

## Site-directed immobilization of antibodies on well-defined polymer brushes

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**INTRODUCTION:** Surface characteristics for biosensor applications require both reducing non-specific biofouling and enhancing specific recognition. Especially in the protein-chip technology, immobilization of proteins in proper orientation is necessary to maintain the biological activities. We have reported that 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers synthesized as biomimetics in biomembrane structures significantly reduce protein adsorption and cell adhesion<sup>1</sup>. Furthermore, manipulation of protein and cell was well performed on well-defined poly(MPC) (PMPC) brushes produced by atom transfer radical polymerization (ATRP)<sup>2</sup>. The block copolymer brushes consisting of PMPC and poly[glycidyl methacrylate (GMA)] were also recently prepared for engineered biomaterial surfaces<sup>3</sup>. We describe here about oriented immobilization of antibodies onto the polymer brushes for biorecognition surfaces.

**METHODS:** Polymer brushes consisting of PMPC and poly(GMA) (PGMA) were formed on silicon wafers by ATRP as described previously<sup>3</sup>. Pyridyl disulfide moieties were then introduced to the polymer brushes via epoxy groups in GMA unit. Fab' fragments (Goat anti-mouse IgG) solution was in contact with polymer brushes with pyridyl disulfide moieties and reacted at room temperature over night. After the immobilization of Fab' fragments and wash with buffer, 1% bovine serum albumin (BSA) was applied to surfaces and incubated for 1h at room temperature. Fluorescein isothiocyanate- (FITC-) labeled immunoglobulin (Mouse anti-rat IgG) was used as antigen. BSA solution was removed and antigen solution was in contact with polymer brushes with Fab' fragments for 1 h at room temperature. After wash and dried, the fluorescence intensity was analyzed.

**RESULTS:** Fab' fragment is one of the antibody fragments and has thiol group in the opposite side of antigen-binding domain. We can immobilize the Fab' fragments in ordered orientation via thiol-disulfide exchange between the thiol groups of Fab' fragments and pyridyl disulfide moieties in polymer brushes. To compare the amount of immobilized antibody, FITC-labeled Fab'

fragments were reacted with each surfaces and the fluorescence intensity was determined. We prepared the organosilane monolayer having epoxy groups (epoxysilane films) as a control surface. The amount of immobilized antibody increased with an increase in the length of GMA unit. PGMA brush immobilized largest amount of antibody. Fig. 1 shows the ratio of fluorescence intensity of each surface after contact with FITC-labeled antigen. The fluorescence intensity of polymer brushes was higher than that of epoxysilane. In PMPC-*b*-PGMA brushes, the fluorescence intensity increased with an increase in the thickness of PGMA.

**DISCUSSION & CONCLUSIONS:** The amount of immobilized Fab' fragments and subsequent reaction with antigen could be controlled by changing the thickness of polymer brushes. Although PGMA brush surface has larger amount of Fab' fragment compared with PMPC-*b*-PGMA brush with longer PGMA, the reactivity with antigen was similar on these surfaces (Fig. 1.). It is considered that the condition of immobilized Fab' fragments are different between PGMA brush and PMPC-*b*-PGMA brush which has biocompatible PMPC under PGMA. It was also shown that polymer brush surface for reaction with antigen was more effective than the epoxysilane films. The characteristics of PMPC and dense immobilization of antibodies in defined orientation are effective in high sensitive biorecognition.

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