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Development of Renewable Surface Modification Technique utilized the Self-Assembling of β-Sheet Peptide

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We propose easily renewable surface modification technique. A self-assembling peptide, RASDA16[CH_3CO-(Arg-Ala-Ser-Ala)$_3$-Arg-Ala-Asp-Ala-$CONH_2$] was used as a component for a surface modification. This peptide forms a self-assembling hydrogel which could contain water as much as 99.5wt%. Then, the peptide was fixed on a silicon substrate to use a silane coupling agent. The peptide hydrogel was constructed on a silicon substrate by immersing in the peptide solution. The obtained substrate showed very high hydrophilicity. Furthermore, the wetting property of the substrate would be recovered by soaking the substrate in the peptide solution again, even if the peptide network on the substrate was removed. The hydrophilicity of the peptide-fixed substrate was efficiently recovered by RASDA16 adsorption on the substrate in a lower concentration of the peptide solution.
Key words: self-assembly / peptide / surface modification / re-construction

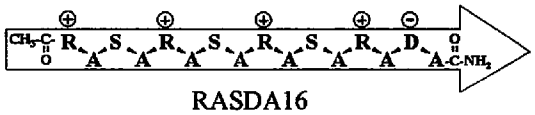
1. INTRODUCTION

A control of the material surface character is important to produce a functional interface for bio-interfaces, medical devices. Many scientists make an effort to produce a functional interface which was modified by various components such as lipids, polymers, and proteins[1-3]. A lot of methods are also suggested as a surface modification technique, such as chemical-vapor-deposition, spin-coating and plasma-induced graft polymerization etc[4-6]. However, it is difficult for these methods to re-construct the modified surface generally if once the function of the surface modification is lost. Then, we propose easily renewable surface modification technique. Self-assembling peptides were applied for the surface modified components. Recently, self-assembling peptides are explored to application of novel functional nano-materials for its specific nano-structures[7-9]. Our previous research showed that RADA16[CH_3CO-(Arg-Ala-Asp-Ala)$_4$-$CONH_2$] forms self-assembled hydrogel consists of nano-fibrous object[10]. The nano-fiber is composed of β-sheet structure which has an ability to form intermolecular hydrogen bonding. It was expected that the spontaneous assembly on the materials surface which was created to have the interactive region for the self-assembling component. However, RADA16 has a tendency to make a precipitation for its electrostatic interaction at neutral pH regions because of RADA16 has equivalent cationic and anionic amino acid residues, so that the peptide sequences were partly displaced Aspartic acid to Serine. In this study, a self-assembling peptide, RASDA16 was fixed on the material surface via silane coupling agent, and its surface morphology was observed by AFM measurements and its wetting property was also investigated by contact angle measurements.

2. EXPERIMENTAL

2.1 Materials

The amphiphilic peptide, RASDA16 was prepared by solid phase method using 9-fluorenylmethoxycarbonyl (Fmoc) strategy (Scheme 1). The peptide chain was synthesized on a CLEAR-Amide Resin (cross-linked ethoxylate acrylate resin, Peptide Institute). The N-terminus of the peptide was acetylated by acetic anhydride. The obtained peptide was identified by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (Applied Biosystems, VoyagerRP, m/z calc. for RASDA16 $[M+H]^+$: 1629.8; found: 1627.7).



RASDA16

Scheme 1 Amino acid sequences of the amphiphilic peptide, RASDA16.

2.2 Preparation of hydrogel

The solution of RASDA16 was prepared by dissolving the peptide powder with Milli-Q water and Tris-HCl buffer (pH 7.5). Final concentration of the peptide in 50 mM Tris-HCl buffer was 1.8 mM or 0.3% (3 mg/ml). The peptide solution was sonicated for 30 min with an ultrasonic cleaner and stayed it for one night before each measurement.

2.3 Peptide-modification substrate

The silicon substrate was sonicated for 30 min in nitric acid and rinsed by milli-Q water. And then, the silicon substrate was cleaned by ozone cleaner

(NL-UV253S, Nippon Laser & Electronics Lab.). The silicon substrate was treated in H_2O/28%NH_3/30%H_2O_2(6/4/1) mixed solution for 30 min at 60°C to give a substrate with hydrophilic surface[11]. It was rinsed by Milli-Q water and dried in vacuum. Next, silane coupling of the silicon substrate was performed by immersing in a solution of 1% 3-glycidoxypropyltriethoxysilane (GPS) in anhydrous ethanol. After 24 hours, the silicon substrate was rinsed by aliquot ethanol, and then dried and incubated at 110°C for 20 min. The resultant substrate was immersed in the peptide solution (contained 30 mM LiCl) for 24 hours. Then, it was rinsed by Milli-Q water, and dried.

2.4 Contact angle measurements

Contact angles were measured by the sessile drop technique using a Drop Master 500(Kyowa Interface Science). A 2 μl droplet of Milli-Q water was placed onto the surface using a microsyringe and the contact angle measurements were taken 30 second after deposition.

2.5 Atomic force microscope observations

The images were obtained by atomic force microscope (AFM)(Nanoscope IV, Digital Instruments) using a silicon cantilever (NCH-10V, Veeco Instruments) operated in tapping mode. All images were obtained in air at room temperature. A 14 μm x 14 μm scanner was used for imaging. The scanning speed was at a line frequency of 1 Hz. Original images were sampled at a resolution of 512 x 512 points. Obtained images were filtered by plan-fit and flatten routine.

3. RESULTS AND DISCUSSION

3.1 Peptide characterizations

The solution of the amphiphilic peptide, RASDA16, transformed into a hydrogel at neutral pH, which contains water as much as 99.5% (Figure 1(a)). Aliquots of RASDA16 hydrogel were diluted with Milli-Q water and a few microliter of sample was immediately deposited onto mica substrate. The sample was left on the mica for 30 seconds, then rinsed with 100 μl of Milli-Q water. The sample was then air-dried, and AFM observation was acquired. The AFM image showed networks composed with nanofibers ranging from a few hundred nanometers to a few microns in length (Figure 1(b)). The higher dots observed in the AFM image, which caused by duplication of the fibers, would be corresponded to crosslinking points.

3.2 Surface morphology of peptide-modified silicon substrate

The surface structure of RASDA16 modified silicon substrate was observed by AFM. The AFM image showed a lot of peptide nanofibers intertwined on the substrate (Figure 2(a)). It was suggested that the peptide thin film was formed on the silicon substrate. The formed thin film would assume nature of hydrogel, since a lot of crosslinking points were observed in the AFM image. Next, the substrate coated with peptide thin layer was sonicated in CH_3CN/H_2O/TFA(50/50/0.1) for 30 min to remove an adsorbed peptide. After sonicating, the crosslinking points disappeared, suggesting that RASDA16 formed the thin film was removed (Figure 2(b)). The substrate displayed many nanofibers on the substrate even after the sonication. The observed nanofibers would be attributed to the peptides covalently tethered on the substrate. When the same process was carried out by the bare silicon substrate, it was showed none of peptide nanofibers on the substrate. This result was also supported that RASDA16 was fixed on the

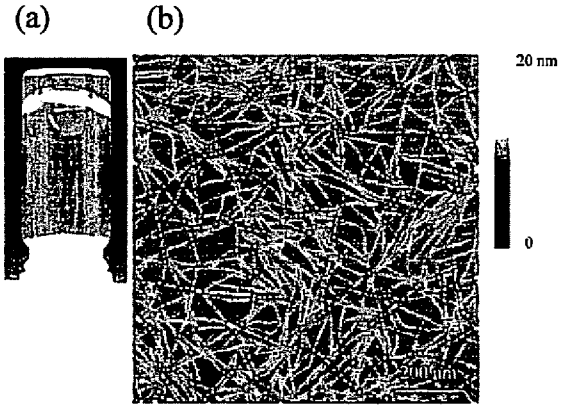


Figure 1 (a) Photograph of the hydrogel constructed by RASDA16 (0.3 wt%, pH 7.2), (b) AFM image of RASDA16 nanofibers.

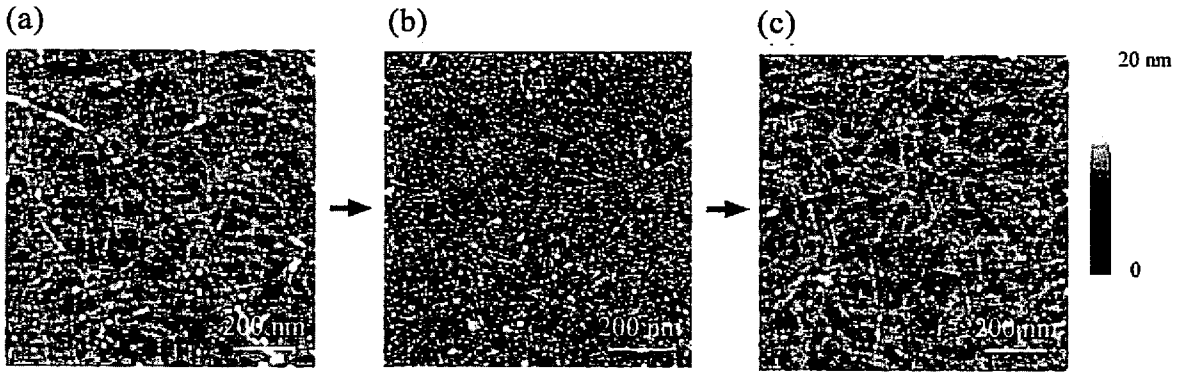


Figure 2 AFM images of surface structure of RASDA16-modified substrate. (a) RASDA16 was coated on GPS-modified substrate by immersing in 0.3 wt% RASDA16 hydrogel at 24 hours, (b) absorbed RASDA16 was removed by sonicatoin, (c) RASDA16 was re-assembled.

GPS-modified substrate via covalent bond between epoxy group and peptide side chain. Next, the substrate was immersed in the solution of the peptide again. Its surface was observed by AFM (Figure 2(c)). The AFM image also showed that the network structure was recovered as much as the network before the sonication. It was suggested that the peptide network on the substrate would be repeatedly re-constructed (Figure 3).

3.3 Surface property of peptide-modified silicon substrate

The wetting property of the peptide-modified substrate was characterized by contact angle measurement. The contact angle of each process of the surface modification was measured (Figure 4). The contact angle of the GPS-modified substrate was 45°. The contact angle of the substrate covered with the peptide was changed to 7°, indicating very high hydrophilic surface. After the peptide adsorbed on the substrate was removed by sonication, the contact angle was estimated to be 30°, suggesting that the wetting property was decreased. When thin layer of the peptide nano-fibers were re-constructed on the substrate, the contact angle showed 8°. It was suggested that the wetting property was recovered. This result indicated that the surface wetting property was repeatedly recovered by immersing the substrate into the solution of the peptide. The recovery of the wetting property was successfully repeated for 3 times at least. The suggested possibility to control the surface property would be applied as a simple surface modification method, which was achieved by only immersing the peptide solution.

The surface property of the peptide fixed substrate was also characterized by immersing to a peptide solution with different concentration and a solution of other component (Table 1). The peptide modified substrate was immersed in the RASDA16 solution(0.01 wt%, 50 mM Tris-HCl buffer pH 7.5, concentration not to form hydrogel) for 24 hours. The contact angle of the obtained substrate estimated to be 6°, indicating highly hydrophilic surface. It was suggested that the peptide modified silicon substrate has an affinity with RASDA16, so that enough amount of peptides were adsorbed on the substrate. Next, poly(ethylene glycol)(PEG), which doesn't have ability of self-assembly, was used for immersing solution. The peptide-modified substrate was immersed into the PEG aqueous solution (0.3wt%). The contact angle of the obtained substrate was 31°. The contact angle didn't change by immersing PEG solution, suggesting that the absorbed PEG on the substrate was easily carried away by rinsing. These results suggested that the combination of the substrate modified with the peptide by covalent bond and the component having a self-assembling ability with the modified peptide has a great advantage for controlling of wetting property.

4. CONCLUSION

We have demonstrated that the surface modification technique using a self-assembling peptide. The substrate covered with the peptide showed high hydrophilicity surface. The surface wetting property and morphology were repeatedly recovered by immersing the peptide solution when the modified peptide removed by some external stimuli. The self-assembling peptide would be selectively adsorbed on the material surface due to the peptide fixed on the surface. The self-assembling peptide has a great advantage for surface modification with an ability to renew the surface property.

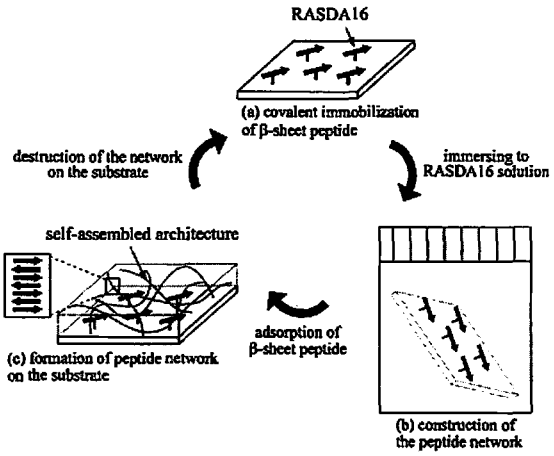


Figure 3 Schematic illustration of re-construction of the peptide network on the substrate.

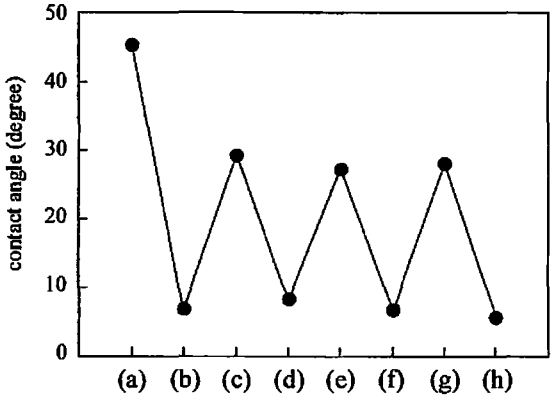


Figure 4 Contact angle measurements of each process of surface modification. (a) GPS-modified substrate; (b, d, f, h) RASDA16 coated substrate by immersing peptide solution; (c, e, g) after sonication.

Table 1 The contact angles (CA) of the peptide-modified silicon substrate before and after immersing in the polymer solutions

substrate	CA before soaking	CA after soaking	
		0.01wt% RASDA16 solution	0.3wt% PEG solution
peptide-modified silicon	31°	6°	31°

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