Long term patency and transmural endothelialisation of small caliber microporous compliant vascular bypass grafts manufactured from poly(carbonate-urea) urethane incorporating polyhedral oligomeric silsesquioxane pendant nanocage within its hard segment in an ovine model

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Statement of Purpose: Poly(carbonate-urea) urethane has been synthesized with polyhedral oligomeric silsesquioxane pendent nanocages covalently bonded to the hard segment, in so doing removing the susceptibility to hydrolytic degradation of the simple polyurethane (Kannan RY. Biomaterials. 2006;27:1971-9) and also affording intrinsic anti-thrombogenicity (Kannan RY. Biomacromolecules. 2006;7:215-23). We aim to demonstrate that phase inversion of the nanocomposite in solution can produce a reproducible, arterial compliance-matched, microporous vascular prosthesis with adequate strength and durability to withstand long term physiological pulsatile flow, and that the desirable traits of the nanocomposite previously shown in vitro can translate to long term patency in a highly thrombogenic animal model.

Methods: A novel automated vertical extrusion-phase inversion device was used to coat 5mm diameter stainless steel mandrels with the nanocomposite dissolved in N,N dimethylacetamide and lower it at 10mm/sec through a 6mm aperture into deionised water at 0°C, where phase inversion took place. The conduit slid off the mandrel after degassing with absolute ethanol. Scanning electron microscopy (SEM) was used to examine the porous structure. Wall uniformity was assessed using image analysis software. Conduits were placed in a flow circuit containing human whole blood using a positive piston actuator as a pulsatile source (pulse pressure 40mmHg) and an ultrasound probe with wall tracking software to map distension and hence compliance. Burst pressure was measured by inflating conduits lined with non-porous latex sleeves with water at 0.2ml/min until failure. Eight such grafts were implanted using end to end anastomoses under 5% longitudinal stretch replacing 50mm resected lengths of right common carotid artery in 8 sheep. 150mg aspirin was administered daily after implantation. The grafts were explanted after 17 to 22 months or when the sheep died and were examined visually and histologically for patency and fibrous capsule. Staining for CD31 identified vascular endothelium. Compliance and burst pressure testing of the explanted grafts was undertaken with and without the fibrous capsule to demonstrate any change of mechanical properties.

Results: In the in-vitro studies, 5 consecutively produced conduits had a mean wall thickness of 0.506mm (SD 0.017mm). SEM revealed a four layered structure comprising from lumen outwards: a luminal skin with small pores(<20μm); densely packed large interconnecting pores(300μm) forming most of the cross section; dense small pores (25μm) near the outer edge; a very low porosity outer skin with microscopic ridges throughout. Compliance was 5%/mmHg x 10^{-2} for mean pressures between 20 and 100mmHg, which was similar to that reported for external iliac artery in the physiological range. Burst pressure testing without a latex lining sleeve resulted in transmural leakage, indicating the expansion of pores on the grafts' outer surface due to inflation. Burst pressure was 357 mmHg (SD 15mmHg). Of the implanted grafts, 2 animals died with fully patent grafts at 29 and 91 days due to unknown reasons and chest infection respectively. The remaining grafts were harvested from healthy animals at 523 – 662 days. One was occluded and another partially occluded. 4 were fully patent. Histological analysis suggested a thin neointima with a confluent endothelial cell lining, as well as neocapillary infiltration through the graft wall. CD31 immunostaining preferentially marked the lumen lining as well as the periphery cells of the supposed neocapillaries. A thick fibrous capsule surrounded each graft, reducing its compliance to 1.8%/mmHg x 10^{-2} . Graft compliance after removal of the capsule was the same as pre-implantation values. Burst pressure with fibrous capsule was 874mmHg. After capsule removal this fell to pre-implantation values.

Conclusions: The novel vertical extrusion/phase inversion method allows production of replicable microporous nanocomposite small caliber bypass prostheses, which are compliance-matched for human artery in the physiological range. Burst pressures are comfortably beyond the physiological range. The nanocomposite has shown no loss of mechanical integrity as a result of biodegradation. Implantation results in graft healing with fibrous capsule formation, restricting compliance greatly. The four lamina differential porosity graft wall allows transmural lumen endothelialisation, despite the minimum unstretched graft pore size being less than that thought optimum for endothelialisation. This has been achieved in an animal model known for its high thrombogenicity and, in common with humans, a reluctance to endothelialise. This is the first report of confluent transmural endothelialisation of a synthetic graft in sheep. In the possible absence of precise compliance matching due to fibrous capsule formation, the long term patency may be due to the anti-thrombogenicity of the nanocomposite initially, allowing time for subsequent confluent endothelialisation. This supports the in vitro anti-thrombogenicity results.