A Novel Endothelial Progenitor Cell Capturing Vascular Prosthesis

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Instruction: While endovascular surgery is well-developed, bypass is still one of indispensable therapies in cardiovascular surgery, in which either auto-grafts or prosthetic vascular grafts has to be used. In the entire history of the development of prosthetic vascular grafts, endothelialization remains the most challenging obstacle and has been extensively researched over the past 50 years, especially for the prosthesis less than 5 mm in diameter. Because mature endothelial cells in adult patients have very limited capacity to proliferate onto the implanted synthetic vascular prostheses, a new strategy is to capture circulating endothelial progenitor cells (EPCs). In this work we synthesized two types of reactive polyurethanes and sprayed them to the inner surface of clinically used polyester vascular prosthesis. These vascular prostheses were bio-activated by reacting with heparin or antibody CD34.

Materials and Methods: Two types of segmented poly(carbonate urethane) with functional poly(ethylene glycol) side chains were synthesized at Sichuan University, China. They are labeled as PU-PEG-NH2 and PU-PEG-EPO according to their reactive group –NH2 and EPO (epoxy). One point three percent (1.3%) of PU-PEG-NH2 or PU-PEG-EPO was dissolved in DMF and then sprayed on the inside surface of the polyester vascular prostheses of 8 mm in diameter (Vascutek, twill woven). After being dried in vacuum oven overnight, the prostheses were cut into specimens of 1x1 cm^2 in size. The specimens were reacted with anti-CD34 FITC (555821, BD Pharmingen™,) and heparin-rhodamine (HP-204, Creativepegworks). After thorough wash, the specimens were observed under a fluorescent microscope. Effort was also made to quantify the polyurethane surface reactive primary amine groups. To do so PU-PEG-NH2 in DMF was reacted with fluorescamine for 10 minutes and with its fluoresce intensity measured by a spectrofluorophotomerter (Bio-Tek FL600) with excitation at 380 nm and emission at 460 nm. Quantification was made by comparing the measured fluoresce intensity with a standard curve obtained by reacting different aliquot solutions of 2-methylbuthylamine (220523, Sigma) in PBS with fluorescamine. The surface chemistry of the polyurethane sprayed prosthesis was measured using an X-ray photoelectron spectroscope (XPS). The surface morphology of the prosthesis was observed with a scanning electron microscope (SEM) and a light microscope.

Results: Polyurethane spray coated on the prostheses surface appeared thin and fairly uniform, with occasional clusters. The water permeability of the polyurethane coated prosthesis was similar to that of the non-coated controls, meaning that polyurethane did

not block the porous structures (Figure 1). The prostheses coated with the reactive polyurethanes showed strong fluorescence intensity after reacting with the FITC conjugated CD34 or rhodamine conjugated heparin, while the fluorescence intensity on the no-coated prostheses was very weak after rinse, indicating that CD34-FITC and heparin-rhodamine cannot be immobilized on the prosthesis surface without being primed with the reactive polyurethanes. Based on the mechanism that fluorescamine reacts with primary amine to form fluorescent pyrrolinone moieties, a linear relationship between primary amine concentration and fluorescence intensity was established, which was used to quantify the surface reactive groups of the polyurethanes.

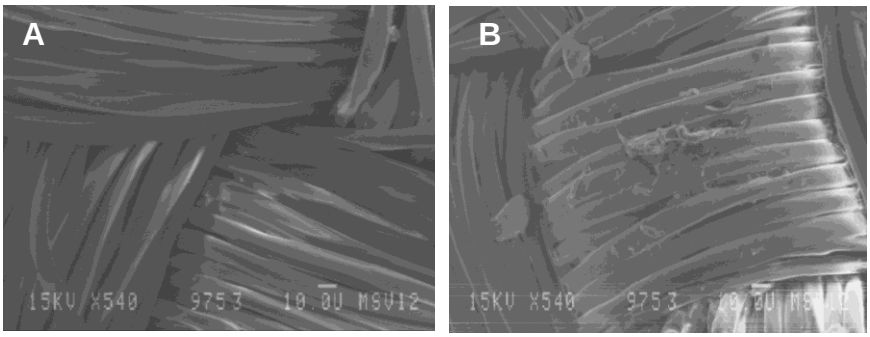

Figure 1. Polyester vascular prosthesis (A) spray coated with reactive polyurethane (B).

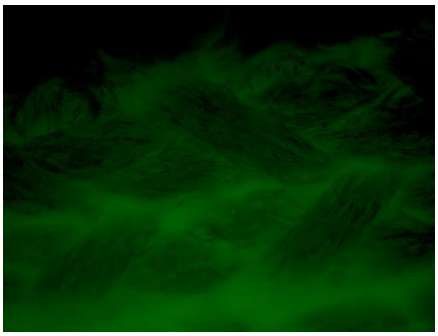

Figure 2. PU-PEG-NH2 coated polyester vascular prosthesis reacted with albumin-FITC

Discussion: The newly synthesized polyurethanes were reactive enough to immobilize proteins or heparin either through covalent bonds or electrostatic interactions. Such immobilization was stable after repeated washes. Their ability to immobilize CD34 and heparin warrants further in vivo assay to test their ability to capture circulating EPCs. The spray technique developed in this work proved effective in applying thin layer of chemicals to vascular prosthesis surface without changing the original water permeability and surface morphology of the prosthesis.

References:

1. J. Ben-Shoshan et al., Pharmacol Ther. 2007; 115:25-36.
2. J. Aoki et al., J Am Coll Cardiol 2005;45:1574 –9.