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In vitro stability study of organosilane self-assemble monolayers and multilayers

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Abstract

The stability of self-assembled monolayers (SAMs) and multilayers formed on silicon surface by amino-terminated silanes and SAMs formed by alkyl and glycidyl terminated silanes were investigated in vitro with saline solution at 37 °C for up to 10 days. FTIR and XPS results indicated that amino-terminated SAMs and multilayers are very unstable if the alkyl chain is short ($(CH_2)_3$), while stable if the alkyl chain is long ($(CH_2)_{11}$). On the other hand, alkyl-terminated SAMs are very stable regardless of the alkyl chain length, and glycidyl terminated SAM retained approximately 77% of the organosilane molecules after 10 days. Hydrogen bonding between the organosilane monomer and silicon surface and among the organosilane monomers is believed to contribute to the instability of the SAM and multilayer formed by amino-terminated silane with a short alkyl chain ($(CH_2)_3$). Therefore, the widely used (3-aminopropyl) trimethoxysilane (APTMS) SAM and multilayer may not be suitable for implantable biomedical applications.

Keywords: Self-assembled monolayer; Multilayer; Organosilane; Silicon; Stability; In vitro

1. Introduction

Modification of solid surfaces has found tremendous applications in many areas. Among the various surface modification techniques, deposition of a self-assembled monolayer (SAM) or multilayer of organosilane is very versatile, and it has demonstrated numerous benefits over others [1]. Many types of organosilanes have been used to form SAMs or multilayers on the surfaces, and among them amino-terminated SAMs and multilayers are of particular interest. They have been used to modulate the nucleation and growth of minerals [2], promote the attaching and spreading of neurons [3], fix antigen/antibody [4], attach peptides to promote cell adhesion [5], tether DNA oligonucleotides [6], immobilize heparin and hyaluronan to enhance biocompatibility [7,8], and maintain the bioactivity of enzymes on alloy surfaces [9]. They have been widely used in the applications of biosensing and electrocatalysis as well [10]. Typically SAMs are formed in anhydrous environments [11], while multilayers are formed if water is present in the reaction solution [4].

The biggest concerns in the field of implantable biomedical devices are their stability and biocompatibility. It has been agreed that the surface properties of materials, such as hydrophilicity, chemical composition, microphase separation, and adsorbed water content, are key factors that mediate the interactions between the physiological environments and the foreign objects [12]. The outermost surfaces of the alien devices were often modified not only to enhance their stability, but also to promote or inhibit specific tissue/cell responses. Depositing an organosilane SAM or multilayer on the device surface has been a popular approach to achieve desired surface properties. Silver et al. [13,14] investigated the effects of the chain length and terminal functional groups of the SAMs on blood biocompatibility. However, the stability of various SAMs and multilayers formed by organosilanes has not been systematically studied [15].

Silicon has been dominant in the biomedical applications of implantable neural prostheses [16,17], but our previous in

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vivo tests in rat brains indicated that the reaction of glial cells and tissues to silicon was elevated as compared with sham control. The silicon surface was also noticeably corroded while staying in the rat brain for 10 days.[1] Therefore, both the biocompatibility and biostability of silicon need to be improved if long-term applications (e.g., neural prostheses) are to be possible. To achieve these tasks, protective and/or biocompatible SAM deposition on the silicon surface is one promising option. Our in vitro tests demonstrated that the biocompatibility of silicon could be enhanced by the deposition of SAMs formed by $NH_2(CH_2)_3Si(OCH_3)_3$ (APTMS) and $CH_3(CH_2)_{17}SiCl_3$ (OTS) [18].

In this paper, we report our systematic stability test results for various SAMs and multilayers deposited on a silicon surface, performed in vitro in saline solution at 37 °C for up to 10 days. The organosilanes used in our study are listed in Table 1. GTMS was chosen because it offers an alternate route to introduce primary amine groups on the surface by reacting with diamines. Although SAMs formed by alkylsilanes (e.g., PTMS and OTS) are typically inert, biopolymers, such as proteins, heparin and hyaluronan, can still be covalently attached on top of them by UV-based photo-immobilization [18]. This serves as our preliminary screening step prior to the in vivo animal experiments. The quality of these SAMs and multilayers was examined by atomic force microscopy (AFM) and contact angle measurement, and their stability was evaluated by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR).

We found that the widely used APTMS was unable to produce stable SAM or multilayer at all. However, SAM and multilayer formed by aminounidecyl trimethoxysilane (AUTMS) are much more stable, and SAMs formed by alkyl silanes or epoxy-terminated silane demonstrate considerably better stability than APTMS as well.

2. Materials and methods

2.1. Materials

One-side polished N type silicon (111) wafers (test grade, with resistivity 1–2 ohm cm and thickness 475–575 microns) were purchased from Wafer World, Inc. (West Palm Beach, FL). Deionized water (DI water) with resistivity of 18 MΩ cm was obtained with a Barnstead Nanopure System (Dubuque, IA). OTS (97.5%) was purchased from United Chemical Technologies (Bristol, PA). Carbon tetrachloride (anhydrous), *n*-hexadecane (anhydrous), APTMS (97%), EDS (97%), PTMS (98%), and GTMS (98%) were purchased from Sigma–Aldrich (St. Louis, MO). AUTMS was a gift from CK Witco Corp. (Greenwich, CT). AUTMS was purified by vacuum distillation before use, while the other organosilanes were used as received. Chloroform (HPLC grade) was purchased from Burdick & Jackson (Muskegon, MI). Sodium chloride (NaCl), dioxane and toluene (anhydrous) were obtained from Fisher Scientific (Pittsburgh, PA).

2.2. Deposition of silane multilayers and monolayers

The pre-cut silicon pieces (approx. 1.2×1.2 cm^2) were rinsed with ethanol, acetone and then cleaned by the RCA method [19,20], with hydroxyl groups on top of the silicon oxide layer (SiO_x) afterwards [21,22]. Thus treated silicon is referred as RCA Si hereafter. They were blown dry with nitrogen before SAM or multilayer deposition.

To obtain an APTMS multilayer, the RCA Si pieces were put in 1% APTMS/dioxane (wt/wt) solution in the presence of 0.2% water at 65 °C for 1 h. They were then cleaned under ultrasonication at room temperature in dioxane for two 5-min cycles and in toluene for two additional 5-min cycles to remove physically adsorbed APTMS monomers and aggregates. To obtain an APTMS SAM, the RCA Si pieces were put in 1% APTMS/toluene (anhydrous) solution for 4 min at 60 °C, followed by cleaning under ultrasonication at room temperature for four 5-min cycles in toluene [4]. The EDS SAM was prepared according to Stile et al.'s approach [23]. The RCA Si pieces were put in 1% (v/v) EDS solution in 94% methanol (containing 1 mM acetic acid) and 5% water for 5 min at room temperature, followed by rinsing three times with methanol and baked for 15 min at 120 °C. The procedures to prepare the AUTMS multilayer and SAM were similar to APTMS, except that the AUTMS concentration was 1.5%.

The OTS SAM was obtained by subjecting the RCA Si pieces to freshly prepared 7 mM OTS solution in 4:1 (v/v) CCl_4:*n*-hexadecane in a sealed Teflon bottle for 10 min at room temperature. The pieces were then rinsed and washed with chloroform 5 times. The PTMS SAM was prepared by putting the RCA Si pieces in 1% PTMS/toluene (anhydrous) solution for 1 h at 65 °C, followed by washing with toluene 5 times. The GTMS SAM was prepared by putting the RCA Si pieces in 2% GTMS/toluene (anhydrous) solution for 1 h at 65 °C, followed by washing with toluene 5 times.

In the presence of water (even trace amount), the organosilane molecule ($R–SiX_3$, X = Cl or OCH_3) quickly hydrolyzes into an intermediate product containing three hydroxyl groups connecting to the silicon atom (i.e. $R–Si(OH)_3$), which is highly reactive. These trihydroxyl intermediate molecules have high tendency to crosslink with one another to build a 3-dimensional network structure (i.e. multilayer), competing with the SAM (commonly viewed as 2-dimensional) formation at the liquid–solid interface. However, in anhydrous environment, the reaction between the organosilane monomers and hydroxyl groups on the RCA Si surface is more competitive than the reaction among the organosilane monomers themselves. Therefore SAMs were typically formed in anhydrous condition. Even if there are

[1] The in vivo biocompatibility and biostability study of silicon was done with our collaborators, Jie Li, Kelley Darren, Carolyn Black, Paul Finlayson and James P. McAllister (School of Medicine, Wayne State University, Detroit, MI).

Table 1
The full names and chemical structures of the organosilanes used in this study

Abbreviation	Full name	Molecular structure
APTMS	(3-aminopropyl) trimethoxysilane	$NH_2(CH_2)_3Si(OCH_3)_3$
AUTMS	(11-aminounidecyl) trimethoxysilane	$NH_2(CH_2)_{11}Si(OCH_3)_3$
PTMS	propyltrimethoxysilane	$CH_3(CH_2)_2Si(OCH_3)_3$
EDS	*N*-(2-aminoethyl)-3-aminopropyl-trimethoxysilane	$NH_2(CH_2)_2NH(CH_2)_3Si(OCH_3)_3$
GTMS	(3-glycidyloxypropyl)trimethoxysilane	$CH_2(O)CH-CH_2O(CH_2)_3Si(OCH_3)_3$
OTS	octadecyltrichlorosilane	$CH_3(CH_2)_{17}SiCl_3$

some excess organosilane aggregates on the surface after SAM preparation, they can be readily removed by using soft cotton cloth or tissue paper and other approaches [24,25].

All of the silane multilayer and SAM deposited surfaces were blown dry with compressed nitrogen before stability test and characterization.

2.3. Stability test of various SAMs and multilayers on silicon

SAMs and multilayers obtained as described above were immersed in a saline solution (0.9% NaCl in DI water) statically at 37 °C for up to 10 days. Afterwards, the samples were washed with saline solution twice and DI water twice, before being blown dry with nitrogen. They were then characterized by XPS, FTIR and AFM.

2.4. Surface characterization

AFM images were acquired using a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) in tapping mode. Only the height images are presented here. An E-scanner (maximum scan size of 16 × 16 μm) and a silicon probe (length: 125 μm, resonance frequency: between 244 and 366 kHz) were used. The probe was washed with ethanol and illuminated under UV light by a fiber optical illuminator (Dolan-Jenner Industries, Inc., Lawrence, MA) for more than 5 min before use, as recommended by the manufacturer.

The surface hydrophobicity was measured with a Ramehart NRL contact angle goniometer (Model 100, Landing, NJ) in the laboratory atmosphere. A 20 μl DI water droplet was placed on the substrate and the static contact angles were measured on both sides of the droplet. Three droplets were placed at various spots on the substrate surface and the average readings and standard deviations are reported.

XPS analysis of the surfaces was conducted with a PHI 5500 Spectrometer (Perkin–Elmer, Wellesley, MA) equipped with an aluminum $K\alpha$ X-ray radiation source (1486.6 eV) and AugerScan system control (RBD Enterprises, Bend, OR). The pressure in the chamber was below 2×10^{-9} Torr before the data were taken, and the voltage and current of the anode were 15 kV and 13.5 mA, respectively. The take-off angle was set at 45°. The pass energies for survey and multiplex scans were 117.40 and 23.50 eV, respectively. The binding energy scale was referenced by setting the C1*s* peak maximum at 285.0 eV.

The FTIR spectra were obtained with a Nicolet Magna-IR 560 single beam spectrometer equipped with a mercury–cadmium–telluride (MCT-A) detector operated at 4 cm^{-1} resolution with 200 scans for the SAMs and multilayers before and after the stability test. Attenuated total reflection (ATR) and transmission modes were used for SAMs and multilayers, respectively. A silicon 45° trapezoidal prism with dimensions of 50 × 10 × 2 mm or 50 × 10 × 3 mm was used to obtain the ATR spectra. The integrated area corresponding to the absorption peaks of the symmetric and asymmetric stretching of C–H bond was used to quantify the amount of organosilane molecules on the surface.

3. Results

3.1. AFM results

The AFM images of selected SAMs and multilayers on RCA Si are shown in Fig. 1. AFM images of the SAMs formed by PTMS, OTS and EDS are not shown, since there is no noticeable morphological difference between them and the RCA Si surface (Fig. 1a). The root-mean-square (RMS) values for these surfaces, which indicate the surface roughness, are listed in Table 2. The deposition of these SAMs and multilayers all increased the roughness of the RCA Si surface, as the RMS of RCA Si is only 0.045 nm. The SAMs formed by PTMS, OTS and EDS are very smooth (RMS < 0.2 nm) at the atomic level, while SAMs formed by APTMS and AUTMS are somewhat rougher (0.23 nm < RMS < 0.25 nm) (Table 2). GTMS produced the roughest surface (RMS ∼ 0.3 nm) among all the SAMs studied, which is attributed to the presence of the bulky epoxy group at the end of GTMS monomer. Meanwhile, the RMS values for APTMS and AUTMS multilayers are 1.226 and 1.132 nm respectively, and significantly larger than all the SAMs. Many aggregates, ranging from 20 to 100 nm in diameter, were observed on these multilayer-covered surfaces.

After the saline test for 10 days, the APTMS multilayer surface became much smoother (AFM image not shown), and the RMS value decreased from 1.226 to 0.371 nm. A few small aggregates were still observed. However, there was no noticeable change of the surface morphology of the AUTMS multilayer after the saline test for 10 days, which still appeared to be relatively rough, with an RMS value over 1 nm.

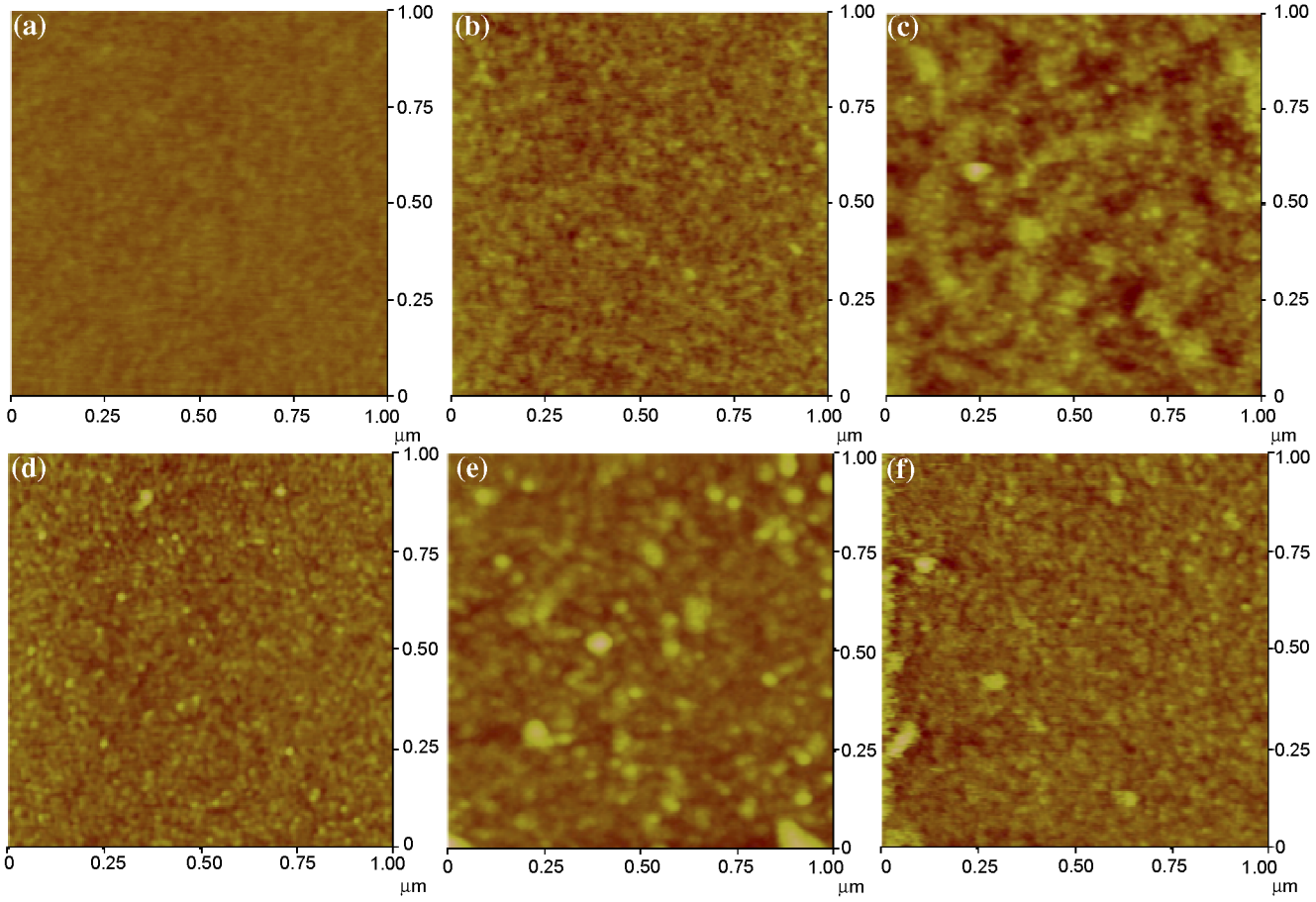

Fig. 1. The AFM images of (a) RCA Si; (b) APTMS SAM; (c) APTMS multilayer; (d) AUTMS SAM; (e) AUTMS multilayer; and (f) GTMS SAM on RCA Si. The scan area is 1 × 1 μm for all the surfaces. The z-range is 5 nm for (a), (b), (d) and (f), and 20 nm for (c) and (e). The multilayers formed by APTMS and AUTMS are significantly rougher than all the SAMs, and many aggregates were observed on them.

Table 2
Static contact angle with water and RMS (obtained from AFM) for various SAMs and multilayers on RCA Si

	RMS (nm)[a]	Contact angle[b] (°)	
		Before	After 10 days
RCA Si	0.045	2.0 ± 0.5	–
APTMS SAM	0.242	48.1 ± 1.3	59.5 ± 1.5
APTMS multilayer	1.226	53.0 ± 1.5	61.2 ± 1.0
AUTMS SAM	0.230	70.8 ± 0.7	77.7 ± 1.3
AUTMS multilayer	1.132	62.1 ± 1.0	79.5 ± 0.7
EDS SAM	0.139	47.1 ± 0.8	58.8 ± 0.7
PTMS SAM	0.173	57.7 ± 0.4	60.2 ± 1.0
OTS SAM	0.183	107.0 ± 0.5	106.7 ± 0.4
GTMS SAM	0.298	43.9 ± 0.7	53.5 ± 1.8

[a] RMS values were obtained on the AFM images with area 1 × 1 μm before stability test.
[b] Static contact angle with DI water.

These results agree well with the XPS results (discussed later).

3.2. Contact angle results

The static contact angle measured with DI water indicates the surface hydrophobicity. The contact angles measured for the SAMs and multilayers on RCA Si are listed in Table 2. RCA Si is very hydrophilic, with a measured contact angle of 2°. OTS SAM deposition increased the surface hydrophobicity most effectively (to 107°), and the other SAMs and multilayers increased the surface hydrophobicity with contact angles ranging between 44° and 71°. Interestingly, the APTMS multilayer is slightly more hydrophobic than its SAM, while the AUTMS multilayer is less hydrophobic than its SAM. Besides the chemical groups on the surface, surface roughness plays an important role in determining the hydrophobicity [26]. The contact angle results for OTS SAM agree well with those reported in the literature [20,27]. For amine-terminated surfaces, the reported contact angles vary widely. For instance, 22° to 68° have been reported for APTMS SAM [11]. The reading may depend on whether the measurement was made after the SAM preparation [11]. All the contact angle measurements on our SAMs and multilayers were performed within 30 min after their preparation.

The static contact angles on the SAM and multilayer surfaces after saline test for 10 days are also listed in Table 2. The increment of hydrophobicity is more apparent on amino-terminated surfaces (i.e. APTMS, AUTMS and EDS), consistent with Petri et al.'s findings [11]. However,

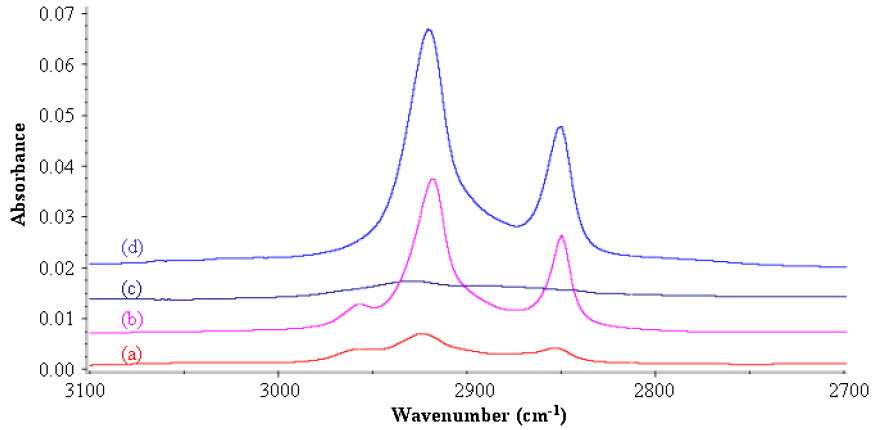

Fig. 2. ATR-FTIR spectra of the SAMs formed by (a) PTMS, (b) OTS, (c) APTMS, and (d) AUTMS. (a) and (b) were obtained on a 50 × 10 × 3 mm silicon ATR crystal, and (c) and (d) were obtained on a 50 × 10 × 2 mm silicon ATR crystal.

Table 3
FTIR adsorption peak assignments for various SAMs on RCA Si (unit: cm^{-1})

	$\nu(CH_3)$	$\nu_a(CH_2)$	$\nu_s(CH_2)$
OTS	2959	2918	2851
PTMS	2957	2924	2854
APTMS		2932	2859
AUTMS		2921	2852

the contact angle essentially did not change on OTS SAM surface, and increased slightly (from 57.7° to 60.2°) on PTMS SAM surface. The increase of hydrophobicity on the GTMS SAM surface was substantial as well.

3.3. FTIR results

Fig. 2 shows the 2700–3100 cm^{-1} region of the ATR-FTIR spectra of SAMs formed by PTMS, OTS, APTMS and AUTMS on RCA Si. The adsorption peaks corresponding to the vibration of CH_3 and symmetric and asymmetric stretching of CH_2 are summarized in Table 3. Our results for PTMS and OTS agree well with the reported data [27]. ATR FTIR spectra for APTMS and AUTMS SAMs are also consistent with their standard spectra obtained in ATR mode (not shown). For APTMS and AUTMS SAMs, the adsorption at 3380–3300 cm^{-1} (observed in the standard spectra of APTMS and AUTMS) corresponding to the primary amine group was too weak to be discerned, and the adsorption at 1585 cm^{-1} for $–NH_2$ was detectable although the signal-to-noise ratios are pretty low.

The integrated areas of C–H stretching vibration (from 3010 to 2778 cm^{-1}), which correlates to the relative amount of organosilane molecules on the surface, were measured after different durations in saline solution to quantitatively evaluate the amount of hydrocarbons remaining on the surface. As shown in Fig. 3, the APTMS SAM is much less stable than AUTMS, PTMS and OTS SAMs while tested in saline for up to 10 days. The APTMS multilayer was also found to be very unstable in saline solution within two days

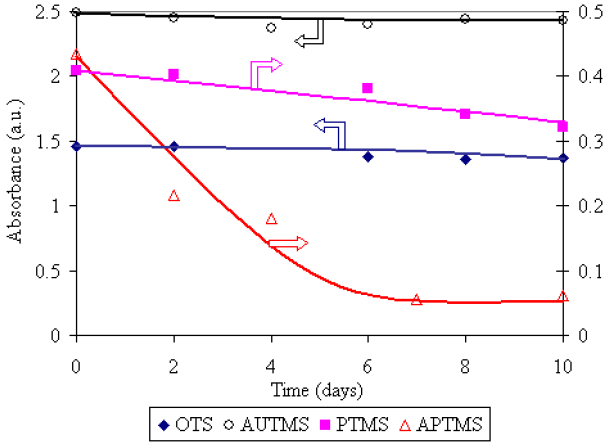

Fig. 3. Integrated area corresponding to the C–H vibration (from 3010 to 2778 cm^{-1}) for SAMs on silicon ATR crystal formed by OTS, PTMS, APTMS and AUTMS versus time in saline solution at 37 °C. OTS and PTMS SAMs were on a 50 × 10 × 3 mm silicon ATR crystal, while APTMS and AUTMS SAMs were on a 50 × 10 × 2 mm silicon ATR crystal.

based on the transmission FTIR data (not shown). Baking the APTMS multilayer and SAM at 120 °C for 30 min, which was commonly believed to be able to complete the crosslinking reaction among the APTMS monomers and effectively enhance the stability, did not enhance their stability in saline at all.

3.4. XPS results

The XPS survey spectra for OTS SAM, PTMS SAM, GTMS SAM, APTMS SAM and multilayer, AUTMS SAM and multilayer, and EDS SAM are shown in Fig. 4. Basically OTS, PTMS and GTMS SAMs have only C, O and Si on the surfaces, while amino-terminated silane SAMs and multilayers have N in addition. For the OTS SAM, no chlorine (Cl) was detected, which originally exists in the OTS monomers. This indicates that all the OTS molecules on the silicon surface were hydrolyzed and linked with each other and with the silicon surface via Si–O–Si bond. Elemental contents

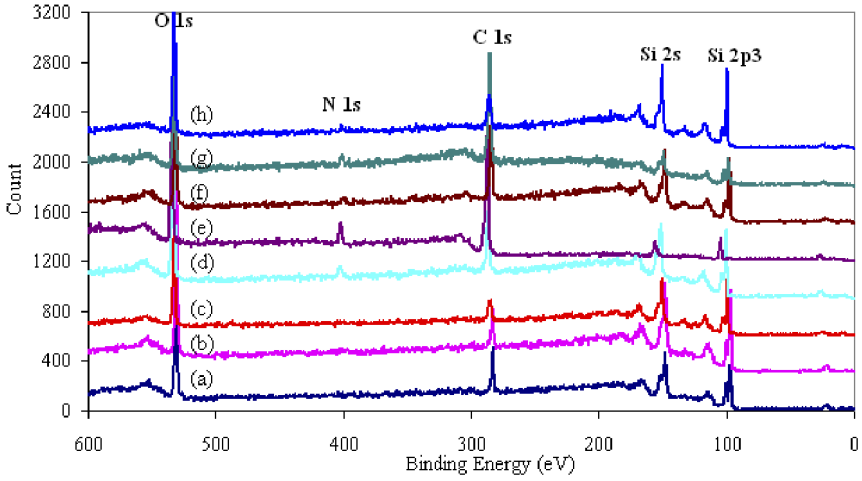

Fig. 4. XPS survey spectra for (a) OTS SAM, (b) PTMS SAM, (c) GTMS SAM, (d) APTMS SAM, (e) APTMS multilayer, (f) AUTMS SAM, (g) AUTMS multilayer, and (h) EDS SAM on silicon.

on the surfaces of various SAMs and multilayers, obtained from XPS detailed scans before the stability test in saline, are summarized in Table 3. Anhydrous reaction media typically leads to the formation of SAMs, while the presence of water will facilitate the formation of multilayers [3]. If an ideal two-dimensional crystalline structure (i.e. a perfect SAM) were formed on the silicon surface, the carbon contents should be in the order OTS > AUTMS > GTMS > EDS > PTMS = APTMS, assuming each silane molecule occupies the same area on the silicon surface. However, this trend was not observed. Moreover, the EDS SAM should possess double the nitrogen content of that in the APTMS and AUTMS SAMs, which was also not observed.

The amount of silane molecules remaining on the silicon surfaces was evaluated by the elemental ratios of C/Si and N/Si (the latter is for amino-terminated SAMs and multilayers only). When more organosilane coating is on the surface, more C and N, while less Si will be detected by XPS, therefore C/Si and N/Si ratios will be higher. On the other hand, if some organosilane molecules are stripped from the surface by the saline solution, the C/Si and N/Si ratios will decrease. Fig. 5 shows the C/Si and N/Si ratios for APTMS SAM and multilayer versus time in saline solution at 37 °C. C/Si and N/Si ratios for the APTMS SAM decreased from 0.94 to 0.38, and from 0.10 to 0.03, respectively, after staying in saline for 10 days at 37 °C. The decreasing effect is even more significant for the APTMS multilayer over the same time period. The major loss took place within the first 2 days for both the APTMS SAM and multilayer. Obviously, both APTMS SAM and multilayer are unstable under such mild test conditions.

The C/Si and N/Si ratios versus time in saline for the AUTMS SAM and multilayer are shown in Fig. 6. Interestingly, both ratios remained almost unchanged after 10 days' staying in saline solution. AUTMS SAM and multilayer clearly demonstrated much better stability than the APTMS counterparts. Fig. 7 shows the C/Si and N/Si ratios for the

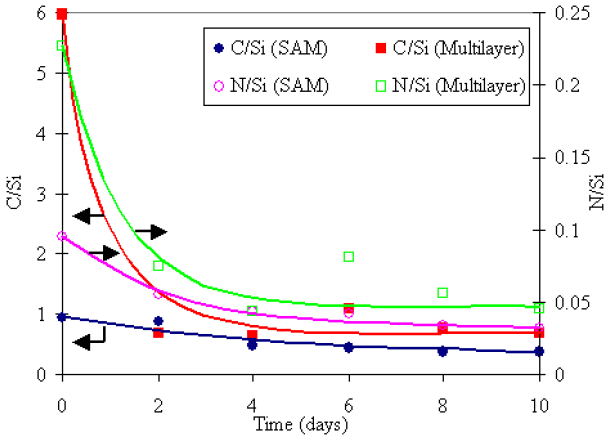

Fig. 5. Elemental ratios of C/Si and N/Si for APTMS SAM and multilayer versus time in saline solution at 37 °C, obtained from XPS detailed scans.

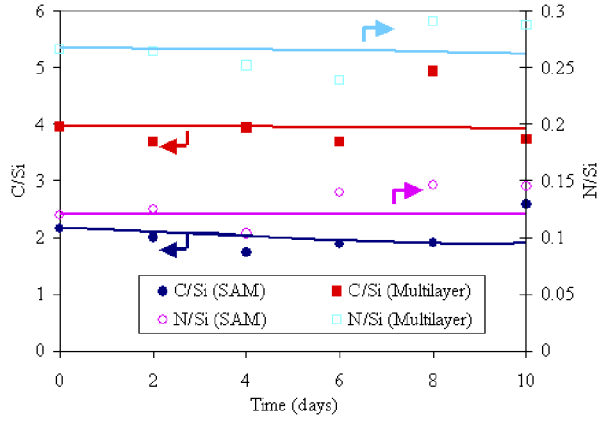

Fig. 6. Elemental ratios of C/Si and N/Si for AUTMS SAM and multilayer versus time in saline solution at 37 °C, obtained from XPS detailed scans.

EDS SAM versus time, which changed from 0.54 to 0.46, and from 0.069 to 0.037, respectively, after 10 days. The stability of EDS SAM is between that of APTMS SAM and AUTMS SAM. The C/Si ratios for OTS, PTMS and GTMS

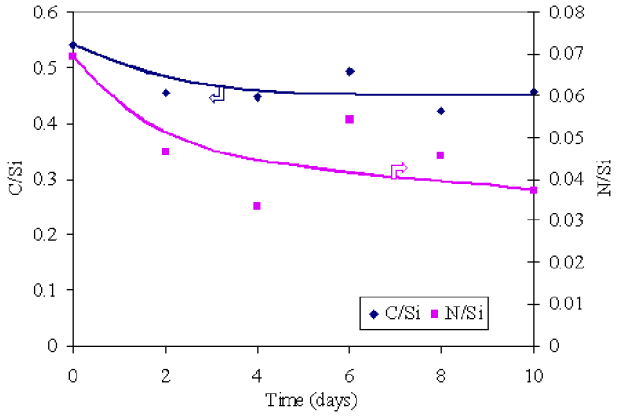

Fig. 7. Elemental ratios of C/Si and N/Si for EDS SAM versus time in saline solution at 37 °C, obtained from XPS detailed scans.

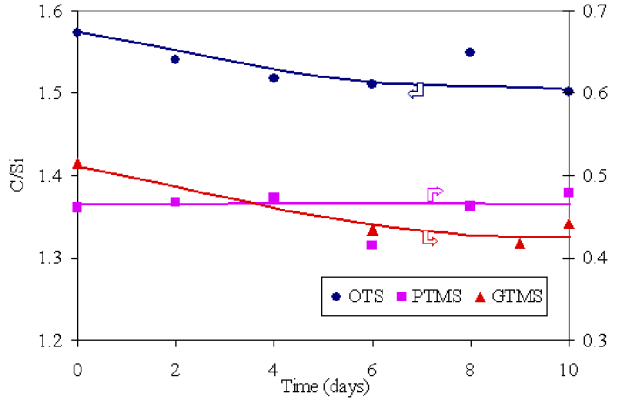

Fig. 8. Elemental ratios of C/Si for OTS, PTMS and GTMS SAMs versus time in saline solution at 37 °C, obtained from XPS detailed scans.

SAMs versus time in saline solution at 37 °C are shown in Fig. 8. The C/Si ratio changed from 1.57 to 1.50, and from 0.52 to 0.44 for OTS and GTMS SAMs, respectively, while this ratio remained nearly unchanged for PTMS SAM. These SAMs with no amine group showed better stability than APTMS SAM as well. The stability results for APTMS, AUTMS and OTS SAMs obtained from XPS agree very well with our FTIR results. However, the PTMS SAM demonstrated a slightly better stability while characterized by XPS than by FTIR.

Taking adventitious carbon contamination into consideration, the percentage of organosilane molecules remaining on the silicon surface after the 10-day saline test at 37 °C are illustrated in Fig. 9. APTMS multilayer and SAM are the worst with regard to the stability, and 92 and 75% of the silane molecules were removed after 10 days, respectively. The degradation rate was calculated as $(C_0 - C_t)/C_0$, where C_0 and C_t are the elemental concentrations of carbon or nitrogen (i.e. C% or N%) before saline test and after saline test for predetermined time, respectively. APTMS multilayer appeared to degrade faster because its C_0 is higher than that of SAM, while the difference of C_t between APTMS multilayer and SAM is pretty small. This resulted in a relatively larger value of $(C_0 - C_t)/C_0$ for APTMS multilayer. Mean-

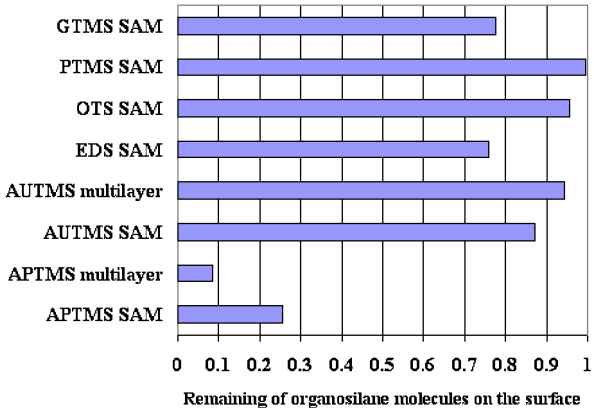

Fig. 9. Fraction of organosilane molecules remained on silicon surface for various SAMs and multilayers after saline test for 10 days at 37 °C.

Table 4
Elemental concentrations on the surfaces of various SAMs and multilayers on RCA Si

	C%	N%	O%	Si%
OTS SAM	49.7	0	18.7	31.6
PTMS SAM	20.7	0	33.8	45.5
GTMS SAM	21.7	0	36.2	42.1
APTMS SAM	33.5	3.4	27.5	35.6
APTMS multilayer	59.4	8.1	22.6	9.9
AUTMS SAM	50.8	2.8	22.8	23.5
AUTMS multilayer	59.4	4.0	21.6	15.0
EDS SAM	23.4	3.0	30.4	43.2

Note. Obtained from XPS detailed scans.

while, only 6 and 13% of the AUTMS molecules was lost from its multilayer and SAM, respectively. PTMS and OTS SAMs demonstrated the best stability among the SAMs and multilayers tested in our study, and only less than 5% of the organosilane molecules were dissolved after 10 days in saline solution. The EDS and GTMS SAMs showed moderate stability.

Another distinct difference between APTMS and AUTMS multilayers is that the former could be more easily built up and made the underlying elemental silicon (Si^0, 97.4 eV) undetectable by XPS. Only silicon at the oxidation state (101.4 eV), mainly from the organosilane molecules was detectable for the APTMS multilayer before the saline test (Fig. 10a). The thickness of APTMS multilayer was previously measured to be between 40 and 60 nm by ellipsometry, while the probing depth of XPS was only 6.9 nm for Si2p [28]. After staying in saline solution for 10 days, due to significant loss of the APTMS molecules, elemental silicon (Si^0) reappeared (Fig. 10b). On the other hand, significant portion of Si^0 was still detected for AUTMS multilayer on silicon, because the combined thickness of AUTMS multilayer and SiO_x on RCA Si is less than 6.9 nm. After staying in saline solution for 10 days, there was no significant change in the Si2p_3 spectrum for AUTMS multilayer (Figs. 10c and 10d). The Si2p_3 spectra can be de-convoluted into three peaks, and their area percentages are listed in

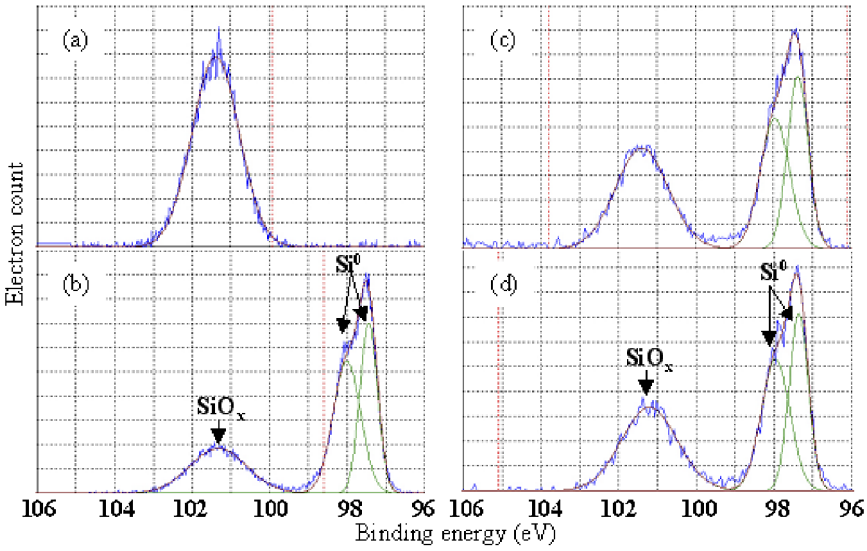

Fig. 10. XPS Si2p_3 spectra of (a) APTMS multilayer on silicon pre-saline test; (b) APTMS multilayer on silicon after 10 days in saline at 37 °C; (c) AUTMS multilayer on silicon pre-saline test; and (d) AUTMS multilayer on silicon after 10 days in saline solution at 37 °C.

Table 5
Curve-fitting results for Si2p_3 XPS spectra of APTMS and AUTMS multilayers on RCA Si

		Si^0 (97.40 eV)	Si^0 (98.00 eV)	SiO_x (101.32 eV)
RCA Si		32.5%	48.3%	19.2%
APTMS	Pre-test	0%	0%	100.0%
Multilayer	Post-test	31.9%	40.6%	27.5%
AUTMS	Pre-test	28.4%	29.8%	41.8%
Multilayer	Post-test	28.8%	31.8%	39.4%

Table 5. There is a SiO_x layer on the RCA Si surface (approximately 1.5–2 nm thick), which corresponds to 19.2% of the area of the whole Si2p_3 spectrum (Table 5). After saline immersion for 10 days, 27.5 and 39.4% of the Si2p_3 area are in the oxidation states (SiO_x) for APTMS and AUTMS multilayers, respectively, which is from the organosilane molecules and SiO_x that originally existed on the RCA Si surface.

4. Discussion

The surface morphology, hydrophobicity and chemical functionality of silicon can be modified by depositing organosilane SAMs and multilayers. The ζ-potentials of these SAM and multilayer-modified surfaces can further be controlled by varying the pH of the surrounding environments [29]. Such modified surfaces are capable of modulating the interaction between the alien materials and the host, with or without further attachment of bio-molecules. Our AFM and contact angle measurement results demonstrated that the SAMs and multilayers were successfully deposited on the RCA Si surface. Such modified surfaces have great potential in the applications of biomedical devices that are in direct contact with human tissues. Therefore their stability is a very crucial criterion in evaluating their in vivo applicability.

Real-life physiological environments are generally harsher than the stability test we conducted at 37 °C in saline solution. Hammerle et al. report that the silicon based biomedical microdevices was stable in saline test for over 21 months, but their surface was corroded within 6 to 12 months while it was tested in vivo on animals [30]. We use 37 °C in saline solution as the initial screening test of the biomaterials to be implanted. If poor stability was observed on the samples, they will not be evaluated in vivo on animals.

Both FTIR and XPS results indicated that the APTMS SAM and multilayer on silicon were very unstable when they were exposed to saline solution at 37 °C, with a significant amount of the APTMS molecules dissolved in two days. AFM imaging also showed that the APTMS multilayer was unstable in saline solution. This is very undesirable if such coatings are intended for applications within the human body. Currently, APTMS is still frequently used to introduce amine groups on material surfaces, which can be utilized to covalently attach peptides and proteins (including antigens, antibodies and enzymes), heparin, hyaluronan, DNA segments, and so on. However, the stability of such coating was not investigated previously. We observed that the instability of the APTMS SAM and multilayer resulted in the instability of subsequently immobilized heparin (via carbodiimide coupling reaction) and hyaluronan on top of them, which is detrimental to the anticipated long-term biocompatibility [18]. Interestingly, Puleo reported that the APTMS multilayer was also unstable on some alloys [7]. About 65% was lost on Co–Cr–Mo alloy surface, and nearly totally lost on Ti–6Al–4V alloy surface after staying in a cell culture medium (humidified, 5% CO_2) at 37 °C for 72 h. Stenger et al. reported that short-chain aminosilane films on solid

surface were highly disordered [31]. However, PTMS SAM is very stable under the same test conditions, although it is poorly ordered on the surface as well. A well defined, highly ordered and oriented SAM can only be obtained when there are more than 10 CH_2 units in the alkyl chain [32]. PTMS and APTMS have the same alkyl chain length, while APTMS has an additional primary amine group at the end. The presence of this amine group is obviously responsible for the instability. We suspect that the hydrogen bonding between the hydrolyzed APTMS monomers and silanol groups (Si–OH) on the RCA Si surface, and among the hydrolyzed APTMS monomers, prevented the formation of stable SAM and multilayer [33]. Baking at 120 °C for 30 min did not improve their stability at all. Further investigation is needed to reveal the causes of the instability of APTMS SAM and multilayer.

On the other hand, amine-terminated silane with longer alkyl chain, AUTMS, generated SAM and multilayer with much better stability than APTMS. Hydrogen bonding among the silane molecules, and between the silane molecules and the hydroxylized surface is still present for AUTMS [24], but stronger van der Waals attraction among the long alkyl chain ($(CH_2)_{11}$) mitigates the disrupting effect from hydrogen bonding, and AUTMS monomers have a greater tendency to form a closely packed two-dimensional structure. Thus closely packed layers are effective in preventing water penetration, and hence demonstrate better stability in saline test. The thickness of the AUTMS multilayer is less than 6.9 nm on RCA Si (based on the XPS results) [28], while the APTMS multilayer could be over 40 nm in thickness. Hydrogen bonding in amino-terminated silanes with 18 CH_2 units made its SAM less well-oriented than the alkyl silane with the same alkyl chain length [24].

APTMS and AUTMS multilayers have higher C and N contents, yet lower Si content than their corresponding SAMs, meaning more organic coatings are present on the multilayer deposited silicon surfaces. The APTMS multilayer is significantly less stable than its SAM, while the AUTMS multilayer is slightly more stable than its SAM. The mechanism for the difference is under further investigation.

GTMS is an important coupling agent because of the reactive epoxy group at the end. It can be used to increase adhesion, and an alternative route to introduce amine groups on surfaces can be designed based on it. However, the bulky epoxy group at the end and the short alkyl chain prevent the formation of well-oriented SAM as well. Approximately 77% of GTMS molecules on the GTMS SAM coated silicon surface were retained after 10-day staying in saline solution.

If SAMs are ideally formed on silicon surface, OTS SAM should have the highest carbon content (C%) on the surface, because OTS has the longest carbon chain. On the contrary, as shown in Table 4, AUTMS SAM has slightly higher C% on the surface than OTS SAM. Meanwhile, APTMS SAM has higher C% on the surface than the SAMs formed by PTMS and GTMS. There are two possible reasons for this: (1) amino-terminated surfaces are more prone to be contaminated by dusts in the atmosphere (size varies from tens of nanometers to a few microns) [11], which are of hydrocarbon nature; (2) due to hydrogen bonding, aggregates are more readily formed on the amine-containing organosilane SAMs. Unlike APTMS and AUTMS SAMs, the C% on EDS SAM surface was much lower, while slightly higher than on the surfaces of PTMS and GTMS SAMs. Due to the presence of amine groups, amino-terminated silanes are self-catalytic to form SAMs. Therefore, some research groups prepared them at room temperature [6,23]. However, a sub-monolayer instead of a complete coverage was often formed [29]. We followed the procedure used by Stile et al. [23] to prepare the EDS SAM at room temperature for 5 min, and acetic acid was used as the catalyst. The EDS SAM obtained by this means is believed by us to be a sub-monolayer as well, because the nitrogen content (N%) in it is not twice of that in the APTMS and AUTMS SAMs.

Among the SAMs and multilayers tested, OTS and PTMS provided the SAMs with the best stability. Nihei et al. reported that hydrophobic organosilane coatings are more effective in enhancing hydrolytic stability than hydrophilic organosilane coatings, while tested in vivo in the oral cavity for years [34]. Water was believed to be able to penetrate SAM and multilayer coatings, especially hydrophilic ones, through imperfections [35,36]. This process caused the surface to reconstruct, and even displaced the organosilane molecules on the surface. Therefore, poor stability was often observed for hydrophilic SAM and multilayer coatings. On the other hand, hydrophobic coatings are typically more effective in preventing water penetration, and thus demonstrate better stability in aqueous environments. Kim et al. found that water penetration even made octadecyltriethoxysilane ($CH_3(CH_2)_{17}Si(OCH_2CH_3)_3$) SAM on mica, which is very hydrophobic, unstable in aqueous media [37].

5. Summary

Although APTMS SAM and multilayer are still widely used in the field of biomedical applications, their poor stability in saline solution at 37 °C makes them inappropriate for long-term applications, especially for implantable biomedical devices. However, AUTMS SAM and multilayer are much more stable, due to the longer alkyl chain ($(CH_2)_{11}$) in AUTMS monomer and stronger van der Waals interaction. Alkyl terminated SAMs demonstrate excellent stability, regardless of the alkyl chain length, and glycidyl terminated SAM and EDS SAM show intermediate stability. The instability of APTMS SAM and multilayer also results in the instability of subsequently attached bio-molecules on top of them [18]. Alternate routes must be sought to produce a stable ultra-thin coating with amino functional groups on the outermost surfaces.

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References

[1] A. Ulman, An Introduction to Ultrathin Organic Films: From Langmuir–Blodgett to Self-Assembly, Academic Press, New York, 1991.
[2] P.X. Zhu, M. Ishikawa, W.S. Seo, A. Hozumi, Y. Yokogawa, K. Koumoto, J. Biomed. Mater. Res. 59 (2002) 294.
[3] D. Kleinfeld, K.H. Kahler, P.E. Hockberger, J. Neurosci. 8 (1988) 4098.
[4] P.B. Chowdhury, P.F. Luckham, Colloids Surf. A: Physicochem. Eng. Aspects 143 (1998) 53.
[5] L. Kam, W. Shain, J.N. Turner, R. Bizios, Biomaterials 23 (2002) 511.
[6] L. Jin, A. Horgan, R. Levicky, Langmuir 19 (2003) 6968.
[7] L. Cen, K.G. Neoh, E.T. Kang, Langmuir 18 (2002) 8633.
[8] S.M. Rowland, K.D. Stanley, Application: US (Cordis Corp (US)), US 1994, p. 11.
[9] D.A. Puleo, J. Biomed. Mater. Res. 37 (1997) 222.
[10] S. Sampath, O. Lev, Adv. Mater. (Weinheim, Germany) 9 (1997) 410.
[11] D.F.S. Petri, G. Wenz, P. Schunk, T. Schimmel, Langmuir 15 (1999) 4520.
[12] J.-C. Lin, W.-H. Chuang, J. Biomed. Mater. Res. 51 (2000) 413.
[13] J.H. Silver, R.W. Hergenrother, J.-C. Lin, F. Lim, H.-B. Lin, T. Okada, M.K. Chaudhury, S.L. Cooper, J. Biomed. Mater. Res. 29 (1995) 535.
[14] J.H. Silver, J.-C. Lin, F. Lim, V.A. Tegoulia, M.K. Chaudhury, S.L. Cooper, Biomaterials 20 (1999) 1533.
[15] D. Wang, Y. Ni, Q. Huo, D.E. Tallman, Thin Solid Films 471 (2005) 177.
[16] D.J. Edell, J.N. Churchill, I.M. Gourley, Biomater. Med. Dev. Artificial Organs 10 (1982) 103.
[17] S.S. Stensaas, L.J. Stensaas, Acta Neuropathologica 41 (1978) 145.
[18] A. Wang, T. Cao, H. Tang, X. Liang, S.O. Salley, K.Y.S. Ng, Colloids Surf. B, submitted for publication.
[19] W. Kern, J. Electrochem. Soc. 137 (1990) 1887.
[20] B. Wu, G. Mao, K.Y.S. Ng, Colloids Surf. A: Physicochem. Eng. Aspects 162 (2000) 203.
[21] O. Sneh, S.M. George, J. Phys. Chem. 99 (1995) 4639.
[22] L.T. Zhuravlev, Langmuir 3 (1987) 316.
[23] R.A. Stile, T.A. Barber, D.G. Castner, K.E. Healy, J. Biomed. Mater. Res. 61 (2002) 391.
[24] K. Bierbaum, M. Kinzler, C. Woell, M. Grunze, G. Haehner, S. Heid, F. Effenberger, Langmuir 11 (1995) 512.
[25] B.Y. Chow, D.W. Mosley, J.M. Jacobson, Langmuir 21 (2005) 4782.
[26] D. Quere, Nature Mater. 1 (2002) 14.
[27] J.E. Headrick, C.L. Berrie, Langmuir 20 (2004) 4124.
[28] D. Briggs, Surface Analysis of Polymers by XPS and Static SIMS, Cambridge Univ. Press, Cambridge, UK, 1998, p. 39.
[29] A. Hozumi, H. Sugimura, Y. Yokogawa, T. Kameyama, O. Takai, Colloids Surf. A: Physicochem. Eng. Aspects 182 (2001) 257.
[30] H. Hammerle, K. Kobuch, K. Kohler, W. Nisch, H. Sachs, M. Stelzle, Biomaterials 23 (2002) 797.
[31] D.A. Stenger, J.H. Georger, C.S. Dulcey, J.J. Hickman, A.S. Rudolph, T.B. Nielsen, S.M. McCort, J.M. Calvert, J. Am. Chem. Soc. 114 (1992) 8435.
[32] C.D. Bain, E.B. Troughton, Y.T. Tao, J. Evall, G.M. Whitesides, R.G. Nuzzo, J. Am. Chem. Soc. 111 (1989) 321.
[33] D. Wang, F.R. Jones, P. Denison, in: K.L. Mittal (Ed.), Silanes and Other Coupling Agents, VSP, Utrecht, 1992.
[34] T. Nihei, S. Kurata, Y. Kondo, K. Umemoto, N. Yoshino, T. Teranaka, J. Dental Res. 81 (2002) 482.
[35] J. Genzer, K. Efimenko, Science 290 (2000) 2130.
[36] M.J. Pellerite, E.J. Wood, V.W. Jones, J. Phys. Chem. B 106 (2002) 4746.
[37] S. Kim, H.K. Christenson, J.E. Curry, Langmuir 18 (2002) 2125.