A Novel Nitric Oxide-eluting Nanocomposite Polymer for Cardiovascular Applications

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Statement of Purpose: Cardiovascular disease is responsible for a majority of deaths worldwide, and thus hightening the critical need for next generation surgical implants, e.g. bypass grafts. In the absence of a fully functional endothelial layer, graft failure occurs due to excessive thrombosis and intimal hyperplasia (IH).[1] Therefore, the development of nitric oxide (NO) releasing biomaterials is of great interest within the physiological concentration range. We have developed a novel nanocomposite polymer based on reacting polyhedral oligomeric silsesquioxane nanocages (1-5nm) with poly(carbonate-urea)urethane (POSS-PCU). The nanocomposite shows enhanced mechanical and haemocompatible properites ideal for cardiovascular applications,[2] and has already been implanted first-in-man as lower limb bypass grafts, tracheal replacement and lacrimal duct conduit. Due to the time interval between graft implantation and endothelialization (6-8 wks), anti-thrombogenic surface properties would be desirable through NO generation and release from bypass grafts to prevent thrombosis. *S*-nitrosothiols are known as preferred NO donors for incorporation in to polymeric implants. The aim of this study was to 1) develop NO-eluting nanocomposite biomaterials using fumed silica nanoparticles (FSNP) conjugated with *S*-nitrosothiols, 2) measure *in situ* NO release profiles under physiological conditions, and 3) study whole blood kinetics to evaluate haemocompatibility.

Methods: 1% (w/w) FS (12nm) (Aerosil 200, Degussa) was amine functionalized by refluxing FS with (3-aminopropyl)trimethoxysilane in iso-propanol and distilled water. Acid titration and Kaiser tests were performed to confirm the functionalization. *N*-acetyl-d-penicillamine (NAP), as a precursor to *S*-nitrosothiol, was attached to amine functionalized FSNPs using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*´,*N*´-tetramethyluronium hexafluorophosphate (HBTU) as a coupling reagent.[3] Ellman's assay and ATR-FTIR were used to confirm the attachment of NAP to FSNPs. The product was converted to *S*-nitrosothiol by reacting the compound with acidified sodium nitrite solution to give a terminal *S*-nitroso-*N*-acetyl-d-penicillamine-FS (SNAP-FS). The synthesis of POSS-PCU has been described elsewhere in detail.[2] SNAP-FS particles were dispersed in to POSS-PCU at 1, 2, and 3 wt%. Small diameter (5mm) grafts were fabricated using polymer extrusion phase inversion technique.[2] Bypass grafts were connected to a pulsatile flow circuit; the NO detection probe (ISO-NOP-2mm, WPI Company) was placed inside the graft lumen, and NO release was measured using the amperometric method. Both POSS-PCU and POSS-PCU-NO grafts were placed in a tube containing 20 mM PBS solution and incubated for 30 min at 37°C and 5% CO_2. The graft lumen was exposed to 5ml citrated whole blood, collected from healthy volunteers. 340µl of blood was collected from grafts, and fresh blood was used as a simple model control. Blood samples were placed in to the thrombelastograph® haemostasis analyzer (TEG® 5000, Medicell Ltd, UK) cups, and 20µl of 0.2M $CaCl_2$ was used to initiate blood coagulation, and evaluate haemocompatibility.

Results: The results confirmed the attachment of SNAP to FSNPs. NO release from modified bypass grafts (POSS-PCU-NO) demonstrates initial burst kinetics followed by a steady release of NO with time. It was found that the release profiles could be controlled by changing the amount of FSNPs in to the polymer matrix. The most steady NO release profile was evident with 1 wt% at physiologically relevant concentrations of ~ 8×10^{-10} mol $cm^{-2}min^{-1}$ over a 7 h period (Fig.1). Figure 2 shows enhanced anti-thrombogenic properties of modified grafts compared to POSS-PCU controls. There was a slight delay in the rate of clot formation (R value), and a 20% decrease in overall clot strength was apparent (MA value).

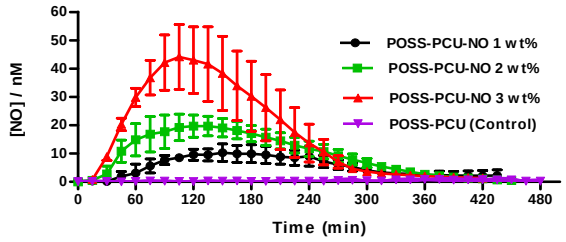

Fig 1. NO release profile from the bypass grafts (n=4).

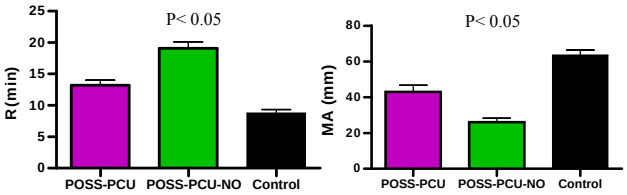

Fig 2. TEG analysis (POSS-PCU-NO Vs POSS-PCU and fresh blood) (n=6).

Conclusions: The nanocomposite polymer demonstrates anti-thrombogenic properties through NO elution within the physiological range to delay thrombosis and reduce clot formation. Such nanocomposites can be used for next generation cardiovascular implants such as bypass grafts, stent coatings, and heart valves where blood coagulation needs to be controlled.

References:

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