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Polymer Scaffolds for Biomaterials Applications

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ABSTRACT: Biomaterials have been used extensively in medical, personal care, and food applications, with many similar polymers being used across disciplines. This Perspective will emphasize polymers used in medicine and specifically those designed as scaffolds for use in tissue engineering and regenerative medicine. The areas of active research in tissue engineering include: biomaterials design—incorporation of the appropriate chemical, physical, and mechanical/structural properties to guide cell and tissue organization; cell/scaffold integration—inclusion into the biomaterial scaffold of either cells for transplantation or biomolecules to attract cells, including stem cells, from the host to promote integration with the tissue after implantation; and biomolecule delivery—inclusion of growth factors and/or small molecules or peptides that promote cell survival and tissue regeneration. While a significant and growing area of regenerative medicine involves the stimulation of endogenous stem cells, this Perspective will emphasize polymer scaffolds used for delivery of cells and biomolecules. The challenges and solutions pursued in designing polymeric biomaterial scaffolds with the appropriate 3-dimensional structure will be explored.

Choice of Polymer

The polymer of choice is dictated by its end application and requires thoughtful consideration of the polymer's physical and chemical properties. The desired longevity of the polymer dictates the use of biostable vs biodegradable polymers, and the desired cellular interactions guide the choice of naturally derived vs synthetic polymers. While base polymer composition influences cellular response, the polymer can be modified with specific proteins and/or peptides to promote desired cellular interactions. The overarching principle for successfully choosing or synthesizing the appropriate polymer is having thoroughly defined design criteria, which are dictated, of course, by the proposed end use.

Biocompatible Polymers. While many polymers have been studied for medical applications, they share certain properties that are fundamental to their use as biomaterials. Their application in tissue engineering requires them to be biocompatible, nontoxic, and noninflammatory, which is particularly important when designing degradable polymers as the degradation products too must meet these criteria. In a recent paper,[1] David Williams proposed the following definition: "*The biocompatibility of a scaffold or matrix for a tissue engineering product refers to the ability to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signalling systems, in order to optimise tissue regeneration, without eliciting any undesirable local or systemic responses in the eventual host*." This definition, while broad, emphasizes the role of tissue engineered scaffolds in supporting cellular function, which leads to tissue generation. For effective integration of engineered tissue with host tissue, the polymer and its degradation products must elicit only a minimal inflammatory response. All foreign materials evoke an inflammatory response; however, the goal is to minimize this reaction because a fibrotic scar will often form at the materials−tissue interface, thereby isolating the implanted polymer from the body. This can be devastating to a

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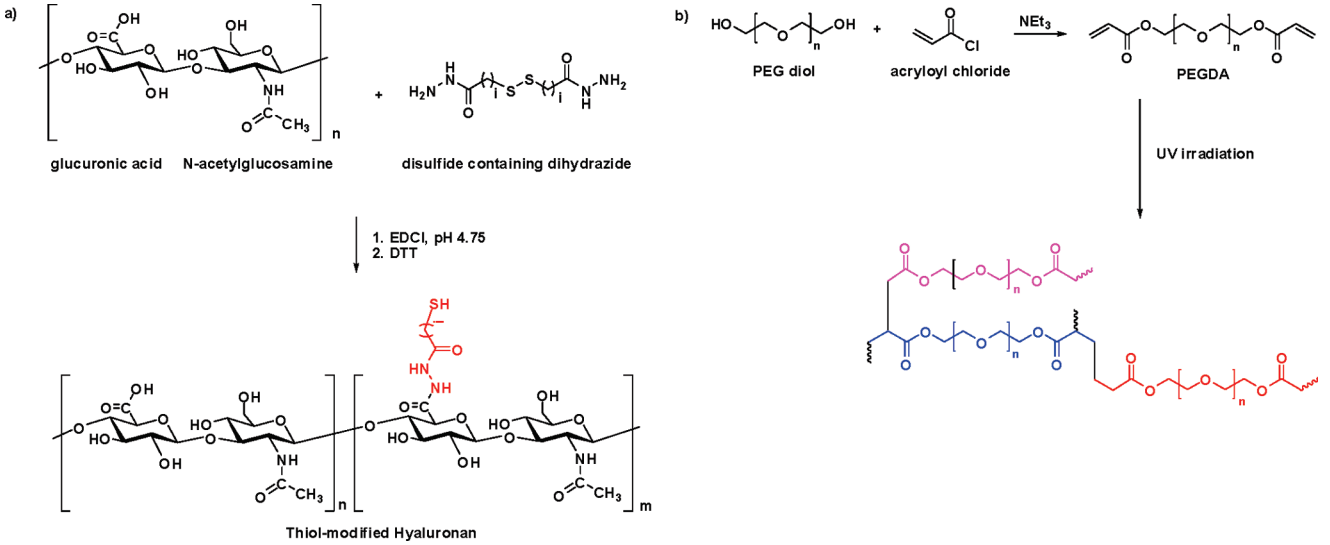

Figure 1. (a) Hyaluronan and (b) PEG represent two common polymers used to limit protein and cell adhesion. Importantly, both can be cross-linked to create 3-dimensional scaffolds for tissue engineering. For example, (a) hyaluronan can be modified using a disulfide containing dihydrazide which produces a thiol-modified HA that is then cross-linked through disulfide bond formation (Adapted from ref 117.) and (b) PEG diol is reacted with acryloyl chloride to give PEG diacrylate (PEGDA), which is cross-linked upon UV irradiation. (Adapted from ref 118.)

cell-based implant, limiting both integration with the host tissue and local supply of blood-borne nutrients.

Polymers that have low protein adsorption and cell adhesion have been thoroughly investigated because they can provide some stealth properties to implanted materials, reducing immune system recognition and clearance. In other applications, these polymers can be specifically modified with proteins and/or peptides to elicit a specific cellular response, thereby providing a clean canvas to which cellular interactions are added. Poly(ethylene glycol) (PEG) is one of the most widely explored polymers, used either alone or as a surface modifier, for its properties of low cell adhesion and protein adsorption.[2] It is proposed that the ordered water surrounding each PEG chain provides a hydrated shell that limits protein adsorption and thus recognition by immune cells such as macrophages.[3] Interestingly, this property of limited protein and cell adhesion to PEG has also been pursued to limit tissue adhesion after surgery.[4,5] In this application, PEG-diacrylates are often used to allow photoinitiated cross-linking. Similarly, cross-linked PEG scaffolds[6,7] have been synthesized where the inclusion of enzyme-degradable peptide cross-links creates a responsive scaffold, the degradation of which is controlled by the biological milieu.[8,9] Thus, a key advantage of polymers such as PEG, which have inherently low protein adsorption and cell adhesion, is the ability to tune adhesive properties through attachment of specific proteins or peptides.

A number of polysaccharides have similar properties to PEG in terms of biocompatibility and low protein and cell adhesion. For example, hyaluronan, a native component of extracellular matrix, has been explored in numerous biomedical applications because it is easily injected into tissue. Hyaluronan is injected in knees for cartilage repair[10,11] and is also used to prevent tissue adhesions.[12,13] Like PEG, hyaluronan can be cross-linked to create a scaffold; yet, unlike PEG, hyaluronan is enzymatically degraded. Hyaluronidase degrades hyaluronan to nontoxic products that are easily processed by the body. Interestingly, hyaluronan has been shown to modulate the inflammatory response,[14,15] thereby highlighting some additional beneficial properties of naturally derived polymers over synthetic polymers. For example, after spinal cord injury, injection into the intrathecal cavity of a physical blend of two polysaccharides—hyaluronan and methylcellulose—showed a decreased inflammatory response relative to saline controls.[16] Here, the hydrophobic interactions between methylcellulose chains result in a physical gel. Figure 1 shows the linear and typical cross-linked chemical structures of PEG and hyaluronan.

Biodegradable Polymers. Polymeric scaffolds are often designed as temporary structures having the desired geometry and the physical, chemical, and mechanical properties required for implantation. The use of degradable polymers is desirable because the need for surgical removal is obviated; however, care must be taken to ensure the compatibility of both intermediate and final degradation products, the timing of the degradation process, and how each of these affects the regenerative process. The rate and mechanism of degradation (surface erosion or bulk) will impact the mechanical properties of the scaffold: bulk eroding polymers maintain their physical structure until the molar mass of the polymer is sufficiently low for polymer dissolution in the aqueous environment, at which point there is a precipitous loss of mechanical properties; surface eroding polymers lose their shape and mechanical properties slowly over time. For both degradation mechanisms, the regenerative process will inevitably be negatively impacted if the degradation products are toxic to the tissue that has formed and/or if the integrity of the scaffold is lost prior to new tissue formation and integration with the host. This narrows the selection of polymers to those that degrade at rates slow enough for cell integration and tissue growth and to those that produce only biocompatible degradation products.

The most commonly used degradable synthetic polymers are poly(lactide-*co*-glycolide) and their respective homopolymers. Poly(glycolide) and poly(lactide) have been used clinically for several decades as suture materials, providing a depth of regulatory experience (for a review see ref 17). This is a significant incentive for their continued use in tissue engineering applications, where the degradation products, while acidic, have been shown to be largely benign. Moreover, the rate of degradation can be tuned by composition, and the generation of 3-dimensional scaffolds has been demonstrated.[18−20] The rate of degradation and the scaffold's physical structure influence the inflammatory

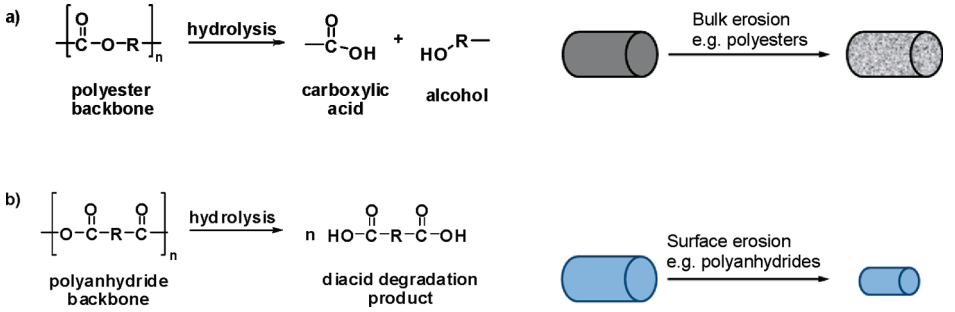

Figure 2. Hydrolysis of polymer scaffolds can occur by bulk or surface erosion. Bulk erosion of polymer scaffolds, such as (a) polyesters, occurs when water is able to penetrate the scaffold and catalyzes degradation from within the scaffold. Alternatively, surface erosion, of (b) polyanhydrides for example, occurs when water is unable to penetrate the core of the scaffold; thus, degradation occurs from the outside in.

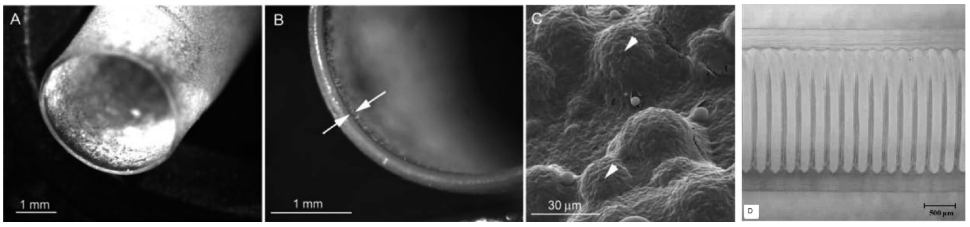

Figure 3. Chitosan channels can be processed into hydrogel tubular structures and used as nerve guidance channels. Chitosan tubes have PLGA microspheres incorporated into their wall structure as shown in (a) and (b) in light micrographs where the transparent chitosan tube appears white due to the PLGA microspheres enmbedded in an inner chitosan layer (~20 μm thick, denoted with arrows). (c) Scanning electron micrograph shows PLGA microspheres (denoted with arrowheads) under the inner chitosan layer. (Reprinted with permission from ref 119. Copyright 2008 American Institute of Chemical Engineers.) (d) Light micrograph shows a chitin tube reinforced with a PLGA coil incorporated into its wall structure. (Reprinted with permission from ref 120. Copyright 2005 Elsevier.)

response, where, for example, faster degradation rates result in higher local concentrations of (potentially) inflammatory molecules. These synthetic poly(α-hydroxy ester)s degrade hydrolytically by bulk erosion whereas another class of well-studied polymers, poly(anhydride)s, degrade by surface erosion (see Figure 2). Poly(anhydride)s have the anhydride incorporated into the backbone, are biocompatible, and degrade to the relevant diacid (for a review see ref 21). Poly(anhydride)s have been explored primarily for drug delivery, and poly(sebacic acid-*co*-1,3-bis(*p*-carboxyphenoxy)propane) (P(CPP-SA)) is used clinically (as Gliadel) to deliver the chemotherapeutic agent BCNU for the treatment of brain cancer.[22]

Naturally derived degradable polymers, such as collagen and chitosan which are the two most abundant naturally derived polymers, have also been explored clinically because they are biocompatible, easily modified, and easily processed into various structures. Collagen I is a structural protein that has been commercialized as an injectable product for both tissue bulking in cosmetic applications[23] and tissue sealing in surgical applications.[24] As an important protein in the extracellular matrix on which many cells grow, collagen is naturally cell-adhesive and provides an environment conducive to cell viability. Moreover, collagen can be designed to have the appropriate properties for cell penetration, resulting in cross-linked porous scaffolds for applications in tissue engineering.[25] Chitosan, derived from chitin found in crustacean exoskeletons, is normally insoluble at physiologic pH (pH 7.4) but has been formulated with glycerol phosphate to be soluble at pH 7.4 and investigated as an injectable, in situ forming gel for cartilage repair.[26,27] An injectable scaffold, such as this, is promising clinically as the surgery itself is less invasive than that required with an implant, and thus the subsequent recovery is faster and less complicated. Chitosan scaffolds have also been explored in numerous tissue engineering applications,[28] and our lab has demonstrated its potential as nerve guidance channels[29] where the processing technique has been manipulated to create coil-reinforced hydrogel tubes or tubes with the drug-eluting microspheres in the wall the structure, as shown in Figure 3.

Biostable Polymers. Biostable (or nondegradable) polymers are of interest for encapsulated cell therapy, where cells are protected in a polymeric membrane from the host immune system. The premise of encapsulated cell therapy is to provide an immune privileged environment in which transplanted cells produce therapeutically relevant molecules for extended periods of time. This strategy overcomes some of the limitations of conventional protein delivery, eliminating multiple dosing requirements and providing extended bioactivity of the desired compounds. Cells have been encapsulated in both hollow fiber membranes[30−32] and microspherical membranes,[33] which allow the passive diffusion of small nutrient and waste molecules and therapeutic cell products, but not larger immunoglobulins, across the membrane. Figure 4 is a conceptual representation of the microencapsulated and macroencapsulated strategies. These membranes are meant to protect the encapsulated cells from rejection by the immune cells, thereby providing the cells with stealth properties and minimizing the immune response. Here the scaffold is porous and has some properties similar to ultrafiltration membranes. While conceptually simple, and some success has been achieved with both synthetic and naturally derived polymeric membranes, significant challenges remain, such as encapsulating sufficiently large numbers of cells for therapeutic benefit,[34] maintaining their long-term viability and function once encapsulated and overcoming any immune-associated response associated with antigens shed from the encapsulated cells or the biomaterials themselves.[35]

The polymers that have been most thoroughly studied for encapsulated cell therapy include poly(acrylonitrile-*co*-vinyl

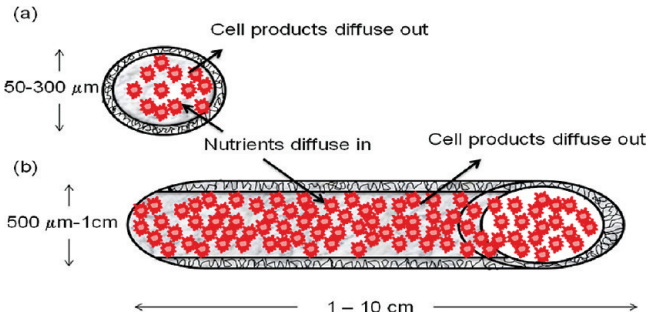

Figure 4. Conceptual representation of encapsulated cell therapy where cells (shown as red star shapes) are incorporated into either (a) microcapsules or (b) macrocapsules. The capsules have wall structures similar to ultrafiltration membranes when they are formed from synthetic polymers that are extruded from an organic solvent into an aqueous solution.

chloride) (PAN/PVC),[36] which has been studied as hollow fiber membranes; poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (PHEMA−MMA), which has been studied as microcapsules;[33] and alginate/poly(lysine),[37] which has been studied as microcapsules and also as scaffolds.[38] These polymers were chosen initially based on biocompatibility and processability. For example, PAN/PVC can be extruded into hollow fiber membranes with the dimensions and porosities suitable for cell encapsulation. Similarly, PHEMA−MMA and alginate can be extruded with cells to form microcapsules around the cells. To stabilize the calcium−alginate cross-linked microcapsules, a poly(lysine) coating is added, followed by another alginate layer for enhanced biocompatibility.[39] The alginate−PLL microcapsules are more fragile than the PAN/PVC hollow fiber membranes, and this can limit their utility.

Synthetic vs Naturally Derived Polymers. Working backward from the end use, a series of design criteria are established where the site of implantation and expected performance define polymer selection. On the one hand, synthetic polymers can be tuned in terms of composition, rate of degradation, mechanical, and chemical properties. On the other hand, naturally derived polymers provide compositional uniqueness, such as stimulating a specific cellular response, which sometimes overrides the advantages of synthetic polymers.

Of the numerous synthetic polymers, a few have been examined in more detail: poly(*N*-isopropylacrylamide) (PNiPAAM), PEG, and PLGA. PNiPAAM, a nondegradable polymer, has a lower critical solution temperature of 32 °C, which has been exploited in both drug delivery and scaffold design. For example, an innovative use of PNiPAAM is in cell culture, where the polymer serves as a temporary scaffold but is not implanted. To avoid the use of trypsin (typically used to remove cells from culture dishes and potentially harmful to cells), retinal cells were grown to confluency, in sheets, on PNiPAAM, and then the temperature was manipulated to collapse the PNiPAAM, resulting in detached cell sheets available for transplantation.[40] This technique preserved existing cell−cell contact and the deposited extracellular matrix, both important for survival and proliferation upon implantation. PEG is another polymer that has been thoroughly examined, and even though PEG alone limits cell adhesion and protein adsorption, it has also been modified with cell adhesive peptides and proteins to promote adhesion and differentiation of specific cell types. By starting with a polymer that is inherently nonadhesive to cells (like PEG), specific cell-adhesion molecules can be incorporated into the scaffold design, affording some control over cell function. While PNiPAAM has interesting thermal properties, and PEG has important non-cell-adhesive properties, PLGA degrades to known products, one of which, lactic acid, is produced in the body by muscle exertion. This idea of designing a polymer that degrades to known safe products stimulated the synthesis of poly(propylene fumarate) (PPF), which degrades hydrolytically to fumaric acid and has been studied as a scaffold in bone tissue engineering applications.[41] Originally designed as an injectable, in situ cross-linking polymer, this process was found to be cytotoxic, necessitating the use of PPF as a preformed solid scaffold.[42] Like PEG, cell-specific interactions have been included in the design of many biodegradable polymers, including the poly(α-hydroxy ester)s, such as poly(lactide) and poly(glycolide).[43]

There are naturally derived polymers that have compelling properties as well—whether low protein-adsorptive or inherently cell-adhesive. Four common naturally derived polymers are highlighted, representing the spectrum of cell adhesion: agarose, hyaluronan, collagen, and fibrin. For example, agarose is inherently nonadhesive to cells but can be modified to include cell-adhesive moieties. This is particularly useful for spatial guidance of cells in 3-dimensional tissue-like constructs where the cell-adhesion peptides are immobilized in defined volumes.[44−46] In contrast, other protein- and polysaccharide-based hydrogels provide natural cell-adhesion sites for specific, beneficial, cell−material interactions. Collagen and hyaluronan are probably the best examples. Both are degraded by enzymes resident in the body (collagenase and hyaluronidase, respectively); both can be cross-linked, allowing mechanical and/or physical properties to be tuned; and both promote specific cellular interactions. Collagen is part of the basement membrane of many cells, and thus there are defined cell surface receptors, called integrins, which interact with collagen and promote cell adhesion. Similarly, some cells have receptors (CD44) for hyaluronan[47] and are stimulated by it, which has been exploited to induce wound healing. Since other cells do not have receptors for hyaluronan, the cell−hyaluronan interaction can be manipulated, depending on either cell type/implantation tissue or the inclusion of specific cellular ligands.[48] This remarkable property of hyaluronan has resulted in its use in multiple platform technologies.[11,16,49−51] Fibrin, produced from fibrinogen and thrombin, forms naturally in the wound healing process and has been used as a tissue sealant[52,53] and a growth factor delivery vehicle for tissue repair.[54] Like some of the other naturally derived polymers, fibrin supports cell adhesion and growth and its physical properties can be tuned by the fibrinogen/thrombin formulation in the design of a tissue engineered scaffold.[55]

Scaffold Design

The physical aspects of scaffold design, as with polymer choice, depend largely on the final application. The scaffold is meant to provide the appropriate chemical, physical, and mechanical properties required for cell survival and tissue formation. Essentially, the polymeric scaffold is designed to define the cellular microenvironment (cell niche) required for optimal function.[56,57] Understanding the series of stimuli provided during development and/or healing is the guide to which tissue engineers most often turn when designing a scaffold. Typically, the scaffold is a 3-dimensional open-cell, interconnected porous structure, allowing facile communication between the biological cells dispersed in the scaffold. Depending on the intended use, these structures are also conducive to cell proliferation, migration, and/or differentiation.

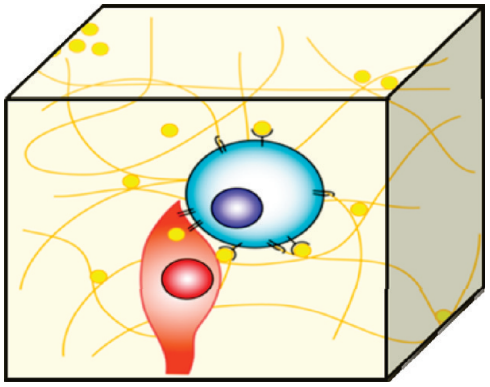

Figure 5. The tissue engineered scaffold is a 3-dimensional structure that provides cells with the appropriate microenvironment of chemical, topographical, and mechanical cues, including cell−cell and cell−matrix interactions.

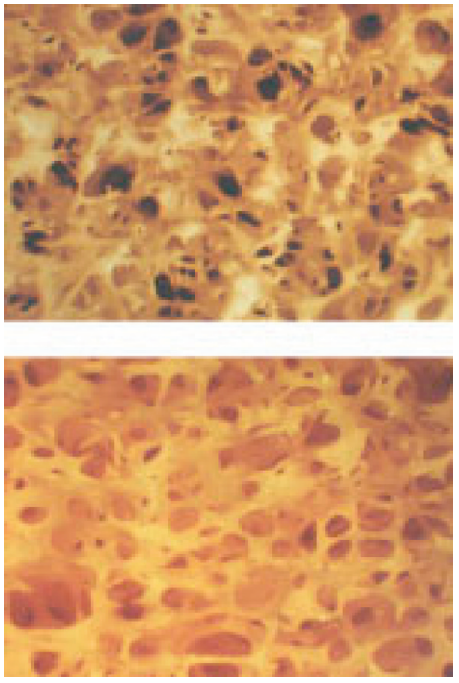

Figure 6. Light micrographs of a cross-sectioned engineered PLGA 75/25 scaffold synthesized by a phase inversion/particulate leaching technique (top) and human trabecular bone (bottom). A similar pore structure, strut size, and distribution can be observed in both images (field widths = 1.8 cm in both images). (Reprinted with permission from ref 59. Copyright 2003 Wiley Periodicals.[59])

The stimuli that define the cellular microenvironment include the chemical, physical, and mechanical properties of the scaffold as well as other cells and signaling molecules incorporated into the scaffold design. The 3-dimensionality of the scaffold is key to its use in tissue engineering, where a 3-D cell construct is meant to integrate into a 3-D tissue. Figure 5 summarizes some of these design features required in a 3D scaffold.

Design Criteria. Determining the appropriate geometry/physical structure of the polymeric scaffold requires an understanding of the tissue into which it is being implanted. For example, polymeric scaffolds designed for implantation into the spinal cord have included elaborate designs of the gray and white matter tracts[58] while implantations into bone have mimicked the porosity of trabecular bone.[59] Figure 6 shows how the physical structure of the tissue (in this case bone) influenced the design of the engineered scaffold.

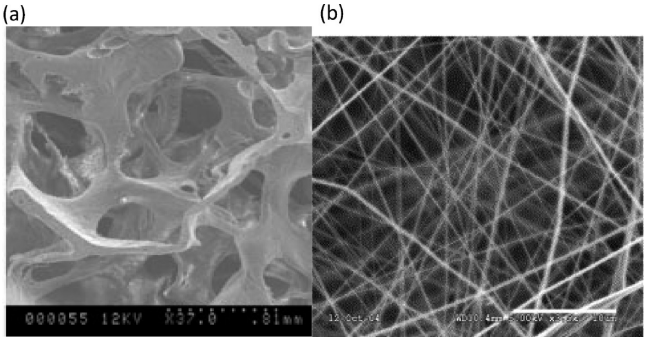

Figure 7. Scanning electron micrographs of PLGA scaffolds prepared by (a) phase inversion/particulate leaching (Reprinted with permission from ref 59. Copyright 2003 Wiley Periodicals.[59]) and (b) electrospun fibers (scale bar is 10 μm). (Reprinted with permission from ref 122. Copyright 2007 Elsevier.[122])

Perhaps the most celebrated example is that of the tissue engineered ear, where the shape of the ear was predesigned into the polymer scaffold to guide cell growth within.[60]

The mechanical properties of the scaffold are dictated by the tissue into which it is implanted. For example, hard tissue, such as bone, necessitates a stiff polymeric scaffold[20] whereas a soft tissue, such as nerve, requires a malleable polymeric scaffold,[29,61,62] and an elastomeric tissue, such as skin (or blood vessel), demands a flexible polymeric scaffold.[63−66] Interestingly, this manifests itself at the cellular level as well, where, for example, neural stem cells[67−69] thrive and differentiate on low-modulus materials whereas mesenchymal stem cells, from which bone develops, thrive on stiffer materials.[70] In addition to defining the modulus of the polymer on (or in) which cells are cultured, there is a burgeoning understanding of the mechanical tension that cells themselves exert on these polymers. By gaining greater insight into the tension that cells themselves exert on each other or the materials in which they are cultured, researchers are exploiting cellular differentiation patterns and the interplay of cells with materials to positive effect.[71,72] For example, cell function can be manipulated by controlling cell shape which is in turn defined by culturing the cells on distinct cell-adhesive patterns. This has been observed with numerous cell types, including stem cells, the differentiation profile of which has been manipulated,[73,74] demonstrating another important way that cell function can be tuned in the design of engineered tissues.

In addition to the mechanical properties, the tissue engineered scaffold is designed for enhanced cell penetration and 3-dimensional tissue formation. This has been achieved by incorporating pores or cell-cleavable groups within the scaffold design. For many years, pores were introduced into scaffolds by a variety of processes involving salt leaching,[75−77] phase inversion,[78,79] and high-pressure gasification[75,80] (Figure 7a). These scaffolds presented 3-dimensional environments on which cells were seeded and throughout which cells grew (depending on the cell-seeding process). These methods have been examined most thoroughly with the PLGA family of polymers. Interestingly, by tuning the lactide/glycolide ratio, the scaffolds were found to be inductive (and conductive) to cell growth and tissue formation. For example, PLGA 75/25 (vs PLGA 85/15 or PLGA50/50) was found to be the most suitable of the PLGA family for bone tissue engineering applications. An alternate way of introducing pores is by forming the scaffolds with electrospun polymers,[81−84] which provide microfibers that can guide cell growth and differentiation (Figure 7b). The introduction of cell-cleavable groups in polymeric hydrogels

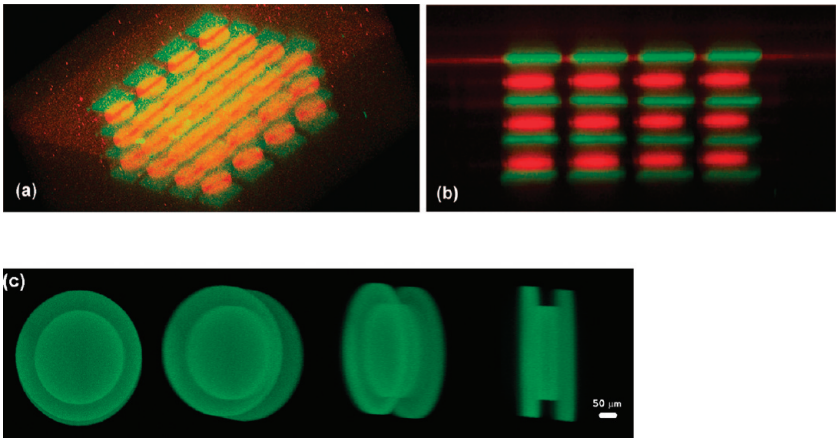

Figure 8. Multiphoton patterning of agarose modified with multiphoton-labile bromo, hydroxycoumarin-protected cysteine thiol groups results in 3-D patterned gels with distinct chemically defined volumes (a) oblique and (b) side view of $4 \times 4 \times 4$ array of 3D patterned squares (ca. 60 μm per side) of AF488-Mal, overpatterned with a second $4 \times 4 \times 4$ array of circles (ca. 50 μm in diameter) of the red fluorescent dye AF546-Mal. (Reprinted from ref 45.) and (c) PEG hydrogels with pendent photoreactive alkene groups are modified with fluorescent cysteine-containing peptides via thiol−ene chemistry in the presence of focused multiphoton laser light. By controlling the intensity and exposure time of the light, the concentration of immobilized peptides can be explicitly controlled within 3D. This method is fully described.[123]

allows cells to penetrate 3-dimensional scaffolds without macroscopic pores. For example, PEG hydrogels have been cross-linked with enzyme-degradable groups,[85,86] allowing cells that produce these enzymes to migrate through the gels. In this example, the rate of tissue formation may be limited by the rate of scaffold degradation.

Controlling Cellular Interactions. Defining the biological interactions of the polymeric scaffolds with cells is equally important as the physical properties of the scaffold. To promote cell adhesion or cell differentiation, scaffolds have been modified with specific biomolecules. For example, scaffolds have often been modified with cell-adhesive ligands targeting the integrin family of cell-surface receptors. The most ubiquitous adhesion ligand is the tripeptide sequence arginine−glysine−aspartic acid (RGD).[87] Derived from fibronectin, the RGD sequence is also present on other extracellular matrix proteins, including laminin. Modifying a polymer with RGD renders it cell-adhesive; however, the specificity of this interaction and its impact on cell function have to be tested in order to fully understand the nature of the cellular response. Polymers have also been modified with two peptide sequences to provide a more specific cellular interaction[88] or a synergistic response, such as promoting neuronal cell adhesion and neurite extension.[89] While cell adhesion is influenced in vitro with peptide modification, the importance of this approach has been questioned in vivo where serum proteins will adsorb to implanted materials, thereby dominating the cell−biomaterial interaction over that of the peptide-modified materials. The peptides, however, may influence which proteins adsorb and/or their conformation, which will in turn impact the cellular response. Notwithstanding this concern, peptide-modified biomaterials are important for in vitro models, which can provide important insights into cell function.

To guide cell migration in two or three dimensions, polymeric scaffolds can be chemically modified with a concentration gradient of one or more factors that spatially guide cell growth (i.e., tropic factors). This mimics an important process in development where cells are guided to their target tissues by a series of attractive and repulsive cues. This has been most thoroughly investigated in the nervous system[90] and has been incorporated into polymeric scaffolds designed for applications therein.[91−93] Here, the goal is to mimic the processes that guide nerve fibers (axons) to their target tissues in development as a way to promote wound healing after injury. One of the advantages of chemically immobilized growth factors, compared to soluble molecules, is the ability to spatially control and guide cell growth; however, it is important that the active site on the growth factor remains available after immobilization to induce the desired cellular response.

Recently, complex 3-D scaffolds have been designed to actively guide cell growth with the use of growth factor concentration gradients and immobilized adhesion factors. Using multiphoton lasers and multiphoton-labile protecting groups, these advanced 3D scaffolds have been designed to promote cell infiltration and differentiation in spatially controlled volumes within the scaffold. The chemistry involved is elegant and promises to allow micrometer scale spatial control over the coculture of multiple cell types.[94] This latter concept is particularly important with the view toward building tissues. It is critical to examine cell behavior in the context of the tissue and thus necessitates that the scaffold design accommodate the interaction of multiple cells. Here, agarose[95,96] and cross-linked PEG gels[97] have been most thoroughly investigated, and representative images of the 3-D patterning technologies are summarized in Figure 8.

Microfluidics is currently being explored to create patterned concentration gradients to guide cell growth in scaffolds. This technique allows small volumes of expensive factors to be patterned into scaffolds and provides control over the gradient created. For example, a concentration gradient of the extracellular matrix protein, laminin, was designed using microfluidics as a way to guide neural cell growth.[98] Similarly growth or trophic factors have been investigated using microfluidics to guide neural cells or bacterial cells to gain insights into the mechanisms involved in development or infection, respectively. Interestingly, these gradients can be created by taking advantage of the laminar flow characteristics, hydrophilicity, or capillary action, among other properties, of the microfluidics channels.[99,100]

A controlled release strategy can also be integrated into the porous scaffold design to deliver factors that may influence stem cell differentiation, promote host tissue infiltration, and/or guide cell growth. There are numerous ways to control release, including enzymatic pathways and polymers that incorporate the bioactive factors into the

polymer backbone.[101] Perhaps the most common is the encapsulation of growth factors or cytokines into biodegradable polymer microspheres or rods from which they diffuse. This controlled release scaffold design has been used with some success to enhance, for example, blood vessel formation.[102] An interesting scaffold design uses the microspheres themselves as the scaffold, sintering them together at low temperature to create the desired geometry.[103] One of the advantages of controlled release is that the growth factors are provided to cells in the soluble form for a defined period of time; however, the timing of this release must to be tuned to optimize the biological response.

Scientific Challenges for Successful Implementation

The key challenges of tissue engineered scaffolds are 3-D penetration of cells, throughout the scaffold, resulting in 3-D tissue formation; continued cell viability within the 3-D construct; and integration of the de novo engineered tissue with the host tissue. Polymer science can influence cell penetration through the design of scaffolds with the porosity, chemistry, and modulus required by the cell of interest. For enhanced cell viability, polymer science can be used to promote the coculture of multiple cell types, thereby better mimicking the tissue. This is important for tissue engineered scaffolds that are designed for both implantation to guide cell growth and in vitro screening. For in vitro screening, the tissue engineered construct is designed to better approximate the in vivo outcome than standard 2D cell culture conditions; thus, the culture of one cell type in 3D is important, and the culture of multiple cell types in 3D is even more compelling toward the creation of tissue analogues.

For implantation, the tissue engineered construct must promote cell viability, function, and integration with the host. Having a vascularized scaffold is critical to all tissues, although perhaps less important in cartilage. Often those cells in the center of the scaffold die due to lack of oxygen and nutrients. To overcome this deficiency, pseudo-vascularized scaffolds have been designed.[104,105] Here endothelial cells, a key cellular component of blood vessels, are included in the scaffold design. While the endothelial cells alone are insufficient to form blood vessels, they provide a framework for integration with the host endothelial cells, with the goal of accelerating blood vessel formation upon implantation and thus enhancing the viability of cells within the scaffold. Building on this strategy, when endothelial cells were cultured with myoblasts (muscle cells) and fibroblasts (support cells for endothelial cells forming blood vessels) in PLGA/PLLA 3-D porous scaffolds, vascularized networks were formed in vitro, promoting structural organization of the other cells and improving their viability both in vitro and in vivo after implantation.[106]

Integration with the host tissue requires not only biocompatible materials but also penetration of host cells into the construct to promote bridging of the implanted constructs and host tissue. This is critical in all applications, whether the construct is initially devoid of cells or full of cells. For example, in nerve tissue engineering, cells have not always been included in the scaffold design because for nerve repair; it is the host axons (nerve extensions) that must connect to their target tissues for function to be restored. Cells can provide tissue bridges for host cell regeneration, or other factors, such as growth factor gradients or cell adhesive paths, can be incorporated in nerve guidance channels. For skin, perhaps the most studied engineered tissue, cells have most often been included in the design, yet there are some acellular biomaterial-based designs that are compelling.[107,108] Acellular matrices are derived from tissues and comprise the extracellular matrix and the associated proteins and growth factors. The small intestinal submucosa has been the mostly widely studied and is used clinically in numerous tissues.[109] Regardless of the scaffold design, integration of the engineered tissue into the host tissue is critical to tissue regeneration; however, the inflammatory response can often limit this host-tissue integration through fibrosis at the implant periphery. Similarly, the immune response to implanted, allogeneic cells often results in their death, which limits their therapeutic benefit. By taking advantage of immune-privileged implantation sites or immune-privileged cells, the immune response may be modulated,[110] and this strategy provides an exciting opportunity.

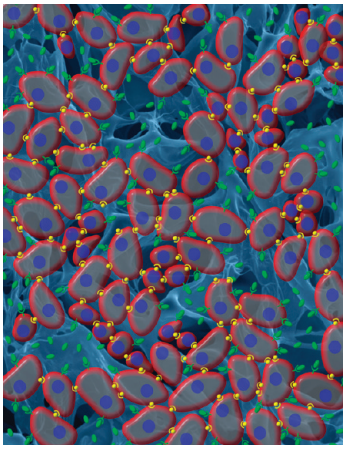

Figure 9. Cells (in red, with blue nuclei) interact with the tissue engineered scaffold through chemical (green ovals) and mechanical stimuli and with each other (yellow circles). These interactions define the cell microenvironment and guide cellular function and differentiation.

Perspective on the Future of Tissue Engineered Scaffolds

With the realization that cell therapy alone is insufficient for successful tissue regeneration, the engineered scaffold has gained importance. Today we understand that the mechanical properties of the scaffold can influence cell proliferation and differentiation similarly to the chemical properties, and thus attention has refocused on the design of the biomaterial. Most of the engineered biomaterial scaffolds are polymeric, and thus the opportunity to design polymers for applications in medicine is great. Importantly, our concept of a scaffold includes both the 3-dimensional traditional geometrically defined construct and the newer injectable material, which does not provide a distinct macroscopic architecture but still provides a controlled microenvironment for the cells. It is this microenvironment which is a key determinant of success and is comprised of cell interactions with other cells, soluble or matrix-bound growth factors and adhesion molecules, and the biomaterial itself through mechanical and chemical stimuli (Figure 9). The underlying strategy for the future is to understand the tissue sufficiently to design a polymeric biomaterial with the appropriate properties for success, whether the application is in vitro or in vivo.

Often the regulatory history of a given polymer serves as an impetus to pursue it over another material. Given the importance of translating fundamental research advances toward clinical application, considering the regulatory pathway is critical and can shorten (or lengthen) the review process. It is important to realize that regulators do not approve a polymer for general use, but only the specific application. Notwithstanding this important distinction, choosing a polymer that is known to the FDA can be an important strategic decision in obtaining a favorable (or faster) review. Yet, limiting the choice of polymer to ones that are already approved may not fulfill the design criteria required for success, and thus the regulatory file cannot be the only determinant.

Innovative biomaterials strategies, whether the choice of polymer or the way it is formulated, continue to drive the field;

however, designing a novel polymer in the absence of specific design criteria often leads to unsatisfactory results. The simpler biomaterials strategies—that is, those that have therapeutic benefit on their own (without cells)—will be commercialized first due to the complexity and expense of cell-based strategies. For example, there are some polymers, such as hyaluronan[111] and methacrylic acid-based polymers,[112] which promote wound healing on their own, likely by inducing angiogenesis or new blood vessel formation. However, biomaterials strategies alone are often insufficient. Incorporation of factors that promote cell interaction and cell guidance into the scaffold design provides a way to foster cellular interactions. Finding the right combination of factors and timing is nontrivial[102] and is made more difficult by the fact that some of these factors are not yet known. Notwithstanding, scaffolds are being designed with growth factors, adhesive peptides, and proteins immobilized to the polymeric scaffold to guide cell migration. These scaffolds hold great promise for both in vitro screening, providing more relevant data on cell and tissue response, and implantation strategies in the future.

Building on this complexity, biodegradable scaffolds impregnated with cells have shown great benefit in bladder replacement.[113] Moreover, multiple cell types are now being incorporated into tissue scaffolds, further emulating the tissue into which they will be implanted. These tissue engineered scaffolds go beyond the cell-based scaffolds and start to approach tissue scaffolds. This has been explored in screening applications where, for example, tissue engineered liver constructs require two cell types for cell survival and functioning. While the tissue engineered liver is far from implantation, it provides an excellent model for drug screening where toxic pharmaceuticals can be tested prior to animal studies.[94,114,115]

To further approximate tissues, the power of stem cells is being harnessed. While no engineered polymers were involved in the recent case of trachea repair, the concept of having a scaffold on which to culture cells for tissue replacement was used. Here, a donor trachea was decellularized and then seeded with the patient's own (autologous) stem cells, taking advantage of both the donor trachea for the appropriate mechanical and topographical features and the patient's stem cells to differentiate into the required cell types, to re-engineer the trachea.[116] The reliance on donor tissue is a primary limitation of this technique, precluding expansion of the procedure beyond the number of human donors. The promise of engineered scaffolds removes this limitation and has been a key driver of the field for the past 20 years.

With the advent of stem cells, biomaterials strategies have been redesigned to mimic the stem cell niche as a way to drive stem/progenitor cell differentiation to the desired cell type.[56,69] Understanding the cellular microenvironment and then incorporating this into biomaterials design strategies is an important focus. Similarly, and significantly, the biomaterials design strategy can be used as a way to better understand the cellular microenvironment. In this way, science and engineering work together to better define the cellular microenvironment and then use this advanced knowledge to engineer better tissue scaffolds. Inevitably, the complexity of this field demands multidisciplinary collaboration for success.

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