Surface phosphorylation for polyelectrolyte complex of chitosan and its sulfonated derivative: surface analysis, blood compatibility and adipose derived stem cell contact properties

Hsi-Yi Yeh, Jui-Che Lin*
Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan, R.O.C.

Statement of Purpose: Many researches tried to look for the application of chitosan in tissue engineering. Previous studies had indicated that the incorporation of sulfonic or phosphonic functionalities would be beneficial to the growth of certain cells. However, no study has explored the effect of incorporation of both abovementioned anionic functionalities onto the chitosan structure. In this study, we have surface-phosphorylated the polyelectrolyte membrane formed by chitosan and water soluble sulfonated chitosan with an aim to incorporate phosphonic and sulfonic functionalities onto the membrane surface. This phosphorylated polyelectrolyte membrane had been evaluated by various surface characterization techniques. In addition, the blood compatibility and stem cell proliferation had been analyzed by *in vitro* platelet adhesion and adipose-derived stem (ADS) cell incubation study.

Methods: 1.5 wt% of water soluble sulfonated chitosan solution was prepared, and then equal volume of 1.5 wt% of chitosan/HCl (pH3) solution was added and mixed well by mechanical blender. After ultrasonication for 30 min, the mixture was added into plastic dishes (40ml/dish), and then dried in oven at 40°C. The formed polyelectrolyte complex (PEC) membrane was cut into proper size for the following experiments. PEC or chitosan membranes were immersed in 200ml tetrahydrofuran (THF) within a four-neck reactor. Followed the addition of $POCl_3$ and triethylamine (TEM) ($POCl_3$/TEM=1/3), the membranes were reacted for 3hrs under ice-bath. Then, 200ml of 0.1M NaOH(aq) was further added, and the reaction was carried on for another 3hrs under ice-bath. As reaction completed, the modified film was washed by 75% ethanol.

Results: For plasma recalcification time (PRT) evaluation (Fig 1), phosphorylated polyelectrolyte complexes (specimens: PECp-0.25%, PECp-0.5%, PECp-1%, and PECp-2.5%) extended the plasma recalcification time as compared with the non-treated chitosan control and direct-phosphorylated chitosan (specimens: C and Cp-2.5%).

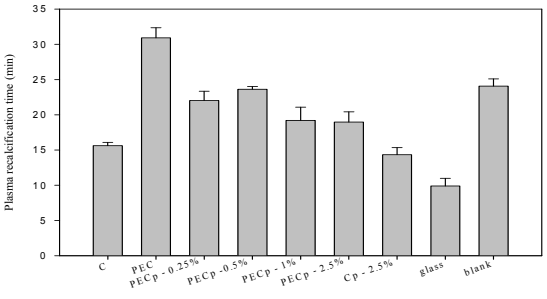

Figure 1 Plasma recalcification time (PRT) of modified chitosan membranes in Falcon™ tube at 37°C

Although, these phosphorylated polyelectrolyte complexes might induce platelet adhesion, but could still keep the platelet slightly activated and non-coagulated (Fig 2). In contrast, significant platelet activation and adhesion were noted on the direct-phosphorylated chitosan.

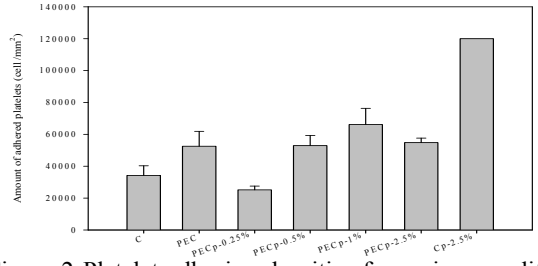

Figure 2 Platelet adhesion densities for various modified chitosan specimens.

In addition, adipose-derived stem cell incubation study has demonstrated, by alamar blue method, that the incorporation of both phosphonic and sulfonic acid-related functionalities onto the chitosan surface could enhance the stem cell proliferation (Fig 3).

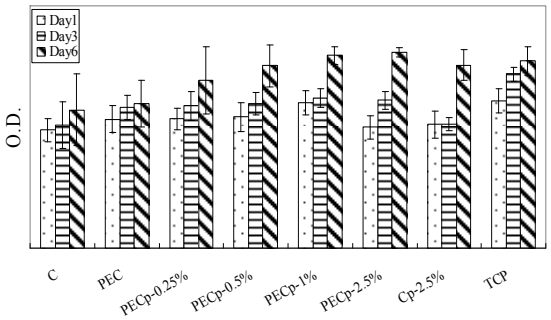

Figure 3 Proliferation of ADS cell evaluated by alamar blue assay.

Conclusions: Experiments of platelet adhesion study and plasma recalcification time showed that the phosphorylated chitosan as well as unmodified chitosan exhibited the hemostatic characteristics. In contrast, the PEC membrane displayed well anticoagulation feature, and the phosphorylated PEC membranes still retained the fair blood compatibility. Furthermore, the ADS cell incubation experiment showed that the phosphorylated PEC membranes could improve ADS cell adhesion and proliferation. From our study, we observed that the sulfonic groups could inhibit the blood coagulation, and the phosphonic groups might improve stem cell adhesion and proliferation. Furthermore, the phosphorylated PEC membrane, containing both sulfonic and phosphonic functional groups, displayed not only well blood compatibility but also stem cell compatibility. Therefore, the phosphorylated PEC membrane could be a novel and useful biomaterial, and worthful for further studies for the

applications in biomaterials and tissue engineering area.