Design, Preparation and in vitro Assay of a Novel Endothelial Progenitor Cell Capturing Vascular Prosthesis

Bin Li[1], Ze Zhang[1], Xingyi Xie[2], Robert Guidoin[1], Yvan Douville[1], Yingping Zhong[2], Qian Fu[2]
[1]Centre de recherché du CHU de Québec, Département de chirurgie, Faculté de médecine, Université laval, Québec (QC), Canada; [2]Department of Polymeric Biomaterials and Artificial Organs, College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu, Sichuan, China

Statement of Purpose: Although endovascular surgery is well-developed, bypass is still an indispensable therapy for cardiovascular surgery, in which autograft or prosthetic vascular graft has to be used. In case where there is no autograft available and so prosthetic graft is needed, the lack of endothelialization becomes the most challenging issue that prevents small diameter prosthetic grafts from clinic use. This is because mature endothelial cells in adult patients seemingly have very limited capacity to proliferate onto implanted prosthetic vascular grafts. In recent years, studies about endothelial progenitor cells (EPCs) revealed their capacity to differentiate into highly proliferative endothelial cells, as well as the very limited number of EPCs in circulation. Nevertheless, there are literatures showing the successful examples of EPC capturing heart valve and coronary stent. In this work, we have designed an EPC capturing vascular graft based on conventional polyester vascular graft and novel functional polyurethanes (PUs). This bioactive vascular graft is expected to immobilize circulating EPCs and hopefully to enhance vascular prosthesis endothelialization.

Methods: Two novel PUs were synthesize and presented elsewhere. In brief, these are polycarbonate urethanes with PEG side chains. At the end of the PEG side chains there are either primary amine (PU-PEG-NH_2) or epoxide (PU-PEG-EPO) groups. The primary amine groups can immobilize negatively charged proteins through electrostatic interactions. The epoxide groups on the other hand are readily react with the primary amine groups in proteins. The PU was evenly coated to the lumen of polyester (Dacron) vascular prosthesis (Vascutek, UK) without blocking the space between microfibres. In this way the water permeability of the PU-coated grafts was not significantly affected. To identify the presence of NH_2 groups on PU-coated grafts, different aliquot solutions of 2-methylbuthylamine (220523, Sigma) in PBS and 0.13% PU-GEP-NH2 in DMF reacted respectively with fluorescamine (3mg/ml in 1,4-dioxane) for 10 minutes. The fluorescence intensity was measured by a spectrofluorophotometer (Bio-Tek FL600) at an excitation wavelength of 380 nm and an emission wavelength of 460 nm. A calibration curve was constructed using the fluorescence intensity of known concentration of 2-methylbuthylamine. The concentration of NH_2 groups in the solution could be calculated.
The solution of PU-PEG-NH2 or PU-PEG-EPO in DMF was evenly applied to the lumen of the prosthetic graft. After being dried in vacuum overnight, the grafts were cut into specimens of 1 cm x 1 cm in size. The PU-coated specimens were reacted with albumin-FITC and heparin to investigate their reactivity with proteins and negatively charged molecules. Furthermore, the specimens were reacted with anti-CD34 FITC (555821, BD Pharmingen™,) and then with heparin-rhodamine (HP-204, Creativepegworks). The grafts were observed under light microscope, scanning electron microscope, and fluorescent microscope.

Results: PU-coated graft showed similar surface morphology as the non-coated graft, meaning thin coating on microfibre surface only (Fig. 1). Based on the mechanism which fluorescamine reacts with primary amine to form fluorescent pyrrolinone moieties, we established a linearity relationship between the concentration of 2-methylbuthylamine and fluorescence intensity (calibration curve), which was used to quantify the primary amine groups on PU-coated graft. Model protein albumin readily reacted with the graft coated with PU-PEG-NH2, as showed in Figure 2, showing strong and uniform absorption of albumin.
The fluorescence intensity of no-coated graft was very weak in comparison with that of the PU-NH2 and PU-EPO coated grafts after being rinsed. This implicated that CD34-FITC and heparin-rhodamine cannot be immobilized on grafts without PU or can be easily be rinsed away.
The grafts, coated with PU-NH2 or PU-EPO, has strong fluorescence intensity using FITC and rhodamine, which indicated that CD34 antibody and heparin can be immobilized on the PU-NH2 and PU-EPO coated graft at the same time. Under fluorescence microscopy, we could not assess the impact of pH on immobilization of CD34 antibody and heparin, nor the efficiency of sequence of CD34-FITC and heparin-rhodamine coating.

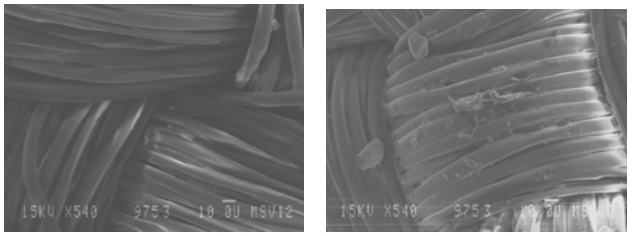

Figure 1. Non-coated (left) and PU-coated vascular graft.

Conclusions: A novel bioactive vascular graft was developed with the capacity to immobilize EPC antibody such as CD34 and heparin at same time.

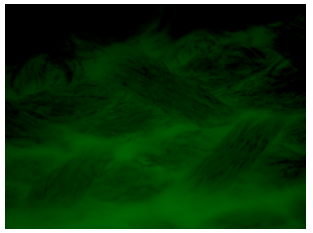

Figure 2. Albumin coated graft

References:

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