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REVIEW ARTICLE

Formation of highly porous biodegradable scaffolds for tissue engineering

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In recent years, lack of donor organs has caused many to consider tissue engineering methods as means to replace diseased or damaged organs. This newly-emerging field uses tissue-specific cells in a three-dimensional organization, provided by a scaffolding material, to return functionality of the organ. For these applications, the choice of scaffolding material is crucial to the success of the technique. In addition to the chemical properties of the material, physical properties such as surface area for cell attachment are essential. Various methods of creating pores in these materials to increase surface area are reviewed here. Scaffolds formed using the different techniques, which include fiber bonding, solvent casting/particulate leaching, gas foaming and phase separation, are compared on the basis of porosity, pore size, and promotion of tissue growth.

Despite recent technological advances, thousands die each year while waiting for organ transplants due to lack of donor organs or efficient organ substitutes (Thomson et al. 1995). Although clinicians have tried to replace the function of failing organs mechanically (dialysis and heart-lung bypass machines), or through implantation of synthetic replacements (blood vessel and joint replacements), these are often only temporary solutions and do not allow the patient to completely resume normal activities (Thomson et al. 1995). Infection and device rejection are also serious concerns in such procedures (Ishaug-Riley et al. 1997).

The emerging field of tissue engineering may help to resolve many of these problems. Tissue engineering involves the use of cells to regenerate the damaged tissue, leaving only natural substances to restore organ function. It has been found that in order for the cells to maintain their tissue-specific functions once implanted, a substrate material must be inserted to aid in organization of the cells in three dimensions (Temenoff et al. in press). In considering substrate materials, it is imperative to chose one that exhibits good biocompatibility. This means that the material must not elicit an unresolved inflammatory response nor demonstrate extreme immunogenicity or cytotoxicity. In addition, the mechanical properties of the scaffold must be sufficient so that it does not collapse during the patient's normal activities. As with all materials in contact with the human body, these scaffolds must be easily sterilizable to prevent infection (Temenoff and Mikos, in press).

Both natural and synthetic materials have been researched for use as tissue engineering scaffolds. Although preliminary results are promising for naturally-derived polymers (Wakitani et al. 1994; Caplan et al. 1997; Grande et al. 1997; Solchaga et al. 1999), concerns about the feasibility of finding the large amounts of material needed for clinical applications has prompted other researchers to investigate the use of synthetic polymers. These materials can be easily mass-produced and their properties can be tailored for specific applications. This includes creating degradable polymers that allow room for tissue growth in

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the construct while eliminating the need for a second surgery to remove the implant (Temenoff and Mikos, 2000).

In particular, many investigators have concentrated on synthetic biodegradable polymers that are already approved by the FDA as suture materials. These are poly (α-hydroxy esters) that are degraded by hydrolysis to products which can be metabolized and excreted (Athanasiou et al. 1996). The most common of these polymers are poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA) and their copolymer, poly(DL-lactic-co-glycolic acid) (PLGA) (Thomson et al. 1995). These polymers offer distinct advantages in that their sterilizability and relative biocompatibility have been well documented. In addition, their degradation rates can be tailored to match that of new tissue formation. PLLA is more hydrophobic and less crystalline than PGA and degrades at a slower rate (Thomson et al. 1995). The degradation rate of the amorphous copolymer can thus be easily controlled by altering the ratio of PLA to PGA in the formulation.

In addition to degradation rate, certain physical characteristics of the scaffolds must be considered when designing a substrate to be used in tissue engineering. In order to promote tissue growth, the scaffold must have large a large surface area to allow cell attachment. This is usually done by creating a highly porous polymer foam. In these foams, the pore size should be large enough so that cells penetrate the pores, and the pores must be interconnected to facilitate nutrient and waste exchange by cells deep within the construct (Temenoff et al. in press). These characteristics (porosity and pore size) are often dependent on the method of scaffold fabrication (Mikos et al. 1993a; Mikos et al. 1994; Mooney et al. 1996a; Nam and Park, 1999b; Nam et al. 2000).

Several methods have been developed to create highly porous scaffolds, including fiber bonding (Mikos et al. 1993a), solvent casting/particulate leaching (Mikos et al. 1993b; Mikos et al. 1994), gas foaming (Mooney et al. 1996a; Nam et al. 2000) and phase separation (Lo et al. 1995; Whang et al. 1995; Lo et al. 1996; Schugens et al. 1996; Nam and Park, 1999a; Nam and Park, 1999b). This review will compare these methods in terms of foam porosity, pore size, efficacy of promoting tissue growth and ease of use in a clinical setting.

Fiber bonding (unwoven meshes)

Scaffolds based on the use of PGA fibers were some of the earliest constructs proposed in tissue engineering (Freed et al. 1993; Mikos et al. 1993a). These fibers, if bonded together in three-dimensions, provide large surface area for cell interaction and growth. The fibers can be attached to each other via two different techniques. In the first, developed by Mikos et al. (Mikos et al. 1993a), PGA fibers are immersed in a PLLA solution. When the solvent evaporates, the network of PGA fibers is embedded in PLLA. The composite is then heated to above the melting temperature of both polymers. The PLLA melts first and fills all voids left by the fibers. This helps retain the spatial arrangement of fibers so that when the PGA begins to melt, the fiber structure does not collapse. Instead, in order to minimize interfacial energy, fibers at the cross-points become "welded" (melted) together, forming a highly porous foam. The PLLA is then removed by dissolution with methylene chloride. This fabrication technique results in foams with porosities as high as 81% and pore diameters of up to 500 μm. Hepatocytes cultured for one week in these foams remained alive and began to interact with each other to form clusters (Mikos et al. 1994).

A second method for bonding PGA fibers uses atomization of PLLA or PLGA to coat the fibers. PLLA or PLGA is dissolved in chloroform and sprayed onto the PGA fibers (Mooney et al. 1996b). Since PGA is only weakly soluble in chloroform, the fibers remain unchanged during this process. The solvent is then evaporated, leaving the fibers glued with PLLA or PLGA. Although porosities were not reported, pore sizes similar to those of the previous technique were attained. When tubes made in this manner were implanted in rats for 17 days, fibrous tissue ingrowth was observed, indicating that constructs with these physical properties could encourage neotissue formation (Mooney et al. 1996b).

Although fiber bonding techniques produce highly porous scaffolds with interconnected pores that are suitable for tissue regeneration (Freed et al. 1993; Mikos et al. 1993a; Mooney et al. 1996b), both methods involve the use of solvents that could be toxic to cells if not completely removed. In order to extract these chemicals, the constructs must be vacuum dried for several hours, making it difficult to be used immediately in a clinical setting. In addition, the first method involves heating to high temperatures. The combination of toxic chemicals and extreme temperature presents difficulties if cells or bioactive molecules, such as growth factors, are to be included in the scaffold during processing.

Solvent casting/particulate leaching

Another method to create pores involves the use of a water-soluble porogen, such as salt (NaCl) (Mikos et al. 1993b; Mikos et al. 1994). The first step in this process is to dissolve the polymer (PLLA or PLGA) in chloroform or methylene chloride and then cast it onto a petri dish filled with the porogen. After evaporation of the solvent, the polymer/salt composite is leached in water for two days to remove the porogen. The resulting scaffold's porosity can be controlled by the amount of salt added, while the pore size is dependent on the size of the salt crystals. With 70 weight percent salt and above, the pores exhibited high interconnectivity (Mikos et al. 1994). Foams fabricated in this manner have been used extensively with various cell

types and have shown no adverse effects on new tissue formation (Freed et al. 1993; Mikos et al. 1993b; Ishaug et al. 1997; Ishaug-Riley et al. 1998; Goldstein et al. 1999). However, due to concerns that the side of the foam exposed to air had a different morphology (rougher) than that exposed to the petri dish, a modification of this technique has been developed (Thomson et al. 1998; Goldstein et al. 1999). In this case, pieces of the polymer/salt composite are compression molded into cylindrical form at temperatures just above the melting (PLLA) or glass transition temperature (PLGA). The cylinder is then cut into discs of desired thickness before leaching in water. This allows more precise control of scaffold thickness and increases uniformity of the foam surface. However, thermal degradation of the polymer during the compression molding step could be a concern.

In an alternate form of the particulate leaching method, Shastri et al. (Shastri et al. 2000) recently reported the fabrication of PLLA and PLGA scaffolds with up to 87% porosity and pores well over 100 μm in diameter using waxy hydrocarbons as porogens. After mixing the porogen and polymer (dissolved in methylene chloride or chloroform) into a paste, the composite is packed in a teflon mold. The mold is immersed in a hydrocarbon solvent (pentane or hexane) to remove the wax without dissolving the PLLA/PLGA. The remaining foam is vacuum-dried for several days to extract any solvents. Thick samples (up to 2.5 cm) with interconnected pores can be created using this technique. This method also offers the possibility of adding a particulate phase to the paste to increase the strength or electrical conductivity of the final structure. When blended with polyethylene glycol (PEG) and seeded with bovine chondrocytes for four weeks, formation of cartilage-like tissue is seen in these foams, demonstrating their biocompatibility.

With any solvent casting/particulate leaching procedure, organic solvents are used, which in many cases precludes the possibility of adding pharmacological agents to the scaffold during fabrication. Also, the leaching step for water-soluble porogens significantly increases the scaffold preparation time. However, in applications where prefabrication of cell-polymer constructs is suitable, promising results using a large range of cell types make these scaffolds very appealing.

Gas foaming

In order to eliminate the need for organic solvents in the pore-making process, a new technique involving gas as a porogen has been introduced (Mooney et al. 1996a). The process begins with the formation of solid discs of PGA, PLLA or PLGA using compression molding with a heated mold. The discs are placed in a chamber and exposed to high pressure CO_2 (5.5 MPa) for three days, at which time the pressure is rapidly decreased to atmospheric pressure. Porosities of up to 93% and pore sizes of up to 100 μm can be obtained using this technique, but the pores are largely unconnected, especially on the surface of the foam. While this fabrication method requires no leaching step and uses no harsh chemical solvents, the high temperatures involved in the disc formation prohibit the incorporation of cells or bioactive molecules and the unconnected pore structure make cell seeding and migration within the foam difficult.

Another approach to using gas a porogen was recently developed by Nam et al. (Park, 1999; Nam et al. 2000). This technique includes both gas foaming and particulate leaching aspects. Ammonium bicarbonate is added to a solution of polymer in methylene chloride or chloroform. The resultant mixture is highly viscous and can be shaped by hand or with a mold. The solvent is then evaporated and the composite is either vacuum dried or immersed in warm water. Vacuum drying causes the ammonium bicarbonate to sublime while immersion in water results in concurrent gas evolution and particle leaching. The latter method is preferred because it does not result in the creation of a nonporous outer skin, as seen in the vacuum-dried scaffolds. Porosities as high as 90% with pore sizes from 200-500 μm are attained using this technique.

Rat liver cells were seeded in these foams and up to 40% remained viable over one week in culture, suggesting the scaffolds are biocompatible and facilitate adequate nutrient exchange (Nam et al. 2000). In addition, the putty-like consistency of the polymer-salt mixture could be useful in molding constructs during surgery. However, concerns about the use of organic solvents and the long-term effects of residues of ammonium bicarbonate on cells may prevent these scaffolds from soon being employed in "on the spot" tissue engineering.

Phase separation/emulsification

Additional techniques proposed for the fabrication of porous polymer scaffolds are based on the concepts of phase separation rather than incorporation of a porogen. They include emulsification/freeze-drying (Whang et al. 1995) and liquid-liquid phase separation (Lo et al. 1995; Lo et al. 1996; Schugens et al. 1996; Nam and Park 1999a; Nam and Park, 1999b). With the first method (Whang et al. 1995), PLGA is dissolved in methylene chloride and then distilled water is added to form an emulsion. The polymer/water mixture is cast into a mold and quenched by placing in liquid nitrogen. After quenching, the scaffolds are freeze-dried at -55°C, resulting in the removal of the dispersed water and polymer solvents. Scaffolds with large porosities (up to 95%), but small pore sizes (13-35 μm) have been fabricated using this technique. These parameters are very dependent on parameters such as the ratio of polymer solution to water and viscosity of the emulsion as these values influence the emulsion's stability prior to quenching (Whang et al. 1995). Therefore, with further adjustment, it is possible that pore size could be increased.

However, although this technique is advantageous as it does not require an extra washing/leaching step, the use of organic solvents remains a concern for the inclusion of cells and bioactive molecules. This, combined with the small pore sizes obtained, indicate that this fabrication method currently has limited usefulness in the field of tissue engineering.

Liquid-liquid phase separation employs thermodynamic principles to create polymer-rich and polymer-poor phases within a polymer solution. The polymer poor phase is then removed, leaving a highly porous polymer network. Both PLLA and PLGA scaffolds have been formulated using this technique (Lo et al. 1995; Lo et al. 1996; Schugens et al. 1996; Nam and Park, 1999a; Nam and Park, 1999b). The polymers are dissolved in a solvent with a low melting point and that is easy to sublime, such as naphthalene, phenol or 1,4 dioxane. In some cases, small amounts of water are added as a non-solvent to induce phase separation (Schugens et al. 1996; Nam and Park, 1999a; Nam and Park, 1999b). The polymer solution is cooled below the melting point of the solvent (polymer poor phase) and then vacuum dried for several days to insure complete solvent sublimation. The cooling parameters for the solution play an important role in determining the morphology of the resultant scaffold. At temperatures just below the critical temperature (or cloud point in the case of polydisperse polymers, such as those used here) the phase separation occurs via a nucleation and growth mechanism. At lower temperatures (beneath the spinodal curve in a phase diagram), the separation occurs via spinodal decomposition. While the nucleation and growth mechanism results in spheroidal domains, spinodal decomposition causes the formation of interconnected cylinders. Annealing can cause enlargement of domains formed by either mechanism (Sperling, 1992).

Based on these thermodynamic principles, spinodal decomposition is preferred as it increases the number of interconnected pores within the network (Nam and Park, 1999a). In addition, it has been found that annealing is important to increase pore size (Nam and Park, 1999a; Nam and Park, 1999b). Various parameters that influence the phase diagram of the system, and thus the resulting pore morphology, are polymer concentration, cooling method and time, solvent/nonsolvent ratio, and the presence of surfactants, which can reduce the interfacial tension between phases and increase pore size and interconnectivity (Nam and Park, 1999a; Nam and Park, 1999b). Foams up to 90% porous, with pores of approximately 100 μm, have been produced using this technique, but no cell culture studies have yet been attempted to determine cell viability within such scaffolds (Nam and Park, 1999b). Although, as in many other methods, the use of harsh organic solvents is a disadvantage, the flexibility afforded by a system in which various parameters can be changed to tailor pore size and porosity for specific applications is appealing. However, the phase diagrams of the polymer-solvent systems must be better characterized before this flexibility can be fully exploited for use in tissue-engineered constructs.

Concluding remarks

Many techniques have been investigated in recent years to form highly porous biodegradable scaffolds suitable for use in tissue engineering. Many of these methods are able to form foams with high porosity to encourage cell attachment. Of these, the methods of fiber bonding, solvent casting/particulate leaching, gas foaming/particulate leaching and liquid-liquid phase separation produce large, interconnected pores to facilitate cell seeding and migration. The fiber bonding, solvent casting/particulate leaching and gas foaming/particulate leaching methods exhibit good biocompatibility, making these techniques especially promising for future use in tissue-engineered cell-polymer constructs. However, almost all techniques described in this review require the use of organic solvents, which could reduce the ability of cells to form new tissues in vivo. Thus, long processing times to fully remove these solvents are necessary. To overcome this problem, other combinations of materials and pore-forming techniques must be explored to create constructs that can be fabricated during surgery and tailored for specific applications. It is only when these clinical design criteria have been addressed that tissue engineered constructs will see widespread use to aid patients suffering from various types of organ and tissue failure.

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