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Injectable poly(lactic-co-glycolic) acid scaffolds with *in situ* pore formation for tissue engineering

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Abstract

Appropriate porosity is an important biomaterial design criterion for scaffolds used in tissue engineering applications as it can permit increased cell adhesion, migration, proliferation and extracellular matrix production within the scaffold at a tissue defect site. Tissue engineering scaffolds can either be injected in a minimally invasive manner or implanted through surgical procedures. Many injectable scaffolds are hydrogel-based; these materials often possess nanoscale porosity, which is suboptimal for cell migration and proliferation. Solid scaffolds with engineered micron-scale porosity are widely used, but these scaffolds are usually pre-formed and then must be implanted. Here we report on the development of a solid, injectable, biomaterial scaffold that solidifies *in situ* via phase inversion with microporous, interconnected architecture on the surface and within the bulk. This injectable system utilizes the biodegradable polymer poly(lactic-co-glycolic acid), a nontoxic FDA-approved solvent, and biocompatible porogens. Various scaffold formulations are examined in terms of morphology, porosity, degradation, elastic modulus, and ability to support cellular adhesion and growth. Furthermore, the ability to form a microporous architecture upon injection *in vivo* is verified. This technology is a promising noninvasive approach for *in vivo* formation of porous biodegradable scaffolds.

Keywords: Biomaterial; Injectable; *In situ* pore formation; Poly(lactic-co-glycolic acid); Scaffold

1. Introduction

Many solid biopolymer scaffolds with microscale porosity used for tissue engineering applications are first formed in the laboratory and then must be surgically implanted. Methods that are commonly used to produce such scaffolds include solvent casting [1,2], gas foaming methods [2,3] and solid free-form fabrication [4–6]. For solvent casting and gas foaming approaches, a porogen is first mixed throughout a polymer, the polymer is fused together to form a continuous network and then the porogen is subsequently leached out from the polymer to provide a porous, interconnected scaffold. The scaffolds fabricated using the solvent casting technique are typically made from polymers that are not water-soluble and require toxic solvents for them to go into solution. The solvents must be thoroughly removed from the polymeric system prior to implantation in a patient, necessitating these scaffolds to be prefabricated on the bench and eliminating the possibility of having an injectable system. Scaffolds formed by gas foaming and free-form fabrication techniques require the use of large, expensive equipment for scaffold manufacturing, which prevents *in situ* formation at a tissue defect site.

In contrast, biomaterials that can be injected to form a scaffold *in vivo* offer a minimally invasive alternative, which

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is valuable in a clinical setting because it eliminates the requirement for surgical implantation and thus reduces both patient risk and treatment expense. Many injectable scaffolds are hydrogel-based; these materials typically have inadequate mechanical properties for load-bearing sites [7]. Furthermore, they often possess nanoscale porosity [8,9] which is suboptimal for cell migration, growth and new extracellular matrix deposition [10]. Previous work by others has demonstrated the potential utility of injectable nonporous poly(lactic-co-glycolic acid) (PLGA) for the purpose of delivering drugs, proteins and DNA to treat diseases such as chronic inflammatory disease [11–13] or for the delivery of bulking agents for the treatment of urinary incontinence [14], but these have not been engineered to possess micron-scale porosity for use in tissue engineering. Poly(propylene fumarate) has been used to form porous injectable scaffolds with high mechanical properties which are quite promising for use in tissue regeneration strategies, but the use of foaming reactions to form this porosity can be uncontrolled and may cause local tissue damage [15,16]. Additionally, the elastic modulus of these scaffolds may be unsuitably high for certain applications.

Appropriate micron-scale scaffold porosity is an important biomaterial design criterion in tissue engineering applications as it can permit increased cell adhesion, migration, proliferation and new extracellular matrix production [17–19]. Biomaterial porosity is especially critical when the regeneration strategy implemented does not include cell transplantation and therefore relies solely on the recruitment of host cells to the defect site [2,20]. In this situation, if the host cells do not migrate into the scaffold, proliferate and secrete extracellular matrix, there will be no tissue repair in the defect. Therefore the scaffold must permit, and ideally promote, cellular infiltration throughout.

We have developed an injectable biomaterial scaffold that solidifies *in situ* with microporous, interconnected architecture on the surface and within the bulk. The choice of the scaffold material is a major consideration; it must be biocompatible and ideally will degrade over time as new tissue is formed. The biomaterial used in this system is PLGA, which is a biodegradable polymer that has been approved by the FDA for use in several clinical applications [21,22]. The rate of degradation of the polymer can be altered by changing the ratio of lactic acid blocks to glycolic acid blocks or the molecular weight of the polymer [23]. In the system described herein, the PLGA is dissolved in tetraglycol, also known as glycofurol, a water-miscible solvent which is used with some FDA-approved pharmaceuticals administered via injection; quantities of tetraglycol up to 0.07 ml per kg body weight per day are deemed safe [24]. When a solution of PLGA in tetraglycol is injected into an aqueous environment, the tetraglycol diffuses out of the polymer into the surrounding solution. As the water-insoluble PLGA comes into contact with the aqueous solution, it precipitates by phase inversion into a nonporous scaffold [25,26]. The injection of pure PLGA in tetraglycol into an aqueous solution will form a solid scaffold with minimal porosity. Advancing this technology for tissue engineering applications may require modifying this injectable PLGA system to allow for *in situ* pore formation to create solid scaffolds with interconnected porosity throughout the polymer surface and bulk. In the work presented here, such scaffolds are successfully created by the addition of porogens and a small amount of water to the PLGA solution, which solidifies *in situ* following injection into an aqueous solution to form a microporous, solid scaffold. This system is characterized *in vitro* by examining the morphologic appearance of resultant scaffolds, measuring their porosity, quantifying their degradation rates and mechanical integrity over time, and evaluating their ability to support cellular growth and infiltration. It is also shown that these scaffolds can be formed with a highly porous structure when injected subcutaneously in the backs of mice. To the best of our knowledge, this is the first demonstration of the formation of solid, biodegradable polymer scaffolds by phase inversion with surface and bulk micron-scale porosity.

2. Materials and methods

2.1. Scaffold fabrication

75:25 7E (i.v. 0.76 dl g^{-1}) or 50:50 6A (i.v. 0.58 dl g^{-1}) PLGA (Lakeshore Biomaterials, Birmingham, AL) was dissolved in tetraglycol (Sigma, St. Louis, MO) at 10 wt./vol.% by stirring overnight at 60 °C. To create porous scaffolds, 210 mg of sodium chloride, sodium bicarbonate, ammonium bicarbonate or sucrose (Fisher Scientific, Fairlawn, NJ) sieved to fall within a size distribution of 106–250 μm was placed in one 3 ml syringe, and 350 μl of the polymer solution was placed in a second 3 ml syringe, followed by the addition of ultrapure, deionized water (diH_2O) to the tip of this syringe (35 μl of diH_2O was added to 75:25 PLGA scaffolds while only 15 μl of diH_2O was added to 50:50 scaffolds). All components of the scaffold were mixed by joining the syringes together with a Luer-Lok connector. The polymer solution was then injected into 10 ml of phosphate-buffered saline (PBS; Hyclone, Logan, UT) in a 20 ml glass vial, where it precipitated by phase inversion to form a solid scaffold as the tetraglycol diffused into the PBS and allowed the porogen to be leached from the scaffold to form a porous structure. These scaffolds were placed in a humidified environment with 5% CO_2 at 37 °C for 24 h, before being lyophilized for 48 h and vacuum-sealed for storage at −20 °C. Other scaffolds were fabricated by leaving out either or both the porogen or diH_2O.

2.2. Scaffold characterization

Scanning electron microscopy (SEM) was used to characterize the surface and bulk morphology of the scaffolds. Scaffolds were sputter coated with palladium for 60 s (Desk II, Denton Vacuum, Moorestown, NJ). A Philips XL30

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