European Polymer Journal 68 (2015) 21–35

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Macromolecular Nanotechnology

Nanofibrous composite scaffolds of poly(ester amides) with tunable physicochemical and degradation properties

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ARTICLE INFO

Article history:
Received 14 January 2015
Received in revised form 31 March 2015
Accepted 19 April 2015
Available online 23 April 2015

Keywords:
Poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate) (APS)
Polycaprolactone (PCL)
Myoblasts
Electrospinning
Nanofibrous scaffolds

ABSTRACT

Polymeric elastomers like Poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate) (APS) have gained importance in soft tissue engineering applications due to their tunable mechanical properties and biodegradability. The fabrication of extracellular matrix (ECM)-mimetic nanofibrous scaffolds using APS is however limited due to its poor solubility in commonly used solvents, low viscosity and high temperatures required for thermal curing. In this study, we have overcome these limitations of APS by blending uncrosslinked APS pre-polymer with polycaprolactone (PCL), and have successfully fabricated ECM-mimetic nanofibrous APS scaffolds for the first time. The developed fibrous scaffolds were further characterized for their physicochemical, thermal, mechanical and degradation properties. Effects of APS:PCL weight ratios (0:1, 1:1, 2:1 and 4:1) and total polymer concentration (15–30% w/v) on the fiber morphology, tensile properties, chemical and thermal properties of the APS–PCL composite scaffolds were investigated. Higher APS concentrations in the polymer blend resulted in formation of fused fibers and thus, increased fiber diameters. The degree of hydration and consequently, degradation rate of the scaffolds increased with the APS concentration. The FTIR and DSC studies showed selective loss of APS polymer from composite scaffolds after degradation. Scaffolds with 1:1 APS:PCL ratio exhibited maximum elastic modulus (EM) of 30 ± 2.5 MPa compared to 0:1, 2:1 and 4:1 ratios. Increasing total polymer concentrations (15–30% w/v) at constant (2:1) APS:PCL ratio increased stiffness and tensile strength of the electrospun scaffolds. Biocompatibility studies using C2C12 mouse myoblast cells showed enhanced cell spreading on APS containing scaffolds after 6 h as compared to PCL-only scaffolds. Thus, the present study demonstrates successful development of APS-based thermoset elastomeric nanofibrous scaffolds by blending with semicrystalline PCL polymer for the first time. Tunable physicochemical, mechanical and degradation properties of these composite APS–PCL scaffolds will be further exploited for skeletal muscle tissue engineering applications.

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http://dx.doi.org/10.1016/j.eurpolymj.2015.04.026
0014-3057/

1. Introduction

Tissue engineering aims to develop functional synthetic or biological substitutes to repair or replace damaged organs/tissues in the body [1]. For successful therapeutic tissue engineering, it is important to recreate biomimetic cellular microenvironments that consist of extracellular matrix (ECM), cells as well as biochemical and mechanical cues to promote tissue regeneration [2,3]. Polymeric scaffold serves as an important component in the initial process of tissue regeneration by providing necessary mechanical support and extracellular matrix (ECM)-mimetic three-dimensional environment to the cells *in vitro* and *in vivo*. A number of natural and synthetic polymers have been used to facilitate scaffold design for tissue engineering which include chitosan [4,5], polyglycerol sebacate (PGS) [6–8], polycaprolactone (PCL) [6,9], polylactic acid (PLLA) [10,11], etc. While natural biopolymers offer advantages like good biocompatibility, batch to batch variability in the properties limits their usefulness. On the other hand, synthetic polymers provide choice of wide variety of methods for scaffold fabrication and allow fine tuning of chemical, physical and mechanical properties suitable for regeneration of target tissue [12]. Of particular interest is the design of synthetic biodegradable elastomers with tunable physicochemical/mechanical/biological properties suitable for soft tissue engineering [7,13–16]. Such elastomeric scaffolds can promote regeneration of damaged soft tissues such as skeletal/cardiac muscles by providing dynamic mechanical environment experienced by cells in these tissues.

To date, biodegradable synthetic elastomer, PGS has been widely explored for various tissue engineering applications [6,8,17–27]. PGS exhibits faster degradation rate of 17% in 9 weeks in PBS, elastic modulus of around 0.282 MPa, and its tensile strength is above 0.5 MPa [7,28]. To prolong the degradation profile of PGS, Bettinger et al. synthesized Poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate)s (APS) [29]. APS elastomers are a class of biodegradable poly(ester amide)s consisting of amino alcohol-based cross-linked networks with tensile strength of 1 MPa, reversible elongations up to 92% and projected *in vivo* degradation half-lives of about 20 months [29,30]. Although APS has better mechanical strength and longer degradation times than PGS, poor solubility of APS polymers in the most commonly used polar solvents such as ethanol, acetone and non-polar solvents like dichloromethane limit its utility. APS pre-polymer is only soluble in hexafluoroisopropyl alcohol (HFIP) [7,16,29]. Moreover, low viscosity of APS pre-polymer solution hampers its processing into fibrous scaffolds that can mimic native ECM of many tissues. In general, poor solubility and amorphous waxy nature of the most thermoset elastomers has restricted their processing only to thermally cured smooth films [30] or microfabrication [31]. Recently, PGS has been blended with other materials such as polycaprolactone (PCL) [6], poly(L-lactide) [32], gelatin [21] and porcine urinary bladder matrix [33] to facilitate its processing into ECM-mimicking nanofibrous scaffolds and to further improve their mechanical, biological and degradation properties.

PCL is a semicrystalline thermoplastic polyester with hydrophobic properties, slow degradation profile (over years) and poor cell attachment [6,24,34]. However, solubility in most common organic solvents and ease of processability into electrospun fibrous scaffolds has led to its widespread use in electrospinning. In our study, addition of small quantity of PCL to amorphous APS elastomer improved the solution viscosity. It was hypothesized that addition of PCL will facilitate fiber formation of APS while presence of APS will enhance hydrophilicity, degradation and cell adhesion properties of hydrophobic PCL. Here, we report fabrication and characterization of APS-based fibrous composite scaffolds by blending with small quantities of PCL. The effect of PCL addition on the physicochemical properties (hydration, *in vitro* degradation) and mechanical properties (elastic modulus and ultimate tensile strength) of APS–PCL composite scaffolds were investigated. Biocompatibility of composite scaffolds was also investigated by seeding C2C12 mouse myoblast cells on the composite scaffolds for future applications in skeletal muscle repair.

2. Materials and methods

1,3-Diamino-2-hydroxy-propane (DAHP), glycerol (G), sebacic acid (SA) and Poly(ε-caprolactone) (PCL, Mw 70–90 kDa) were purchased from Sigma–Aldrich (St. Louis, MO) and used as received. Hexafluoroisopropanol (HFIP) was obtained from Acros Organics. All other chemicals were purchased from Sigma–Aldrich unless otherwise mentioned. Cell culture supplies including media, trypsin–EDTA and antibiotics were obtained from Corning, unless otherwise mentioned.

2.1. Synthesis of Poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate) (APS) elastomer

APS elastomer with DAHP:G:SA ratio of 2:1:3 was synthesized as shown in Fig. 1, following the procedure described earlier [29]. Briefly, DAHP, glycerol (G) and sebacic acid (SA) were charged in a dry round bottom flask in 2:1:3 M ratios. The flask was sealed and heated in oil bath at 120 °C for 1 h under inert atmosphere using Argon gas. The pressure was then slowly dropped to approximately 100 mTorr and the reactants were allowed to react at 120 °C for 10 h under constant stirring. At the end of the reaction, a semisolid, light yellow colored APS pre-polymer elastomer was obtained and it was stored at 4 °C until further use.