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Biomimetic modified clinical-grade POSS-PCU nanocomposite polymer for bypass graft applications: A preliminary assessment of endothelial cell adhesion and haemocompatibility

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ABSTRACT

Background: To date, there are no small internal diameter (<5 mm) vascular grafts that are FDA approved for clinical use due to high failure rates from thrombosis and unwanted cell proliferation. The ideal conditions to enhance bioengineered grafts would be the blood contacting lumen of the bypass graft fully covered by endothelial cells (ECs). As a strategy towards this aim, we hypothesized that by immobilising biomolecules on the surface of the polyhedral oligomeric silsesquioxane-poly(carbonate-urea)urethane (POSS-PCU) nanocomposite polymers, which contain binding sites and ligands for cell surface receptors similar to extracellular matrix (ECM) will positively influence the attachment and proliferation of ECs. Since, the surface of POSS-PCU is inert and not directly suitable for immobilisation of biomolecules, plasma graft polymerisation is a suitable method to modify the surface properties ready for immobilisation and biofunctionalisation.
Methods: POSS-PCU was activated by plasma treatment in air/O_2 to from hydroperoxides (–OH, –OOH), and then carboxylated via plasma polymerisation of a 30% acrylic acid solution (Poly-AA) using a two-step plasma treatment (TSPT) process. Collagen type I, a major component of ECM, was covalently immobilised to mimic the ECM structures to ECs (5 mg/ml) using a two-step chemical reaction using EDC chemistry. Successful immobilisation of poly-AA and collagen on to the nanocomposites was confirmed using Toluidine Blue staining and the Bradford assay. Un-treated POSS-PCU served as a simple control. The impact of collagen grafting on the physical, mechanical and biological properties of POSS-PCU was evaluated via contact angle (θ) measurements, scanning electron microscopy (SEM), atomic force microscopy (AFM), dynamic mechanical thermal analysis (DMTA), ECs adhesion and proliferation followed by platelet adhesion and haemolysis ratio (HR) tests.
Results: Poly-AA content on each of the plasma treated nanocomposite films increased on Low, Med and High samples due to more carboxylic acid (–COOH) groups at the surface forming amide (–NH_2) bonds. The amount of –COOH groups on each of the Low, Med and High nanocomposites correlated with Poly-AA grafting density at 14.7 ± 0.9, 18.9 ± 0.9, and 34.2 ± 2.4 μg/cm^2. Immobilisation of collagen type I on to nanocomposite surface was also found to increase significantly on the Low, Med and High samples from 22 ± 4, 150 ± 15, and 219 ± 17 μg/cm^2, respectively. The level of ECs and their adhesion efficiency were improved with increasing amounts of grafted collagen I. The maximum adhesion of ECs was found on the highest collagen type I coated nanocomposites. Platelet adhesion and activation also increased with increasing collagen density. The obtained HR values for all of the treated samples were well within the acceptable standards for biomaterials (<5% HR).
Conclusion: Poly-AA-g-POSS-PCU surfaces offer binding sites for the covalent bonding of collagen type I and other biomolecules such as fibronectin by exposure of RGD cell binding domains and growth factors using EDC cross-linking chemistry. Collagen type I modification can yield accelerated EC growth and enhance the endothelialisation of POSS-PCU nanocomposites, and the amount of immobilised collagen can control the level of platelet adhesion on functionalized POSS-PCU via TSPT and poly acrylic acid (poly-AA) treatment. Such surface modification procedures

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of polymeric surfaces can improve the patency rate of POSS-PCU nanocomposites as vascular bypass grafts in the preparation of a range of medical devices ready for pre-clinical and in vivo evaluation.

1. Introduction

Atherosclerosis and coronary arterial restenosis are the two major consequences of cardiovascular disease (CVD), which are responsible for high morbidity and mortality rates. However, due to the availability of autologous vessels through prior removal, disease, or inadequate size miss-matching, patients do not possess suitable replacement blood vessels. Hence, the use of artificial bypass graft materials used to treat occluded vessels is recommended, but such small-diameter grafts present weak patency due to thrombosis and intimal hyperplasia (IH) induced mainly by the lack of the confluent layer of endothelial cells (ECs) [1]. The endothelium is an active organ lining the blood vessels maintaining integrity with dynamic mechanisms and unique biochemistry, which prevents thrombosis and IH. Studies involving in vitro endothelialisation of grafts with cultured EC prior to implantation have shown that fully functional endothelium prevents chromogenic complications, and improves long-term patency. In vitro endothelialisation procedures are labour intensive and making the process costly and limited to specialty centres. Hence, materials that promote in situ endothelialisation of cardiovascular implants (without IH or thrombus formation during endothelium development) would be highly desirable. In vivo circulating ECs, endothelial progenitor stem cells (EPCs), and the less well-defined $CD133^+$, $CD34^+$, and $CD45^+$ progenitor cells could potentially be recruited on to bypass graft materials to enhance in situ endothelialisation [2–4].

A family of nanocomposite polymers based on polyhedral oligomeric silsesquioxane (POSS) nanoparticles incorporated in to poly (carbonate-urea) urethane (PCU) has been synthesized for use in a wide range of surgical implants, and was developed and patented by the University College London (POSS-PCU) [5]. The introduction of unique nanoscale POSS moieties incorporated in to PCU acts as a cross-linking agent, which tailors the physicochemical properties of this nanocomposite polymer when compared with their conventional constituents [6–9]. Since 2006, POSS-PCU has been developed for many important biological applications such as word's first synthetic trachea [10], lacrimal duct conduits [11], and lower limb bypass grafts all of which have been used first-in-man, and about to enter clinical trials [12]. Their anti-thrombogenic and viscoelastic properties similar to the native vessels make POSS-PCU an ideal candidate for blood-contacting device applications. Although, POSS-PCU can encourage cell attachment due to surface chemistry and nanotopography, the materials are hydrophobicity and chemically inert, and lack cell-recognition moieties, which are a limiting factor to achieve a fully confluent layer of ECs in earliest possible time frame (<6 weeks). In most cases, it is impossible to find a single material that meets all the demands required to maintain graft patency, whilst preventing platelet adhesion, and thrombosis whilst enhancing endothelialisation and a common strategy to improve the surface properties of biomaterials is to modify the surface or interfacial properties using different strategies through physical or chemical modification. Plasma-based approaches have gained considerable interest and popularity [13–17]. In fact, the attractive feature of the plasma surface modification process is that the extent of modification can be precisely controlled by the proper selection of involved parameters [18]. Surface modification of POSS-PCU has been achieved using UV light treatment [19], oxygen (O_2) plasma treatment [17], physical adsorption of collagen type I on plasma treated POSS-PCU [13], immobilisation of the RGD binding sequence from fibronectin [20], and nitric oxide (NO) delivery [21]. Collagen type I is one of the major common proteins involved in the extracellular matrix (ECM) responsible for cell maintenance and binding to the surface. Many studies have shown that the incorporation of collagen in to synthetic polymer can enhance cell adhesion, proliferation of ECs, and differentiation of stem cells such as EPCs and MSCs [22].

In earlier attempts on improving blood compatibility of POSS-PCU, collagen type I was directly reacted with plasma activated POSS-PCU without using low molecular weight reagents such as acrylic acid (AA). Although, POSS-PCU with an adsorbed layer of collagen type I showed more cell attachment, proliferation and growth of ECs, the stability of collagen layer on the surface was not enough, and the majority of it was washed away during rinsing and sterilization processes prior to cell culture [13]. In the present investigation, biomimetic surface modification of POSS-PCU (e.g. collagen type I grafted on to POSS-PCU) was performed by means of a fast and easy three-step reaction procedure by, 1) creation of −COOH functionality, 2) grafting coupling agents on to the surface, and 3) immobilisation of collagen. The efficiency of this strategy for biomimetic modification of POSS-PCU surfaces was demonstrated by measuring cell adhesion and behaviour of ECs. However, it is well known that collagen is pro-thrombogenic [23]. Therefore, improved endothelialisation without causing severe thrombosis due to the presence of collagen is considered in the present study to enhance the long-term patency of POSS-PCU as synthetic vascular grafts. To reach this goal, we modified POSS-PCU surfaces with three different amounts of collagen type I termed low, medium (med) and high in order to accelerate in situ endothelialisation whilst retaining their anti-thrombogenic surface properties before reaching a confluent layer of ECs.

2. Materials and methods

2.1. Preparation of POSS-PCU thin films

The synthesis of POSS-PCU has been described elsewhere in detail [24]. Briefly, a mixture of polycarbonate polyol and *trans*-cyclohexanediol isobutyl-silsesquioxane (POSS, Hybrid Plastics, USA) was placed in a reaction vessel equipped with a stirrer and purged with nitrogen gas and heated to 125 °C to dissolve POSS in polycarbonate polyol. Then, 4, 4′-methylenebis(phenyl isocyanate) (MDI) was added to the cooled mixture and reacted at 75 °C for 90 min to form a pre-polymer. The prepared pre-polymer was subsequently dissolved by the addition of *N*, *N*-dimethylacetamide (DMAC). Chain extension was carried out by drop-wise adding of a mixture of ethylenediamine and diethylamine (in DMAC) to form a final polymer solution of POSS-modified poly(carbonate-urea)urethane (in DMAc). Then, 4 g of 1-butanol in DMAC was added to form a 2% (w/w) POSS-PCU solution. All chemicals and reagents were purchased from Sigma Aldrich Ltd, U.K. Glass Petri dishes (40 mm diameter) were coated with 4 ml of POSS-PCU (15% w/w in DMAC) and cured overnight (18 h) at 55 °C. The thin films were rinsed with sterile 20 mM phosphate buffered saline (PBS), immersed in 70% (v/v) ethanol and placed in a vacuum oven to dry at 25 °C. Each film was the cut in to rectangular shaped samples ($10 \times 40 \times 0.015$ mm^2) with a specific surface area (SSA) of 801.5 mm^2 (~8.15 cm^2) and used for experimentation. In all subsequent reaction steps, the polymer will be referred to as nanocomposite.

2.2. Surface functionalization

The technique used in this study is based on grafting of poly acrylic acid (Poly-AA) to the substrate surface through a plasma-induced graft polymerisation process, followed by coupling of collagen type I layer on to the carboxyl acid (–COOH) functional groups of acrylic acid