Eur J Vasc Endovasc Surg (2015) 49, 335–343

Nanotopography and Plasma Treatment: Redesigning the Surface for Vascular Graft Endothelialisation

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WHAT THIS PAPER ADDS

This multidisciplinary approach targeted on the luminal surface brings together new engineering technologies to established vascular graft development of small diameter vascular grafts. Results from this study confirm the potential of these surface modification techniques for wider adoption forming the basis towards producing 'self-endothelialising' vascular grafts, enhancing long-term patencies, and benefitting vascular patients in the future.

Introduction: Vascular graft materials in clinical use, such as polytetrafluoroethylene (PTFE) and Dacron, do not endothelialise and have low patency rates. The importance of an endothelial cell layer on the luminal surface of a vascular graft is well-known with surface topography and chemistry playing an important role. The aim of this study was to investigate the potential of plasma treatment and topographical structures on the luminal graft surface to enhance the self-endothelialisation potential of a nanocomposite vascular graft.

Methods: POSS-PCU is a polycarbonate urea urethane (PCU) with a nanoparticle, polyhedral oligomeric silsesquioxane (POSS) incorporated within it. Planar, microgrooved, and nanopit patterned polymer films were fabricated using photolithography, electron beam lithography, reactive ion etching, and replication by solvent casting. Films were then exposed to oxygen plasma treatment at different powers for a fixed time (40W, 60W, 80W/60 seconds). Effects of plasma treatment were assessed using scanning electron microscopy, atomic force microscopy and water contact angle analysis. Human umbilical vein endothelial cell (HUVEC) proliferation and morphology were characterised using immunostaining, live/dead staining, and Coomassie blue staining.

Results: Successful embossing of the micro- and nanostructures was confirmed. Oxygen plasma treatment of the different samples showed that increasing power significantly increased the hydrophilicity of the samples ($p < .0001$). Improved HUVEC adhesion was seen on plasma modified compared with untreated samples ($p < .0001$). Coomassie blue staining showed that after 5 days, cells started to form monolayers and live/dead staining showed the cells were viable. Immunostaining showed that HUVECs expressed nitric oxide synthase on all topographies with focal adhesions appearing more pronounced on nanopit surfaces, showing retention of morphology and function.

Conclusion: These encouraging results indicate a future important role for plasma treatment and nanotopography in the development of endothelialised vascular grafts.

Article history: Received 4 September 2014, Accepted 5 December 2014, Available online 8 January 2015

Keywords: Nanotopography, Vascular grafts, Surface modification

INTRODUCTION

Current developments in the fabrication of small diameter vascular grafts have benefited from new areas of investigation, namely nanotechnology and vascular tissue engineering. Small diameter vascular grafts in clinical use still suffer from early failure from thrombosis, and the majority of these use polytetrafluorethylene (PTFE). A major clinical trial conducted in Vienna showed the benefits of an endothelial layer within the luminal surface of PTFE grafts implanted in the infrainguinal region.[1] At 5 years, the primary patency rate for pre-seeded PTFE grafts was 78% compared with previous studies which showed 49–57.4% patencies at 5 years for infrainguinal vascular grafts.[2,3] Despite these encouraging results, the logistics of pre-seeding still hamper wider usage of this technique. The main problems are the length of time taken to

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1078-5884/

http://dx.doi.org/10.1016/j.ejvs.2014.12.008

'endothelialise' the grafts, the need for specialised cell culture facilities, and invasive procedures to harvest cells from patients. Integrated provision of cell culture facilities and operating theatres limit this method to university or specialist hospitals.

POSS-PCU is a novel nanocomposite polymer, which has been developed as a vascular bypass graft material, and is composed of a nanoparticle, polyhedral oligomeric silsesquioxane (POSS), and polycarbonate urea urethane (PCU). The beneficial effects of the nanoparticle when combined with the polyurethane-based polymer include its antithrombogenicity and mechanical properties allowing it to be compliant while retaining strength.[4–6] Despite these advantages, recent large animal studies conducted to Good Laboratory Practice (GLP) standards, have shown that after insertion as a carotid interposition graft and subsequent explantation there is an incomplete endothelialisation within the graft.[7] This raises concern in terms of the long-term patency potential of the graft and has led us to consider other techniques and methods that may promote endothelialisation.

The ability to influence inward migration of vascular endothelial cells (ECS) from the neighbouring vessel resulting in endothelialisation offers an exciting prospect. However, scepticism arose when it was noted that in PTFE and Dacron grafts the inward migration is never more than 2–3 cm and initial enthusiasm quickly wavered.[8] However, advances in technology, namely plasma modification and surface patterning, have renewed interest in this approach to self-endothelialisation.

Surface chemistry is known to play an important part in the responsiveness of cells to biomaterials. Plasma treatment has been used to help enhance surface chemistry by incorporating chemical groups into the material that are known to enhance cell attachment, such as —OH, $-NH_2$, and —COOH groups, lowering the water contact angle (WCA) of the material. O_2 plasma technology produces a mixture of —OH and —COOH groups on the polymeric surfaces optimising surface hydrophilicity thus providing a more conducive surface to cell attachment and spreading. Furthermore it is believed that uncharged moderately hydrophilic surfaces have the potential benefit of low levels of interaction with plasma, proteins, and blood.[9] Plasma treatment has several potential advantages over other methods of surface chemical modification such as wet chemical treatments — it is solvent-free, conserves the original mechanical properties of the bulk material, and uniformly changes the surface chemistry.[10]

The modulation of surface structures to provide biophysical cues to ECs has been an area of recent focus.[11] Surface topography works synergistically with surface chemistry to provide the cell with instructional cues to modify cell behaviours.

Emerging technologies such as nanotechnology and nanoengineering have shown initial promise in providing biophysical cues at the micro- and nanoscale to cells that influence cellular migration, differentiation, and adhesion.[12–14] Surface material structuring techniques used in the life sciences have been borrowed from the semiconductor industry and these technologies enable the production and optimisation of their fabrication.[15] Electron beam lithography (EBL) and photolithography (PL) fabrication techniques (Fig. 1) have been widely used in the engineering industry and remain the 'standard' for producing ordered micro- and nanostructured surfaces.[16] Over the last 10 years, these techniques have been modified for use in the biomedical devices industry, including the production of vascular devices.[11] A topography of particular interest over the last few years has been the near-square 50 (NSQ) surface that has been demonstrated to increase mesenchymal stem cell adhesion and function.[13,14,17] It comprises 120 nm diameter pits (100 nm deep) in a square lattice. The pits are placed with 300 nm centre-centre spacing but with up to ±50 nm offset from the true centre, that is it is deliberately disordered rather than random.

Thus surface modulation of the vascular graft to promote endothelialisation is now an important aspect of vascular engineering technologies. Understanding the role of both surface topography and chemistry in endothelial cell biology is an integral part to engineering the luminal surface. In this study, the synergistic role of surface topography and chemistry is investigated in promoting endothelialisation within a nanocomposite vascular graft material.

MATERIALS AND METHODS

Cell culture

Human umbilical vein endothelial cells (Life Technologies, UK) (HUVECs) were cultured in M200 media supplemented with Low Serum Growth Serum (Life Technologies, UK) and for experiments, cells were used between passages 3 and 6.

POSS-PCU preparation

Preparation of the POSS-PCU polymer has been reported extensively.[4] In brief, polycarbonate diol and trans-cyclohexanechlorohydrinisobutyl-POSS were added to a reaction vessel then heated to 130 °C while being stirred under nitrogen gas. The reactants were then cooled before 4,4′-methylenebis(phenyl isocyanate) (MDI) was added and all components reacted for 30 minutes under nitrogen gas. This reaction then forms a prepolymer before dimethylacetamide (DMAC) was added to convert this prepolymer into a solution. This solution was then cooled before the chain extender, ethylenediamine, was added dropwise until the reaction was completed. The chain stopper, 1-butanol, was then used to prevent further unwanted polymerisation. All of the reagents were supplied by Sigma Aldrich (Dorset, UK) and used as provided with the exception of POSS, which was supplied by Hybrid Plastics Inc (Mississippi, USA).

Fabrication of planar, micro- and nanostructured POSS-PCU substrates

Microgroove (MG) and nanopit (NP, i.e. NSQ) silicon masters were fabricated via photolithography and electron beam lithography (EBL), respectively, followed by reactive