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Cell-Interactive Polymers for Tissue Engineering

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Abstract: Tissue engineering is one exciting approach to treat patients who need a new organ or tissue. A critical element in this approach is the polymer scaffold, as it provides a space for new tissue formation and mimics many roles of natural extracellular matrices. In this review, we describe several design parameters of polymer matrices that can significantly affect cellular behavior, as well as various polymers which are frequently used to date or potentially useful in many tissue engineering applications. Interactions between cells and polymer scaffolds, including specific receptor-ligand interactions, physical and degradation feature of the scaffolds, and delivery of soluble factors, should be considered in the design and tailoring of appropriate polymer matrices to be used in tissue engineering applications, as these interactions control the function and structure of engineered tissues.

Keywords: Tissue engineering, Scaffold, Ligand-receptor, Physical properties, Degradation, Soluble factor

Introduction

Millions of surgical procedures are performed in the US every year to treat patients who suffer the loss or failure of an organ or tissue, resulting from accidents or disease[1]. Tissue or organ transplantation is one generally accepted therapy to replace damaged tissues or organs in these patients. However, the number of donors is extremely limited[2]. Tissue engineering is one exciting approach to treat patients who need a new organ or tissue with man-made organs or tissues. In this approach, tissues are engineered using a combination of a patient's own cells and polymer scaffolds. Tissue-specific cells are isolated from a small biopsy from the patient and expanded *in vitro*. The cells are subsequently incorporated into three-dimensionally designed polymer scaffolds. In this approach, the polymer potentially mimics many roles of natural extracellular matrices, which bring cells together and control the tissue structure, regulate the function of the cells, and allow the diffusion of nutrients, metabolites, and soluble factors[3,4]. Many tissues, including skin, artery, bladder, cartilage, and bone, are being engineered using this approach, and several of them are at or near clinical uses[5].

One critical element in this tissue engineering approach is the regulation of interactions between the cells and polymer scaffolds. The interactions can be regulated by controlling specific ligand-receptor interactions, physical properties of the scaffolds (e.g., mechanical properties and degradation rate), and the release of soluble factors (i.e., growth factor and DNA) from the scaffolds. All of these signals can alter the gene expression of cells (Figure 1). A variety of polymers have been studied and utilized to date in tissue engineering approaches. However, no single polymer has been considered ideal for all tissues and approaches. For example, alginate has been widely used as a delivery vehicle of cells in many tissue engineering applications, due to its biocompatibility and easy gelation. However, it has no specific interactions with cells and its degradation is very uncontrollable. In any case, fundamental investigations of the interactions between cells and polymers should precede the design and tailoring of polymers to be used in tissue engineering applications.

In this review, we discuss cell-interactive polymeric materials potentially useful in tissue engineering applications. We describe the role of physical (e.g., mechanical properties of matrices) and chemical (e.g., ligand-receptor interaction) signals in controlling cellular gene expression and eventually the function and structure of engineered tissues. This review will mainly focus on approaches taken in our laboratory to modify conventional polymers to introduce desired cell interactive features, and to fabricate scaffolds for tissue engineering applications.

Potential Polymeric Materials for Tissue Engineering Applications

Cell/polymer constructs are generally transplanted back into the body in order to engineer tissues, either by an open surgical procedure (implantable material) or in a minimally invasive manner using syringes or endoscopes (injectable materials)[6]. Implantable scaffolds are typically in the physical form of foams, sponges, and films. Many synthetic polymers, such as aliphatic polyesters, polyanhydrides, poly (ortho ester)s, and polypeptides are potential implantable materials. The injectable materials include natural polymers such as alginate, chitosan, hyaluronic acid, and collagen, which can form hydrogels or micro beads. Certain polymers such as polyanhydrides and collagen can be also used in both forms[7,8].

Implantable Materials

Aliphatic polyesters such as poly(glycolic acid) (PGA),

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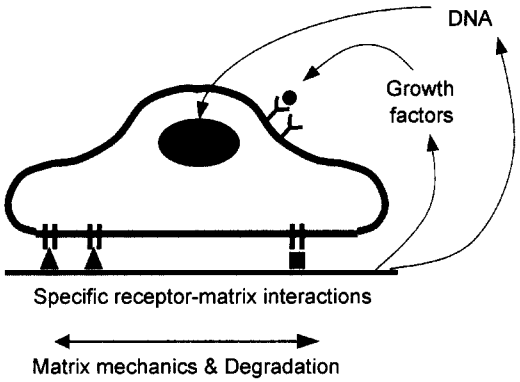

Figure 1. Various signals generated from cell/polymer interactions that can be used to regulate cellular gene expression.

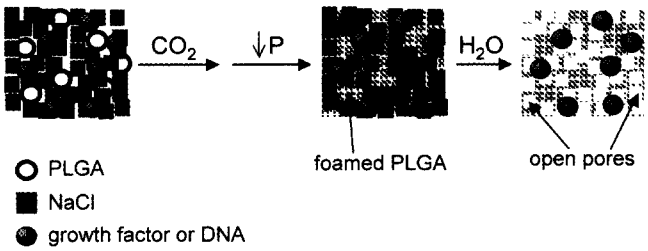

Figure 2. Schematic description of gas foaming/particulate leaching process.

poly(lactic acid) (PLA), and their copolymers (PLGA) are representative synthetic polymers, which have been widely used in many tissue engineering applications[9]. PGA has a high crystallinity and low solubility in organic solvents, while PLA has better solubility in organic solvents than PGA due to the methyl group in PLA. However, PLA is less labile to hydrolysis due to steric hindrance of the methyl group, resulting in slower degradation. PLGA can be readily synthesized, and their physical properties as well as degradation rate are controlled by the ratio of glycolic acid to lactic acid[10].

We have adopted a unique processing technique to generate highly porous polymer scaffolds by a gas foaming/ particulate leaching method[11,12]. This technique has provided an efficient means to generate inter-connected porous structure, which enables the penetration of cells into these scaffolds (Figure 2). Since the gas foaming process does not require any organic solvents or high processing temperature, biologically active molecules such as growth factors and plasmid DNA can be incorporated into these matrices without denaturation[13,14]. Alternatively, many different approaches such as phase separation[15], emulsion freeze-drying[16], and fiber extrusion and fabric formation [17] have been also reported by other research groups to fabricate scaffolds from these polymers. Non-woven fabrics of PGA have been stabilized by physically bonding with PLLA to increase the resistance to compressive forces, and used to successfully engineer smooth muscle tissues[18].

A number of other synthetic polymers could be also utilized to fabricate scaffolds for tissue regeneration. Polycaprolactone (PCL) is also one of the aliphatic polyesters, and is a semi-crystalline polymer with high solubility in organic solvents and low melting temperature. However, the degradation rate of PCL is much slower than PGA or PLA. Polyanhydrides are usually copolymers of aromatic diacids and aliphatic diacids. They usually degrade by surface erosion, and their degradation rate can be controlled depending on the choice of diacids[19]. Poly(ortho ester)s are biodegradable polymers, which degrade by gradual surface erosion, and have been known as useful materials for controlled drug delivery[20]. There has been wide interest in synthesizing polypeptides to mimic natural proteins, as they are major components of natural matrices of tissues. However, it is generally very difficult to precisely control the sequence of amino acids of polypeptides, in addition to poor solubility in common organic solvents. Recently, new polymerization strategies to synthesize polypeptides with well-defined amino acid sequences and a wide range of molecular weights was reported using various organonickel initiators[21] or by synthesizing genetically engineered polypeptides[22,23].

Injectable Materials

A number of natural polymers can be utilized in an injectable form for tissue engineering applications. Hydrogels are highly attractive injectable materials for these applications due to their structural similarity to the macromolecule-based materials in the body, as well as their potential to be introduced into the body in a minimally invasive manner. We have been interested in the utilization of alginate as an injectable cell delivery vehicle as well as a drug delivery system[24] due to its simple gelation when ionically cross-linked with divalent cation such as Ca^{2+}. There have been many attempts to utilize alginate in medical applications, including its use as a wound dressing, dental impression, immobilization matrix, and tissue engineering scaffold due to its biocompatibility, hydrophilicity, and relatively low cost [25-27]. Alginate can be used in an injectable form by either being preformed into small beads[28], or by simple injection after gelation[29]. We have prepared macroporous alginate beads to allow for the migration of cells throughout the beads and integration of the engineered tissue with the surrounding host tissue[28]. In brief, macroporous beads were formed by incorporation of gas pockets within the beads, stabilization of the gas bubbles with surfactants, and subsequent removal of the gas in a vacuum. The interconnected macroporous alginate beads have found to support cell invasion *in vivo* (Figure 3).

Other natural polymers including chitosan, hyaluronic acid, dextran, fibrin, and collagen are potential candidates to

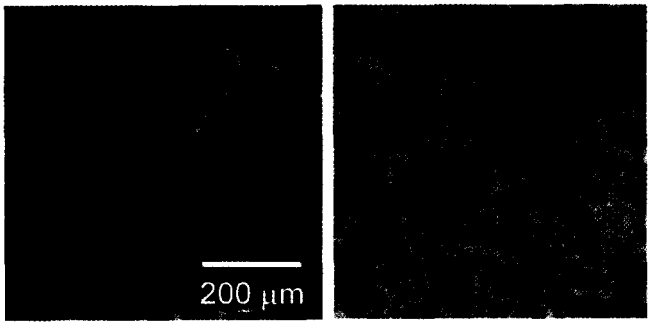

Figure 3. (a) Environmental scanning electron micrograph of the surface of porous alginate beads, (b) *In vivo* response to the porous alginate beads after subcutaneous implantation into rats (2 weeks, 100× magnification). Photomicrographs have labels for alginate and (A) granulation tissue (G)[28].

form gels. Chitosan has been known to be biocompatible, biodegradable, and has low toxicity[30,31]. Chitosan has been used as a cell substrate to engineer liver[32] and bone [33]. Hyaluronic acid is known to be one of glycosaminoglycan components of natural extracellular matrices, and is degraded by hyaluronidase[34]. Hyaluronic acid has been used in many biomedical applications. However, it has poor physical strength and this has limited its applications. Fibrin gels can be produced from the patient's own blood, and have also been utilized to engineer tissues with skeletal muscle cells [35], smooth muscle cells[36], and chondrocytes[37]. No toxic degradation or inflammatory reactions are expected from this natural component of the body. However, fibrin gels are limited in mechanical strength, and this prevents their use in certain applications. Collagen is the best-known tissue-derived natural polymer. It is the main component of many mammalian tissues including skin, bone, cartilage, tendon, and ligament. Collagen has been used as a tissue culture scaffold or artificial skin due to the easy attachment of many different cell types, regardless of its limited range of physical properties and high cost[38]. Covalent cross-linking of hyaluronic acid and collagen gels has been widely investigated as a means to broaden the range of mechanical properties available from these materials[39,40].

Specific Ligand-Receptor Interaction

The adhesive interactions of cells with matrices may significantly affect their proliferation, migration, and differentiation. The adhesion of cells to matrices may be cell-type specific, and is dependent on the interaction of specific cell receptors, which recognize adhesion molecules (i.e., ligand) at the surface of materials[41]. These ligand molecules can be either inherent components of materials or artificially introduced onto the materials. We have introduced small peptides containing RGD sequences (Arginine-Glycine-

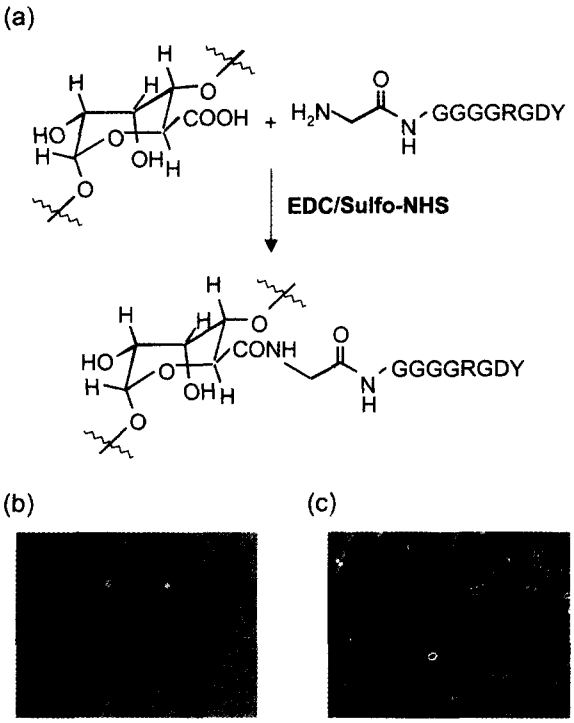

Figure 4. (a) Synthetic scheme of RGD-modified alginate. Myoblast adhesion onto (b) unmodified and (c) RGD-modified alginate hydrogels (seeding density, 25000 cells/cm^2; 4 hr post-seeding)[43].

Aspartic acid) to alginate, as alginate is known to discourage protein adsorption due to its hydrophilic character, and this may decrease the survival of many cell types in alginate matrices[42]. In brief, alginate was modified with the GRGDY peptide in the presence of water-soluble carbodiimide (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS). The optimum reaction condition was found to be slightly acidic (pH 6.0-7.5, 0.1 M MES buffer). Carboxylic acid groups of alginate offer the potential reaction site for covalent modification with RGD-containing peptides (Figure 4). Mouse skeletal myoblasts adhered to the RGD-modified alginate gels, proliferated, fused into multinucleated myofibrils, and expressed heavy-chain myosin (i.e., a differentiation marker for skeletal muscle)[43].

Physical Properties of Matrices

The physical properties of polymer matrices are also important factors to design matrices for tissue engineering applications. The adhesion and gene expression of interacting cells may be related to the physical properties of polymer scaffolds[44], as well as the specific receptor-ligand interactions described earlier. The physical properties of polymer matrices mainly depend on the inherent physical characteristics of the polymer chains as well as on the processing technique used to form a three-dimensional structure from the polymer.

Useful polymers may arise from the synthesis of new types of polymers or from the modification of conventional polymers that have an established history of biocompatibility. We have taken the latter approach as the following two examples demonstrate.

Nonwoven fabrics of PGA fibers are attractive matrices in tissue engineering approaches, and have found many applications in engineering various tissues. However, these matrices lack structural stability, and often cannot maintain their original structure during tissue development. This makes it difficult to engineer tissues with predefined configurations and dimensions using these materials. We and others have prepared PLLA-bonded nonwoven fabrics to resist cellular contractile forces, and maintain their predefined structure during tissue development *in vitro* and *in vivo*[18,45]. These physically bonded matrices exhibited 10-35-fold increase in the compressive modulus, compared to unbonded matrices, and their use resulted in higher cellularity and synthesis of new matrix molecules by cells (e.g., elastin) in an engineered tissue[18].

We have also introduced covalent cross-links to alginate gels to control their physical properties, as ionically cross-linked alginate gels release divalent ions in an uncontrollable manner, and this deteriorates the mechanical properties of the gels in an unpredictable manner[46]. We have prepared alginate gels covalently cross-linked with various cross-linking molecules, including adipic acid dihydrazide, L-lysine, and amino-poly(ethylene glycol)[47]. We have found that the physical properties of the gels are mainly controlled by the cross-linking density, but are also moderately dependent on the type of cross-linking molecule. The chemistry of the cross-linking molecules significantly contributes to the swelling of the gels. The introduction of hydrophilic cross-linking molecules as a second macromolecule (e.g., PEG) can compensate for the loss of the hydrophilic character of the gels resulting from the consumption of carboxyl groups of alginate during cross-linking (Figure 5).

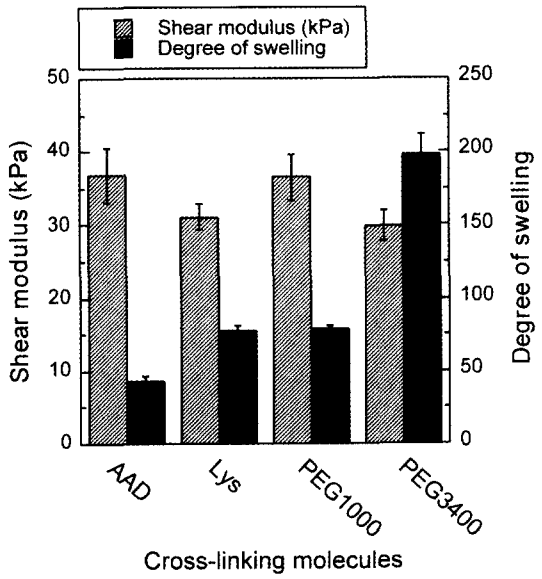

Figure 5. Effect of cross-linking molecules on the maximum shear modulus and swelling degree of alginate gels in water at the maximum shear modulus. AAD and Lys represent adipic acid dihydrazide and methyl ester L-lysine, respectively[47].

Controlled Degradation

The controlled degradation of polymer matrices is critical in many tissue engineering applications, as one may want to time the degradation rate of the matrices to the rate of new tissue formation. This time will be dependent on the tissue to be engineered[4]. We have focused on strategies to control the degradation of polymer matrices. First, one can utilize a polymer that undergoes main chain scission by hydrolysis and/or enzyme action (e.g., PLGA) to fabricate a three-dimensional structure. Second, one can fabricate a matrix from non-degradable polymers that are coupled with degradable cross-links. In this situation, the polymer should be of sufficiently low molecular weight that it can be readily solubilized and released from the implant site and subsequently be cleared from the body.

As an example of the first approach, we have reported that alginate may be rendered susceptible to hydrolytic degradation by partial oxidation[48]. The molecular weight of commercially available alginate is typically above the renal clearance threshold of the kidney[49] and alginate is not degradable in physiological conditions. To bypass these limitations, commercially available high molecular weight alginate was partially oxidized using sodium periodate, and this resulted in a conformational change of uronic acid residues to an open-chain adduct, which behaves like an acetal group susceptible to hydrolysis. The degradation rate of the oxidized alginate was dependent on the pH and temperature of the solution. These partially oxidized alginates have been successfully used to engineer cartilage-like tissue *in vivo*, suggesting these materials have potential as a cell transplantation vehicle[48].

We have also controlled the degradation of alginate-derived hydrogels by covalently cross-linking low molecular weight alginate derivatives (second approach described above). Polyguluronate (6000 Da) was isolated from alginate, oxidized, and cross-linked with adipic dihydrazide to form gels[50]. The mechanical properties of the resultant gels were regulated by the extent of cross-linking, and the hydrazone bond coupling the polymers is hydrolytically labile. One interesting finding is that the degradation rate and mechanical properties of these gels could be decoupled [51], unlike conventional gels. This decoupling of the degradation rate and mechanical properties could be inferred by the introduction of excess amounts of the cross-linking agent. This resulted in large numbers of these molecules

reacting only partially with the polymer chains, leaving these molecules coupled on only one end to the polymers. This results in the formation of weak gels, due to these network imperfections. However, these dangling cross-linking molecules can form effective cross-links as the original cross-links degrade, which results in very stable gels for long time periods[51]. These gels have shown utility in engineering bone-like tissue with pre-osteoblast transplantation[29].

Delivery of Soluble Factors

One critical element to engineer large tissues is the development of new vascular network, which enables the delivery of sufficient oxygen and other nutrient to the engineered tissues. This vascular network should be rapidly formed during the process of tissue development. A lack of rapid vascularization currently limits our ability to engineer many tissues, including liver[52] and bone[29]. One recent and exciting approach to promote angiogenesis in engineered tissues is the delivery of angiogenic molecules and/or blood vessel forming cells (endothelial cells) to the site at which the tissue is being engineered[53,54]. In this section, we focus on the delivery of angiogenic promoters, as their delivery can be tightly regulated by characteristics of the polymer.

Growth Factor Delivery

Controlled and sustained release of angiogenic molecules from polymer scaffolds may enable one to optimize the vascularization process. Various growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF-2), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) can be incorporated into polymer matrices, and released at controlled and sustained rates for extended periods of time[13,55].

An important issue in growth factor delivery is that many tissues (e.g., bone, muscle, and blood vessels) exist in a mechanically dynamic environment. Conventional delivery systems for growth factors and/or cells have been designed to operate under static conditions, and the effect of a mechanically dynamic environment on the factor delivery has not been systemically exploited yet. We have recently demonstrated that mechanical signals can be exploited to modulate growth factor release from polymer matrices, and to provide a novel approach to guide tissue formation in mechanically stressed environments[56,57]. In brief, VEGF-incorporated alginate hydrogels were prepared, and mechanically stimulated *in vitro* with different strain amplitudes. The release of VEGF was remarkably upregulated by mechanical stimulation, and the increase of local concentration of VEGF affected the vascularization process in animals (Figure 6)[56].

DNA Delivery

Delivery of plasmid DNA encoding angiogenic proteins may provide an alternative approach to generate new vascular networks in engineered tissues. The difficulty of protein stabilization has led to the development of polymer systems for the DNA delivery[58]. We have prepared porous PLGA scaffolds containing a plasmid encoding for PDGF, a potent angiogenesis promoter, by the gas foaming/particulate leaching technique described earlier. This delivery vehicle greatly increased the number of blood vessels and granulation tissue formed in animals, compared to the direct injection of the plasmid[59]. Since transfection is transient, the sustained release provides high levels of expression over controlled time scales.

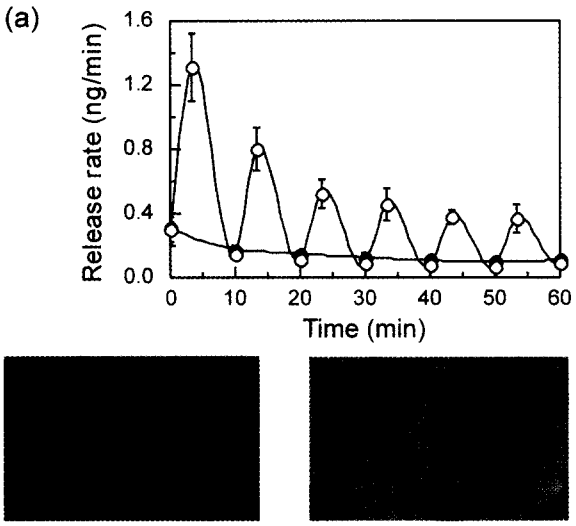

Figure 6. (a) *In vitro* release rate of VEGF from alginate gels under mechanical stimulation (*l*, no compression as a control; ○, 25% strain amplitude). Mechanical stimulation involves six cycles of compression for 2 min followed by relaxation for 8 min. Photomicrographs of representative tissue section surrounding VEGF-loaded gels, which have been implanted into the femoral artery ligation site of nonobese diabetic mice for 2 weeks (b) without mechanical stimulation as a control and (c) under cyclic stimulation. Original pictures were taken at 1000× magnification, and arrows indicate blood vessels[56].

Concluding Remarks

We have summarized several critical design parameters of polymer matrices that can significantly affect cellular behavior in tissue engineering approaches, as well as a variety of polymers which are frequently used to date or potentially useful. It is important to note that some of the design parameters should be considered together to design and tailor appropriate polymer systems for engineering specific tissues. For example, it is clear that mechanical stimulation plays a significant role in controlling the proliferation and differentiation of cells, as well as their organization into tissues. This factor can result in the increased mechanical

properties of the tissues[60]. In addition, however, this type of stimulation also affects the release behavior of growth factors from polymer matrices, and enhances new vascular network formation *in vivo* due to the increased local concentration of growth factors in the engineered tissue [56,57]. We believe that there is no single ideal material satisfying all the requirements to engineer all tissue types with all strategies. However, the design parameters described in this review will likely be important regardless of the particular polymeric materials used in various tissue engineering applications.

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