Scaffolds for Tissue Engineering

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Abstract

Devices for tissue engineering comprise scaffolds with the appropriate chemistry and architecture to promote cell infiltration and colonization. The scaffold is designed with biology in mind, and thus the architecture and chemistry differ according to tissue type. In this review, we focus on scaffolds for two tissue types—bone and nervous tissue—and describe different approaches used to create them. The appropriate scaffold for a hard tissue such as bone has a high degree of interconnected macroporosity and allows the rapid invasion of cells while maintaining a rigid structure. Several approaches are described for constructing tissue-engineering scaffolds for bone. The appropriate scaffold for soft tissues like nerve fibers (e.g., axons, which conduct nerve impulses) also has a high degree of interconnected pores; however, the pores may require orientation and may be smaller. Homogeneous, high-water-content hydrogels with mechanical properties that match the soft nerve tissue are commonly used as a scaffold, and the methods used to make these are reviewed.

Keywords: *cellular solids, open-cell foam, polymers, scaffolds, tissue engineering.*

Introduction

Tissue engineering has evolved out of the need to repair organs and tissues damaged by disease or injury. While the "gold standard" for regeneration and healing is the autograft, this approach is inherently limited by the amount of available donor tissue and necessitates a second injury site, resulting in additional trauma to the patient and associated risks such as pain, infection, and donor-site morbidity (dead tissue at the donor site). The concept of tissue engineering embodies the creation of a scaffold structure that has the appropriate physical, chemical, and mechanical properties to enable cell penetration and tissue formation in three dimensions. The appropriate scaffold for tissue engineering will be one that is created with biology in mind. The goal is for the new tissue grown in the scaffold to integrate with the host tissue. Ideally, the scaffold provides a temporary pathway for regeneration and will degrade either during or after healing, thereby obviating the need to remove the material later and eliminating possible side effects associated with leaving materials in the body. Of course, attention must be paid to ensure that degradation products are non-cytotoxic.

While there are numerous methods for creating scaffolds, most of these do not take biology into consideration and thus have limited efficacy. Perhaps one of the greatest challenges faced in tissue-engineered devices, regardless of tissue type, is promoting healing in three dimensions. Allowing blood-vessel formation (angiogenesis) throughout the scaffold is also critical to the success of the scaffold.

In this article, we review the most promising scaffold approaches for the regeneration of two tissue types: bone and neural tissue. We chose to focus on these tissues for two important reasons: First, they represent two very different types of tissue—hard (bone) and soft (nerves). Second, they require two regenerative medical strategies that can overlap: seeding cells on a scaffold prior to implantation versus enhancing regeneration along a pathway with therapeutic agents.

Bone-Tissue Engineering

The current standard for the treatment of bone defects of a critical size that do not heal on their own is an autologous graft. However, the supply of suitable donor bone is limited and harvesting this bone subjects the patient to additional trauma and risk. The emerging field of bone engineering attempts to replace or augment the current approaches by using porous scaffolds that are designed to support the migration, proliferation, and differentiation of osteoprogenitor cells and aid in the organization of these cells in three dimensions. These scaffolds may be made from a wide variety of both natural and synthetic materials. Aside from autografts and allografts of cancellous and cortical bone,[1–4] naturally derived materials include cornstarch-based polymers,[5] chitosan (a polysaccharide derived from chitin, found in crab shells),[6,7] collagen,[8] and coral.[9,10] Of these materials, coral has proven to be an effective clinical alternative to autogenic and allogenic bone grafts for certain applications.[11,12] Scaffolds created from marine coral exoskeletons that are hydrothermally converted to hydroxyapatite, the mineral component of bone, have approval from the U.S. Food and Drug Administration (FDA) for the repair of metaphyseal long-bone cyst and tumor defects, which occur at the junction (metaphysis) of the growth plate and shaft of long bones. Synthetic materials include inorganic materials such as calcium phosphates[13–15] and organic materials such as poly(phosphazenes),[16] poly(tyrosine carbonates),[17] poly(caprolactones),[18] poly(propylene fumarates),[19] and poly(α-hydroxy acids).[20–23] Composites of inorganic and organic materials have also been successfully used to create scaffolds for bone grafts.[24–27]

Poly(α-hydroxy acids) are the most commonly used polymeric materials for the creation of tissue-engineering scaffolds for bone, as they were the first synthetic biodegradable materials to receive FDA approval for *in vivo* applications such as resorbable sutures and implants. The most common of the poly(α-hydroxy acids) are poly(glycolic acid), poly(lactic acid) (PLA), and copolymers of poly(lactic-*co*-glycolic acid) (PLGA). The degradation products of these materials are easily metabolized and excreted.

The properties of scaffolds that may affect bone healing include pore size, pore shape, pore-wall thickness, pore interconnectivity, pore-wall surface area, porosity, surface morphology, rate of degradation, surface chemistry, and mechanical stability. These properties must be tailored for the specific application, which is dependent on factors such as anatomical location, severity of trauma, and age of the patient as well as the presence of other pathological conditions. The mechanical strength of the scaffold must be able to withstand physiological stresses and minimize stress shielding (bone loss

that occurs when the scaffold assumes more than its share of the weight burden) in the surrounding host bone.[28] The scaffold material and its degradation products should not provoke inflammation or toxicity *in vivo*.

It is generally believed that scaffolds with a high degree of interconnected porosity are necessary in order to support the in-growth of cells as well as to allow a sufficient nutrient supply to reach the center of the scaffold. Neovascularization in the scaffold is perhaps the biggest limitation to tissue regeneration. This is of paramount importance, since cells must be within several hundred microns of the nearest blood supply in order to survive.[29] Angiogenesis occurs at a rate of <1 mm per day, and it can take up to 1–2 weeks for complete vascularization of relatively thin (3 mm) scaffolds.[30,31] Strategies that involve using the scaffold itself to deliver cell growth factors such as vascular endothelial growth factor,[32] platelet-derived growth factor,[33] and recombinant human bone morphogenic protein[34,35] have been investigated to enhance and accelerate the wound healing process. These studies further demonstrate the importance of having a highly porous, interconnected morphology for neovascularization.

Many methods exist for tailoring various properties of porous synthetic scaffolds during the fabrication process. Scaffolds can be created using solvent casting,[36] membrane lamination,[37] freeze-drying,[38] phase separation,[39] gas-foam processing,[40] fiber bonding,[41] rapid prototyping,[42] solvent casting/particulate leaching,[23] and phase inversion/particulate leaching.[21] Each of these techniques has advantages and disadvantages; only the latter three techniques—rapid prototyping, solvent casting/particulate leaching, and phase inversion/particulate leaching—are described in the following sections.

Rapid Prototyping

Rapid prototyping enables scaffolds to be fabricated with precise control over micro- and macrostructure. This free-form solid fabrication method is capable of directly producing complex, three-dimensional scaffolds by joining liquids, powders, and sheet materials one layer at a time using computer-aided design.[42] Rapid prototyping offers the potential to precisely control the morphology, geometry, and overall shape of the scaffold (see Figures 1a and 1b) and may enable the creation of scaffolds that match the anatomical defect site.

Several rapid-prototyping processes have been developed based on the unique properties of the raw materials used. Such processes include sheet lamination, adhesion bonding, laser sintering, photopolymerization, and droplet deposition.[43] Fused-deposition modeling is a specific droplet-deposition process that has recently been described for tissue-engineering applications.[42,44] Although each of these processes has limitations (e.g., cost, thermal degradation of materials), rapid-prototyping processes offer potential in the field of tissue engineering where a custom-made, precisely controlled scaffold may be required.

Solvent Casting/Particulate Leaching

The process of solvent casting and particulate leaching is one of the most common techniques used to fabricate scaffolds.[45–51] This technique, originally developed by Mikos and co-workers,[45,51] involves dissolving a biodegradable polymer in a volatile solvent and casting the solution in a mold filled with a porogen—small crystalline molecules, such as a salt, used to form pores. The solvent is evaporated, and the porogen is leached out with water. Although this process may result in scaf-

Clinical Use of Porous Scaffolds for Tissue Engineering of Skin

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A tissue-engineering scaffold is a critical component of a clinically successful and commercially available system used for the surgical replacement of lost skin. The inventors, Burke and Yannas, called this skin-replacement system "artificial skin."[1–5] However, the tissue engineering of a skin-replacement system is more complex than the engineering of a scaffold for cellular in-growth.

The purpose of artificial skin is to allow a surgeon to close, without forming a scar, a clean surgical wound that is too large in area to be closed by suturing.[6] Such wounds are created during the surgical removal of necrotic tissue following severe burn injury or during reconstructive surgery. The surgeon's primary intention is to heal the surgical wound without the wound contraction and formation of granulation tissue that characterize natural wound-healing physiology.

The engineering of artificial skin for this purpose was based on both design inputs and experimental data.[1] The scaffold component of this artificial skin is a porous copolymer composed of purified collagen and a glycosaminoglycan (GAG), chondroitin-6-sulfate. The choice of collagen-GAG for the scaffold component was based on reasoning by the inventors that both collagen and GAG are components of the normal extracellular matrix, so it was expected that these materials would be inherently biocompatible, weakly immunogenic, and degradable by normal physiological mechanisms. The covalent cross-linking of collagen and GAG was used to control the biodegradation rate of the scaffold to ensure a residence time in the body of several weeks. Animal implantation studies demonstrated that this cross-linked collagen-GAG scaffold showed minimal inflammatory and encapsulation responses and non-fibrotic cellular in-growth. Encapsulation is an undesired process in which a foreign body or implant is surrounded by scar tissue; non-fibrotic tissue has the architecture of normal skin, as opposed to that of scar tissue. They were unable to achieve this same combination of properties with cross-linked collagen alone. Pore size and void volume fraction are quantitative characteristics of the collagen-GAG scaffold that were shown to be critical to its *in vivo* performance.

folds with a low degree of interconnectivity, the interconnectivity can be increased by filling the mold with porogen in a humid environment prior to adding the polymer solution.[52] The humidity helps to fuse the porogen particles together to ensure interconnected structures when the porogen is removed. NaCl is the most common porogen used for solvent casting/particulate leaching, and leads to scaffolds with a non-uniform pore morphology.

The solvent-casting/particulate-leaching process was recently modified to create scaffolds containing spherical pores with a high degree of uniformity (Figure 1c). For this process, paraffin spheres are bonded together through heat treatment to form a 3D structure in a mold, creating a high degree of interconnectivity. Biodegradable polymers, such as PLA and PLGA, are dissolved in a pyridine solution, poured on the bonded paraffin spheres, and hardened. After dissolving the paraffin with hexane, a highly porous (95% porosity) polymer scaffold forms. Although this technique is simple and requires no sophisticated equipment, it can take up to three weeks to fabricate

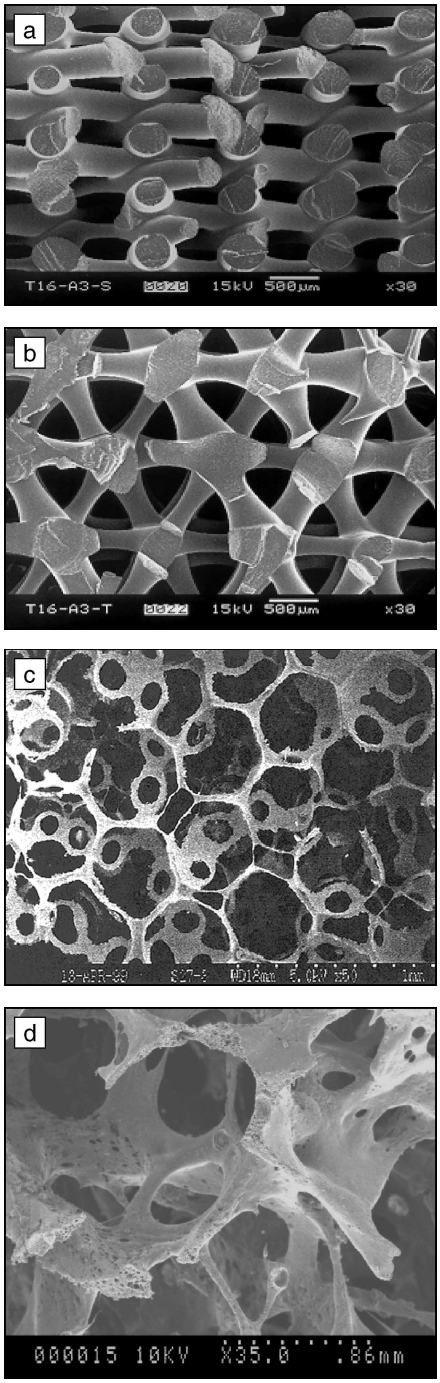

Figure 1. Many methods exist for fabricating highly porous scaffolds for bone tissue-engineering applications. Shown are scaffolds produced by means of (a), (b) rapid prototyping, (c) solvent casting/porogen leaching, and (d) phase inversion/particulate leaching. Figures 1a and 1b appear courtesy of S.H. Teoh, National University of Singapore; Figure 1c is from Reference 23; and Figure 1d is from Reference 72.

The collagen-GAG scaffold does not function alone, however. It is firmly bound to a membrane of silicone elastomer, which serves as a temporary epidermal covering and is also critical to the clinical performance of the artificial skin. Other essential requirements for this skin-replacement system are proper surgical preparation of the wound, proper postoperative care, and the establishment of a permanent epithelial cover by a second procedure that applies an autograft of epidermal tissue. The two-step artificial skin/autograft process requires only a thin layer of skin to be removed from the donor site, minimizing damage to the donor site and resulting in less pain and risk to the patient.

Skin-replacement surgery begins with the removal of any necrotic tissue and the creation of a clean open wound. The natural physiological response to an open wound includes inflammation, fluid loss, wound contraction, and granulation-tissue formation (which matures into scar tissue). The application of artificial skin establishes the physiology of a closed wound, with minimal inflammation, contraction, and granulation-tissue formation. The initial wound closure is followed by vascularization of the collagen-GAG scaffold and the regeneration of a permanent dermal tissue, while the original scaffold material degrades and is remodeled. Upon adequate vascularization of the scaffold layer, the second surgical procedure removes the temporary silicone layer, and an epidermal autograft is placed over the newly synthesized dermal tissue. Cells from this epidermal autograft migrate and grow to form an intact epidermis.

It is easy to recognize the function of the collagen-GAG scaffold in supporting the in-growth of connective-tissue cells and inducing them to regenerate a tissue that provides the critical physiological functions of dermis. Less obvious is how a scaffold contributes to the initial wound-closure physiology and the inhibition of wound contraction, since these functions are achieved rapidly and well before connective tissue in-growth into the scaffold begins. Another illustration that the physiological response to artificial skin is more complex than tissue in-growth into a scaffold is its clinical success in wounds such as burns that cover a large portion of the total body surface area. A large wound must be closed rapidly to avoid a life-threatening hypermetabolic systemic response. Again, when patients with large wounds are treated with artificial skin, the systemic physiology of a closed wound is achieved rapidly and before new tissue growth in the scaffold begins. These physiological responses to artificial skin are dependent on its bilayer system design and not on the scaffold alone.

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a scaffold, largely because of the lengthy solvent-removal process.

Phase Inversion/Particulate Leaching

The phase inversion/particulate leaching process is used to fabricate scaffolds with a high degree of interconnecting macroporosity that mimics the structure of trabecular bone (Figure 1d).[21,53] The process involves dispersing a porogen (typically glucose crystals) in a solution of PLGA in dimethylsulfoxide. The polymer slurry is frozen in a mold and then placed into a nonsolvent for PLGA (e.g., water). The nonsolvent causes PLGA to phase-invert and solidify. Water also solvates the porogen, resulting in a particulate-leaching process. Together, this phase inversion and leaching of particulates impacts pore size, interconnectivity, and porosity across the pore walls. The process parameters can be manipulated to create an open-cell foam structure with a connectivity that resembles trabecular bone; properties such as pore size, porosity, and degradation rate can be modulated by varying the PLGA comonomer ratio and the porogen size and concentration. While the resulting structure is predictable and homogeneous, it is not precisely ordered (in contrast to the structures achievable with the rapid-prototyping method). The entire fabrication process requires about three days.

Neural-Tissue Engineering

As in bone-tissue engineering, the autograft is the method of choice to promote healing in peripheral nerves after an injury; however, there is no method available to promote healing after injury to the spinal cord. While the autograft strategy is limited, tissue-engineering strategies offer great promise to those with nerve injuries. There are numerous cellular and molecular therapies under investigation; however, this article addresses only those that involve scaffolding structures and focuses particularly on strategies to achieve spinal-cord repair.

Unlike bone and peripheral-nerve tissue, the spinal cord does not spontaneously regenerate, probably because an inhibitory chemical and physical environment results after injury. While spinal-cord regeneration was once thought to be impossible, we know now that the spinal cord can regenerate, with the best results obtained by grafting peripheral nerves into the spinal cord.[54,55]

Since regeneration is difficult to achieve, the nature and morphology of cell-invasive scaffolds for the treatment of spinal-cord injuries is open to debate and conjecture. There is a consensus that providing a pathway along which nerve fibers can regenerate is important and that this pathway should incorporate molecules that stimulate regeneration. Hydrogels are particularly interesting materials for nerve regeneration, as their properties can be tuned to match the mechanical properties of the soft, viscoelastic neural tissue.[56] Gross mismatching of the flexibility between tissue and implant can result in tissue death at the interface. The following sections highlight some of the approaches available to create scaffolds that have two types of architecture: an oriented structure to direct neurite outgrowth and regeneration,[57] or a random distribution of pores to increase surface area and promote regeneration.[58] Some nerve-regeneration approaches include encasing the scaffold in a porous tube as a nerve guidance channel; our emphasis here is on the scaffold.

Oriented Porous Scaffolds

The fibers in scaffolds of both fibrin and collagen can be aligned using very strong magnetic fields.[59,60] These naturally derived, degradable biopolymers can be oriented during their formation, and *in vitro* experiments indicate that these scaffolds guide extending neurites. While these scaffolds are intended for use in regenerating peripheral nerves, it would be interesting to study and compare them in the spinal cord to better understand the stimulatory molecules required for regeneration in the central nervous system.

Oriented structures can be created in poly(α-hydroxy acids) by thermally induced, polymer-solvent phase separation.[61,62] For example, when PLA is dissolved in dioxane and then quenched in liquid nitrogen, various oriented structures are achieved through solvent crystallization. When the solution is quenched at one end, oriented fibers grow from that end in a direction perpendicular to the end immersed in liquid nitrogen. Thus, scaffold orientation can be manipulated. Furthermore, PLA and its degradation products are biocompatible in the spinal cord.[63,64]

Perhaps a simpler way to create an oriented scaffold is to use fiber bundles formed inside a cellular suspension and then placed into a transected spinal cord.[65] For example, a composite of PGA fibers and cells promoted spinal-cord regeneration and functional recovery in paraplegic rats.[65]

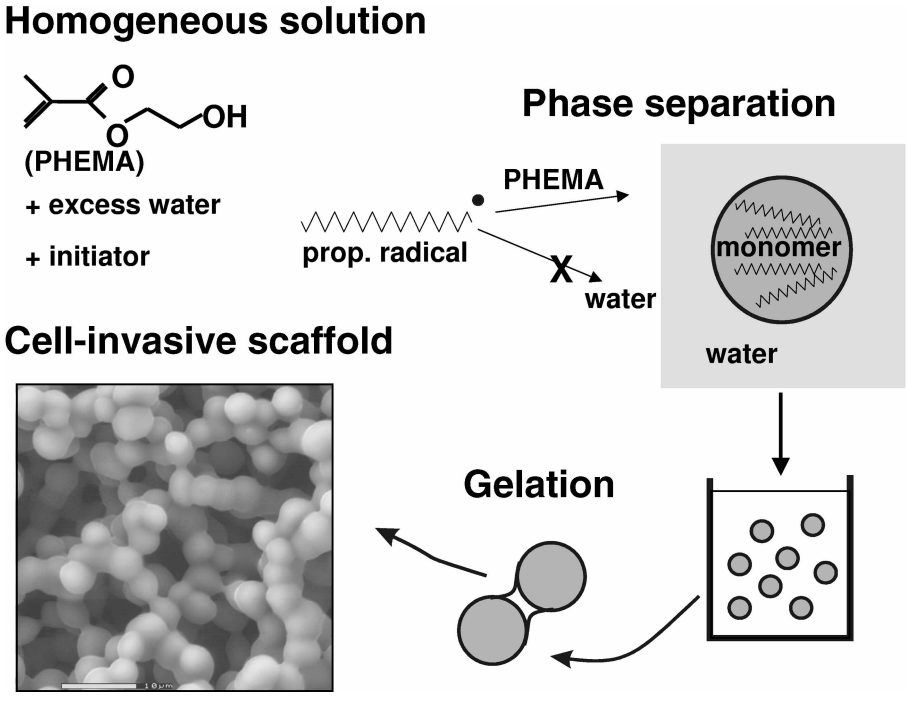

Figure 2. Example of a hydrogel scaffold formed by polymerization-induced phase separation of poly(2-hydroxyethyl methacrylate) (PHEMA) in water. The monomer is the preferred solvent for the propagating polymeric radical after it becomes insoluble in water. The initiator is a redox initiator: ammonium persulfate and sodium metabisulfite. Phase-separation of monomeric droplets containing the propagating polymer chains results in a series of spheres gelled together with interconnecting pores, creating a cell-invasive scaffold.

Heterogeneous Porous Scaffolds

Scaffolds formed by phase separation during polymerization result in cell-invasive structures with random porosities. Hydrogel scaffolds formed by this process include poly(hydroxypropyl methacrylamide) (PHPMA) in acetone[66] and poly(2-hydroxyethyl methacrylate) (PHEMA) in water.[67,68] In both cases, the monomer behaves as a solvent for the propagating radical at the end of a growing polymer chain that becomes insoluble in water but soluble in the remaining monomer as it grows. Phase separation of these monomer/polymer droplets results in spheres that gel together and result in an interconnected, cell-invasive scaffold (Figure 2). These porous regions support neural ingrowth, particularly when the monomer is modified to include adhesive proteins or peptide sequences.[69]

These hydrogels are very soft and have low moduli similar to that of native spinal-cord tissue; however, certain formulations cannot support their own weight, making them difficult to handle. One solution to maintaining structural integrity is to prepare the scaffolds within nerve guidance channels—two nerve ends are inserted in either end of a tube that both supports the scaffold and guides the nerves as they grow—which can provide a regenerative environment free of non-neuronal cells and inhibitory molecules. The promise of nerve guidance channels is evidenced by the regenerative capacity that has been demonstrated with empty hydrogel tubes.[70,71]

Because the requirements for spinal-cord regeneration are not fully understood, a definitive description of the most useful tissue-engineering scaffolds is not yet possible. To define the requirements for a neural scaffold's morphological structure, a polymeric system that can be manipulated into a range of scaffold shapes, with control of the pore size, would be invaluable. Additionally, the incorporation of surface and diffusible cues—molecules that promote axon adhesion and elongation—would be desirable. Eventually, a combinatory approach is likely to result in the successful reversal of spinal-cord injury. Such treatment is many years in the future, but may include cell transplantation, drug and molecular delivery, electrical stimulation, and cell-invasive scaffolds.

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