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Polymers to direct cell fate by controlling the microenvironment

R Warren Sands and David J Mooney

Enhanced understanding of the signals within the microenvironment that regulate cell fate has led to the development of increasingly sophisticated polymeric biomaterials for tissue engineering and regenerative medicine applications. This advancement is exemplified by biomaterials with precisely controlled scaffold architecture that regulate the spatio-temporal release of growth factors and morphogens, and respond dynamically to microenvironmental cues. Further understanding of the biology, qualitatively and quantitatively, of cells within their microenvironments and at the tissue–material interface will expand the design space of future biomaterials.

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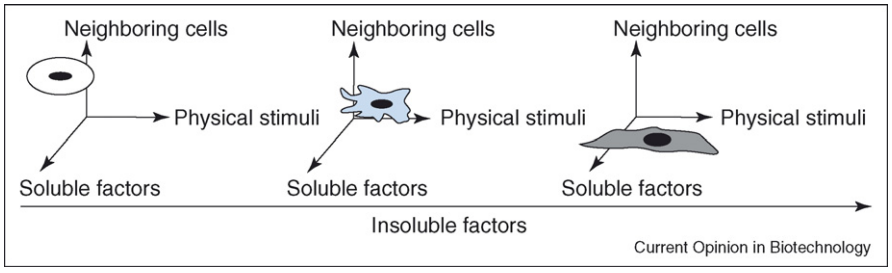
Introduction

Tissue engineering and regenerative medicine hold the promise to treat and even cure a wide range of diseases ranging from acute pathology (e.g. traumatic injury) to chronic diseases (e.g. cardiovascular disease, cancer, and diabetes) and materials will typically play a prominent role in these therapies. In addition to maintaining, replacing or regenerating lost, diseased, or damaged tissues, tissue engineering, and regenerative medicine may provide artificial tissues for extracorporeal support, pharmacologic testing, or the enhancement of normal tissue function. The field has already contributed model systems to facilitate basic biological studies and led to the development of FDA-approved therapies [1]. Materials are a cornerstone of tissue engineering and regenerative medicine and have grown from inert mechanical supports, physical bridges, or cell and drug delivery vehicles with poorly understood biologic functions or limited control to dynamic substrates that serve as cell instructive materials by directing interactions at the tissue–material interface. By better understanding and incorporating elements of the cellular microenvironment, this new generation of materials promises to allow greater control over cell fate and ultimate tissue structure and function. This review will address the recent progress in material designs and fabrication approaches that are leading to the development of increasingly multifunctional mimics of the native cellular microenvironment. As the fundamental biology of the cellular microenvironment is often the inspiration for material design, this review will begin with a brief discussion of the cellular milieu, and then highlight polymers that direct the tissue–material interface through the controlled presentation of specific cues in time or space, or in response to external signals. Finally, a discussion of current and potential future approaches to further develop a quantitative understanding of the biology within cellular microenvironments and at the tissue–material interface will be provided as this knowledge will enrich the palette for future polymer design. The chemistry and physical properties of materials are critical parameters for directing cell fate and are discussed as they pertain to the spatial control of the scaffold architecture, the spatial and temporal control of morphogen and growth factor delivery, and dynamic polymer design; for a more comprehensive review on these topics the reader is encouraged to refer to other recent articles [2,3].

The cellular microenvironment

The cell is immersed in a dynamic landscape composed of insoluble macromolecules of the extracellular matrix (ECM), soluble bioactive factors, and neighboring cells (Figure 1). The landscape varies from tissue to tissue and changes during disease and aging. Three of the principle molecular signals within this environment include insoluble hydrated macromolecules, soluble molecules, and cell surface proteins [4]. These signals are sensed, integrated, and processed by the cell to determine behavior and function, and information is passed bi-directionally as the microenvironment is remodeled by the cells. Information is encoded within the chemical identity, localization, duration, and context of these molecular cues. Spatial cues are displayed in 3D [5] and can include signaling gradients such as that observed during chemotaxis, haptotaxis, and mechanotaxis. Moreover, spatial cues are found at many length scales. Architectures range from the nanometer to the centimeter length scales [2,6–10], exemplified by ECM fibers, cells, and tissue tubes, folds, and bends. In addition, the concentration, duration, and context of the molecular cues contain information that dictates cell fate. As exemplified by angiogenesis, certain growth factors initiate angiogenesis, while a second group of growth factors induces maturation. Later, a third cohort of molecules maintains the integrity of the established vasculature [11,12]. If the

Figure 1



4D pseudo-phase diagram of cell fate. The cellular microenvironment is composed of signals from neighboring cells, physical stimuli, soluble factors including growth factors, and insoluble molecules such as the extracellular matrix. The effects of these variables are plotted as different axes on this 4D diagram of cell fate (e.g. differentiation) and are symbolized in this illustration by the different shapes and colors of the cells located at different positions in space. In advanced tissue engineering and regenerative medicine the biomaterial may direct cell fate through any of the variables. The signals from the biomaterial may change over time as a result of pre-programmed spatio-temporal control or in response to the microenvironment, allowing for the recapitulation of complex signaling pathways.

appropriate concentration, duration, and context (e.g. presence and sequence of multiple factors) are not achieved, poor vascularization results. Finally, the microenvironment changes dynamically over time. The ECM is continually processed, degraded, and synthesized anew, altering the matrix's presentation of chemical cues and elasticity, and leaving behind proteolytic fragments and cryptic domains that in turn affect cellular activity [13,14]. Meanwhile, soluble bioactive factors are secreted and destroyed as the cells migrate, differentiate, proliferate, and undergo apoptosis.

Spatial control of the scaffold architecture

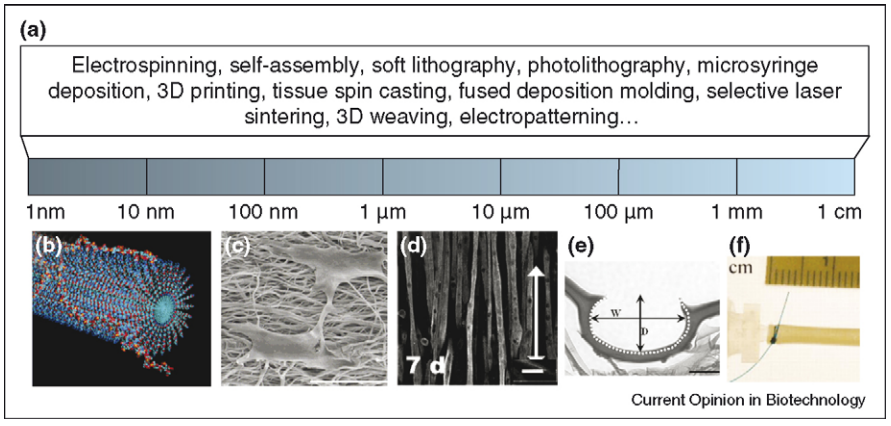
Polymeric materials designed in the past incorporated many signals found within the microenvironment but typically only provided one or two cues in a static manner. The focus of current efforts is to dynamically encode the localization, duration, and context of multiple cues to adequately integrate signaling and direct cell fate in light of the host microenvironment. This aim requires control over architecture at multiple size scales, spatiotemporally regulated release of signaling molecules, and dynamic polymer design.

Within the past decade, a dramatic increase in the resolution of control over scaffold architecture has been achieved through microfabrication technologies and has led to the recent widespread use of this technology for tissue engineering and regenerative medicine applications. In comparison to traditional polymer processing methods, microfabrication, and more recently nanofabrication, may allow for design at the nanometer to the supramillimeter length scale (Figure 2) [15,16] and for exquisite control of the internal architecture of the material *a priori*, facilitating the patterning of immobilized chemicals, cells, and mechanical gradients [17]. High-resolution spatial control could benefit a wide range of applications from artificial blood vessels with compliances that match host tissue to directional nerve guidance channels to aid neural regeneration. In many fabrication procedures, however, increased spatial resolution comes at the expense of long fabrication times. Thus, efforts are underway to create high throughput technologies suitable for bulk fabrication of a wide range of materials that can maintain spatial resolution.

Soft lithography and multiphoton photocrosslinking allow for submicrometer to supramillimeter spatial control. Soft lithography has been used with microfluidics to create hydrogels with gradients of adhesive ligands and mechanical properties [18] and complex spatial gradients can be readily generated [19]. Increased 3D resolution down to the submicron range can be achieved with multiphoton photocrosslinking but at the expense of fabrication time. To decrease processing time, mask-based methods for rapid prototyping have been developed [20] and could be further improved if used in tandem with a micro-mirror-based masking device [21•]. The utility of microfabrication techniques has been demonstrated in the design of 3D skin substitutes with an artificial epidermis containing micrometer scale features similar to the rete ridges and dermal papillae found in normal skin at the epidermal–dermal junction [22]. The depth and width of the engineered invaginations was demonstrated to influence keratinocyte stratification and differentiation.

Electrospinning has emerged as a prominent method for nanometer scale design not addressable with traditional microfabrication techniques, at the expense of control over internal scaffold architecture, void space connectivity, and mechanical properties. However, both synthetic and natural polymers can be electrospun into fibers with diameters ranging from the 10s of nanometers to several micrometers [23,24]. The goal of recent work has been to create more complex materials with defined internal architectures. To this end, composite materials and aligned fibers have been synthesized and utilized for vascular [25] and meniscal [26] tissue engineering applications,

Figure 2



Tissue engineering and regenerative medicine at many length scales. Many techniques are available to design biomaterials from the nanometer to centimeter length scales, some of which are listed above **(A)**. Examples of biomaterials mentioned in the review can be seen approximately beneath their respective length scale (**B–F**). An illustration of a heparin-nucleating self-assembled peptide amphiphile **(B)** [29]. The fatty acid tail segregates to the center of the nanofiber, while the heparin binding sequence bonds to heparin leading to charge shielding and gel formation. The nanofibers have a diameter of 6–7.5 nm and further aggregate to higher order structures with diameters of 50–100 nm. Mesenchymal stem cells seeded on submicrometer electrospun poly(ε-caprolactone) meshes **(C)** [26]. Anisotropic skeletal myotube formation after seven days of culture (7 d) on poly(dimethylsiloxane) substrates patterned using soft lithography **(D)** [15]. Hematoxylin and eosin staining of a micropatterned dermal analog composed of a collagen-glycosaminoglycan membrane laminated to a collagen sponge **(E)** [22]. Tissue-engineered blood vessel produced by seeding smooth muscle cells onto a polyglycolic acid substrate and then culturing the substrate in a pulsatile bioreactor **(F)** [57••]. The vessel is approximately 5.5 cm in length and 3 mm in diameter. The scale bar in C, D, and E is 50 μm. The scale bar in D added to scale from the control sample. W and D represent the width and depth of the invagination. The original image in F was cut to fit into the figure. Images in B and D reproduced with permission, copyright 2006, American Chemical Society. Image in C reproduced with permission, copyright 2007, Elsevier. Image in E reprinted with permission of John Wiley & Sons, Inc., Journal of Biomedical Materials Research Part A, 72A, 2005, 53, copyright 2004, John Wiley & Sons, Inc. Image in F reproduced with permission, copyright 2006, National Academy of Sciences, USA.

respectively. The modulus of tissues formed from aligned nanofibers can be dramatically increased as compared to unaligned fibers [26]. Electric fields can be utilized during the electrospinning process to create oriented polymers within the nanofibers that further recapitulate the hierarchical design found within natural anisotropic ECM [27]. Finally, soft lithography, multiphoton photocrosslinking, and electrospinning may be used in combination with other materials and processing methods to create scaffolds with even greater control over spatial properties to potentially enhance material performance.

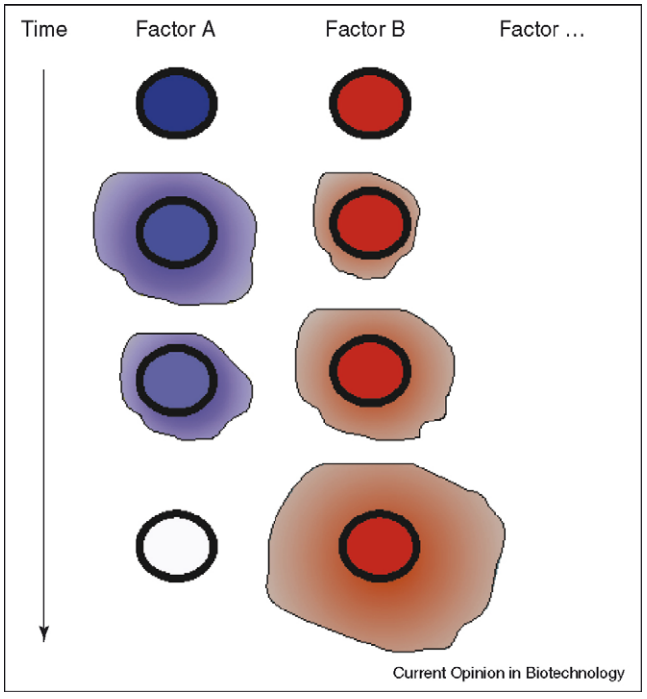
Spatial and temporal control of morphogen and growth factor delivery

Additional direction over cell fate, beyond control of scaffold architecture, can be achieved through the spatio-temporal control of morphogen and growth factor delivery from the scaffolding material (Figure 3). Polymeric systems allow for independent regulation of the localization, duration, and availability of one or more soluble factors [28–30], while limiting the overall quantity of drug and minimizing the potential side effects of systemic dosing. Many techniques have been developed to regulate the kinetics and distribution of soluble factors, including multiple levels of encapsulation [31] as well as noncovalently bonding the bioactive factors to peptides with a range of dissociation constants mimicking ECM immobilization of growth factors [32]. With respect to the latter, phage display has been a powerful technique for identifying ligands that can tightly control the kinetics of factor release [32]. Dual delivery of multiple growth factors and morphogens has been achieved in multiple systems including poly(-lactide-*co*-glycolide) (PLG) [31], oligo(poly(ethylene glycol) fumarate) [33], and alginate [34] scaffolds. Multiple factors can be delivered sequentially, simultaneously, or both, and these techniques can be combined with DNA or RNAi delivery to enable long-standing protein expression [35] or abrogation of expression [36]. The physiologic relevance of control over factors availability in time and space has been demonstrated by differentiation of stem cells [37], regulation over the extent of vascular network maturation with time [31], enhanced delivery and integration of transplanted cells [38], and the simultaneous formation of vascular networks at distinct stages of maturation in distinct spatial compartments [28].

Dynamic polymer design

One goal of biomaterial design is to develop systems that respond dynamically and reversibly to external or microenvironmental cues. At the extreme, a material may act as a microprocessor, a closed-loop system that surveys the external cues and the microenvironment, integrates the

Figure 3



Spatial and temporal control of morphogen and growth factor delivery. Polymeric systems allow for independent regulation of the localization, duration, and availability of one or more soluble factors, exemplified above by the delivery of two factors, A and B, with different release kinetics. Factor A is released locally from the biomaterial (circle) within a short duration of time. By contrast, factor B is released over a large volume for a long period of time.

information, and responds accordingly in real-time. It is unclear what level of complexity will be required for advanced tissue engineering and regenerative medicine applications; however, progress has been achieved to create polymeric systems that alter their properties in response to external or microenvironmental effectors. Polypyrrole-based neural interfaces [39] and electroactive self-assembled monolayers [40] respond dynamically to external controls by functioning as electrical relays or surfaces that change conformation, respectively, in response to an electric potential. Other materials have been designed to respond directly to microenvironmental effectors. In the context of material degradation, materials have been developed that incorporate proteolytic domains [41] or bioactive linkers [42], and their degradation and function is altered by contacting cells. Other bioresponsive polymers change physical properties irreversibly with or without material degradation in response to small molecule receptors/ligands, or cell-secreted factors such as enzymes or enzymatic substrates [43]. Reversibility may often be desired and has been demonstrated in response to cellular ligands [44] and enzymes [45••]. In the former, the structure of calmodulin was altered by ligand binding, leading to macroscopic shape changes in the hydrogel. In the latter, a pentapeptidic hydrogelator reversibly self-assembled or disassembled as a result of its phosphorylation state.

Expanding the design space and developing tomorrow's biomaterials

A bottleneck in the successful clinical implementation for many polymeric materials rests in our limited understanding of biological principles, both in the fundamental biology relating how cells interact with their environment, as well as in the quantitative aspects of these interactions. The increasingly important question is not how to make a material, but what and how biological cues should be incorporated into the material. To this end, the work of many interdisciplinary teams is helping to define the future landscape of polymer design.

A quantitative understanding of biological processes is and will be of great importance for material design. Specifically, a quantitative understanding of the concentration, distribution, and interaction of molecules within normal, diseased, and regenerating systems as they change with time is important to be able to adequately design polymeric materials that can recapitulate these events. For example, correlation microscopy studies are defining integrin dynamics and focal adhesion-actin coupling [46,47]. Other studies are teasing apart the environmental factors that influence 3D cellular migration [48•]; however, the quantitative relationship between ligand bonding and integrin dynamics, focal adhesion-actin coupling, and motility is largely unknown. This relationship can be explored using techniques such as fluorescence resonance energy transfer (FRET) to quantify bond formation in real-time 3D culture [49••], and the subsequent dynamic process of cell-mediated ECM remodeling [50]. These events can ultimately be correlated with cellular activity, such as the mechanism of motility, and fabrication methods could then be applied to incorporate the appropriate signaling cues for the desired material performance. Additionally, recent studies suggest that the cellular reprogramming of somatic cells requires a delicate balance of factors [50–54]. As the expression of these factors becomes known, controlled delivery may allow for *in situ* reprogramming to generate stem cell and differentiated cell populations. Detailed knowledge of cues regulating cell fate is already being exploited to design materials that direct stem cell fate *in vivo* [38].

A number of studies have also revealed complex biological subtleties at the tissue–material interface that may greatly affect material performance. First, the influences of the material on the host, such as cell migration into a scaffold or differentiation of autologous stem cells within a polymer, will probably depend upon the sex, disease state, and age of the patient [55,56,57••]. Second, most materials elicit immunogenic responses following

transplantation, and even some polymers that are typically considered biocompatible may harbor subtle, but important immunogenic activity [58]. Understanding the role of these activities for a given material in a specific tissue engineering/regenerative medicine context may provide routes to minimize detrimental immunologic activity. Conversely, harnessing immunologic activity may provide additional opportunities.

Conclusion

A deeper understanding of the interactions between cells and their microenvironments has led to the development of increasingly sophisticated polymeric materials for tissue engineering and regenerative medicine. Future advances in the biological sciences, particularly with respect to qualitative and quantitative biology of cells within their microenvironments and at the tissue–material interface, will increase the design space of future biomaterials.

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