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Biodegradable, thermoplastic polyurethane grafts for small diameter vascular replacements

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ABSTRACT

Biodegradable vascular grafts with sufficient in vivo performance would be more advantageous than permanent non-degradable prostheses. These constructs would be continuously replaced by host tissue, leading to an endogenous functional implant which would adapt to the need of the patient and exhibit only limited risk of microbiological graft contamination. Adequate biomechanical strength and a wall structure which promotes rapid host remodeling are prerequisites for biodegradable approaches. Current approaches often reveal limited tensile strength and therefore require thicker or reinforced graft walls. In this study we investigated the in vitro and in vivo biocompatibility of thin host-vessel-matched grafts ($n = 34$) formed from hard-block biodegradable thermoplastic polyurethane (TPU). Expanded polytetrafluoroethylene (ePTFE) conduits ($n = 34$) served as control grafts. Grafts were analyzed by various techniques after retrieval at different time points (1 week; 1, 6, 12 months). TPU grafts showed significantly increased endothelial cell proliferation in vitro ($P < 0.001$). Population by host cells increased significantly in the TPU conduits within 1 month of implantation ($P = 0.01$). After long-term implantation, TPU implants showed 100% patency (ePTFE: 93%) with no signs of aneurysmal dilatation. Substantial remodeling of the degradable grafts was observed but varied between subjects. Intimal hyperplasia was limited to ePTFE conduits (29%). Thin-walled TPU grafts offer a new and desirable form of biodegradable vascular implant. Degradable grafts showed equivalent long-term performance characteristics compared to the clinically used, non-degradable material with improvements in intimal hyperplasia and ingrowth of host cells.

1. Introduction

During the last decades different approaches and biomaterials have been applied to solve problems associated with small diameter grafts, notably thrombogenecity, biomechanical mismatch with the host vessel and intimal hyperplasia. Although efforts to find the ideal graft candidate persist, currently available substitutes are not yet comparable to native tissues in small diameter applications [1–3].

Considerable research is currently focused on designing biodegradable vascular grafts which could provide a suitable vascular substitute for small diameter (<6 mm) applications in coronary and vascular surgery [4–8]. These grafts are intended to serve as temporary scaffolds until a fully functional new vessel has been created by the host. Such short-lived vascular templates allow adaptation in the growing individual and limit the time when

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the organism is confronted with an artificial biomaterial, thus reducing the risk of microbiological contamination.

Studies have shown that naturally derived biomaterials such as decellularized matrices have superior functional attributes due to their extracellular matrix (ECM) composition [9]. In addition to biomechanical compatibility with the host vessel, these implants also attract cells from the recipient and promote efficient scaffold remodeling [10,11]. However, these biologically derived substitutes have not found their way into clinical practice because of safety issues and the lack of regulatory approval [12,13].

Electrospun conduits can largely mimic the desirable properties of decellularized matrix grafts. The graft wall is a web of randomly orientated micro and/or nanofibers with an architecture very similar to that of the native ECM. With appropriate porosity it facilitates host cell migration [14,15].

Many groups have spun a variety of biodegradable polymers [16]. Most of those currently in use are natural (collagen, elastin) or approved suture materials such as polylactic acid (PLA), polyglycolic acid (PGA) or poly-epsilon-caprolactone (PCL). Many of these materials when applied as vascular grafts show poor biomechanical properties. Most of these grafts have been formed for and used in small animal models. They had thick graft walls or were designed as multilayered scaffolds to bolster their mechanical strength, particularly to prevent aneurysmal dilatation [17].

Recently we developed a thermoplastic polyurethane (TPU) polymer containing a biodegradable chain extender which has biomechanical properties similar to polyurethane and which can be manufactured by electrospinning [18]. Biomechanical analysis and in vitro cell studies show that this polymer has satisfying biomechanical properties and that its degradation by-products are non-toxic. The polymer has a low degradation time, 10 times less than that of polylactic acid.

In this study we used TPU to fabricate biodegradable, porous grafts with a very thin wall, as a small diameter vascular substitute. The matrix design was optimized for cell migration in a previous study [19]. Biocompatibility and physical characterization of the grafts were studied in vitro. Protein and lipid adsorption were investigated. The grafts were then studied in vivo using a small rodent model for short- and long-term applications in direct comparison to expanded polytetrafluoroethylene (ePTFE) controls. ePTFE has been chosen as a control graft. It is currently the standard artificial graft for small-diameter vascular repair. Our aim was to compare a synthetic, non-degradable, clinically used prosthesis with our synthetic, degradable prosthesis and to show that this degradable thin-walled prosthesis has a sufficient long-term performance and can resist biomechanical failures.

2. Materials and methods

2.1. Polymer synthesis

The thermoplastic polymer was synthesized by the prepolymer method as described previously [19]. Briefly, dried poly(tetrahydrofuran) (M_n = 1000 g mol^{-1}, 3.65 g, 3.65 mmol, Sigma–Aldrich, Vienna, Austria) was weighed directly into the reaction flask. Freshly distilled hexamethylene diisocyanate (1.228 g, 7.30 mmol, Sigma–Aldrich, Vienna, Austria) in 5 ml dry dimethylformamide (<50 ppm water, Acros, Renningen, Germany) was added to poly(tetrahydrofuran) under argon atmosphere. 0.15 ml of tin(II)-2-ethyl hexanoate (stored over 5 Å molecular sieves; Sigma–Aldrich, Vienna, Austria) were added to the reaction mixture. After 2 h of stirring at 90 °C bis(2-hydroxyethyl) terephthalate (Sigma–Aldrich, Vienna, Austria, dried over $CaCl_2$, 0.982 g, 3.65 mmol), dissolved in 5 ml dry dimethylformamide, was added. After 1 h of stirring at 90 °C the reaction mixture was stirred overnight at room temperature. The viscous mixture was diluted with ~70 ml of dimethylformamide and the polymer was precipitated in ~1.5 l methanol, filtered, reprecipitated from dimethylformamide in methanol and dried in vacuo.

2.2. Graft fabrication

The synthesized polymer was dissolved at 6 wt.% in 1,1,1,3,3,3-hexafluoro-2-propanol; (Sigma–Aldrich, Vienna, Austria) and electrospun on a PTFE mandrel as previously described [20]. The conditions for the graft fabrication were as follows: 9 cm needle–target distance, 2 cm target–back electrode distance, 0.02 ml min^{-1} flow rate, 10 kV at the needle and 250 rotations min^{-1} of the mandrel. To remove residual solvent, electrospun conduits were dried under vacuum for 2 h at 40 °C.

ePTFE prostheses (1.5 mm inner diameter (ID), 100 μm wall thickness, 5–25 μm internodal distance (IND); Zeuss, Orangeburg, USA) were used as controls. Electrospun grafts and controls were sterilized with ethylene oxide and soaked in heparinized saline for 15 min before application.

2.3. Graft characterization

Fiber morphology and fiber size distribution were determined by scanning electron microscopy (SEM; JEOL JSM-5400, Japan). Fiber diameters were manually measured by ImageJ (ImageJ 1.45k, National Institutes of Health Bethesda, USA). Fiber size distribution was evaluated in three grafts by measuring 20 fiber diameters at a magnification of 3500.

The accessible porosity of the electrospun fabrics and of the ePTFE conduits was determined by the liquid intrusion method at room temperature as described by De Valence et al. [21]. Pore size was determined using scanning electron micrographs (3500×) of the lumina of three electrospun grafts. Pore size of the grafts was also calculated by an empirical model developed by Tomadakis and Robertson [22].

The water contact angle was measured using a drop-shape analyzer (DSA 30, Krüss GmbH, Hamburg, Germany). Four measurements were performed on each graft before (n = 4) and after (n = 4) sterilization.

Image acquisition was performed using micro-computed tomography (μCT) (μCT-35, SCANCO Medical, Zurich, Switzerland). Visualization was performed using Matlab (MathWorks, Natick, Massachusetts, USA) and matVTK [23].

2.4. Mechanical characterization

Biomechanical attributes of the grafts (n = 4/time point) were measured prior to and after long-term (6 and 12 months) in vivo implantation and compared with ePTFE controls (n = 4/time point). The maximum tensile force, the compliance in the physiological range and the suture retention were investigated as described previously [19].

2.5. Biocompatibility testing in vitro

The use of primary human vein endothelial cells (HUVECS), isolated from the umbilical cord, was approved by the Ethics Committee of the Medical University of Vienna (EK Nr.: 1183/2012). HUVECS were cultivated in 48 well plates on 0.35 cm^2 slices from sterilized electrospun conduits and ePTFE grafts as described previously [19]. In brief, seeded vascular scaffolds (50,000 cells per well, n = 8 per time) were cultivated on a shaking device for 12 and 24 h, respectively. The number of metabolically active and viable cells was determined with a colorimetric XTT cell viability and proliferation kit (Biomol, Hamburg, Germany) as previously described [19].

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