Physical Adsorption of Human Thrombomodulin onto Biomaterals for Developing Blood Compatibible Materials

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Statement of Purpose: Polymeric materials have been used as blood-contacting biomaterials such as artificial vessels and catheters, because of their suitable mechanical and chemical properties. However, the serious problem of blood-contacting materials is the lack of blood compatibility, especially antithrombogenicity. Many methodologies for preparing antithrombogenic materials have been performed[1)]. One of the most practical methods is the immobilization of antithrombogenic active substances, such as heparin, urokinase and prostaglandin derivatives[2)]. We reported the immobilization of human thrombomodulin (hTM) is an endothelial cell-associated protein having a potent natural anticoagulant activity (Figure 1a)[3)]. However, the chemical immobilization of bioactive proteins has a risk of denaturation and remaining chemical reagents. We focused on physical adsorption of bioactive proteins onto polymeric biomaterials surface because physical adsorption can prevent those problems. Although the amount of adsorbed bioactive proteins is about nano- to micro-gram, these amounts of highly active proteins will provide enough antithrombogenicity.

In this study, we report the physical adsorption of hTM onto polysulfon (PSF) films, and its biochemical and physicochemical activities for blood compatible materials.

Methods:<u>Adsorption of hTM onto PSF films.</u>
PSF film on QCM was prepared by spin-casting at 1500 rpm for 60 s with 1 wt% PSF chloroform solution. They were immersed in various concentration of 10 mM Tris-HCl buffer (pH=7.4) at 37 °C. After a predetermind time, they were rinsed with ultrapure water and dried with nitogen gas. The amount of adsorbed hTM on PSF films was quantified by frequency shift ($-\Delta F=1.15\times\Delta m$).

<u>Blood Clotting Experiment.</u>
The hTM adsorbed on PSF films were set in a test tube, human blood poured into the test tube. The films were incubated at 37°C for predetermind time, and then the films were removed from the tube and washed by gentle immersion in PBS.

Results/Discussion: Figure 1b shows hTM adsorption isotherm onto PSF film by quartz crystal microbalance (QCM) analysis. The maximum amount of adsorbed hTM was 240 ng/cm^{-2}, it showed that the adsorption of hTM onto PSF films was Langmuir-type adsorption and hTM adsorbed with a relatively weak interaction onto the PSF surface. Surface coverage was ca. 32%. AFM analyses of before and after adsorption of hTM onto PSF films indicated that hTM was homogeneously adsorbed onto PSF films (Figure 1c). Physically adsorbed hTM onto PSF films retained about 52% of its activity without denaturation by the protein C activity assay (Figure 1d).

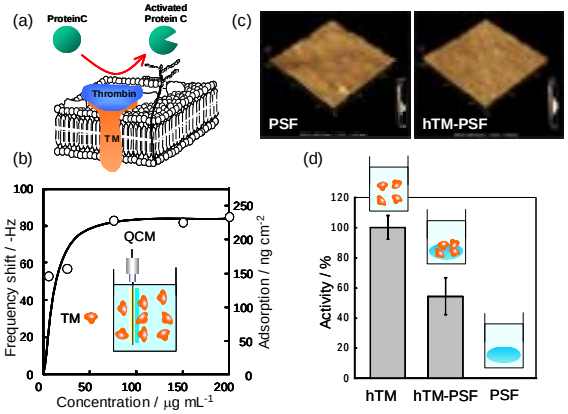

Figure 1. Antithrombogenicity of TM on Endothelical cell surface (a). Adsorption isotherm of hTM onto PSF film in 10 mM Tris buffer solution (pH=7.4) at 37°C (b). AFM images of PSF films (left) and PSF-hTM (right). Both areas were 500 nm x 500 nm (c). Protein C activity of hTM solution, PSF-hTM and PSF film at 37 °C (d).

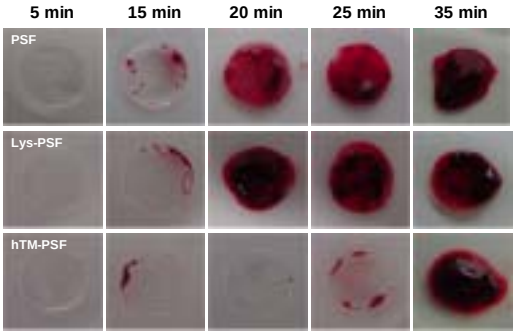

Figure 2. Photographs of blood clots formed on various samples.

Figure 2 shows blood clooting experiment of PSF films, lysozyme adsorbed PSF films (Lys-PSF) and hTM adsorbed PSF films (hTM-PSF). Although, Lys-PSF surface has almost same hydrophilicity (contact angle: 67.3 ± 4.5), only the hTM-PSF (contact angle: 66.6 ± 2.2) had a good anticoagulant activity.

Conclusions: We successfully prepared blood compatible materials by physical adsorption of hTM onto PSF films. Physical adsorption of bioactive molecules can be useful for preparing antithrombogenic biomaterials.

References:

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