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Silane-coupling silicon substrate that fixes protein without adsorption of protein buffer components for device fabrication

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1. Introduction

The fixation of proteins on substrates have been researched. In the medical field, protein detection devices that rely on the fixation of proteins, such as protein sensors and protein chips, have become essential instruments. Also, in the field of nano-electronic device fabrication, proteins are fixed on substrates and serve as scaffolds and/or templates for making nano-functional structures. Salt and alkali ions of the buffer contaminate substrates, inhibiting the sensing and fabrication of nano-electronic devices. To avoid such undesirable effects, buffer solutions have been replaced by pure water [1,2] or alkali-metal-ion-free buffer [3]. However, proteins in such solutions are sometimes denatured. Therefore, a new method of salt-free protein fixation using buffer solutions is needed. We analyzed correlation between substrate properties, adsorption of proteins, and buffer components such as metal contamination. We developed a substrate which adsorbs proteins but does not adsorb contaminants such as salts and alkali metal-ions contained in the buffers.

2. Materials and methods

Silicon substrate was dipped in acetone for a few minutes, rinsed with ultrapure water, immersed in piranha solution at 100℃ for 15min, and carefully rinsed with ultrapure water for a few minutes. After washing, the silicon substrate was dried using nitrogen gas blow and cleaned using UV-Ozone cleaner for 15min. After cleaning, silicon substrates were modified by vapor method with 3-aminopyopyltrimethoxysilane (APTMS), mercaptomethyltrimethoxysilane (MMTS) and methyltrimethoxysilane (MTMS). Silane-coupling was performed as follows. The amounts of silane per vapor volume, and per silicon substrate were 1 volppm, 0.058 ul/cm^2 (1volppm); 10 volppm, 0.58 ul/cm^2 (10volppm); and 100 volppm, 5.8 ul/cm^2 (100volppm). The 0.01 M Phosphate Buffered Saline (PBS) and 0.01M HEPES were adjusted to pH 7.4. Apo-ferritin was diluted with buffer to a final concentration of 20 ug/ml. The substrate was dipped in buffer solution for 1 min. the substrate was taken out and dried using nitrogen gas blowing. The fixation of ferritin was observed by atomic force microscopy (AFM). The adsorption of buffer components was analyzed by X-ray photoelectron spectroscopy (XPS).

3. Results and discussion

To study the correlation between substrate properties, adsorption of protein, and buffer components, we modified silicon substrates by APTMS, which is hydrophilic and positively charged, MMTS, which is hydrophilic and negatively charged, and MTMS, which is hydrophobic. As buffer solutions, PBS and HEPES were selected because they are widely used with proteins. Ferritin in HEPES and PBS solution adsorbed on APTMS-coupled silicon substrate. On the other hand, MMTS- and MTMS-coupled silicon substrate could not adsorb ferritin in HEPES solution. Ferritin in PBS solution adsorbed on MMTS- and MTMS-coupled silicon substrate. We thought that these differences were influenced by ion strength of buffer solutions because ion strength influences Debye length. The ion strength of HEPES and PBS buffer solutions were 0.014 M and 0.172 M, respectively. The calculated Debye length of HEPES and PBS were 2.57 nm and 0.73 nm, respectively. In the case of PBS buffer solution, the short Debye length of both ferritin and the MMTS-, MTMS-coupled silicon substrate cause extreme closeness between ferritin and the substrate. We considered that adsorption of ferritin to be controlled by ion strength of ferritin solution even when the substrate has no positive charge.

Adsorption of materials other than ferritin on silane-coupled silicon substrate (100volppm) was analyzed by XPS (Table 1). APTMS-HEPES had sodium other than elements of APTMS-coupled silicon substrate. Chlorine and sodium adsorbed on APTMS-PBS. Although reproducibility was poor, chlorine and phosphate were detected on the APTMS-coupled silicon substrate. For these reasons, we considered that Cl^-, PO_4^{2-} and/or PO_4^{3-} of the PBS buffer were adsorbed onto the APTMS-coupled silicon substrate because APTMS has a positive charge in solution. MMTS-HEPES and -PBS have sodium other than elements of MMTS-coupled silicon substrate. Adsorption of sodium may be caused by metal contamination onto silicon oxide. Only MTMS-coupled silicon substrate did not adsorb components of buffer solution.

The correlation between coverage of silane and amount of sodium adsorption were examined by XPS to confirm whether the sodium adsorption is derived from metal contamination (Table 2 and 3). As the treatment amount of MMTS increases, the adsorption amount of sodium in-

Table 1 Component ratios of elements on APTMS- MMTS- and MTMS-coupled silicon substrate after dipping in HEPES and PBS by XPS analysis. The amount of silane is 100volppm.

Elements	Composition (at.%)						
	APTMS			MMTS		MTMS	
	HEPES	PBS-1	PBS-2	HEPES	PBS	HEPES	PBS
Cl2p	0	0.4	0.6	0	0	0	0
Na1s	0.3	0.4	0	0.2	0.5	0	0
P2p	0	0	0.6	0	0	0	0
S2p	0	0	0	1.3	1.4	0	0
N1s	5.6	2.7	5.1	0	0	0	0
K2p	0	0	0	0	0	0	0
C1s	31.7	38.8	30.4	11.4	15.0	6.7	13.5
O1s	40.2	43.4	43.5	53.5	52.3	57.0	54.2
Si2p(silicon)	9.0	9.6	8.6	14.6	14.6	16.7	14.5
Si2p(SiO2	13.2	6.3	11.1	16.8	16.8	19.6	17.8

Table 2 Component ratios of S1s and Na1s per Si2p of silicon substrate by XPS analysis of the treatment amounts on MMTS-coupled silicon substrate after dipping in HEPES and PBS.

Elements	HEPES + MMTS			PBS + MMTS		
	1 volppm	10 volppm	100 volppm	1 volppm	10 volppm	100 volppm
S2p / Si2p	0.025	0.045	0.081	0.027	0.051	0.093
Na1s / Si2p	0.005	0.008	0.014	0.016	0.020	0.032

Table 3 Component ratios of C1s and Na1s per Si2p of silicon substrate by XPS analysis of the treatment amounts on MTMS-coupled silicon substrate after dipping in HEPES and PBS.

Elements	HEPES + MTMS			PBS + MTMS		
	1 volppm	10 volppm	100 volppm	1 volppm	10 volppm	100 volppm
C1s / Si2p	0.181	0.124	0.403	0.162	0.250	0.928
Na1s / Si2p	0.006	0.004	0.000	0.009	0.003	0.000

creases for HEPES- and PBS-dipped MMTS-coupled silicon substrates (Table 2). These results showed that sodium increases with the decrease of silicon oxide exposure area. This phenomena suggested that the increase of sodium is not metal contamination. Accordingly, we supposed that the increase of sodium was derived from the -SNa group of MMTS. On the other hand, the sodium adsorption amount decreases with increased coverage of MTMS (Table 3). When the treatment amount of MTMS is 100 volppm and 5.8 ul/cm^2, we assumed that the substrate had no sodium metal contamination because silicon oxide of the substrate was fully covered. The APTMS- and MMTS-coupled silicon substrates after dipping in HEPES and PBS had adsorbed buffer components. Adsorption of buffer components is influenced by ionization of silane molecule. To avoid metal contamination, elimination of exposure of silicon oxide which easily adsorbs sodium is needed.

3. Conclusions

Adsorption of ferritin was achieved by using PBS which had short Debye length by high ion strength. Silane-coupled silicon substrate such as APTMS and MMTS which ionizes in solution, adsorbs Na, Cl and phosphate ions of buffer solution. We clearly showed by experiments that the high coverage methyl-silane-coupled silicon substrate achieves adsorption of ferritin with no buffer components even if the protein solution contains metal ions or salts. This methyl-silane coupled substrate is suited for device fabrication using protein.

References

[1] K. Yamada, S. Yoshii, S. Kumagai, A. Miura, Y. Uraoka, T. Fuyuki, I. Yamashita, Jpn. J. Appl. Phys., **46** (2007) 7549-7553.

[2] Miura, Y. Uraoka, T. Fuyuki, S. Yoshii, I. Yamashita, J. Appl. Phys. **103** (2008) 074503.

[3] S. Yoshii, K. Yamada, N. Matsukawa, I. Yamashita, Jpn. J. Appl. Phys., **44** (2005) 1518-1523.