A New Method to Synthesize Porous 3D PLGA Scaffolds for Tissue Engineering

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Introduction: Synthesis of a porous 3D resorbable implant matrix that can stimulate tissue regeneration is of primary importance in musculoskeletal tissue engineering. Poly(lactic-co-glycolic acid) (PLGA) copolymer is currently being used in a host of therapeutic devices due to its biocompatibility and biodegradability. Methods currently used to produce porous PLGA scaffolds do not provide enough flexibility in engineering the scaffold's architectural characteristics, and/or they require expensive/sophisticated instruments. In this study, we describe a method to synthesize porous PLGA scaffolds that can be used for controlled drug and cell delivery applications in bone and cartilage tissue reconstruction.

Methods: PLGA (50:50, terminated with carboxylic acid) microspheres, blank or loaded with 2 wt% lysozyme (labeled with Alexa 350 fluorophore), were synthesized by the conventional w/o/w double emulsion technique described previously. Sucrose was chosen as excipient to protect the protein from hydrophobic polymer and solvent. The microspheres were sieved in the size range of 50-150 µm. Salt (NaCl) particles (~200 µm) were used as the porogen. Three separate PLGA-salt mixtures containing 50, 60 and 70 wt% salt were prepared and mixed homogeneously for 24 h. PLGA-salt mixtures (0.5 g) were subsequently compressed mechanically at 500 MPa for 2 min into discs 13 mm in dia. and 3 mm in thickness. These discs were then heat-treated in air for 2 days at 42°C, which is just above the T_g of PLGA. The discs were subsequently placed in a stirred deionized water-bath to leach out the salt completely, with water being exchanged every 6 h. To ensure complete salt removal, the conductivity of water was constantly monitored. The scaffolds were air-dried at room temperature. The porosity and pore-size distribution were analyzed by mercury intrusion porosimetry. Scanning electron microscopy (SEM) was used to evaluate the 3D architecture of the polymer scaffold. Scaffold degradation (mass loss) was investigated over a 21d period by incubating the scaffolds in 6 ml phosphate buffered saline (PBS) shaken at 60 rpm at 37°C. The solution pH was measured before it was exchanged every 3 d. The release kinetics of protein in the scaffolds was analyzed by measuring the fluorescence in the supernatant.

Results: Fig. 1 illustrates the cross sectional view of the PLGA scaffolds (61% porous) prepared by this method. The scaffolds were characterized by a homogenous distribution of interconnected pores through the entire thickness of the scaffold. Moreover, SEM and mercury porosimetry results demonstrated that the scaffolds contained interconnected pores in a wide size-range (3nm–700µm), with 70-85% pores larger than 100 µm. In addition, by varying the initial porogen content, scaffolds were synthesized with different porosities (40-70%) and high surface areas (18-40 m^2/g) [Table 1]. The pH of PBS incubated with PLGA scaffolds (40% porosity) varied from 7.2 after 4 d to 6.14 after 21 d. Physical integrity of the scaffolds was preserved during the entire 21 days. However, hydrolytic shortening of the polymer chains and subsequent erosion resulted in mass loss of the scaffolds (Fig. 2). The mass loss of the scaffolds (40% porous) was comparable after 4, 8 and 12 days (~7%), but increased significantly after 16 (16.3%) and 21 days (27%). Ongoing experiments are determining the mechanical properties of the scaffolds as well as the release kinetics and bioactivity of the protein molecules in the scaffold.

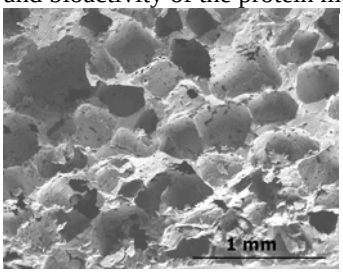

Table 1. Porosity in PLGA scaffolds

NaCl (wt %)	50	60	70
porosity (%)	39	63	60
surface area (m2/g)	19	23	28
700-1 µ (%)	84	96	94
1 µ-3 nm (%)	16	4	6

Fig.1. Representative SEM image of the cross-section of porous PLGA (61% porous).

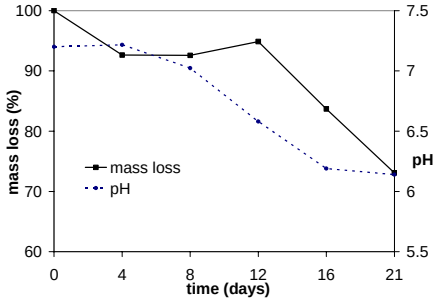

Fig.2. Mass loss and pH of PBS after incubation with the PLGA scaffolds (40% porous),

Discussion: This method provides an alternative to gas foaming and solvent casting approaches for synthesis of porous polymer scaffolds. Compared to solvent casting, scaffolds prepared by this method were characterized by greater uniformity in porosity, pore size, and size distribution throughout their thickness. Moreover, use of CO_2 at high pressures was avoided as small pores on the microsphere surfaces and in pore walls provided the microporosity in the scaffolds. As has been previously published, the macroporosity in scaffolds can be controlled by the amount and size of salt particles. The scaffolds thus prepared were characterized by an interconnected porous structure that should facilitate cellular invasion and fluid circulation at the implant site.

Conclusion: This method for synthesizing 3D porous, mechanically stable bioresorbable scaffolds for drug and cell delivery may be useful in tissue engineering applications.