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Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering

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New generations of synthetic biomaterials are being developed at a rapid pace for use as three-dimensional extracellular microenvironments to mimic the regulatory characteristics of natural extracellular matrices (ECMs) and ECM-bound growth factors, both for therapeutic applications and basic biological studies. Recent advances include nanofibrillar networks formed by self-assembly of small building blocks, artificial ECM networks from protein polymers or peptide-conjugated synthetic polymers that present bioactive ligands and respond to cell-secreted signals to enable proteolytic remodeling. These materials have already found application in differentiating stem cells into neurons, repairing bone and inducing angiogenesis. Although modern synthetic biomaterials represent oversimplified mimics of natural ECMs lacking the essential natural temporal and spatial complexity, a growing symbiosis of materials engineering and cell biology may ultimately result in synthetic materials that contain the necessary signals to recapitulate developmental processes in tissue- and organ-specific differentiation and morphogenesis.

Biomaterials play central roles in modern strategies in regenerative medicine and tissue engineering as designable biophysical and biochemical milieus that direct cellular behavior and function[1–3]. The guidance provided by biomaterials may facilitate restoration of structure and function of damaged or dysfunctional tissues, both in cell-based therapies, such as those where carriers deliver transplanted cells or matrices induce morphogenesis in bioengineered tissues constructed *ex vivo*, and in acellular therapies, such as those where materials induce ingrowth and differentiation of cells from healthy residual tissues *in situ*. Such materials should provide a provisional three-dimensional (3-D) support to interact biomolecularly with cells to control their function, guiding the spatially and temporally complex multicellular processes of tissue formation and regeneration.

Both biologically derived and synthetic materials have been extensively explored in regenerative medicine and tissue engineering. In general, materials from natural sources (e.g., purified protein components such as collagens from animal tissues) are advantageous because of their inherent properties of biological recognition, including presentation of receptor-binding ligands and susceptibility to cell-triggered proteolytic degradation and remodeling. Despite these advantages, many issues have spurred the development of synthetic biomaterials as cellular substrates, including complexities associated with purification, immunogenicity and pathogen transmission. Although some of these limitations can be overcome by recombinant protein expression technologies[4], greater control over materials properties and tissue responses could be achieved were synthetic analogs available.

The last few years have marked a substantial paradigm shift in design criteria for modern synthetic biomaterials, fully integrating principles from cell and molecular biology: materials equipped with molecular cues mimicking certain aspects of structure or function of natural extracellular microenvironments are quickly being developed. This review considers the design and application of such synthetic biomaterials originating from a symbiosis of materials engineering and molecular cell biology. We highlight the role of the ECM and its interactions with cells in natural processes of tissue dynamics, examine the basic principles of materials science that could be applied to address mimicry and exploitation of those interactions for tissue engineering, and finally discuss more sophisticated synthetic materials that can interact with their biological environment to a level that allows them to participate actively in pathways of tissue morphogenesis. The materials focus is limited to 3-D applications and is on emerging classes of polymeric biomimetic materials, such as nanofibrillar, supramolecular materials formed by self-assembly processes, and matrices presenting individual or multiple biochemical ECM-derived signals. For a more comprehensive overview of biomaterials, also including nonpolymeric and naturally derived materials, as well as their successful application in biomedicine we refer to several excellent recent reviews[3,5,6].

Importance of cell-matrix interactions

Tissue dynamics, that is, its formation, function and regeneration after damage, as well as its function in pathology, is the result of an intricate

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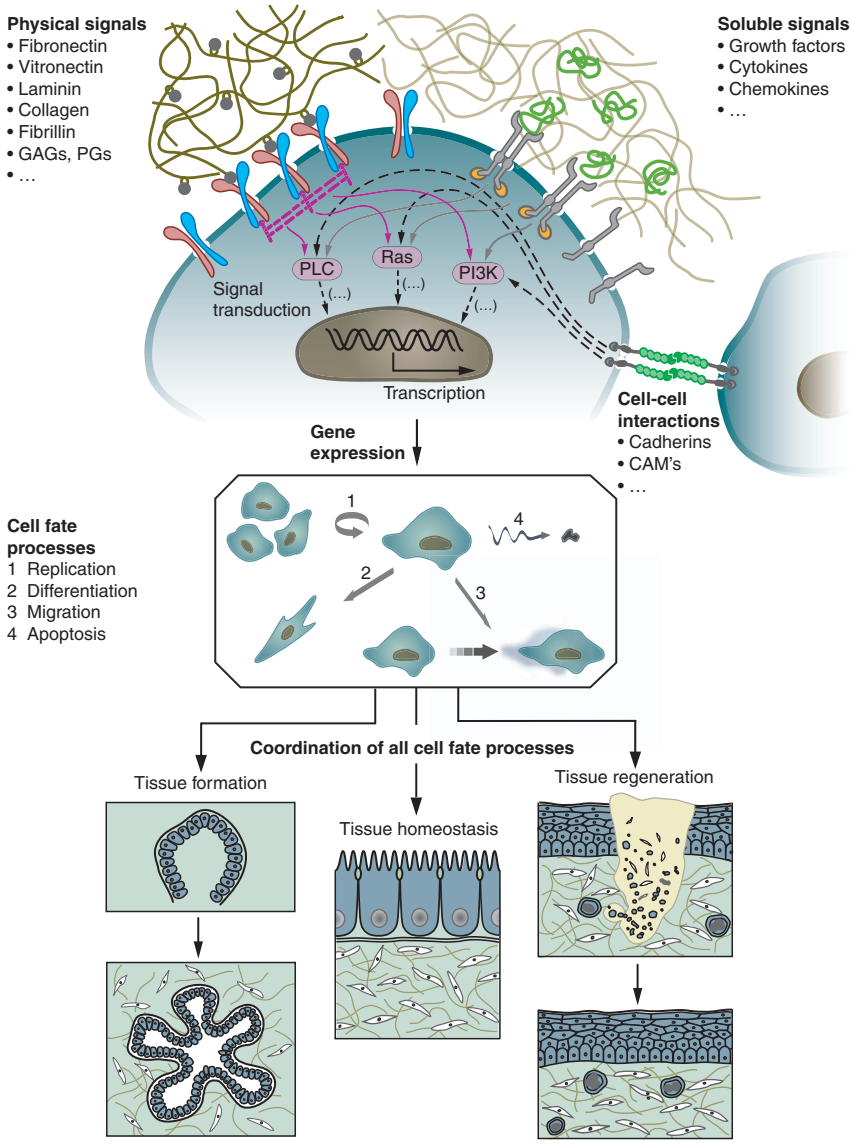

Figure 1 The behavior of individual cells and the dynamic state of multicellular tissues is regulated by intricate reciprocal molecular interactions between cells and their surroundings. This extracellular microenvironment is a hydrated protein- and proteoglycan-based gel network comprising soluble and physically bound signals as well as signals arising from cell-cell interactions. Adapted from ref. 112. Specific binding of these signaling cues with cell-surface receptors induces complex intracellular signaling cascades that converge to regulate gene expression, establish cell phenotype and direct tissue formation, homeostasis and regeneration. Ellipsis (…) indicates that the lists of signals are not intended to be complete. PLC, phospholipase C; GAGs, glycosaminoglycans; PGs, proteoglycans; CAMs, cell adhesion molecules.

temporal and spatial coordination of numerous individual cell fate processes, each of which is induced by a myriad of signals originating from the extracellular microenvironment (**Fig. 1**)[7]. In brief, a highly dynamic and complex array of biophysical and biochemical signals, transmitted from the outside of a cell by various cell surface receptors and integrated by intracellular signaling pathways, converge to regulate gene expression and ultimately establish cell phenotype. The extracellular microenvironment, which surrounds cells and comprises the molecular signals, is a highly hydrated network hosting three main effectors: (i) insoluble hydrated macromolecules (fibrillar proteins such as collagens, non-collagenous glycoproteins such as elastin, laminin or fibronectin, and hydrophilic proteoglycans with large glycosaminoglycan side chains) called physical signals in **Figure 1**, (ii) soluble macromolecules (growth factors, chemokines and cytokines) and (iii) proteins on the surfaces of neighboring cells. Thus, the ultimate decision of a cell to differentiate, proliferate, migrate, apoptose or perform other specific functions is a coordinated response to the molecular interactions with these ECM effectors. It is noteworthy that the flow of information between cells and their ECM is highly bidirectional, as, for example, observed in processes involving ECM degradation and remodeling.

Naturally derived model ECMs

Cell and matrix biologists have long realized that understanding cell behavior within complex multicellular tissues requires systematically studying cells within the context of specific model microenvironments. These model systems mimic to a certain degree the *in vivo* situation and at the same time may significantly reduce its complexity. Although two-dimensional (2-D) *in vitro* assays are still applied in many cell culture studies, there is increasing agreement that 3-D matrices provide better model systems for physiologic situations[8–13]. Indeed, many physiological (examples exist in morphogenesis and organogenesis) and pathological (e.g., in tumor growth) cellular processes have been demonstrated to occur exclusively when cells are organized in a 3-D fashion.

Cell biologists have a number of experimental model systems at their disposal[13]. These range from multicomponent matrices derived from cells or tissues (e.g., Matrigel, commercially available from BD Biosciences (San Jose, CA, USA), which is solubilized basement membrane preparations extracted from mouse tumors that contains several components of basement membranes enriched with laminin), to matrices composed of individual purified or recombinantly produced ECM proteins, and modified versions of these ECM components, as well as proteolytic or recombinant fragments. These matrices have been used in cell culture studies to recapitulate some aspects of both the organization and multicellular complexity of tissues and to gain insight into functions of the ECMs within diverse tissues and organs.

Therapeutic strategies in regenerative medicine and tissue engineering have greatly benefited from the above studies[14]. Natural ECM-derived biomaterials can be used as carriers for transplanted cells that are subsequently grafted into tissue defects[15,16], and also as cell infiltration matrices to induce regeneration and remodeling *in vivo*[17,18]. For example, collagen and fibrin are clinically well-established and FDA-approved matrices for wound healing to treat burns and chronic wounds, and as tissue sealants, respectively. More pertinent for biomedical and materials engineers, naturally derived materials represent valuable models from which one can derive engineering principles to create artificial materials with similar biological function[19,20].

Building biomimetic elements into synthetic materials

Although naturally derived biomaterials have proved effective in many basic and clinical applications, the need for custom-made matrices for tissue-specific cell biological investigations[12] drives recapitulation of their key characteristics in synthetic materials. These materials are still being developed to gain more control over the material and thus over the cellular behaviors they induce.

Natural ECMs. From a structural perspective, natural ECMs are gels composed of various protein fibrils and fibers interwoven within a hydrated network of glycosaminoglycan chains. In their most elemental function, ECMs thus provide a structural scaffold that, in combination with interstitial fluid, can resist tensile (via the fibrils) and compressive (via the hydrated network) stresses. In this context it is worth mentioning just how small a proportion of solid material is needed to build mechanically quite robust structures: in many cases less than 1%. Structural ECM proteins include collagens, some of which are long and stiff and thus serve structural functions, whereas others of which serve connecting and recognition functions, and elastin, which forms an extensive crosslinked network of elastic fibers and sheets. The anisotropic fibrillar architecture of natural ECMs has apparent consequences for cell behavior. Because of a tight connection between the cytoskeleton and the ECM through cell-surface receptors, cells sense and respond to the mechanical properties of their environment by converting mechanical signals into chemical signals[21,22]. Consequently, the biophysical properties of ECMs influence various cell functions, including adhesion and migration. Moreover, the fibrillar structure of matrix components brings about adhesion ligand clustering, which has been demonstrated to alter cell behavior[23]. Structural ECM features, such as fibrils and pores, are often of a size compatible with cellular processes involved in migration, which may influence the strategy by which cells migrate through ECMs[24].

Micro- and nanofibrillar synthetic biomaterials. The intricate fibrillar architecture of natural ECM components has inspired several researchers to produce materials with similar structure. Upon fibers that are tens of microns in diameter, cells seem to respond as though to a 2-D substrate, acquiring an unnatural flat shape, leading to a nonphysiological, asymmetrical occupation of adhesion receptors; notwithstanding, such matrices have already shown remarkable success in tissue engineering applications, such as in the reconstruction of a dog urinary bladder[25] or as scaffolds for neural stem cells to facilitate regeneration after brain injury in a mouse stroke model[26]. Polymer processing technologies such as electrospinning[27] allow fiber formation down to the 10 nm scale. One difficulty in nanofiber technology has been in placing cells within a nanofibrillar structure with pore spaces much smaller than a cellular diameter; somehow the network must be formed *in situ*, around the cells, without cellular damage.

Important progress has been made using supramolecular self-assembly to form nanofibrillar matrices *in situ*[28]. Inspired by the understanding of protein self-assembly, these approaches use noncovalent intermolecular interactions to fabricate higher order structures by self-assembly of oligomeric peptide, nucleotide and nonbiological amphiphilic building blocks[29,30]. Whereas many of these systems require self-assembly under conditions that are intolerable to cells, several can gel at near-physiologic conditions. For example, Zhang and coworkers developed a class of nanofibrillar gels with very high water content (>99%) crosslinked by self-assembling of self-complementary amphiphilic peptides in physiological medium. Under appropriate culture conditions, these matrices have been demonstrated to maintain the functions of differentiated neural cells[31] and chondrocytes[32], and to promote the differentiation of liver progenitor cells[33]. Although not equipped with any specific biofunctional ligands, these gels are scaffolds that biomechanically organize cells in a 3-D fashion. Deming and coworkers have presented fibrillar hydrogels from diblock copolypeptide amphiphiles[34]; self-assembly occurs at low solid content and the mild gelation conditions support cell encapsulation[35]. Rational design principles have been put forward to control fiber morphology (e.g., to produce kinked, wavy or branched fibers) and thus scaffold architecture[36]. Stupp and coworkers have presented the next step in such supramolecular gels by synthesizing self-assembling oligomeric-amphiphiles that allow incorporation of specific biomolecular signals[37]; encapsulated neural progenitor cells were observed to differentiate into neurons within scaffolds presenting the laminin-derived peptide IKVAV[38]. This very promising result underscores the potential of incorporating both biomechanical and biomolecular cues.

Nonfibrillar synthetic polymer hydrogels. As the hydrogel character of the natural ECM is one of its key features, it is not surprising that synthetic hydrogels have found important roles in biology and medicine[3,39–41]. Several distinctive features make synthetic hydrogels excellent physicochemical mimetics of natural ECMs. The molecular architecture of crosslinked, hydrophilic polymers can result in tissue-like viscoelastic, diffusive transport, and interstitial flow characteristics. Of critical importance for cell-containing hydrogels, reaction schemes have been developed that are sufficiently gentle to allow formation *in situ*, in the presence of cells, just as gels from ECM components are formed[42]. Such mild chemistries can even be carried out *in vivo*, for example, using minimally invasive surgical techniques, directly within tissue defects. It is possible to incorporate a number of biological characteristics within synthetic hydrogels, including cell adhesion ligands, proteolytic susceptibility and biologically relevant elasticity, as described below.

Materials that present insoluble ligands. The ECM provides bound multifunctional adhesion ligands, including fibronectin, vitronectin and laminin that guide the development and maintenance of cell function. The integrins, a large family of transmembrane, heterodimeric, cell-surface molecules, function as the principle receptors of animal cells for many of these ECM adhesion molecules. Integrins primarily link the macromolecules of the ECM with the cell's cytoskeleton, but are involved as well in cell-cell adhesion and binding to proteases. When bound to ECM ligands, integrins cluster and form associations with various signal transducing molecules to activate specific signaling pathways including those regulated by protein kinase C, the small GTPases Rac and Rho, and MAP kinase. As such, integrins transmit information across the cell membrane and are critical regulators of cell adhesion and migration as well as many other cell functions[43,44].

The pioneering identification of small oligopeptide sequences within ECM adhesion proteins[45] opened an important door to creation of ligand-functionalized materials. Indeed, numerous cell-adhesive ligands have been grafted to materials, as reviewed elsewhere[46,47]. The creation of such highly defined synthetic ECM analogs, in which ligand type, concentration and spatial distribution can be modulated upon a passive background, may help in deciphering the complexity of signaling in cell-ECM interactions. Relevant studies include work on the quantitative information on the ligand density required for a particular cellular response[48]; the influence of adhesion ligand density on cell migration (that is, the discovery of intermediate adhesion strength for optimal cell migration) in 2-D[49,50] and also in 3-D, in modified biopolymer matrices[51–53] and synthetic gels[54,55]; the finding that cells respond to the nanoscale spatial organization of adhesion ligands[23,56]; the relevance of ligand gradients[57] and finally studies on the coregulation of signals[58,59]. These studies provide several examples that well-controlled biomaterial matrices can yield insight into basic cell biological principles.

Materials that enable binding and release of soluble effectors. Natural ECMs modulate tissue dynamics through their ability to locally bind, store and release soluble bioactive ECM effectors such as growth factors to direct them to the right place at the right time[60]. When many growth

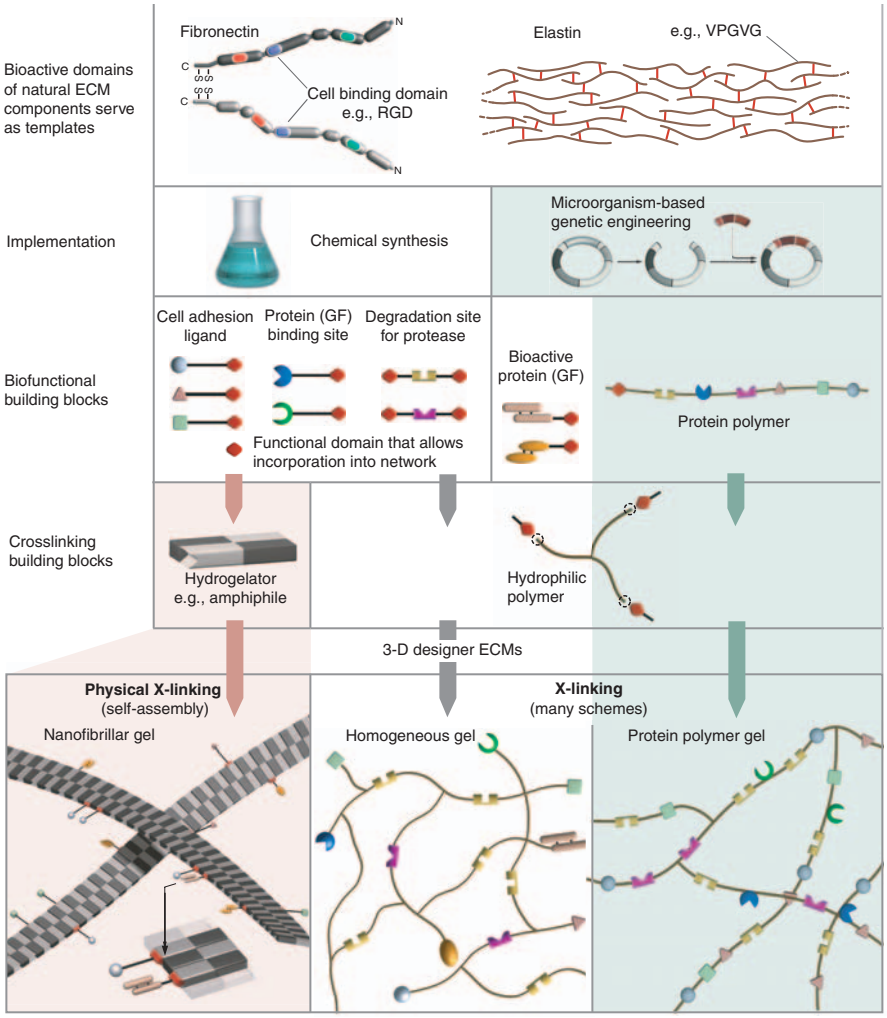

Figure 2 Design strategies for the creation of synthetic biomolecular materials that mimic the complexity of natural ECMs. Bioactive domains of naturally occurring proteins are identified as building blocks (top) and synthesized by either chemical strategies or by protein engineering (recombinant technology). The most important components include cell-adhesive ligands (such as integrin-binding peptides of the prototypical RGD family), binding sites for growth factor (GF) proteins, domains with susceptibility to degradation by cell-secreted or cell-activated proteases to facilitate bidirectional cell-matrix interactions, but also domains with structural function (such as the elastin-derived peptide sequence VPGVG). Synthetic networks can than be obtained by crosslinking of these biofunctional components (from an entire array of building blocks) by distinct crosslinking schemes, involving physical (self-assembly to produce nanofibrillar gels) or chemical mechanisms. The use of such synthetic approaches in ECM design may allow matrices to be tailor-made for a specific cell or tissue.

factors bind to ECM molecules through, for example, electrostatic interactions to heparan sulfate proteoglycans, it raises their local concentration to levels appropriate for signaling, localizes their morphogenetic activity, protects them from enzymatic degradation and in some cases may increase their biological activity by optimizing receptor-ligand interactions. As growth factors are required in only very tiny quantities to elicit a biological response, the main focus in designing synthetic matrices for growth factor presentation has been to control local growth factor concentration. Several strategies to engineer growth factor release from biomaterials have been presented over the past years and some initial success has been reported in animal models for the regeneration of bone and skin as well as the induction of vascularization, as reviewed elsewhere[61–63]. As many cellular processes involved in morphogenesis require a complex network of several signaling pathways and usually more than one growth factor, recent research efforts have focused on schemes for sequential delivery of multiple growth factors[64]. The use of biological feedback mechanisms in growth factor delivery has also been explored[65]. In this case, a growth factor is bound to the matrix and released upon cellular demand through cell-mediated localized proteolytic cleavage from the matrix[66,67]; this approach substantially mimics the mechanism by which these factors are released *in vivo* from stores in the natural ECM by invading cells in tissue repair.

Stimulus-sensitive materials. Whereas many synthetic biomaterials have been designed to degrade by ester hydrolysis, such nonenzymatic hydrolysis of matrices is uncommon *in vivo*. Rather, the macromolecular components of natural ECMs are degraded by cell-secreted and cell-activated proteases, mainly by matrix metalloproteinases (MMP) and serine proteases. This creates a dynamic reciprocal response, with the ECM stimulating the cells within it and cellular proteases remodeling the ECM and releasing bioactive components from it. As discussed later in more detail, cell-induced proteolysis is often required for 3-D cell migration and invasion, because the porosity of the ECM may lead to barrier function and thus impede migration[68].

Exciting progress has been made in mimicking the proteolytic recognition of natural ECMs in synthetic polymer gels, as examples of a larger class of biomolecule-sensitive networks[69]. Pioneered by early work of Kopecek and coworkers[70], several novel methodologies to create synthetic hydrogels with sensitivity to proteases, including plasmin[71,72], MMPs[55,73] or both of these protease families[54,74,75] have been developed. Proteolytic sensitivity in these materials has been achieved either by stepwise copolymerization of hydrophilic polymers and proteolytically sensitive oligopeptide or protein building blocks or by photo-crosslinking of telechelic peptide-flanked PEG copolymers.

Principles of morphogenesis applied to tissue engineering

Much of the preceding discussion has focused on the structure and function of natural ECMs and how it can be mimicked in synthetic materials to control distinct and relatively simple cell fate processes. As the dynamic state of a tissue is regulated by a highly complex temporal and spatial coordination of many different cell-matrix and cell-cell interactions, such reductionist materials approaches may fail in imitating the complexity of natural ECMs. Therefore, more complex biomaterial systems may be required that contain molecular cues to recapitulate or induce developmental processes in tissue- and organ-specific differentiation and morphogenesis.

Morphogenesis during tissue development is regulated by a number of protein families including hedgehog proteins (Hhgs), Wnt proteins,

Notch ligands, members of the transforming growth factor beta (TGFβ) superfamily such as bone morphogenetic proteins (BMPs), or fibroblast growth factors (FGFs) and fibronectin. These morphogens control self-renewal, migration, differentiation as well as other cell fate processes of uncommitted stem or progenitor cells. The question of how the right quantity of a signaling molecule is detected by the right cells at the right time is the subject of extensive investigation. Once differentiation by the interpretation of morphogenetic signals and gradients has occurred, local cell-cell interactions establish boundaries between different populations of cells.

Intriguingly, morphogenetic processes similar to the ones observed during embryonic development also occur during regeneration of some adult human tissues and therefore regeneration may be regarded as a postembryonic recapitulation of fundamental developmental processes[76,77]. Tissues that have the capacity to regenerate include epithelia, liver, blood, and to a limited extent bone and muscle. In many other tissues, however, the response to damage is the formation of collagen-rich scar tissue following an acute inflammatory response. Although rapid scar tissue formation represents a powerful defense mechanism against infection, it may severely compromise tissue function (e.g., in spinal cord injuries or myocardial infarction). In very general terms, functional tissue recovery can only occur when (i) regeneration-competent progenitor and stem cells are present (e.g., satellite cells in the muscle), and (ii) when these cells are conducted into a regeneration pathway by the presence of relevant morphogenetic signals, or alternatively (iii) when the regenerative process is not suppressed by signals that give way to rapid scar formation. An increased understanding of the cellular and molecular foundations of tissue development and regeneration has paved the way towards more effective therapies for regenerative medicine and tissue engineering[78,79]. A potential lack of regeneration-competent progenitor or stem cells in a particular tissue defect can be overcome by transplanting such cells, either isolated or after integration (and eventual differentiation) into bioengineered tissues constructed from cells and matrices *in vitro*. Several sources of adult stem cells are now readily available and significant progress has been made in controlling their differentiation into multiple lineage pathways. Alternatively, tissue regeneration may be promoted by the application of appropriate biomaterial-based microenvironments to stimulate regeneration *in vivo*. Such matrices should contain signals to attract regeneration-competent cells and to stimulate their proliferation and differentiation into tissue-specific cells, or to block regeneration-suppressing signals.

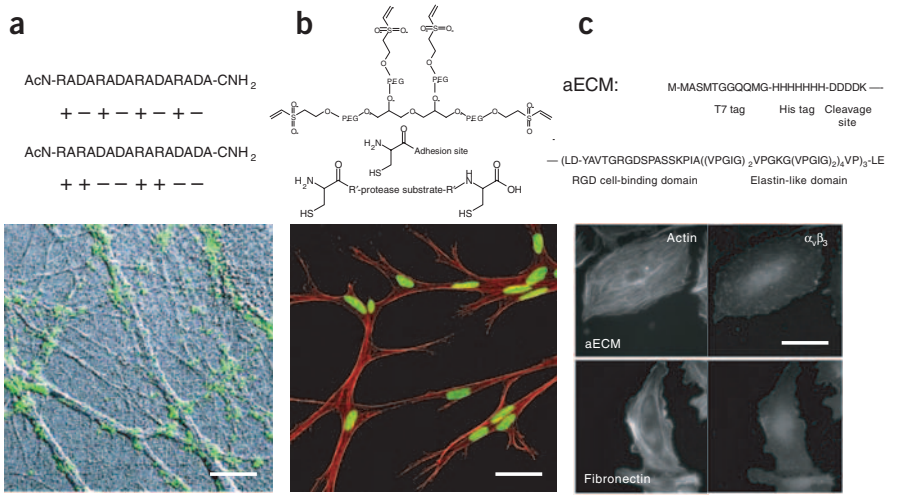

Figure 3 Examples of complex synthetic ECM mimetics proposed in **Figure 2**. (**a**) Nanofibrillar hydrogels formed under physiological conditions from ionic self-assembling peptides (top). These networks support neuronal cell differentiation and extensive neurite outgrowth (bottom). Scale bar, 10 μm. Adapted and reprinted with permission from T.C. Holmes *et al*[31]. © 2003 National Academy of Sciences, USA. (**b**) Hybrid gels formed from cysteine-bearing cell-adhesive and proteolytically degradable peptide building blocks and vinylsulfone-functionalized PEG macromers (top). These gels enable extensive 3-D migration of primary fibroblasts by matrix metalloproteinase- and integrin-dependent mechanisms and, because of localized matrix proteolysis, the morphogenesis of single cells into multicellular structures (bottom). Scale bar, 40 μm. Adapted and reprinted with permission from M.P. Lutolf *et al*[80]. © 2004 Wiley-VCH. (**c**) Creation of synthetic ECMs from artificial protein polymers (aECMs, represented here by one example of a broader family) containing bioactive domains derived from elastin and fibronectin (top). Sequence-specific adhesion of human umbilical vein endothelial cells to bioactive proteins can be achieved by this approach. A similar adhesion behavior of the aECM compared to fibronectin can be observed (bottom). Responses to an artifical ECM (aECM, upper panels) are remarkably similar to responses to a natural extracellular matrix molecule, fibronectin (lower panels). This is true at both the level of the cytoskeleton (left panels) and at the level of the adhesion receptors (right panels). Scale bar, 25 μm. Adapted and reprinted with permission from Liu J.C. *et al*[85]. © 2004 American Chemical Society.

Recapitulating natural ECMs in synthetics: biological cues

Mimicking natural ECMs that regulate complex morphogenetic processes in tissue formation and regeneration necessitates novel design strategies for synthetic biomaterials (**Fig. 2**). These synthetic materials should be biologically multifunctional hydrogel networks, synthesized under physiological conditions, that both biochemically and biophysically mimic natural ECMs. Their functionality should be adjustable to a particular biological environment to obtain cell- and tissue-specificity. Ideally, one would create them from an array of biologically functional building blocks, in some form of a modular design. The precursor building blocks could be crosslinked into solid networks by several means (**Fig. 2** and **Fig. 3**): (i) Small organic gel-formers, such as peptides or peptide-amphiphiles, containing binding sites for biologically functional ligands[38], can be designed to self-assemble into supramolecular structures, allowing the creation of heterogeneous nanofibrillar ECM mimetics[28] (**Fig. 2**, bottom left; **Fig. 3**, left). (ii) Hybrid gels can be formed from bioactive building blocks bearing chemically reactive functional groups (such as amines or thiols)[80] or physically interactive groups[81] and end-functionalized hydrophilic polymers such as N-(2-hydroxypropyl)-methacrylamide (HPMA) or PEG that act as chemical or physical crosslinkers (**Fig. 2**, bottom middle; **Fig. 3**, middle). (iii) Recombinant DNA technology can be used to create artificial protein polymers with desired bioactive domains *de novo*[82–85] (**Fig. 2**, bottom right; **Fig. 3**, right). Genes corresponding to structural and functional elements found in natural ECMs can be synthesized, cloned and expressed in a convenient production host. Such protein polymers can be covalently crosslinked into a network, for example, by reaction with functionalized hydrophilic polymers[71] or other chemical crosslinkers[86] targeting amines or thiols on the protein polymer, by radiation crosslinking[87] or through self-assembly by protein-protein interactions[88].

Synthetic ECMs that enable 3-D cell migration. Cell migration through extracellular matrices is fundamental to morphogenetic

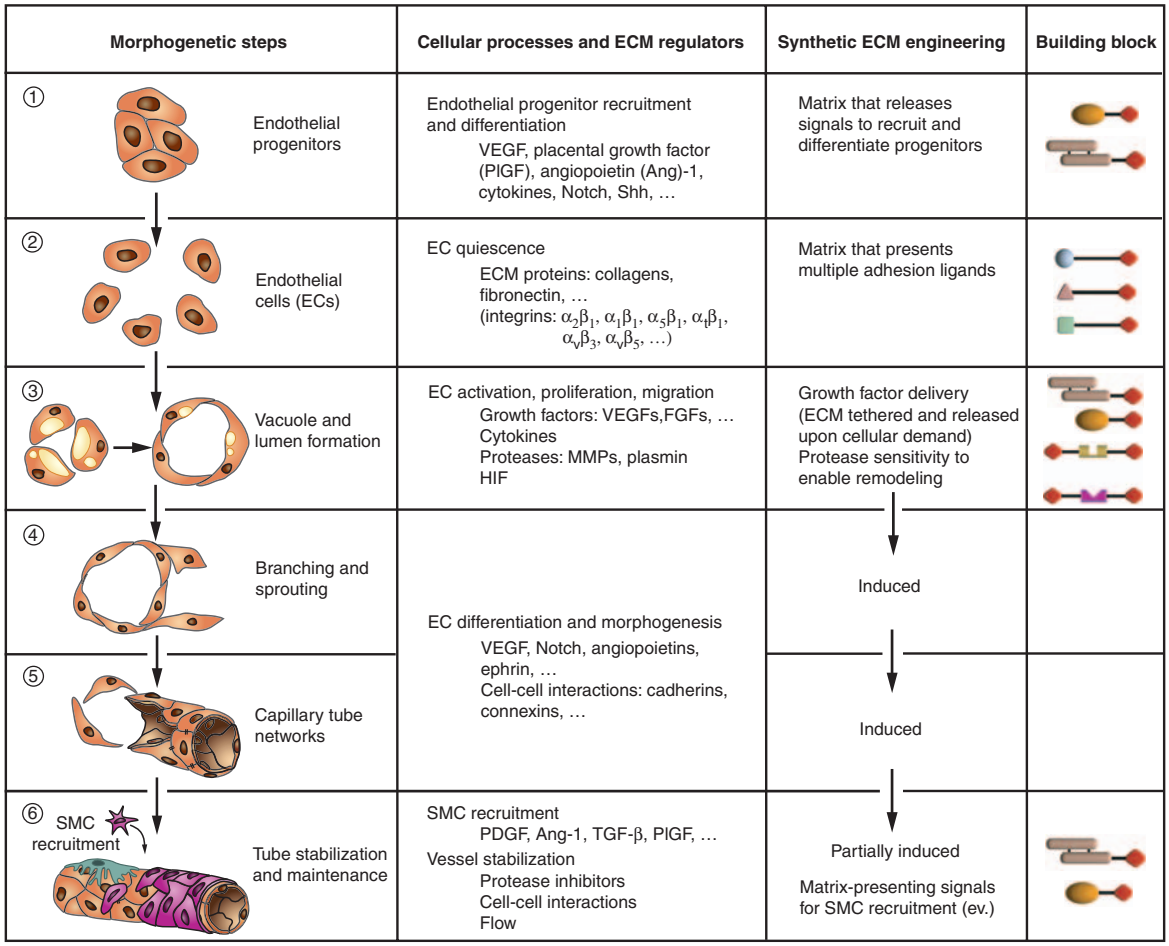

Figure 4 Morphogenetic steps and underlying regulatory molecules involved in endothelial cell assembly into capillary tube structures, and subsequent stabilization of tubes into mature blood vessels. Highly complex, multifunctional synthetic matrices (with hypothetical building blocks corresponding to the one in **Figure 2**) will be required to recapitulate these processes in the future.

processes in tissue development, homeostasis and regeneration[89]. Migration occurs, for example, in response to gradients of soluble or insoluble signals and changes in cell-cell contacts. On planar surfaces, many key aspects of the underlying regulatory pathways have been identified[90]. 2-D migration can be regarded as a cyclic process involving multiple steps: polarization of the cell and extension of protrusions in the direction of migration, stabilization of protrusions by adhesion to the ECM, forward movement by contraction and cell detachment at the rear. Not surprisingly, integrins play an important role by providing traction for the forward movement and also by transmitting ECM guidance signals. In 3-D, migration is further complicated by the need to overcome the biophysical resistance of the surrounding ECM. Depending on cell and ECM type, migration of single cells in 3-D can involve proteolytic (most commonly) and nonproteolytic (e.g., in leukocytes) strategies[24]; recent studies on tumor cell migration provide evidence for a cellular plasticity in selecting a particular migration strategy[91]. During proteolytic migration, cells clear a path by secreting and activating proteases, including MMPs, serine proteases and hyaluronidase, that specifically degrade protein or proteoglycan components of the pericellular matrix. Degradation is highly localized because of the involvement of membrane-bound proteases (e.g., MT-MMPs), complexation of soluble proteases to cell surface receptors (e.g., MMP-2 interaction with integrin $\alpha_v\beta_3$, and binding of urokinase plasminogen activator to urokinase plasminogen activator receptor), and a tightly regulated balance between active proteases and their natural inhibitors (such as inhibition of MMPs by TIMPs). On the other hand, amoeboid migration is driven by cell shape adaptation (that is, squeezing through preexisting matrix pores) and deformation of the ECM network.

Synthetic materials must contain cell-adhesive ligands for traction, as well as space for forward movement. Nonproteolytic migration is thus enabled by cell-adhesive matrices with preformed macroscopic pores (e.g., ref. 92), however, only when the minimal pore size is larger than the cell diameter. Nonproteolytic migration may also occur in soft (physically crosslinked) fibrillar networks[31,38], probably by a combination of squeezing through pores and fiber deformation or even rupture.

In perhaps the most biomimetic concept for 3-D cell migration in synthetic ECMs, proteolytic pathways have been exploited. In one approach, photopolymerized hydrogels have been synthesized by polymerization of acrylated PEG derivatives containing peptide substrates for plasmin and MMPs in their backbone, and cell adhesive peptides grafted on one end in a pendant fashion[54,74,75]. A related system has been described based on conjugate addition reactions[72,80]. Crosslinking occurs upon stepwise copolymerization of two building blocks, namely biologically active peptide sequences as protease substrates (for MMPs or plasmin) that contain flanking cysteine residues with the reactive thiols, and end-functionalized multiarm polyethylene glycol (PEG) macromers serving

as a crosslinking entity responsible for the networks biophysical characteristics. The simultaneous incorporation of adhesion ligands as pendant functionalities into the networks has been demonstrated to enable integrin-dependent proteolytic (as demonstrated independently for MMPs and plasmin) 3-D migration of fibroblasts and endothelial cells. The cell migration rate in these networks can be controlled almost independently by several characteristics of the matrix[55], including its physicochemical characteristics, adhesion ligand density or proteolytic sensitivity of the cysteine-containing peptide. Synthetic materials that support proteolytic migration have also been engineered from protein polymers, by grafting PEG diacrylate onto artificial, cell adhesive and plasmin-sensitive protein polymers containing multiple cysteine residues[71].

Synthetic ECMs that can control stem cell fate: engineering stem cell niches. Tissue formation, homeostasis and regeneration are critically dependent on stem cells and their commitment to differentiated lineages. Although knowledge about signals and the underlying pathways regulating stem cell fate are being identified rapidly, significant technical obstacles need to be overcome before stem cells can be used efficiently and safely in the clinic[93,94]. One of the main challenges appears to be the control of stem cell fate outside of the cells' natural environment. Adult stem cells normally reside within specific extracellular regulatory microenvironments—stem cell niches—consisting of a complex mixture of soluble and insoluble, short- and long-range ECM signals, which regulate their behavior[95,96]. These multiple, local environmental cues are integrated by cells that respond by choosing self-renewal or a pathway of differentiation. Outside of their niche, adult stem cells lose their developmental potential quickly[97]. The design of synthetic materials that mimic natural stem cell microenvironments may be a potentially powerful tool to both understand and control stem cell function.

A variety of artificial stem cell microenvironments are being explored in the context of neural stem cell fate control. Mahoney and Saltzman have designed a synthetic microenvironment useful as a transplantation vehicle based on polylysine-coated poly(lactide-co-glycolide) microparticles loaded with nerve growth factor-beta[98]. The combination of a cell-adhesive matrix and a controlled-release scheme for a morphogenetic factor allowed them to control fetal brain cell survival and differentiation in a rat model. Liu and colleagues have pioneered the concept of engineered stem cell niches by developing a family of artificial ECM proteins to control neural stem cell function[99,100]. Protein polymers were expressed in *Escherichia coli*, consisting of an elastin backbone along with two ligands to the Notch receptor, namely the active domain of hJagged1 and hDelta1. Synthetic biomaterials with less biological functionality may also be beneficial because they function as a mechanical scaffold for stem cells to support cell growth and to bridge large tissue defects[26,101]. Combinatorial and microarray approaches have been adopted recently in high-throughput screening of combinatorial materials[102,103] to control embryonic stem cell fate on polymer surfaces[104]. Such high-throughput approaches may also hold much promise in 3-D screening of libraries[105] of biofunctional groups such as morphogenetic proteins incorporated within synthetic 3-D materials.

Synthetic ECMs permit cellular remodeling and tissue regeneration *in vivo*. Synthetic biomimetic materials have been developed to serve as provisional matrices for tissue regeneration *in vivo*. Based upon the paradigm of fibrin's function as a temporary matrix in tissue repair, biomimetic characteristics for synthetic materials needed to induce regeneration *in vivo* are the presence of (i) ligands for cell adhesion (ii) a mechanism of relatively rapid and localized matrix dissolution, ideally in temporal and spatial synchrony with cell invasion, and (iii) the delivery of morphogenetic signals to attract endogenous progenitor cells and induce their differentiation to a tissue-specific pathway. When bioactive materials were designed, based on this rationale, to be sensitive to MMPs or plasmin, and also contained an integrin-binding adhesion ligand and the bone-inducing growth factor BMP-2, complete matrix remodeling into bone was observed when the materials were implanted in bone defects[72,106]. This design of a cell-adhesive and cell-responsive (that is, provisional) matrix may be extended to other applications in regeneration.

Blood vessel growth represents another major challenge. The lack of a functional vasculature is the cause of numerous pathologies and is also a major stumbling block for successful cell-transplantation tissue engineering therapies[107]. Three distinct mechanisms of vessel growth are known: vasculogenesis (*de novo* formation of blood vessels by endothelial progenitors), angiogenesis (sprouting of vessels from preexisting ones) and arteriogenesis (stabilization of blood vessels by mural cells). Many regulating soluble signals and their receptors have been identified. The major signals include vascular endothelial growth factors (VEGFs), FGFs, TGFs, angiopoietins, ephrins, placental growth factors and various chemokines[108]. As mentioned above, synthetic biomaterials have been developed that are able to deliver such factors with controlled pharmacokinetics. However, an entire cascade of morphogenetic processes is required for blood vessel formation within a biomaterial matrix, with many signals being provided by the extracellular milieu[109] (**Fig. 4**). Recent progress has been made by our group with biomimetic matrices toward this end as well: MMP-sensitive, integrin-binding and VEGF-containing matrices have been replaced by well-formed new blood vessels in animal models[65]. In this case, an important relationship between local growth factor dose and blood vessel morphology seems to be apparent, with only low local concentrations leading to well-formed structures and higher concentrations leading to hyperpermeable vasculature[110,111]. Thus in this application the concept of the cell directing the release of growth factor seems particularly valuable since the tissues responded in a physiological manner, forming healthy vessels in response to the matrix-bound VEGF.

Future challenges

Although considerable progress has been made already, much work remains to develop biomaterial and biomolecular approaches that recapitulate the elaborate biological recognition and signaling functions of the extracellular milieu for application in tissue engineering, repair and regeneration. Ongoing challenges remain in controlling the dynamics and spatial organization of presentation of multiple signals.

Toward modulation of dynamics, the use of stimulus-sensitive linkers, protecting groups and exposing mechanisms may provide paths forward. Combination of display approaches using both bioresponsive and time-sensitive mechanisms may be particularly powerful. It may be possible to exploit biomechanical and biochemical stimuli to expose cryptic biomolecular signals in synthetic biomaterials, as also occurs in some natural ECM molecules.

Toward modulation of spatial arrangement, hierarchically complex tissues may require the establishment of boundaries between different tissue and cell types. Accomplishing this could be imagined by incorporating cell-type-specific chemotactic factors in a spatially controlled manner, by using or upregulating self-segregating molecules, such as cadherins, or by incorporating potentially boundary-forming signals, such as the ephrins. Coculture and cotransplantation of different cell types may also enable hierarchical segmentation.

As to the signals themselves, the number of molecule families and even entire biomolecule classes remain underexploited in tissue engineering and regeneration. Many signals involved in embryonic development, such as Wnts, hedgehog proteins or Notch ligands, may be important to control adult stem cell self-renewal. Likewise, it may be particularly interesting to manipulate transcription directly, for example,

by biomaterial matrix-controlled delivery of molecules that manipulate expression of transcription factors regulating development or morphogen expression.

As to more basic investigations, until recently, cell and matrix biologists have almost exclusively used natural ECM-derived materials as 3-D model systems. Precise control over the extracellular microenvironment may be useful not only in tissue engineering and regeneration, but also in more basic studies of development and pathogenesis and it is likely that we will see many payoffs of such strategies in the near future.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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