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Fabrication, mechanical properties and cytocompatibility of elastomeric nanofibrous mats of poly(glycerol sebacate)

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ABSTRACT

It is often difficult to achieve a satisfactory balance of compliance and biocompatibility simultaneously in a pure elastomeric material. In this work, we successfully fabricated an elastomeric fibrous mat from poly(glycerol sebacate) (PGS) with a 2:3 mol ratio of glycerol to sebacic acid, using the core/shell electrospinning technology of poly(vinyl alcohol) (PVA) as a temporary shell and PGS prepolymer as the core. After the core/shell electrospinning, the PGS core was crosslinked by thermal treatment and the PVA shell was partially removed by dissolution in water. The resulting PGS fibre mat was as soft as many soft tissues (e.g., muscle), and demonstrated nonlinear, J-shaped stress–strain curves (the slope continuously increased with increasing strain) when saturated with water. *In vitro* evaluations showed that the spun $PGS_{2:3}$ mat had good cytocompatibility, better than the culture medium and the control material, PLLA fibrous mat. Hence, the newly developed electrospun $PGS_{2:3}$ fibre mats may offer a much better choice of scaffolds than existing products for engineering of soft tissues working in dynamic conditions.

1. Introduction

Soft biological tissues typically undergo elastic deformation with high extensibility and they exhibit a unique behaviour in that stress rises at an increasing rate with increasing strain, i.e., a J-shaped stress–strain curve or convex to the strain axis. As such, a biomaterial serving to replace damaged tissues (perhaps as scaffolds) should be nonlinearly elastic and have similar mechanical properties to the biological tissues. However, it is often difficult to achieve a satisfactory balance of compliance and biocompatibility simultaneously in a pure elastomeric material) [1]. With poly(glycerol sebacate) (PGS), for example, the PGS synthesized with a stoichiometric 2:3 mol ratio of glycerol (trifunctional) to sebacic acid (difunctional) has much better biocompatibility (due to a low concentration of carboxyl groups) than the PGS synthesized with a 1:1 mol ratio, but the polymer is mechanically more rigid and less extensible [2]. A version of PGS material that is as soft as muscle and yet has a satisfactory cytocompatibility is still not available. Since the stiffness of a material can be greatly reduced when fabricated into a fibrous mat, we hypothesise that a fibrous network of PGS synthesised at the 2:3 mol ratio may offer an opportunity to achieve a satisfactory balance of flexibility and compatibility simultaneously in an elastomeric material.

Electrospinning is a widely used process to produce a fibrous network. Studies have demonstrated that the electrospun nanofibrous scaffolds are good at supporting cellular attachment and proliferation [3]. Electrospinning of thermoplastics, such as polylactic acid, polyglycolic acid, poly(ε-caprolactone) and their copolymers, is a well-established process. However, thermoplastic materials

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are generally unsuitable for use in the repair of organs/tissues working under dynamic mechanical conditions, such as heart and lung, because they lack the ability to recover their shape after deformation [4]. Hence, there is a need of electrospinning of elastomeric biomaterials, such as PGS [1,5].

The production of nanofibres from chemically crosslinked elastomers is technically challenging. A major hurdle is that these polymers cannot dissolve in any solvents once cross-linked, and that fibres spun from uncrosslinked prepolymers would flow when they underwent the crosslinking esterification treatment at elevated temperatures. This problem could be addressed by core/shell electrospinning [2,6–8]. In the core/shell electrospinning process, a non-crosslinked prepolymer, e.g. poly(glycerol sebacate) [2,8–10], can be sheathed by a rigid thermoplastic, such as poly (L-lactic acid) (PLLA), when both materials are fed to the electrospinner simultaneously but via separate flow streams; in this case, the uncrosslinked PGS solution can be directed through an inner tube to form a core, while the PLLA solution flows through the outer tube to form a shell [2,8]. During the spinning process, much of the solvent could evaporate from the shell, leaving a semi-solid casing around the liquid PGS core. In the subsequent thermal crosslinking treatment, the solid thermoplastic PLLA shell should maintain the tube shape and contain the core PGS material which loses the solvent via evaporation through the thin shell and undergoes the cross-linking reaction. Previously, PGS/PLLA core/shell fibres have been fabricated using the above procedures [2,6–8]. The drawback with the use of PLLA as the shell polymer is that this thermoplastic can only be dissolved in solvents such as chloroform, tetrahydrofuran, and dioxane, which are not only environmentally unfriendly, but more seriously, can cause the swelling of PGS core polymer leading to a very fragile fibre. As such, we have found it to be impossible to retain elastic PGS fibres after removing the PLLA shell [2].

To eliminate the above drawback, the PLLA shell could be replaced by polyvinyl alcohol (PVA), an inexpensive and biocompatible polymer. Unlike PLLA, which must be dissolved in an organic solvent [7], PVA is water soluble. PVA has been electrospun by a number of research groups [11–13], and PVA hydrogels have been utilised in regenerating artificial articular cartilage [14] and tendon regeneration [15]. Therefore, the primary objective of this work was to produce elastomeric fibres from PGS with a 2:3 monomer mole ratio, using the core/shell electrospinning technique with PVA used as a temporary shell. In addition, the ultimate goal of this work was to produce elastomeric materials that are biocompatible, degradable and mechanically as soft as biological soft tissues.

2. Materials and experimental procedures

2.1. Materials

Glycerol (purity 99%), sebacic acid (purity 99%), poly (vinyl alcohol) (PVA) with different weight average molecular weights (M_w), varying from 13,000–23,000 (87–89% hydrolyzed), 31,000–50,000 (98–99% hydrolyzed), 89,000–98,000 (99 + % hydrolyzed) and 146,000–186,000 (99 + % hydrolyzed) g/mol were purchased from Sigma–Aldrich (Castle Hill, NSW, AU). Tetrahydrofuran (THF) was purchased from Merck (Kilsyth, VIC, AU). Poly-L-lactic acid (PLLA) was purchased from Sigma–Aldrich (Castle Hill, NSW, AU) and electrospun as described previously [2].

2.2. Synthesis of $PGS_{2:3}$ solid sheets

The synthesis of PGS solid sheets followed procedures that we have used previously [2]. Glycerol and sebacic acid were thoroughly mixed in glass beakers at a glycerol: sebacate (G:S) molar ratio of 2:3, and the monomer mixture was then heat-treated under a nitrogen atmosphere at 130 °C for 24 h. After removal of the water of esterification, the resultant PGS prepolymer was cooled to room temperature under nitrogen [2]. To distinguish this polymer from the $PGS_{1:1}$ produced at a 1:1 mol ratio in our previous work, the present PGS is noted as $PGS_{2:3}$. A previous study [10] of the polymerization of $PGS_{1:1}$ shows that the loss of glycerol under these conditions is about 1.4% and so the loss for the $PGS_{2:3}$ would be expected to be even less and thus negligible. The conversion of the carboxylic acid groups for the 2:3 M glycerol/sebacic acid prepolymer was found to be 56.6 ± 0.1%. If one assumes that all the hydroxyl groups are equally reactive, then theoretically [16] this system should gel at a conversion of 70.7% and this conversion is much higher than that of the non-gelled prepolymer. The pre-polymer was then dissolved in THF, cast onto glass slides, dried under ambient conditions overnight, and then dried a second time under a vacuum overnight at room temperature. The dried slides were then heated at 130 °C under a vacuum for 72 h to cause crosslinking of the $PGS_{2:3}$. After cooling to room temperature in the vacuum, the polymer sheets were soaked in water for 15 min, and then peeled off the glass slides and thoroughly dried under a vacuum for 4 days at room temperature. The conversion of carboxylic acid groups in the cured film was 94.4 ± 0.1%. As expected, this value is considerably higher than the theoretical gel point conversion of 70.7%.

2.3. Rheology of PVA solutions

The steady shear viscosity of the PVA solutions in water and of the $PGS_{2:3}$ prepolymer in THF were measured with a MCR501 Physica rheometer (Anton Paar) in parallel plate (50 mm diameter) mode with 0.5 mm gap and operated at 23 °C using shear rates varying from 1 to 1000 s^{-1}.

2.4. Electrospinning of PVA

PVA polymers of four different weight average molecular weight ranges: 13,000–23,000, 31,000–50,000, 89,000–98,000, and 146,000–186,000 g/mol, were dissolved in hot water (95 °C) at concentrations of 35, 20, 12 and 8 g/100 ml solvent, respectively. These particular concentrations were chosen because the steady shear viscosities of the aqueous solutions were very close (within a factor of two). One of the factors that affects electrospinning is the polymer solution is the solution viscosity [17,18]. Because preliminary experiments indicated that fibres could be