Regulation of Protein Adsorption and Cellular Response on Phase Separated Biofouling and Anti-fouling Surfaces

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Introduction: The adsorbed proteins on materials surface have been believed to mediate cellular adhesion and activation which involved with the main drawbacks of artificial biomaterials such as platelet adhesion and thrombosis. Consequently, adsorption behaviors of proteins on material surfaces have to be primarily considered when designing a biomaterial. Meanwhile, 2-methacryloyloxyethyl phosphorylcholine (MPC) is biomimetic material which shows excellent biocompatibility due to the thick hydrated layer forming around phosphorylcholine groups. Throughout a number of researches, it is well known that the biomaterials formed by proper compositions of MPC polymers are effective to prepare non-biofouling, thus biocompatible surfaces. However, only a little attention has been paid to the effect of surface morphologies formed by MPC polymers. Other types of MPC polymers such as polymer blend or block copolymers usually form a heterogeneous surface induced by phase separation. For that reason, to investigate what kind of relationships are between such a heterogeneous morphology and biocompatibility is important for designing polymer blend or block copolymer containing MPC polymers. In this research, we prepared different kind of nano-ordered morphologies by phase separation of block copolymers composed of MPC and dimethylsiloxane. Poly(dimethylsiloxane) (PDMS) is a hydrophobic material which is strongly interact with proteins by hydrophobic interactions. Thus the phase separated surfaces were expected to form a coexistence of non-biofouling and bioufouling domains. The protein adsorption and cell adhesion behavior on these biomimetic heterogeneous surfaces were investigated.

Methods: The block copolymers were synthesized by means of previously reported method [1]. The samples were cast, heat treated and stained with OsO_4 before or after contact with Au labeled IgG solutions for TEM observation. Surface characterization was performed by static water contact angle and XPS measurement. The amount of adsorbed proteins (IgG and fibronectin) on polymer film was calculated by micro-BCA method. In order to see the effect of serum proteins, L929 mouse fibroblasts were cultured with or without 10% FBS. The cell morphologies were observed by confocal microscope before and after staining with DAPI and Allexa fluoro phalloidin. The each number of cells on samples was calculated by using cell counting kit #8.

Results and Discussions: Four kinds of block copolymers were successfully synthesized with almost same molecular weight (about 30 kDa) with different compositions. (% PDMS of PM1, 2, 3, and 4 is 12.0, 40.7, 55.4, and 75.8 %, respectively) PDMS domain ratio on resulting cast films are increased as PDMS compositions are increased. (Fig.1) The result of static contact angle and quantitative analysis of surface element performed by XPS showed that the hydrophilicity and P/Si value was continuously decreased as the compositions of

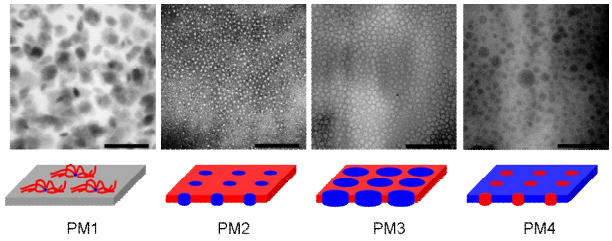

Figure 1. TEM images of block copolymer films. Dark region is OsO4 stained pMPC domains and bright region is PDMS. Scale bar = 300 nm Red region in scheme-PMPC, Blue scheme-PDMS

PDMS was increased. This result indicates that the size of phosphorylcholine domains was successfully controlled by the block copolymer compositions. As a result of protein adsorption test, protein molecules were selectively adsorbed only onto the PDMS domains. (Fig. 2) The adsorption tendency of cell adhesive protein (fibronectin, in this research) was same as IgG molecules and confirmed by micro-BCA method. This result indicates that the phase separated hydrophobic domains remained biofouling natures even the compositions of MPC is over 50 %. The cell adhesion was observed only on the non-coated and 100% PDMS surface with same cellular morphologies in no-FBS medium. This indicates that the adhesion factors, secreted by fibroblast itself, do not interact with the biofouling nanodomains (PDMS). However, the number of adhered cells on samples was increased as PDMS domains were increased until the reversed PDMS/PMPC domain structure (PM4) was formed in 10 % FBS medium. (Fig. 3) This indicates that the biofouling nanodomains were strongly interacts with serum proteins such as fibronectin, and finally cause the cell adhesioin.

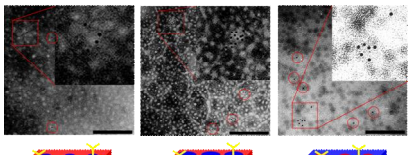

Figure 2. TEM images taken after protein adsorption. Small dot indicate gold colloid labeled IgG molecules. Scale bar = 300 nm

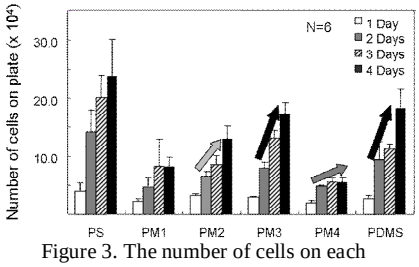

Figure 3. The number of cells on each block copolymer films.(10 % FBS state)

Conclusions: The selective adsorption of proteins only onto the biofouling nanodomains was confirmed even under the high composition of phosphorylcholine groups. This adsorption behavior has an effect to the cell adhesion in the present of serum proteins. The adhesion factors from ground substance secreted by fibroblast do not give an effect to the cell adhesion on polymer surface even under the existence of biofouling nanodomains.

Reference: [1] Seo, J. -H. et al. Biomaterials 2008:29:1367-1376