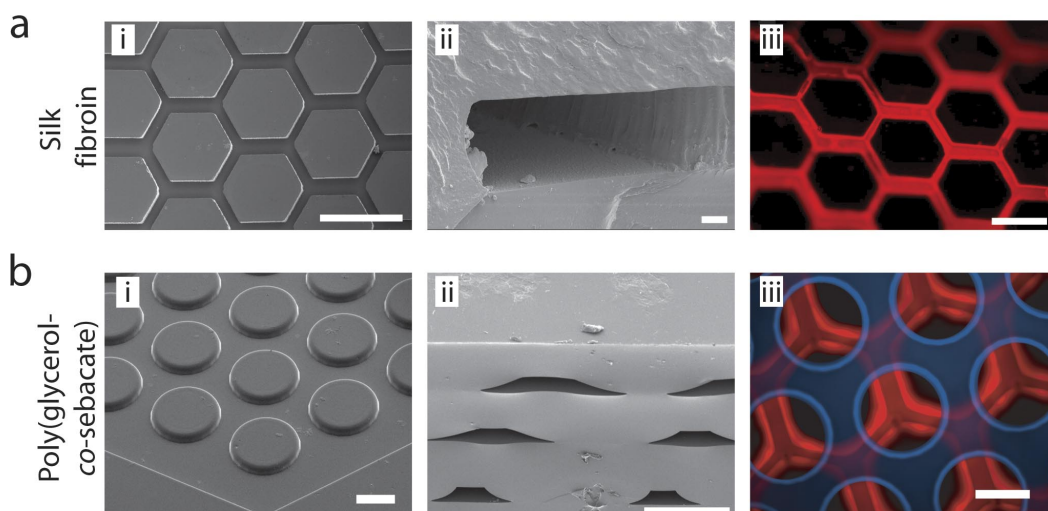


Fig. 6 Biodegradable microfluidic devices for use as tissue engineering scaffolds. Biodegradable microfluidic networks have been fabricated from numerous biopolymers, including: (a) silk fibroin, a natural protein; and (b) PGS, a synthetic elastomer. Replica-molded films of polymer (i) are laminated using a variety of chemical or physical processes in order to form microfluidic devices. The cross-section of single silk fibroin devices (a, ii) and multilayer PGS devices (b, ii) suggest that the films are robustly assembled and able to preserve the microstructures. Devices are also able to support perfusion (iii) and long-term culture of cells. Adapted from: C. J. Bettinger, E. J. Weinberg, K. M. Kulig, J. P. Vacanti, Y. Wang, J. T. Borenstein, R. Langer. *Adv. Mater.* **18**, 165 (2006); C. J. Bettinger, K. M. Cyr, A. Matsumoto, R. Langer, J. T. Borenstein, D. L. Kaplan. *Adv. Mater.* **19**, 2847 (2007), copyright © 2007 Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Microfluidic assembly of tissue structures

Initial studies in microfluidic biomaterials for tissue engineering applications have focused on cell seeding and perfusion within the lumen of a microfluidic network fabricated from a solid bulk material. More recent work has pursued the notion of using hydrogels to create microfluidic networks which can enable convective transport of solutes throughout the bulk of the device. Initial work in this field de-

veloped microfluidic structures using calcium alginate hydrogel films that were cross-linked through the addition of calcium chloride. This device was operated in different modes, including assisted delivery, where the supply of a solute to the bulk material is enhanced by perfusion through the microfluidic channels, as well as assisted extraction, where solute is removed from the bulk by perfusion of pure solvent. Assisted delivery and extraction was also found to depend upon the molecular weight of the solute. Microfluidic hydrogel networks have also been seeded with cells to demonstrate further potential for tissue engineering applications. Calcium alginate hydrogels have been fabricated into microfluidic networks with primary chondrocytes seeded into the bulk phase of the material [85]. These networks are able to control the concentration of multiple solutes throughout the bulk and support cell viability (Fig. 7). Microfluidic networks were also fabricated using agarose and seeded with murine hepatocytes

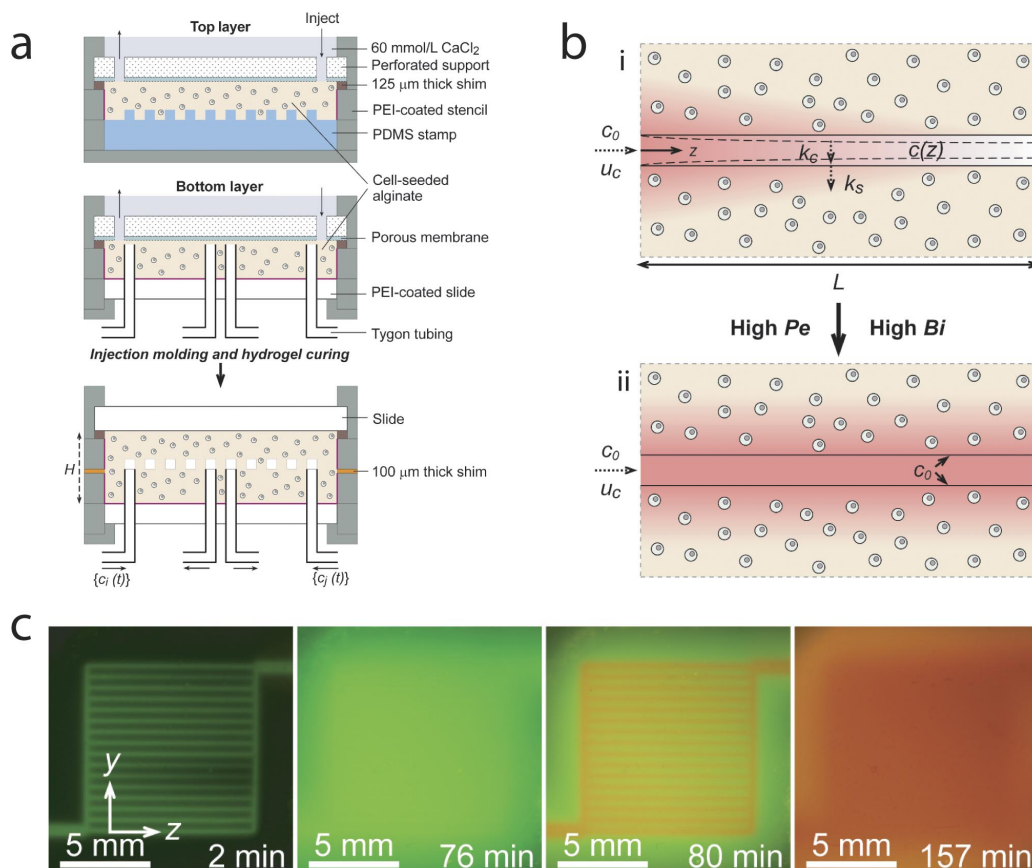


Fig. 7 Fabrication and characterization of mass transport properties of microfluidic alginate hydrogel networks. (a) The fabrication of alginate microfluidic networks begins with the fabrication of one layer that contains micromolded features and a substrate layer that contains premolded tubing inlets for perfusion. Each alginate layer is seeded with cells and the molded layer is cross-linked using calcium solution. The two layers are then brought together and cured to form the final device. H (mm) is the thickness of the scaffold and $c_i(t)$ and $c_j(t)$ denote the instantaneous concentration of solutes i and j , respectively, in injected solutions. (b) Schematic diagram showing convective mass transfer mediated by embedded microchannels at low (i) and high (ii) flow speeds. L (cm) is the microchannel length. (c) Fluorescence micrographs of the microfluidic scaffold at time points during sequential delivery of solutes (fluorescein, green, and rhodamine B, red) throughout the scaffold via embedded microchannels. Reprinted by permission from: *Nat. Mater.* **6**, 908 (2007), copyright © 2007 Macmillan Publishers, Ltd.:

throughout the bulk phase [86]. Cells cultured in the bulk with adequate network perfusion exhibited increased viability compared to those cultured in the bulk under static conditions. These data suggest that perfusion of cell-seeded microfluidic hydrogel scaffolds is necessary for maintaining viability. Assembling more complex networks of biomaterials is another active area of research that is aimed at creating biomimetic microenvironments. Many native tissue structures employ multiple ECM molecules and heterotypic cell populations within close proximity to one another with micron-scale spatial resolution. Toward developing artificial biomimetic analogs of these structures, cell-seeded microfluidic biomaterials with multiple phases of ECM molecules and cell types have been assembled using several combinations of natural polymers, including alginate, collagen I, matrigel, and fibrin [87] (Fig. 8). Microfluidic hydrogel networks were formed using RM techniques to gel an alginate matrix loaded with collagen I molecules. A solution containing a second ECM doped with collagen I is then perfused throughout the network. The collagen from the second collagen I-doped ECM solution interfaces with the collagen I from the alginate bulk to form a coherent two-phase ECM network. Cells can also be in-

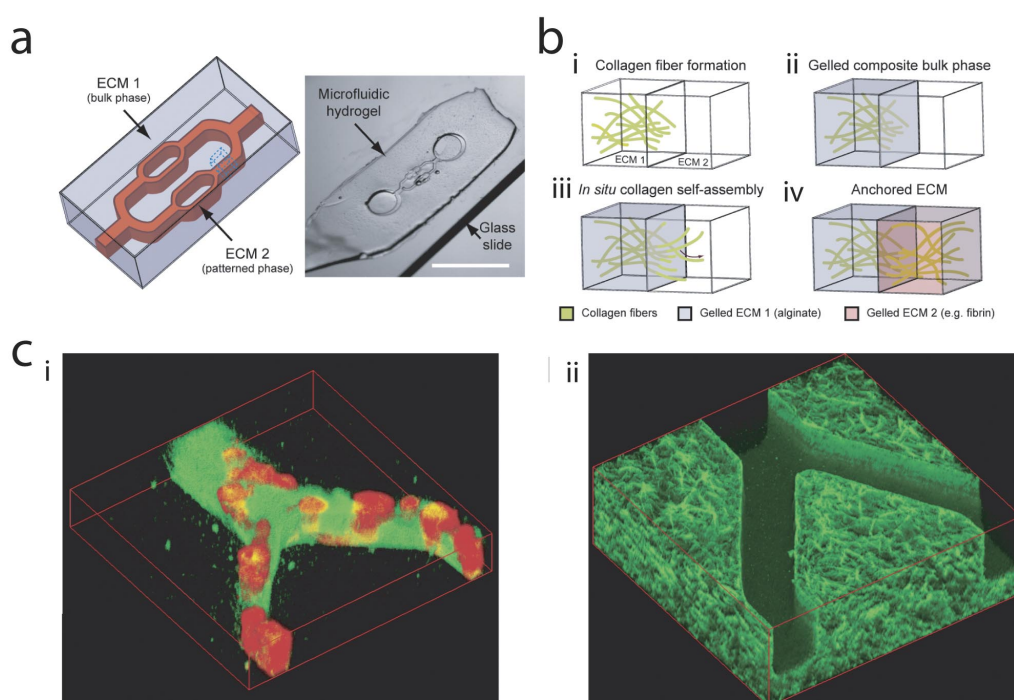


Fig. 8 In situ microfluidic assembly of ECM molecules to create patterned cell-seeded tissue-engineered constructs. (a) Schematic diagram of a construct consisting of multiple 3D matrices: a microfluidically patterned phase and a bulk microfluidic hydrogel phase. An image of the corresponding microfluidic hydrogel is at right. (b) Magnified view of the interface (boxed region in a) showing the formation of each phase. (i) The bulk phase is formed by doping collagen into an alginate solution and allowing a collagen fiber network to form (by increasing temperature). (ii) The alginate is gelled (by ionic cross-linking) around the collagen fiber network to complete the formation of the bulk matrix. (iii) A second collagen-doped ECM solution is then patterned within the bulk phase. Collagen precursors in the second ECM nucleate and assembled from exposed collagen fibers at the interface. (iv) Formation of the patterned ECM is completed on gelling the second ECM solution. (c) (i) Confocal microscopy demonstrates well-partitioned phases of microfluidically patterned collagen (green) seeded with HUVECs (red) in a bare alginate bulk phase. (ii) Collagen-doped alginate bulk phases also demonstrated well-defined structures and uniform distributions of collagen fibers (green). Box dimensions are $230 \times 230 \times 30 \mu\text{m}$ in (c, i) and $230 \times 230 \times 75 \mu\text{m}$ in (c, ii). Reprinted by permission from: *Nat. Mater.* **7**, 636 (2008), copyright © 2008 Macmillan Publishers, Ltd.

corporated into the respective phases: in this case, fibroblasts are loaded into the bulk alginate and endothelial cells are loaded into the patterned matrix. This platform has the unique potential to study a variety of microenvironmental effects such as of ECM pattern geometry and co-culture conditions.

SUMMARY AND OUTLOOK

The importance of tissue engineering and regenerative medicine will continue to grow as it provides a set of concrete objectives that drive certain aspects of biomedical research. These objectives include: (1) long-term goals to address clinically relevant issues of organ loss or function in patients; (2) mid-term goals of combining biology with engineering principles that lead to elucidating the fundamentals of biological processes; and (3) short-term goals to develop new materials, structures, and devices for potential use in tissue engineering systems. With respect to the latter, this article has given some perspective and outlined specific examples regarding advancement in biomaterials and scaffold fabrication strategies. The development of biodegradable synthetic polymers is a relatively mature field in which a wide range of properties has been explored and developed rather extensively. Hence, future synthetic biomaterials development should focus on potentially engineering polymers to more accurately control specific cell function by virtue of these tunable properties. Incorporation of bioactive monomers into synthetic polymers is a potentially versatile method to realize this goal. Biomaterials development should also remain cognizant of the constraints of potential fabrication methods, which may serve as additional design constraints. Hence, the development of novel biomaterials will likely move forward in a synergistic manner with the development of novel fabrication methods to process these materials into systems with micro- and nanoscale structures. Of particular interest is the potential utilization of biomaterials interfaces to control cell function. Although the study of cell–nanotopography interactions is currently principally phenomenological, mechanisms of action and predictive responses of cells to nanotopography will be elucidated as the basic science governing these interactions is unveiled. By far, the most challenging aspects of tissue engineering is the design of large-scale systems in which biomimetic approaches lead to arbitrary and increasing levels of complexity. Although this aspect of tissue engineering remains in its infancy, there is a corresponding demand for developing creative solutions. Biomaterials-based systems with unique properties, many of which are reviewed here, should continue to evolve and mature as tissue engineering continues to serve as a prominent technology driver. The examples presented here suggest that it is possible to engineer systems to control cell function across many length scales, including the molecular, nanoscale, and systems level. The disciplines of tissue engineering and biology will benefit immensely as cell-biomaterial systems are cogently engineered in order to ultimately address the growing clinical demand for therapies based on regenerative medicine.

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