In Vitro and *In Vivo* Models for the Reconstruction of Intercellular Signaling[a]

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ABSTRACT: A critical need in both tissue-engineering applications and basic cell culture studies is the development of synthetic extracellular matrices (ECMs) and experimental systems that reconstitute three-dimensional cell–cell interactions and control tissue formation *in vitro* and *in vivo*. We have fabricated synthetic ECMs in the form of fiber-based fabrics, highly porous sponges, and hydrogels from biodegradable polymers (*e.g.*, polyglycolic acid) and tested their ability to regulate tissue formation. Both cell seeding onto these synthetic ECMs and subsequent culture conditions can be varied to control initial cell–cell interactions and subsequent cell growth and tissue development. Three-dimensional tissues composed of cells of interest, matrix produced by these cells, and the synthetic ECM (until it degrades) can be created with these systems. For example, smooth muscle cells can be grown on polyglycolic acid fiber-based synthetic ECMs to produce tissues with cell densities in excess of 10^8 cells/mL. These tissues contain extensive elastin and collagen, and the smooth muscle cells within the tissue express the contractile phenotype (*e.g.*, α-actin staining). Similar approaches can be used to grow a number of other tissues (*e.g.*, dental pulp) that resemble the native tissue. These engineered tissues may provide novel experimental systems to study the role of three-dimensional intercellular signaling in tissue development and may also find clinical application as replacements to lost or damaged tissues.

INTRODUCTION

Clinicians faced with replacing tissue lost to disease, trauma, or congenital defects currently have two options. The first, use of synthetic materials as a prosthesis, has a long history. The prosthesis typically does not replace the entire range of tissue functions and can serve as a site for infections and other chronic problems. The other option, use of autologous or allogenic tissue/organ transplantation is often very effective at replacing lost tissue structure and function. However, there exists a significant shortage of donor tissues available for tissue reconstruction and organ transplantation. For example, in 1988, only around 10% of the number of patients suffering from liver failure received a liver transplant.[1] This is mainly due to the limited number of organs available for transplantation every year. These limitations have led to the development of the tissue-engineering field, in which new tissues are created from cultured cells and biomaterial matrices.[2] This field has been defined as a "combination of the principles and methods of the life sciences with those of engineering to elucidate fundamental understanding of structure-function relationships in normal and diseased tissues, to develop materials and methods to repair damaged or diseased tissues, and to create en-

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tire tissue replacement."[3] It may be possible to treat a large number of patients by developing tissue-engineering strategies that use cell transplantation. One such strategy uses a combination of synthetic polymer matrices and cells to recreate three-dimensional cell–cell interactions and, hence, tissue formation. These matrices serve as both a transplant delivery system for the cells and templates guiding tissue proliferation and organization. In addition, these systems may provide new models to study tissue development and disease progression in well-controlled, *in vitro* experiments. There is a critical need for cell-culture models that, in contrast to standard tissue culture models, create three-dimensional cell interactions.

Natural extracellular matrices (ECMs) are used as a model for the development of synthetic matrices for tissue engineering. The natural ECM is composed of a complex network of macromolecules that hold cells together in an organized fashion to form tissues. These organized networks allow cells within to migrate and interact with one another. Moreover, nutrients, metabolites, and hormones from neighboring blood vessels diffuse into the ECM to provide nourishment and communication to the cells. The two main classes of extracellular macromolecules that form ECMs are proteoglycans (PG) and fibrous proteins. Proteoglycans contain long unbranched polysaccharide side chains (glycosaminoglycans) covalently tethered to a protein backbone. Proteoglycans form networks of hydrated gels in which cells are embedded, thus providing a three-dimensional space for tissue formation. Structural fibrous proteins like collagen provide the tensile strength of ECMs, whereas proteoglycans provide compressive strength. Hence, critical functions of ECMs include providing potential space for tissue development and mechanical support during this process. Recently, it has been become clear that the ECM also plays an essential role in such cellular functions as growth, differentiation, and motility. The cell–ECM interactions are mediated by the affinity of cell-membrane receptors for specific peptide sequences present in ECM molecules. The best-studied sequence is the tripeptide Arg-Gly-Asp (RGD), which is responsible for cell adhesion of many ECM proteins.[4] Cell adhesion to ECMs by way of ligand-receptor affinity is the first step in a series of biological reactions, and communications between cells and the ECM, and between cells. Cell–ECM interactions ultimately guide the generation of new tissues.

SYNTHETIC BIODEGRADABLE MATRICES

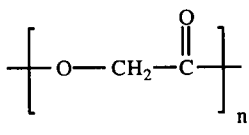
A variety of synthetic ECMs have shown promise in tissue-engineering applications. Natural biodegradable matrices (collagen, fibrin) have been widely used as cell immobilization matrices; however, they suffers from batch to batch inconsistency, which is a major concern in production scale-up. As a result, synthetic polymers have been alternatively used as cell-delivery devices due to their well-controlled structures and properties. It is particularly attractive if these matrices are biodegradable. This eliminates the need to remove the matrix following tissue development. The rate at which the matrix degrades should be compatible with the rate of new tissue development and regeneration. In addition, the degradation products should be biocompatible. In this paper, we will discuss several examples where biodegradable synthetic matrices have been successfully used for tissue engineering.

Polyglycolic acid (PGA), poly-L-lactic acid (PLLA), and copolymers of lactic and glycolic acid (PLGA) are the most widely used biodegradable polymers to fabricate synthetic ECMs. These synthetic polymers have been used in a variety of biomedical applications (*e.g.*, suture materials) for over twenty years.[5] PGA, PLLA, and PLGA contain ester linkages (FIG. 1) and are susceptible to hydrolysis in aqueous environments. Hydrolytic cleavage of the ester linkage yields naturally occurring metabolic by-

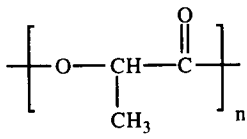
products that are biocompatible and cause minimal inflammatory responses. PGA is a highly crystalline polymer with a high melting point and low solubility in organic solvents and is relatively hydrophilic. PGA implants lose their structural integrity within two weeks and are absorbed after about four weeks of implantation.[6,7] PLLA, on the other hand, is more hydrophobic and more soluble in organic solvents. As a result, PLLA is less labile to aqueous hydrolysis and degrades at a much slower rate compared to PGA.[8] Copolymers of varying ratios of L-lactic acid and glycolic acid yield polymers with a wide range of degradation rates and mechanical properties, making them more attractive candidates for cell-delivery devices in tissue engineering.[12]

Several techniques have been developed to process these polymers into devices suitable for tissue-engineering applications.[9] PGA is typically processed by melting and extruding into fibers (diameter, 10–15 μm) that are used to construct woven and nonwoven fiber-based meshes.[20,21] Nonwoven meshes of PGA fibers are highly porous, permitting diffusion of nutrients throughout the scaffold, and are capable of delivering high densities of cells. However, such constructs lack the structural stability to withstand compressive forces *in vivo*. To improve upon their mechanical properties, PGA fibers in these matrices have been physically bonded with PLLA and PLGA.[21] A wide range of mechanical strengths and rates of degradation can be achieved by varying the amount of physical bonding.

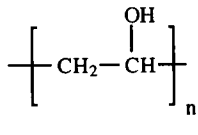
To further improve upon the mechanical strength of synthetic ECMs, highly porous sponges have been fabricated from PLLA and poly-D,L-lactic-co-glycolic acid (PLGA). These polymers are commonly processed into sponges with various pore sizes by a variety of methods involving a phase transition. Typical methods include solvent casting



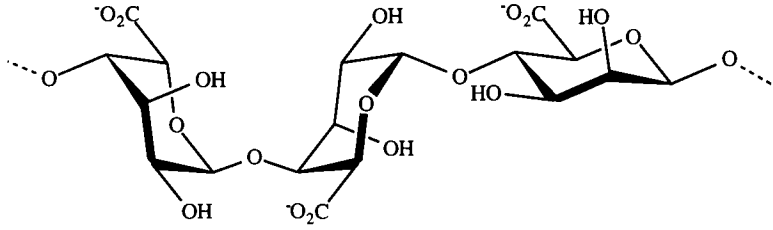
PGA



PLA



PVA



Sodium alginate

FIGURE 1. Chemical structures of polyglycolic acid (PGA), poly-L-lactic acid (PLLA), polyvinyl alcohol (PVA), and sodium alginate.

and particulate leaching or a gas-foaming method.[10-13] In the former method, the polymer is dissolved in an organic solvent and cast into cylindrical molds packed with sodium chloride particles of a desired size range. The organic solvent is evaporated, and the entrapped salt particles are leached out in aqueous solutions. The dimensions of the sponge can be controlled by the size and shape of the mold. Moreover, the pore size distribution can be controlled by the size of the salt particles used in packing the mold, and the porosity of the sponges can be varied by altering the ratio of polymer/salt particles.[10,12] To avoid the use of organic solvents, another approach to fabricate highly porous sponges was developed using high-pressure gases to induce nucleation and growth of gas cells within the polymer matrix. Highly porous matrices (up to 93%) can be fabricated with this approach.[13]

Other materials that are promising for cell immobilization matrices in tissue engineering include alginates. Algal alginate, a naturally occurring polysaccharide isolated from seaweed, has been extensively used in the chemical and food industry as a thickening, emulsifying, and stabilizing agent. Biomedical applications of alginates include wound dressings, dental impression materials, and, more recently, cell immobilization matrices.[14,22,23] Alginates are linear polysaccharides that contains variable amounts of the uronic acids D-mannuronic acid (M) and L-guluronic acid (G) (FIG. 1). The two monosaccharides present are in random sequences of poly-mannuronate (M-M-M-M), poly-guluronate (G-G-G-G), and an alternating sequence of both uronic acids (-M-G-M-G).[15] An attractive feature of alginates is their ability to form hydrogels in the presence of divalent cations (*e.g.*, Ca^{2+}, Sr^{2+}, and Ba^{2+}). It is well established that this affinity and selectivity for divalent cations is related to the poly-guluronate content of the polymer. Sodium alginates with a wide range of G and M content are commercially available. Hydrogels of alginates seeded with cells have the potential to be transplanted endoscopically into a patient, thus providing a minimally invasive method for cell transplantation.[17,18]

IN VITRO TISSUE DEVELOPMENT

The development of cell seeding and cell-culture conditions for the cell-polymer constructs is essential to efficiently engineer tissues. To optimize the number of adherent cells onto fiber-based polymer constructs, different methods for cell seeding have been investigated.[16] Dynamic seeding methods (*e.g.*, seeding in stirred bioreactors) produces a higher number of adherent cells as compared to static seeding methods.[16] Cell seeding can also be enhanced by surface modifications of matrices.[17] The importance of culture conditions has been demonstrated by studies comparing the development of smooth muscle tissue in static and stirred culture conditions. Optimal tissue development is typically achieved in bioreactors with nutrient flow, as indicated by higher cell densities and matrix compositions. Tissues developed in bioreactors contained more elastin and collagen, and the amount of elastin was comparable to that of native tissue.[6] Similar results have been found when engineering dental pulp tissue (unpublished data). Tissues (*e.g.*, dental pulp) developed under appropriate conditions (FIG. 2) can resemble the native tissues.[18] Nonwoven PGA fiber-based scaffolds have also been seeded with bovine chondrocytes and used to engineer new cartilage *in vitro*.[19]

IN VIVO EVALUATION OF CELL-POLYMER CONSTRUCTS

The potential of tissue engineering to create tissues *in vivo* has also been demonstrated with a number of tissue types. Cartilage in various sizes and shapes (*e.g.*, nasal

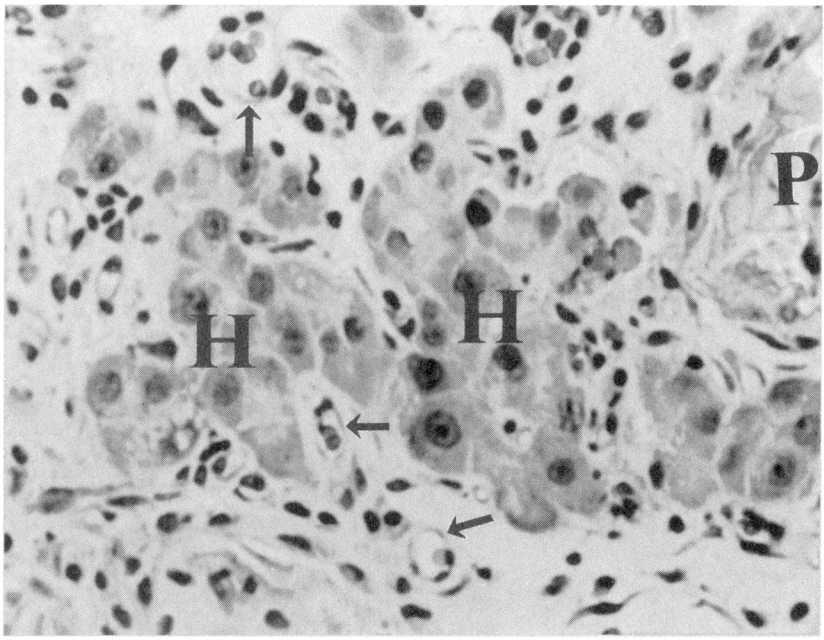


FIGURE 2. Photomicrograph of new liver-like tissue engineered *in vivo*.[17] Hepatocytes (H), residual polymer matrix (P), and new capillaries (arrows) are visible in the photomicrograph (D. J. Mooney, unpublished data).

septal cartilage) has been engineered in various animal models using polyglycolic acid matrices.[20,21] Cartilage regeneration has also been demonstrated with such other biodegradable polymer materials as the sodium alginates.[22] The newly formed cartilage in these studies resembled native cartilage biochemically and structurally.[23] The feasibility of using injectable chondrocyte-alginate gel suspensions has also been demonstrated in the treatment of vesicoureteral reflux in pigs.[24,25] Sponge-based matrices have been used to transplant hepatocytes and engineer new liver-like tissues.[17] A variety of other tissues, including intestine, have also been partially engineered with this type of matrix.[26,27] Implantation of these highly porous matrices typically leads to the ingrowth of blood vessels that support the metabolic needs of the new tissue (FIG. 3).

APPLICATION TO SALIVARY TISSUE

The principles of tissue engineering have not yet been applied to salivary tissue. However, the matrices and systems developed with other tissues will likely be directly applicable to salivary tissue. Matrices developed to transplant hepatocytes, another secretory cell type, will likely be useful for *in vitro* and *in vivo* models of salivary tissue engineering. Matrices to create tubular structures, which would be a critical component of engineered salivary tissue, have also been demonstrated.[26,27] Creation of three-

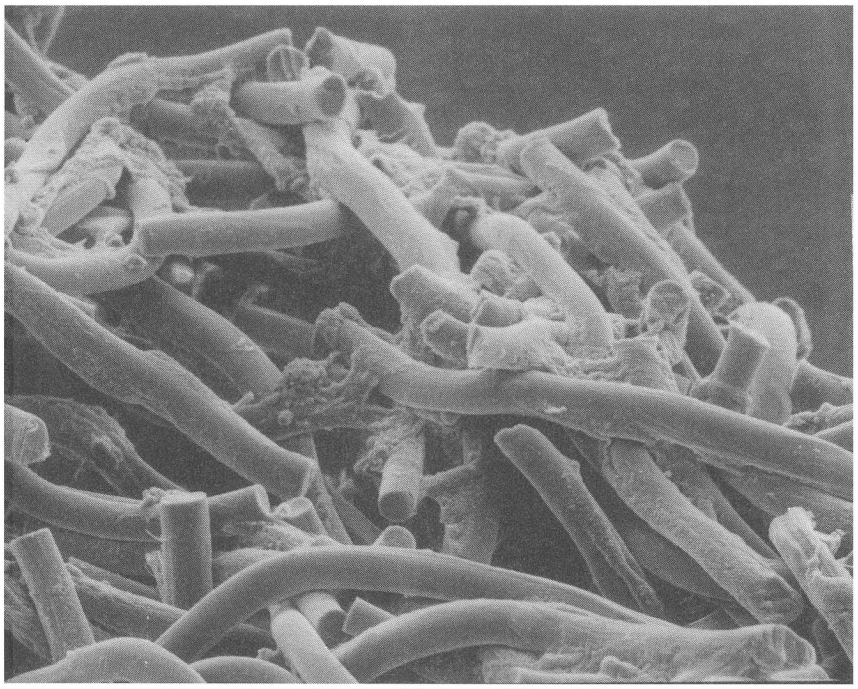


FIGURE 3. Human pulp-derived fibroblasts adherent to fibers of a polyglycolic acid matrix. The cells will proliferate, secrete extracellular matrix, and fill the pores while the matrix degrades. A new, pulp-like tissue will result.[18]

dimensional salivary tissue culture systems may also lead to an improved understanding of the role of cell–cell interactions in salivary development, function, and dysfunction in various disease states.

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