Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm

Effect of cryomilling times on the resultant properties of porous biodegradable poly(e-caprolactone)/poly(glycolic acid) scaffolds for articular cartilage tissue engineering

John B. Jonnalagadda[a], *Iris V. Rivero*[b,*]

[a]*Texas Tech University, Department of Industrial Engineering, Lubbock, TX 79409-3061, United States*
[b]*Iowa State University, Department of Industrial and Manufacturing Systems Engineering, Ames, IA 50011-2164, United States*

ARTICLE INFO

Article history:
Received 19 June 2014
Received in revised form
6 August 2014
Accepted 10 August 2014
Available online 19 August 2014

Keywords:
Articular cartilage
Scaffolds
Tissue engineering
Poly(e-caprolactone)
Poly(glycolic acid)

ABSTRACT

The aim of this research is to develop a parametric investigation of the fabrication of poly (e-caprolactone) (PCL)/poly(glycolic acid) (PGA) scaffolds to decipher the influence of cryomilling time on the scaffolds' resultant physical, morphological and mechanical characteristics. Scaffolds were fabricated via solid-state cryomilling to prepare a homogeneous blend along with conventional compression molding and porogen leaching yielding interconnected porous scaffolds. PCL/PGA scaffolds fabricated through this technique demonstrated high porosity at all cryomilling times. Morphological analysis revealed a co-continuous interconnected pore network. While mean pore size decreased, water uptake and compressive properties increased with increasing cryomilling times. Porous scaffolds cryomilled for 12 min exhibited a mean pore size within the optimal range for tissue engineering and chondrocyte ingrowth. And the compressive modulus of scaffolds cryomilled for 12, 30 and 60 min matched the compressive modulus of human articular cartilage. In addition, scaffolds exhibited water uptake, a key requirement in tissue engineering. A 60 day *in vitro* degradation study revealed mass loss starting from day 10 and increasing through day 60, while notable reduction in compressive properties was observed. The results indicated that cryomilling times affected the resultant properties of PCL/PGA scaffolds and will be interesting candidates for articular cartilage tissue engineering.

1. Introduction

Scaffolds play a critical role in tissue engineering by supporting the cellular responses related to populating the afflicted region until attaining a healthy living tissue. A scaffold should promote biocompatibility to avoid release of toxic substances preventing immunogenicity (Puppi et al., 2010). It should be biodegradable such that neotissue growth synchronizes with the degradation rate of the scaffold (Puppi et al., 2010). The surface characteristics of a scaffold should

*Corresponding author. Tel.: +1 515 294 7944; fax: +1 515 294 3524.
E-mail address: rivero@iastate.edu (I.V. Rivero).

http://dx.doi.org/10.1016/j.jmbbm.2014.08.009
1751-6161/

promote cellular adhesion, proliferation, migration, differentiation, and extracellular matrix production (Vial and Andreopoulos, 2009; Holzapfel, et al., 2012). Porous scaffolds with porosity greater than 90% enhance cell adhesion, cell in-growth and reorganization, and provide adequate space for neovascularization (Puppi et al., 2010). Pore interconnectivity with an optimal pore size of 100–500 μm supports the exchange of nutrients and waste to and from the scaffold besides enhancing cell proliferation and migration (Ikada, 2006; Gross and Rodriguez-Lorenzo, 2004). Lastly, the mechanical properties of the scaffold should be comparable to the new tissue being formed and remain sufficiently strong until the tissue is completely functional.

The factors affecting the performance of the scaffolds include monomer selection, fabrication technique and processing conditions (Daniels et al., 1990). Selecting an appropriate fabrication technique plays a vital role in determining the scaffold architecture. To fabricate scaffolds current techniques include solvent casting with particulate leaching, fiber bonding, melt molding with particulate leaching, gas foaming, freeze drying and solid free-form procedures to name a few (Puppi et al., 2010). However, scaffolds produced by these techniques have limitations for their use in tissue engineering. Limitations include poor morphology, limited thickness and porosity, use of organic solvents, poor mechanical properties, porogen entrapment in the polymer matrix and poor cell-polymer interactions (Puppi et al., 2010). In particular, achieving homogeneous blending seemed to be a concern when processing immiscible polymers in melt or in solution state when using highly viscous polymers with high filler content (Zhu et al., 2006; Chen and Wang, 2001). Melt blending uses harmful solvents and has led to undesirable chemical reactions, unstable scaffold morphologies, and polymer degradation at high temperatures (Smith et al., 2000a, 2000b).

The current study incorporates solid-state cryomilling to the fabrication of biodegradable scaffolds comprised of immiscible polymers. Previously, the suggested manufacturing process was used to successfully fabricate interconnected porous scaffolds with co-continuous morphology consisting of only PCL (Allaf and Rivero, 2011). The scaffolds co-continuous morphology was attained by using water soluble poly(ethylene oxide) (PEO) as porogen. Two cryomilling times, 36 and 90 min were investigated then to examine the resultant properties. The results indicated that PCL/PEO scaffolds produced pore sizes which decreased with increasing milling time, and compressive properties which increased with increased milling time. Porosity and PEO continuity were slightly lowered with increasing milling time. These outcomes implied that cryomilling times could be a critical factor on influencing the scaffold properties for cartilage tissue engineering applications.

To increase the efficacy of PCL based scaffolds with respect to porosity, pore size, water uptake, mechanical properties and controlled degradation (Cui and Sinko, 2012; Mehdinavaz et al., 2011), this study uses a blend of two polymer systems, PCL and PGA combined with the porogen PEO. PCL and PGA are semi-crystalline biodegradable poly(α-hydroxyesters) which have been used to fabricate tissue engineered scaffolds (Wan et al., 2008; Eastmond, 2000) and for medical device applications such as bioresorbable sutures and tissue engineered electrospun scaffolds (Cui and Sinko, 2012). PCL has a slow degradation rate *in vivo* which makes it appropriate for applications requiring longer healing periods (Mano et al., 2004). PGA is more hydrophilic and degrades faster hydrolytically *in vivo* when compared to PCL (Ishaug-Riley et al., 1999; Eastmond, 2000). PCL/PGA blends in various proportions have been used for soft tissue engineering purposes (Cui and Sinko, 2012; Mehdinavaz et al., 2011; Otten et al., 2005).

The objective of this research was to develop a parametric investigation of the fabrication of articular cartilage scaffolds produced by mixture of two immiscible polymers blended via four distinct cryomilling times (12, 30, 60 and 180 min). Overall fabrication of scaffolds included cryomilling followed by compression molding and particulate leaching. It is expected that the resulting polymer blend will be a viable candidate to be used for articular cartilage tissue engineering.

2. Materials and methods

2.1. Materials

PCL (CAPA 6506) obtained from Perstorp UK Limited had a number average molecular weight (M_n) of approximately 47,500; a density of 1.1 g/cm^3 (at 60 °C); a melt flow index (MI) of 5.20–11.3 g/10 min (1" PVC die, 160 °C, 2.16 kg); and melting temperature range of 58–60 °C. PGA (PURASORB PG 20) was obtained from Purac, Amsterdam, Netherlands and it is a GMP grade homopolymer of glycolide suitable for medical device applications with an inherent viscosity midpoint of 1.4 dl/g in 0.1 g/dl Hexafluoroisopropanol (HFIP) at 25 °C. PEO was purchased from Sigma-Aldrich, USA in a fine powder form (Mesh −20, 96–100%) with a viscosity average molecular weight (M_v) of ~100,000, density of 1.13 g/ml (at 25 °C), a viscosity of 12–50 cP (at 25 °C, C=5%, H_2O), T_g of −67 °C, and T_m of 65 °C. Phosphate buffer solution (PBS) without calcium chloride and magnesium chloride with a pH of 7.4 was used for the degradation study. Silica gel was used for desiccating the ground polymers.

2.2. Polymer milling

PGA granules were cryomilled in a SPEX 6770 Freezer mill (SPEX SamplePrep, Metuchen, NJ, USA) for 18 min to refine particle size approximately to the size of PCL and PEO. Prior to cryomilling, PCL, PGA and PEO were vacuum desiccated for at least 48 h to remove excess moisture. 1.5 g of polymer mixture was weighed such that the PCL/$PGA_{(80:20)}$ to PEO ratio was 50:50 wt% blend. The polymer mixture was initially mixed manually by shaking the vial back and forth. Precooling time (15 min), cooling time (2 min) and impactor frequency of 10 cycles per second were kept constant throughout the process. The polymer mixture was cryomilled for 12, 30, 60 and 180 min in separate batches. These cryomilling times were selected based on the previous studies that investigated the effect of cryomilling for combining polymer powders. Cryogenic mechanical alloying for 12 min had a strong influence on structural behavior of the polymer powders, which increased with increase in milling time for up to 30 min and grinding under these cryogenic conditions resulted in fine polymer powders for milling in as short time