Study about a Progenitor Cell Capturing Polyester Vascular Prosthesis

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Statement of Purpose: The inability to endothelialise implanted vascular prosthesis in human subjects remains the principal obstacle in the development of small diameter vascular prosthesis. Taking advantage of circulating endothelial progenitor cells (EPCs), we have designed, fabricated and tested a progenitor cell capturing vascular prosthesis. The objective is to accelerate in situ endothelialisation on commercial vascular prosthesis made of polyethylene terephthalate (PET). Functional polyurethanes (PUs) were synthesized at Sichuan University and tested for their reactivity with proteins. Polyester vascular prosthesis was treated in the lumen with the functional PU and subsequently reacted with antibodies that recognize EPC surface markers. Following in vitro characterizations, the bio-activated prostheses were implanted as thoracoabdominal bypass in canines. This work therefore demonstrates for the first time the potential of capturing circulating progenitor cells on commercial polyester vascular prosthesis.

Methods: Functional polycarbonate based PUs were synthesized using 4, 4'-methylene-bisphenyl diisocyanate (MDI) as hard segment and lysine as chain extender, as described previously (1). The polyethylene glycol (PEG) side chains on the PU were capped with either primary amine or epoxy groups. Solution of the PU was applied uniformly to the lumen of a low water permeability woven PET vascular prosthesis (Vascutek, UK), forming a thin layer on the PET microfibers without blocking the porous space between the microfibers. The uniformity of PU coating was verified with fluorescent dye and with scanning electron microscope (SEM). The reactivity of the functional PU towards proteins was analyzed using bovine albumin tagged with TRITC. CD34 and CD133 antibodies were grafted onto the lumen of the PU treated vascular prostheses for animal implantation. For animal experiment, 15 dogs of 25 kg or above were implanted with the antibody grafted prostheses and control prostheses using a thoracoabdominal model. The implants were harvested at 4h, 2 days, 10 days, 1 month and 3 months, followed by various analyses.

Results: Fluorescent dye showed a uniform distribution of PU on the lumen of the PET prosthesis. SEM revealed no accumulation of PU between the PET microfibers, meaning no significant effect on the water permeability of the prosthesis, as showed in Figure 1. The amount of surface exposed amine groups were quantified by reacting with 2-methylbuthylamine and reading the emission intensity at 460 nm. The immobilization of antibody was confirmed by reacting with CD34-FITC antibody. About 25 cm long PET prosthesis was divided into six segments composed by 4 experimental segments in the middle and 2 isolative segments at both ends. Among the 4 experimental segments were the ones immobilized with CD34, CD133, PU alone, and without any treatment (original PET). The 2 isolative segments were designed to prevent the migration of endothelial cells from both anastomoses. In this way any endothelial cells in the middle must be from circulation. This segmental design also eliminated the effect of individual difference among animals. The percentage of the lumen with thrombosis was calculated as illustrated in Figure 2. H&E stain and immunological stains for endothelium, smooth muscle cells and inflammatory cells were performed.

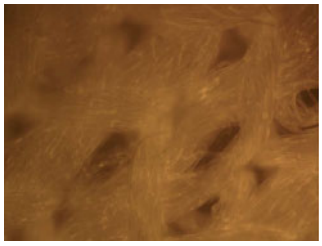

Figure 1. PU with fluorescent dye coated on the lumen of PET vascular prosthesis, showing the uniform and thin coating.

Figure 2. Thrombogenic surface was selected and quantified as percentage of the total surface area.

The two types of functional PUs were found easily dissolved and applied to PET surface, forming a thin layer strongly adhered to PET microfibers. The functional groups were found exposed at PU surface and readily react with proteins. Antibodies were immobilized to the lumen through these functional groups and remained active. In vivo analysis showed that both CD34 and CD133 antibodies recorded reduced thrombosis, particularly at 2 days. However, at 1 and 3 months, the difference in thrombogenic surface area between the antibody immobilized segments and controls was not significant, which warrants further investigation.

Conclusions: PET vascular prosthesis can be readily functionalized with the functional PUs and become reactive to proteins. The immobilization of CD34 and CD133 proved effective in reducing thrombosis, which was likely through capturing circulating cells.

References: 1. Xu Y et al. Polymer 2013; 54: 5363-5373.

Acknowledgement: This project was supported by the Quebec-China Collaborative Program of the Ministère du Développement économique, de l'innovation et de l'exportation du Québec, Canada. The polyester prostheses were generously donated by the Vascutek, a Terumo Company.