Fabrication of Permeable Tubular Structure from Chemically Modified Chitosan with Anticoagulant Activities for Small Blood Vessel Engineering

Yongzhi Qiu [1], Ning Zhang [1,2], Qian Kang [3], Yuehuei An [3], Xuejun Wen [1, 2]

[1] Clemson-MUSC Bioengineering program; Department of Bioengineering, Clemson University, Charleston, SC, 29425,
[2] Department of Cell Biology & Anatomy, Medical university of South Carolina, Charleston, SC, 29425
[3] Department of Orthopedic Surgery, Medical University of South Carolina, Charleston, SC, 29425

Statement of Purpose: An increasing number of incidences of cardiovascular diseases have warranted a compelling need for vascular grafts. While success has been achieved with medium- and large-diameter artificial vascular grafts, small-diameter artificial grafts with inner diameter less than 5~6 mm are not available for clinical uses. These grafts are critical in coronary artery bypass graft (CABG) surgery, lower extremity bypasses, and tissue transfer [1]. The challenges posed by small-diameter vascular engineering have been largely due to the lack of biomaterials with ideal hemocompatibility. As a result, upon contacting with blood, engineered small-diameter blood vessels oftentimes undergo rapid occlusion associated with thrombus and plaque formation, and unfavorable healing at the graft and native vessel interface that lead to problems like incomplete endothelialization and intimal hyperplasia.

In the search for optimal biomaterials for small-diameter vascular engineering, natural biopolymers have gained extensive attentions. In particular, chitosan, a polysaccharide has a molecular structure similar to that of heparin, which may suggest its excellent antithrombogenic potential [2]. However, raw chitosan is highly thrombogenic and has a poor processibility/solubility perhaps due to the existence of amino group in the side chain, therefore, requires modifications before it can be fashioned into tubular structures for small-diameter vascular replacement. To this end, the amino group of chitosan was reacted by the formation of a polar amide bond.

Methods: Chitosan was heated in 50% NaOH aqueous solution under nitrogen flux for 8 hrs, then washed with DI water until pH value turned back to 7 and dried in vacuum. A mixture of chitosan and phthalic anhydride in extra dry DMF was heated with stirring at 130 oC under a nitrogen atmosphere for 12 hours. Products were purified by Soxhlet's extraction with ethanol and dried in vacuum to yield phthalized chitosan. Modified chitosan was characterized with regard to solubility in a wide range of solvents, molecular weight, contact angle, XPS, FTIR, biocompatibility, hemocompatibility, and endothelial growth. Permeable tubular structure was then fabricated using a phase-inversion method.

Results / Discussion: Molecular weight of phthalized chitosan range from 300 KDa to 700 KDa. It has a contact angle of 52 ± 3^{o}, which is significantly less than raw chitosan (92 ± 5^{o}). Fibroblasts on phthalized-chitosan are more spread out than on raw chitosan film. Cells on phthalized-chitosan attached, extended and proliferated very well. Quantitative proliferation assay showed that the fibroblasts proliferation on raw chitosan, modified chitosan and PLGA are in the same range. Live/dead cell assay indicated that both of materials are cyto-compatible. The number of adhered platelet on modified chitosan is significantly fewer than that on raw chitosan ($P<0.0001$, Fig. 1). Moreover, from high magnification (5,000X), morphological appearance of platelet on raw chitosan surface can be classified as spread dendritic or intermediate pseudopodial (Stage III). Platelets on phthalized-chitosan are less reactive and can be classified as dendritic or early pseudopodial (Stage II). Phthalized-chitosan was shown to promote human umbilical vein endothelial cells adhesion and proliferation. Phthalized-chitosan can be dissolved in many solvents, such as DMAC, DMF, DMSO, etc, but not in water. And the solution possesses certain viscidity. These properties allow the further fabrication of different types of permeable scaffolds for guided tissue regeneration. Particular, in this work, this material allows for the fabrication of permeable tubular scaffolds (Fig. 2) ranging from several hundred microns to several millimeters in outer diameter for small-diameter vascular grafts. In addition, the permeability can be well-controlled ranging from non-permeable, permeable to small molecular nutrients, to even permeable to cells.

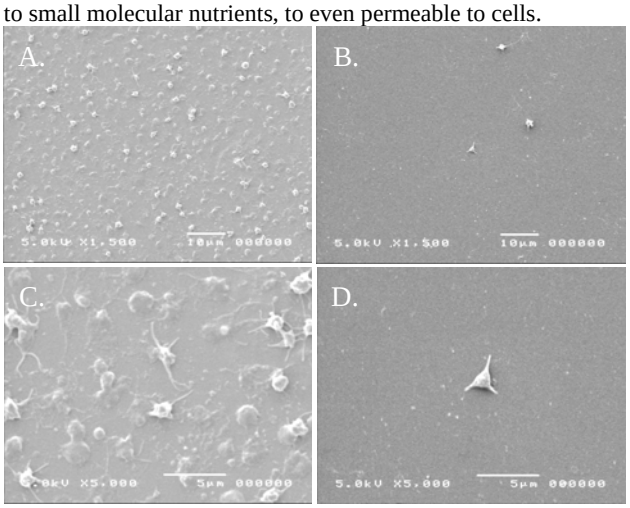

Fig. 1: SEM pictures of the platelet attachment on 2 hr attachment time. Raw chitosan (**A & C**), Phth-chitosan (**B&D**).

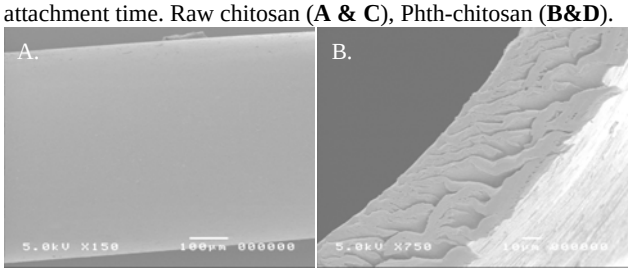

Fig. 2: SEM pictures of the modified chitosan A: the surface of hollow fiber membrane (X150) B: the cross-section of the hollow fiber membrane (X750)

Conclusions: Compared to the raw chitosan, phthalized chitosan exhibited good hemocompatibility (inhibiting fibrinogen adsorption and platelet adhesion), promoted endothelial cell growth and attachment, enhanced solubility, and processibility. Through a wet phase-inversion process, selective permeable tubular constructs of varying sizes, morphology, and properties were fabricated from phthalized chitosan, suggesting its potential as a novel biomaterial for small-diameter vascular engineering. The further research on the smooth muscle cells on the fabricated HFMs is in progress.

References

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