Site-specific dense immobilization of F(ab') on polymer brushes supported by organosilane nanofilaments

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Introduction: Protein immobilization onto solid supports is an important aspect of proteomic and diagnostic assays in obtaining information about protein functions and interactions, and for screening complex protein samples. For these applications, proteins are employed as molecular recognition elements because of their specific affinity. The control of the surface properties of the solid supports, which can enrich specific proteins, maintain native protein structure, and control ordered orientation, must therefore be a key technology.

Accumulating proteins on a limited surface is an effective means of obtaining definite responses. Polymer brushes have been studied because the number of binding sites for proteins can be increased in the polymer chain. However, the density of protein immobilization on dense polymer brushes is limited because of the high chain density. In this study, site-specific dense immobilization of antibodies on polymer brushes supported with organosilane nanofilaments was first performed.

Methods: Silicon wafers (1.2 cm x 1.2 cm) were cleaned by O_2 plasma treatment and placed in an oven at 120°C for 2 h. The wafers were then immediately soaked in toluene solution containing $MeSiCl_3$ and stored under ambient relative humidity for 3 h organosilane nanofilaments were formed. O_2 plasma was treated on the nanofilaments to produce Si-OH groups. A silane-coupling reagent having bromoisobutyryl group was then immobilized through the liquid phase reaction. Random copolymer brushes of 2-methacryloyloxyethy phosphorylcholine (MPC) and glycidyl methacrylate (GMA), namely PMG brushes, were prepared by surface–initiated atom transfer radical polymerization (SI-ATRP).[1)] PMG brushes having pyridyl disulfide moieties (PMG-SS) were prepared via a reaction of epoxy groups in GMA units and the reaction was confirmed through the detection of a sulfur signal determined by X-ray photoelectron spectroscopy. The fluorescence isothiocyanate (FITC)-labeled F(ab')$_2$ fragments were split into F(ab') fragments and the fragments were in contact with PMG-SS brushes on nanofilaments. The fluorescence intensity was then measured to identify the density of immobilized F(ab').

Results: The surface morphology as observed by scanning electron microscope is shown in Figure 1(a). Highly dense nanofilaments were formed on the surface. Both the advancing (θ_A) and receding (θ_R) water contact angles were above 165° and super hydrophobic surfaces were obtained.

Figure 1(b) shows chemical structure of F(ab')-immobilized polymer brushes. The polymerization ability and monomer reactivity of MPC corresponded to those of GMA. The mole fraction was stable during the polymerization periods.

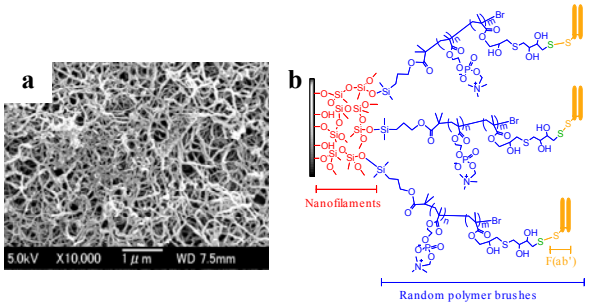

Figure 1. A micrograph (a) of organosilane nanofilaments and the dense immobilization (b) of F(ab').

The apparent thickness and density of the 3 h-polymerized brushes determined using smooth wafer surfaces were 15.6 nm and 0.32 chains/nm^2, respectively. The chain density of a random polymer brush nearly corresponded to that of the poly(MPC) brush (0.35 chains/nm^2) and was slightly lower than that of the poly(GMA) brush (0.43 chains/nm^2).[2)] The bulky MPC influenced the brush formation. θ_A/θ_R of the copolymer brushes supported by nanofilaments was effectively reduced to 25°/19° due to the hydrophilic MPC unit and water adsorbed well onto the surface.

Figure 2 shows fluorescence micrographs and quantitative data for fluorescence isothiocyanate (FITC)-labeled F(ab') fragments immobilized on the polymer brushes supported by the nanofilaments. The fluorescence intensity of surface was significantly ($p < 0.005$) greater than that on the PMG brush surfaces (data not shown). The reason for this result is that the thiol-disulfide interchange reaction occurred preferentially. In a comparison of surface morphologies, the fluorescence intensity on the copolymer brushes having the pyridyl disulfide moieties prepared on the nanofilaments was 65 times greater than that on a smooth surface.

Conclusions: The organosilane nanofilaments were very effective in increasing the capacity for protein immobilization. MPC units in the polymer brushes acted to reduce protein denaturation and preserve their function. The surface modification with silicone nanofilaments can be performed to a variety of substrates. Subsequently, nanofilament-supported MPC copolymer brush surfaces might prove to be useful as diagnostic substrates.

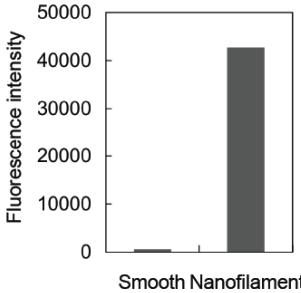

Figure 2. Fluorescence intensity of a unit surface after FITC-labeled F(ab') immobilization.

References: 1) Iwasaki Y et al., *Langmuir* 2008;24:8427-8430. 2) Iwata R et al., *Colloids and Surface B: Biointerfaces* 2008;62:288-298.