Biomaterials 35 (2014) 9033–9040

Contents lists available at ScienceDirect

Biomaterials

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

The performance of a small-calibre graft for vascular reconstructions in a senescent sheep model

Maqsood Ahmed [a, b], George Hamilton [c], Alexander M. Seifalian [a, c, *]

[a] UCL Centre for Nanotechnology & Regenerative Medicine, Division of Surgery and Interventional Science, University College London, United Kingdom
[b] Centre of Mathematics and Physics in The Life Sciences and Experimental Biology (CoMPLEX), University College London, United Kingdom
[c] Royal Free London NHS Foundation Trust Hospital, London, United Kingdom

ARTICLE INFO

Article history:
Received 22 May 2014
Accepted 10 July 2014
Available online 5 August 2014

Keywords:
In vivo test
Biocompatibility
Vascular graft
Polyurethane
Nanocomposite

ABSTRACT

There is an acute clinical need for small-calibre (<6 mm) vascular grafts for surgery. The aim of this study was to evaluate the long-term performance of a small-calibre graft produced from a nanocomposite biomaterial, polyhedral oligomeric silsesquioxane poly(carbonate-urea)urethane (POSS-PCU), in a large animal model following Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP) protocols. Grafts were characterised and implanted into the left carotid artery (LCA) of senescent sheep ($n = 11$) for a period of 9 months. *In vivo* compliance and blood flow rates were measured using ultrasound wall tracking software and a Transonic flow meter. Graft patency and degree of intimal hyperplasia (IH) were examined at the study end point. Seven of the POSS-PCU grafts were free from thrombosis, IH, calcification and aneurysmal dilation, with 4 occluding within 14 days. All of the ePTFE controls ($n = 4$) were found to be occluded by day 32. The lumen of the patent POSS-PCU grafts was free from any cellular deposits, whilst perigraft tissue could be seen to be infiltrating into the body of the graft from the adventitia. No significant differences were detected between the blood flow rates ($p = 0.3693$) and compliance ($p = 0.9706$) of the POSS-PCU grafts and the native artery, either post-operatively or after 9 months implantation. Small-calibre vascular grafts produced from POSS-PCU offer a viable option for the clinical use in revascularisation procedures with a patency rate of 64%.

1. Introduction

Occlusion of the blood vessels is a leading cause of morbidity and mortality. Treatment options to restore perfusion are often limited to surgical interventions. In more severe occlusions, 70% or more, or in cases where stenosis is multi-focal or when multiple vessels are involved; bypass surgery is often the only choice. Approximately 600 000 coronary or peripheral artery bypass grafting operations are performed annually in the USA alone [1]. Currently, autologous vessels, such as the saphenous vein, are the conduits of choice for revascularisation. However, up to 30% of patients who require surgery do not have suitable or sufficient blood vessels to be used for transplantation due to vascular disease, amputation or previous harvest [2]. Further, harvesting autologous vessels requires a second surgical procedure, with potential concomitant morbidities.

Developing clinically viable small-calibre vascular grafts, as an alternative to autologous vessels, is the subject of intense research. Whilst a number of prosthetic grafts have been successfully developed for the replacement of large-calibre (>6 mm) vessels, the small-calibre (<6 mm) counterparts fail due to thrombosis and intimal hyperplasia (IH) [3]. The two most common prosthetic grafts used clinically are produced from polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE). Both materials are rigid compared to the native artery. In small-diameter, low flow positions, such as the coronary or infrainguinal arteries, viscoelasticity of graft is necessary to minimise the impedance to flow and the risk of stasis and thrombosis [4]. Additionally, the viscoelastic mismatch between graft and host artery has been implicated in the development of IH. Pulsatile stretching at the site of anastomosis can trigger the excessive proliferation of smooth muscle cells (SMC) resulting in the narrowing or stenosis of the graft [5].

* Corresponding author. UCL Centre for Nanotechnology & Regenerative Medicine, Division of Surgery and Interventional Science, University College London, United Kingdom. Tel.: +44 020 7830 2901 (direct).
E-mail address: A.Seifalian@UCL.AC.UK (A.M. Seifalian).

http://dx.doi.org/10.1016/j.biomaterials.2014.07.008
0142-9612/

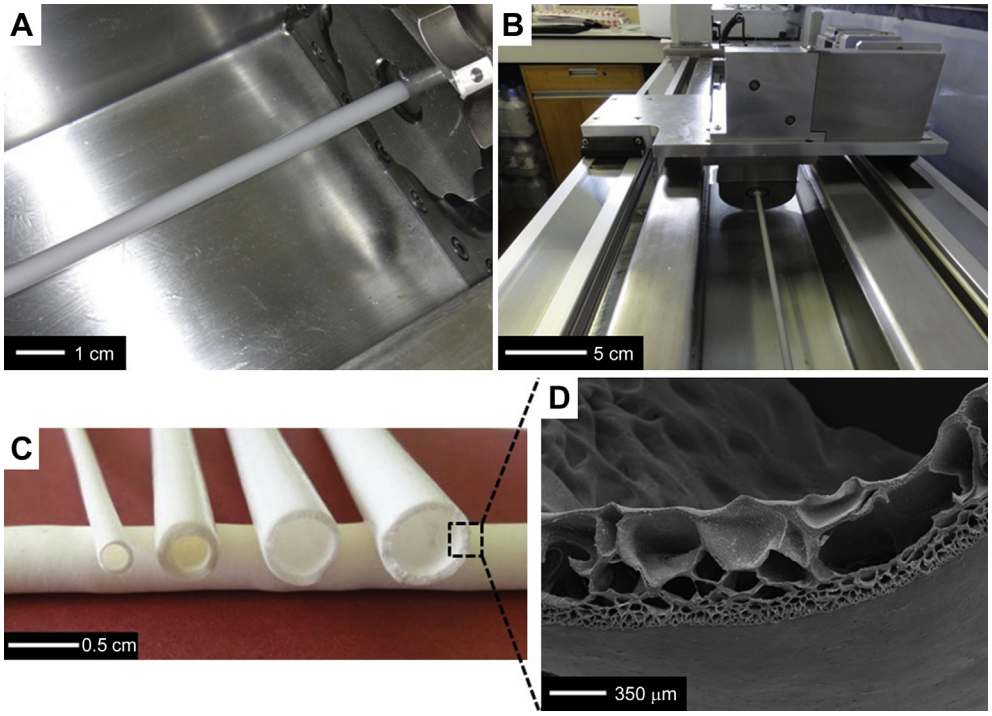

Fig. 1. Digital images of POSS-PCU graft being extruded through a die and phase separating in a water bath (A and B); fully formed vascular grafts in a range of diameters (C) and an scanning electron microscopy (SEM) image of the graft cross-section demonstrating the porous graft wall (D).

Our lab has previously developed a nanocomposite biomaterial, polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane (POSS-PCU) with the aim of improving the poor *in vivo* biostability of polyurethanes [6]. Polyurethanes have been proposed as an alternative prosthetic graft material as they have excellent mechanical properties, namely a compliance profile similar to that of the native artery [7]. However, this initial enthusiasm was dampened somewhat due to the lack of long term durability, leading to mechanical and chemical degradation resulting in radial dilation and the formation of aneurysms. By covalently attaching the chemically robust POSS nanocage to the PCU backbone, a highly durable and biostable material was created which was shown to be resistant to hydrolytic and oxidative degradation both *in vitro* and *in vivo* [8,9]. Following subcutaneous implantation in an ovine model for 36 months, no sign of degradation or inflammation was noted. Constructs from POSS-PCU have already found clinical use as a synthetic tracheal transplant [10]. Additionally, POSS-PCU displayed improved anti-thrombogenic properties *in vitro* compared to ePTFE making it a promising biomaterial for cardiovascular applications [11].

The purpose of this study was to explore the hypothesis that with improved *in vivo* stability, blood compatibility and a compliance profile similar to the native artery, POSS-PCU grafts will yield superior patency rates compared to PTFE counterparts. The long term performance of the grafts exposed to the arterial circulation was evaluated in a senescent ovine model for a period of 9 months following GMP/GLP protocols. The patency, structural integrity, compliance and perfusion efficiency were assessed as key determinants of graft performance.

2. Materials and methods

The study was performed in compliance with the GLP Regulations 1999 (S.I. No. 3106) as amended by the 2004 regulations (S.I. 994) which are based on the principles of GLP as adopted by the Organisation for Economic Co-operation and Development (OECD), ENV/MC/CHEM (98) 17.

2.1. Graft fabrication

Graft fabrication has been described in detail previously [12]. Briefly, a mandrel 5 mm in diameter was used to extrude an 18% POSS-PCU solution in DMAc, through a 7 mm die, into a water bath at room temperature (Fig. 1A and B). The grafts were left in the water bath for a period of 30 min allowing the phase separation process to take place, after which they were removed from the mandrel, and left in distilled water for a period of 48 h ensuring complete removal of solvent. The resultant grafts had an internal diameter of approximately 5 mm and wall thickness of 0.7 mm (Fig. 1C and D). Finally, the grafts were sterilised via autoclaving, thoroughly rinsed and stored in sterile distilled water at a temperature of 4 °C.

2.2. Graft characterisation

Contact angle analysis was carried out using a goniometer (DSA100E, Kruss, Hamburg, Germany) equipped with a high speed framing video recording system with a CCD camera. A 3 μL Milli-Q water drop was placed on the surface of the sample, in air, at room temperature. Manufacturer provided drop shape analysis software was used to determine the air–water contact angle. A minimum of 10 droplets per sample were measured ($n = 6$).

Surface roughness was evaluated using atomic force microscopy (AFM) (XE 100, Park Systems) operating in contact mode. The luminal surface of the graft ($n = 6$) was positioned on a metallic disc and magnetically mounted in the AFM chamber. Surface images for quantitative analysis were obtained from 24 × 24 μm scans at a scan rate of 0.8 Hz. The planar surface roughness values of the scans were calculated using XEI analysis programme (Park Systems Corp).

For platelet adhesion we incubated the grafts in platelet rich plasma (PRP) for 30 min at 37 °C ($n = 6$). PRP was prepared by collecting human blood from consenting volunteers by venepuncture in citrated blood tubes constituting 3.8% citrated whole blood at 1:10 v/v, pH7.4 (Sarstedt, UK) and centrifuging at 400 g for 10 min to remove the red blood cells. Following incubation, grafts were fixed in 4% paraformaldehyde and dehydrated through a degrading ethanol series prior to being placed in hexamethyldisilazane (HMDS, Sigma) for 10 min and then allowed to air dry.

Grafts were imaged using scanning electron microscopy (SEM) by attaching to aluminium stubs with double sided sticky tabs (TAAB) and coating with gold using a SC500 (EMScope) sputter coater for electrical conductance. The stubs were examined and photographed using a Phillips 501 scanning electron microscope.

Suture retention force was evaluated by passing a suture (6.0 Prolene) through the graft wall 2 mm from the end. The graft was placed in the top grip of an uniaxial load machine (Instron 5565) and the free end of the suture was placed in the bottom grip. The specimen was pulled at a speed of 50 mm min^{-1} until either the suture ripped or the graft failed. The force required to pull the suture through the prosthesis