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Structural variants of biodegradable polyesterurethane in vivo evoke a cellular and angiogenic response that is dictated by architecture

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Abstract

The aim of this study was to investigate an in vivo tissue response to a biodegradable polyesterurethane, specifically the cellular and angiogenic response evoked by varying implant architectures in a subcutaneous rabbit implant model. A synthetic biodegradable polyesterurethane was synthesized and processed into three different configurations: a non-porous film, a porous mesh and a porous membrane. Glutaraldehyde cross-linked bovine pericardium was used as a control. Sterile polyesterurethane and control samples were implanted subcutaneously in six rabbits ($n = 12$). The rabbits were killed at 21 and 63 days and the implant sites were sectioned and histologically stained using haemotoxylin and eosin (H&E), Masson's trichrome, picosirius red and immunostain CD31. The tissue–implant interface thickness was measured from the H&E slides. Stereological techniques were used to quantify the tissue reaction at each time point that included volume fraction of inflammatory cells, fibroblasts, fibrocytes, collagen and the degree of vascularization. Stereological analysis inferred that porous scaffolds with regular topography are better tolerated in vivo compared to non-porous scaffolds, while increasing scaffold porosity promotes angiogenesis and cellular infiltration. The results suggest that this biodegradable polyesterurethane is better tolerated in vivo than the control and that structural variants of biodegradable polyesterurethane in vivo evoke a cellular and angiogenic response that is dictated by architecture.

Keywords: Polyesterurethane; Tissue response; Scaffold

1. Introduction

Synthetic biodegradable polymers are becoming increasingly popular for clinical and surgical applications as it is possible to control their mechanical properties and degradation rates, depending on the particular application. Furthermore, these polymers may be processed into a variety of porous and non-porous structures, using processes such as rapid-prototyping, electrospinning, freeze drying, phase inversion and solvent casting [1,2]. The degree of tissue infiltration can be controlled by the morphology and porosity of the scaffold [3]. The porous architecture of the former allows the cells to penetrate the structure, thus increasing the surface area over which they can proliferate.

Implant architecture is known to influence the inflammatory and angiogenic response in vivo. Rough implant architectures have been reported to evoke a greater macrophage response and induce greater numbers of giant cells compared to smooth implant surfaces [4–6]. Electrospun meshes with a fibre diameter less than 10 μm were shown to be less susceptible to encapsulation compared to meshes with a fibre diameter greater than 10 μm [7,8]. It was also noted that electrospun meshes with an inter-fibre distance of less than 100 μm experience less tissue infiltration and greater encapsulation compared to meshes with an inter-fibre distance longer than 100 μm [7,8]. The interstitial inter-fibre distance is

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determinant with regards to the degree of angiogenesis within a porous implant, as arterioles have a diameter of 30–500 μm [9]. Furthermore, implant architecture affects the degradation rate of biodegradable polymers, since porous scaffolds have a larger surface area in contact with fluids than non-porous scaffolds.

Biodegradable polyesterurethanes have been investigated in vitro and in vivo for various tissue-engineering applications [2,3,10–12]. A slowly degrading polyesterurethane with a crystalline segment of poly((*R*)-3-hydroxybutyric acid)-diol linked with a diisocyanate to an amorphous segment of poly(ε-caprolactone-*co*-glycolide)-diol has been developed [13]. The ratio of the soft to hard segments has been based on a predicted long-term degradation period derived from earlier data on DegraPol™ which indicated the degradation rate and the respective stoichiometric ratios of the crystalline to amorphous segments [11,14,15]. Our earlier study characterized and reported the in vitro behaviour of this polymer with respect to change in mechanical properties, molecular weight and cell viability [13]. This is the first study to characterize the in vivo response of this specific blend of polyesterurethane.

The aim of this study was to investigate the cellular and angiogenic response evoked by varying implant architectures in a subcutaneous rabbit implant model at 21 and 63 days. In this study, three scaffolds were processed from the same polyesterurethane: a non-porous film using solvent casting, an electrospun porous mesh and a porous membrane fabricated by a phase-inversion method. The control was bovine pericardium. Haemotoxylin and eosin (H&E) and Masson's trichrome (MT) stains were used to assess the tissue response at both time points for all four variants. Picosirius red stain and polarized light microscopy were used to examine collagen orientation around the four implants. Immunostain CD31 highlighted endothelial cells to subsequently quantify angiogenesis. Stereological techniques quantified the tissue–implant interface thickness, the volume fraction of inflammatory cells, fibroblasts, fibrocytes, collagen and degree of angiogenesis specific to each scaffold.

2. Materials and methods

2.1. Polymer synthesis

A biodegradable polyesterurethane polymer with poly(hydroxybutyrate) for the crystalline segment and diglycolide and ε-caprolactone for the amorphous segments was used in this study [13]. The stoichiometric ratio of the crystalline to amorphous segments was 1:1.5, respectively. The crystalline and amorphous segments were cross-linked with 2,2,4-trimethyl hexamethylene diisocyanate (TMDI). Throughout the synthesis, Fourier transform infrared (FTIR) (Bruker Vertex 70, Bruker Optics GmbH, Faellanden, Switzerland) measurements were taken at regular intervals to identify the presence of the unreacted cross-linker TMDI (–NCO) groups at 2250 cm^{-1}. When the FTIR spectra no longer detected the TMDI, the polymer synthesis was considered complete. The polymer synthesis was concluded after 120 h. Subsequently, the synthesized polymer was precipitated into −70 °C methanol to prevent premature hydrolysis. Following precipitation, the polymer was vacuum-filtered to remove excess solvent and later dried in a vacuum oven at −10 mbar for 72 h prior to processing.

2.2. Scaffold manufacture

2.2.1. Fabrication of film

A 20% (w/w) polymer solution was prepared in chloroform (Merck KgaA, Germany) [13]. The solution was cast using a stencil with a 500 μm gap on a polytetrafluoroethylene-coated plate. The wet films were left under a fume hood to allow solvent evaporation.

2.2.2. Fabrication of mesh

A 27% (w/w) polymer solution was dissolved in chloroform [13]. Ten milliliter of the solution was extracted with a syringe and secured in a syringe pump (Aladdin-220 Programmable Syringe Pump, World Precision Instruments, Stevenage, UK). A cooled (−70 °C) cylindrical target was positioned 200 mm away from the needle. A high-voltage supply (Glassman Europe Ltd., UK) was attached to the needle. The target was rotated at 300 rpm, the syringe flow-rate was 4 ml h^{-1}, and the high-voltage supply was 18 kV. The processing time was 2.5 h. The processed electrospun mesh was removed from the target and stored in a vacuum chamber to remove excess solvent and moisture accumulated during processing.

2.2.3. Fabrication of membrane

A 15% (w/w) solution was dissolved in 1,4-dioxane, this concentration was determined from previous studies, and cast into a wet film using a stencil with a 500 μm gap. This film was immersed in containers of methanol and ethanol for 5 min each. Finally, the glass plate and membrane were then transferred to a container of distilled water for 5 min. The immersion of the film in the non-solvents created a porous membrane. The porous membranes were stored in a vacuum chamber to remove excess moisture.

2.2.4. Control

Glutaraldehyde-treated bovine pericardium (Peri-Strip®, Biovascular Inc., Saint Paul, MN) was used as a control, as this is FDA-approved for reinforcing surgical staples. This biomaterial also has a long degradation rate but must be pre-treated for enhanced mechanical properties and degradation rate. Hence a slowly degrading polyesterurethane that requires no pretreatment prior to processing may be an alternative. In addition, tissue response to glutaraldehyde cross-linked pericardium is well known and will hence act as a control.

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