# Tissue Engineering

## Biodegradable Polymers

Many strategies aimed at generating completely natural living tissue that could replace failing or malfunctioning organs have focused on the use of biodegradable polymers as temporary scaffolds for cell transplantation. The concept is that cells obtained from patients can be expanded in culture, seeded onto an appropriate polymer scaffold, and then transplanted.

The success of the strategy is highly dependent on the properties of the polymer, requiring minimally that it be biocompatible, easily sterilizable, and degradable into products that can be metabolized or excreted. A polymer scaffold should be easily and reproducibly processed into a desired shape and structure that can be maintained after implantation so that it defines the ultimate shape of the regenerated tissue. Mechanical properties are important in designing polymer scaffolds for the regeneration of load-bearing tissues such as bone.

The degradation rate should be optimized to allow transplanted cells to proliferate and secrete their own extracellular matrix, while the polymer scaffold can vanish after a desired time period to leave space for new tissue ingrowth. The rate of scaffold degradation should match the rate of tissue regeneration in order to maintain the mechanical strength of the transplant and to avoid collapse or stress shielding. Biodegradability of a polymer is determined by its composition, molecular weight, degree of crystallinity, scaffold configuration, and environmental conditions such as temperature, pH, and mechanical loading.

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Most mammalian cell types are anchorage-dependent and require a suitable substrate to survive and retain their ability to function and proliferate. Cell morphology correlates with function, and it is possible to control cell shape and induce the expression of a differentiated cell phenotype by engineering specific surface patterns on the polymer substrate. For example, rounded cell morphology could be maintained by confining cells in physical or chemical microstructures. However, topological constraints may affect cell proliferation and migration.

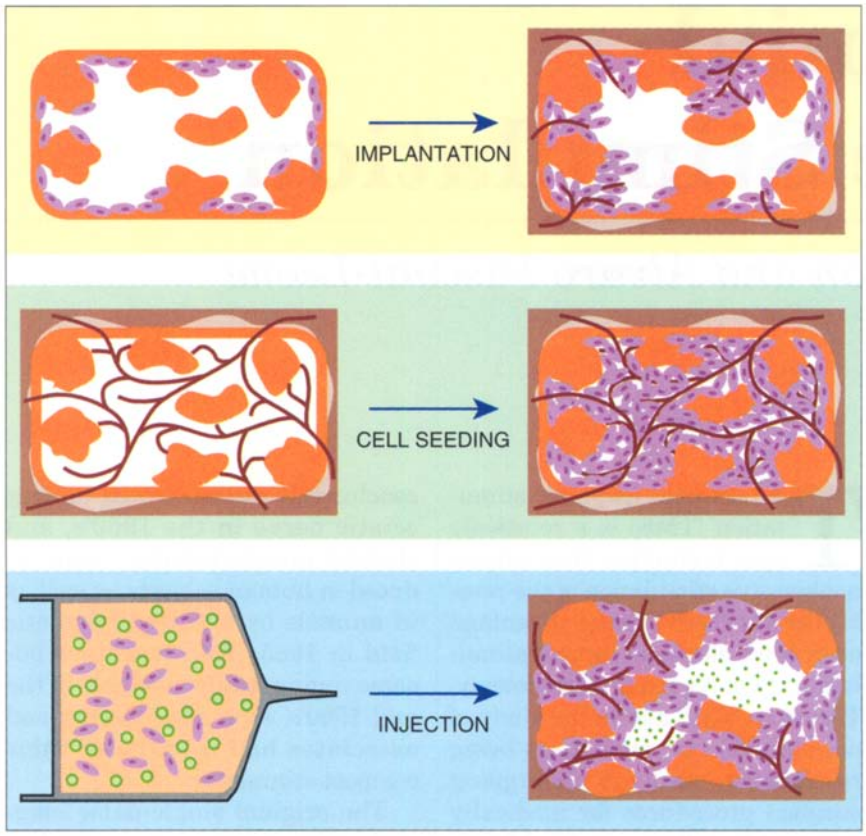
Cellular activity could also be modulated by grafting ligands onto the scaffold surface to allow specific interactions with cell surface receptors. For example, the arginine-lysine-aspartic acid sequence, the site for cell attachment to fibronectin, could be coupled to the polymer with controlled density to influence cell behavior.

In addition to signals from the extracellular matrix, cells respond to soluble bioactive molecules such as cytokines, growth factors, and angiogenic factors. Although these molecules alone can be used for tissue induction, tissue regeneration can be better achieved by combining cell transplantation and drug delivery. Tissue inductive factors can be incorporated into biodegradable polymers during scaffold processing. Alternatively, biodegradable microparticles or nanoparticles loaded with these molecules can be impregnated into the substrate. Release of bioactive molecules in vivo is governed by both diffusion and polymer degradation. Retention of activity has been a major concern in delivering large molecules such as proteins.

Porosity, pore size, and pore structure are important factors in nutrient supply to transplanted cells. To regenerate highly vascular organs such as liver, a porous scaffold with a large void volume and a large ratio of surface area to volume is desirable for maximal cell seeding, extracellular matrix production, and vascularization. Small-diameter pores are preferable to achieve high surface area per volume, as long as the pore size is greater than the diameter of a cell in suspension (typically 10 μm). However, larger pores may be required for cell growth. For example, an optimal pore size of 200 to 400 μm is required for maximum bone ingrowth.

The rate of fibrovascular tissue invasion in porous biodegradable polymers also depends on the pore size. Compared to isolated pores, an interconnected pore network enhances diffusion rates to and from the center of the scaffold and facilitates vascularization, thus improving oxygen and nutrient supply and waste removal. Vascularization of an implant is a prerequisite for regeneration of most three-dimensional tissues, except for cartilage, which is avascular.

Besides cell morphology, the function of many cells is dependent on the three-dimensional spatial relationship of cells and extracellular matrix. The shape of a skeletal tissue such as bone or cartilage is also critical to its function. It is often difficult to achieve uniform cell seeding into three-dimensional scaffolds. In the case of bone regeneration, the cell seeding density also has to be maximized while appreciable space is left for cell proliferation and vascularization in vivo before significant polymer degradation takes place. The major obstacle to the in vitro development of three-dimensional poly-




**Biodegradable polymers** are used for cell transplantation in three ways. They can be processed into porous scaffolds to serve as substrates for in vitro cell culture and subsequent transplantation. For most tissues, the survival and differentiation of the cells rely on formation of new blood vessels in vivo (*upper panel*). To regenerate highly vascular tissue, the scaffold is implanted in vivo to allow angiogenesis to occur before cells are seeded by injection (*middle panel*). Finally, to fill an irregular defect, a cell-polymer construct containing a porogen is injected (*lower panel*). After polymerization in situ, the hardened material acts as a template for cell attachment and growth, while the porogen is eventually leached out. In all cases, the polymer scaffold degrades gradually in vivo.

mer-cell constructs is nutrient diffusion, because cells will not survive farther than a few hundred microns from the nutrient supply.

Some complex osseous defects created by bone tumor removal or extensive tissue damage exceed critical size for normal healing and require a large transplant to restore function. A novel strategy is to prefabricate a vascularized bone flap by implanting a mold containing biodegradable polymers with osteoinductive elements onto a periosteal site remote from the defect, where prevascularization and ectopic bone formation can occur over a period of time as the scaffold degrades. The newly created autologous bone can then be transplanted to the defect site, where its vascular supply can be attached via microsurgery to existing vessels.

A similar approach may be applied to the regeneration of other tissues by injecting cells into a prevascularized biodegradable scaffold. A scaffold containing fibrovascular tissue or uncommitted vascular tissue such as periosteum will provide a substrate for cell attachment, growth, and function. The extent of prevascularization has to be controlled to allow sufficient nutrient diffusion as well as enough space for cell seeding.

Injectable biodegradable materials that polymerize in situ can be used to fill defects of any shape with minimal surgical intervention. These materials must meet additional requirements because polymerization or cross-linking reactions occur in vivo. All reagents and products must be biocompatible, and the reaction conditions such as temperature and pH should not damage the implanted cells or surrounding tissue. One such material, poly(propylene fumarate), has been developed as an injectable bone cement that hardens within 10 to 15 minutes. This material can also serve as a delivery vehicle for bone cells and bone growth factors to induce a bone regeneration cascade.

In order to serve as a template for cell growth and differentiation, the hardened material must be highly porous and have an interconnected pore structure, which is achieved by adding a porogen such as gelatin microspheres or sodium chloride crystals. The porogen is eventually leached out, leaving a porous polymer matrix.

The design criteria for scaffolds in cell transplantation are specific to the structure and function of the tissue of interest. Human tissues and organs are highly organized and dynamic units, involving many cell types and complex interactions between cells and their environment. The limited supply of autograft tissue, the potential for pathogen transfer with allografts, and problems with nondegradable materials are inspirations for overcoming the challenges that remain in the development of successful long-term implants.

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