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Adhesion of Mouse Fibroblasts on Hexamethyldisiloxane Surfaces with Wide Range of Wettability

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Running Heads: Adhesion of Fibroblast on Surfaces with Wide Range of Wettability

Abstract: Surface wettability is an important physicochemical property of biomaterials, and it would be more helpful for understanding this property if a wide range of wettability are employed. This study focused on the effect of surface wettability on fibroblast adhesion over a wide range of wettability using a single material without changing surface topography. Plasma polymerization with hexamethyldisiloxane followed by oxygen (O_2) plasma treatment was employed to modify the surfaces. The water contact angle of sample surfaces varied from 106 degrees (hydrophobicity) to almost 0 degrees (super-hydrophilicity). O_2-functional groups were introduced on polymer surfaces during O_2-plasma treatment. The cell attachment study confirmed that the more hydrophilic the surface, the more fibroblasts adhered in the initial stage that includes on super-hydrophilic surfaces. Cells spread much more widely on the hydrophilic surfaces than on the hydrophobic surfaces. There was no significant difference in fibroblast proliferation, but cell spreading was much greater on the hydrophilic surfaces. These findings suggest the importance of the surface wettability of biomaterials on initial cell attachment and spreading. The degree of wettability should be taken into account when a new biomaterial is to be employed. Further research of surface wettability on adhesive molecules is necessary for a better understanding of this property.

Key words:

44.00 cell adhesion, 100.00 fibroblasts, 115.00 glow discharge (RF/Plasma),

132.00 hydrophilic, 250.00 surface modification

INTRODUCTION

These days, the use of biomaterials keeps increasing, especially in the field of orthopedic implantation and tissue engineering. The surface properties of an implanted biomaterial are of the utmost importance for its biocompatibility. As for a biomaterial surface, the cell-material interaction is strongly influenced by not only surface topography but also by surface chemistry (physicochemical property) including surface wettability (surface energy) and surface charge[1-5].

Surface wettability is an important physicochemical property that could regulate the cell behavior, and many studies have been done to assess the effects of surface wettability on cell behavior by different methods. A large number of studies indicated that cells tended to attach onto hydrophilic surfaces than onto hydrophobic surfaces[6-10]. On the other hand, it has been reported that cells adhered and proliferated at the highest rate when cultured on a substrate with hydrophobic surfaces[11] or a contact angle of around 70 degrees, and cell adhesion and proliferation rates were lower on more hydrophilic or more hydrophobic surfaces[3, 12,13]. These confusing results were considered to be based on the different materials used, different surface topographies employed, and the narrow ranges of surface wettability employed[11, 14, 15]. It should be noted that quite different topographies or substrate materials employed in some of these studies made it difficult to base the results on a single factor[14, 15]. It is considered to be more helpful for understanding this property if a wide range of wettability were employed with fewer confusing factors.

Therefore, we intended to clarify this old issue regarding the influence of surface wettability on cell attachment using HMDSO coatings as a single substrate with oxygen plasma treatment that enable altering the wide range of surface wettability by introducing hydrophilic groups without changing surface topography.

Plasma surface modification is an effective and economical way to modify the surface

physicochemical properties including surface wettability of a material. This technique is of growing interest in the treatment of biomaterial surfaces for its' ease of controlling parameters to meet the different needs for surface physicochemical modification. Several research reports have claimed changes in surface biocompatibility in terms of cell attachment and protein adsorption upon plasma treatment.[3, 14, 16, 17]. Plasma polymerization of an organosilicon monomer, hexamethyldisiloxane (HMDSO), has been used by many researchers for its facile and safe monomer, high deposition rate, and the capability of varying deposition conditions. The HMDSO films by plasma polymerization were generally formed free of pin-holes, adhere strongly to a wide range of materials, and are higher resistant to chemical and physical treatment due to their high degree of cross-linking[18-20]. Our previous studies have demonstrated that the hydrophobic surface of HMDSO could be altered into a super-hydrophilic surface by means of O_2 plasma treatment[21]. Modification of organic polymers by O_2 plasma treatment introduces some hydrophilic groups into the surface but do not change the surface topography and the composition to a great degree. Accordingly, plasma surface modification is useful for clarifying the influence of surface wettability on cell behavior.

The purpose of this study was to use model surfaces with different degrees of wettability to examine their influence on cell attachment, cell proliferation and cell morphology. The goal of this work is to understand how material surface properties influence cellular responses at the material interface so as to develop general principles that can be used to engineer clinically useful implantable devices and tissue-engineered constructs. To obtain more insight into the influence of surface wettability on cell behavior in this study, we prepared a series of HMDSO surfaces with a wide range of wettability by means of O_2 plasma treatment. The initial attachment and proliferation of the fibroblasts on these surfaces were studied.

MATERIALS AND METHODS

Surface Preparation

Plasma polymerization

Thin films of HMDSO [$(CH_3)_3SiOSi(CH_3)_3$, Wako Chemical, Japan] were plasma-polymerized on 15-mm round Thermanox coverslips (Nalge Nunc International, USA) by a plasma-surface modification apparatus (VEP-1000, ULVAC Inc, Japan) using the methods mentioned previously[21]. Briefly, the coverslips were put into the equipment chamber and heated to 125°C before plasma treatment. After a high vacuum (5×10^{-3} Pa) was reached in the chamber, the heated HMDSO monomer (70°C) was introduced into the chamber with a flow rate of 45 sccm (standard cubic centimeter/min, mL/min). When the pressure reached 10 Pa and became stable, the plasma began to be generated by a radio frequency generator at 13.56 MHz. The output power was maintained at 200 W for 10 minutes. After the plasma treatment, nitrogen was used to purge the chamber. The film thickness under these conditions was approximately 100 nm as determined by a QCM-D instrument (QCM-D300, Q-Sense AB, Göteborg, Sweden). Briefly, a frequency shift (? f) of coated HMDSO was converted into the mass (Δm) using the following equation; $\Delta m = (-C?f)/n$. Here C = 17.7 ng Hz^{-1} cm^{-2} for a 5 MHz quartz crystal, n = 1,3,5,7 is the overtone number. The mass was then divided by a density of HMDSO of 0.76g/cm^3 and was finally converted into film thickness of 100 nm. There are no differences in film thickness after O_2 plasma treatment. The sample surfaces were then cleaned with a nitrogen flow and stored in air in clean cell culture disks.

O_2 plasma treatment

The polymerized samples were again introduced into the chamber of the plasma-surface modification apparatus and treated under a low-energy O_2 plasma treatment (10 W, 1.8 Pa, gas flow rate 50 sccm) at room temperature for different time durations (10 s, 20 s, 30 s, 40 s, 60 s, and 80 s).

Surface Characterization

Surface wettability

The surface wettability of these samples was characterized by contact angle measurement with double distilled water using a contact angle meter (CA-P, Kyowa Interface Co. Ltd., Japan). Measurements were made at three different locations on the sample and averaged. The volume of the drop was maintained at 10 μL. A standard contact angle-O2 treatment time curve was drawn. The contact angles of different surfaces were measured hourly for the degree of stability in air and in water.

Surface roughness

The surface roughness of the coverslip surface, the as-coated HMDSO surface, and the O_2-plasma treated HMDSO surface were measured by a profilometer (Handy Surf E-30A, Tokyo Seimitsu, Japan). Measurement was done on three randomly selected fields of each sample and the mean surface roughness (Ra) averaged. Three samples of each type were studied.

Surface topography

The as-coated surface and the 0-degree O_2 plasma surface were coated with Au-Pd alloy. The surface topography was observed with a field emission scanning electron microscope (JSM-6340F; JEOL, Japan) at an accelerating voltage of 15 kV.

XPS analysis

An X-ray photoelectron spectroscopy (XPS) analysis (ESCA-750, Shimadzu, Japan) was performed for the outermost surface composition. The XPS analysis was performed with Mg-Ka as an X-ray source at 8 kV and 30 mA to determine the intensity of Si2p, O1s, and C1s at surfaces with contact angles from 0 to 106 degrees. The binding energy of each spectrum was calibrated with C1s of 284.6 eV for charging correction. Resolution of the instrument was 1.15 eV of FWHM at Ag 3d 5/2. Baseline correction was done with a conventional Shirley method. The intensity ratio of O/C, O/Si and C/Si on the surfaces was then calculated.

Cell Culture Assay

Cell culture conditions

Mouse fibroblast cell lines L929 was used in the following studies. Cells were cultured in culture flasks at 37°C in a 5% CO_2 supplied incubator. The culture media was MEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco). One percent penicillin/streptomycin was added to the media. The culture media was changed the day after the cell seeding and then every three days.

Cell attachment and proliferation

By controlling the time of O_2 treatment, six kinds of discs with surface contact angles of 0, 20, 40, 60, 80, and 106 degrees were produced (106 degrees was used to represent the as-coated HMDSO surface). The sample preparation methods using plasma polymerization and plasma treatment included a sterilization process because these processes were carried out under a high vacuum atmosphere, heating at 125°C and the plasma process. The specimens were aseptically stored until the cell seeding. The cell seeding was done within 1 hour after all samples were prepared. The samples were put into a 24-well culture dish. PMMA rings (inner diameter: 13 mm) were put on the coverslips to avoid possible floating during the cell culture. Freshly confluent flasks of L929 cells were incubated in 1% trypsin (Gibco) for 5 minutes, and then suspended in full culture medium. The cell dilution was at a concentration of 2.5×10^4 cells /mL. Then the cells were seeded into the wells at a density of 2.5×10^4/well (1 mL). After 6, 12, and 24 hours of incubation, cell attachment was measured with the following steps. First, PMMA rings were removed. Second, the discs were washed gently three times with the culture media in the wells to remove any unattached cells; the media was pipetted out; and the discs were washed once with 2 mL PBS. Third, the washed discs were put into a new 24-well culture dish, 0.5 mL trypsin was added, and another 0.5 mL MEM + 10% FBS was added after 5 min. Finally, the cells in each well were counted three times by a Coulter Counter Z1 (Beckman

Coulter, Inc. USA) with the mean representing the attached cells. Before cell counting, cell morphology was observed by an inverted optical microscope.

Cell proliferation on these surfaces was also observed in same manner as the measurement of cell attachment, but the cell density for incubation was changed to 5 x 10^3/well (1 mL), and the culture times were 3 days and 7 days.

CLSM observation (Immunocytochemistry)

The cells were fixed with 1% paraformaldehyde at 4°C for 1 hour, then washed with PBS twice for 5 minutes. The cells were then permeabilized with 0.5% Triton X-100 for 10 min at room temperature. After being washed with PBS, the cells were stained with FITC conjugated phalloidin for 1 hour in the dark at room temperature. The cells were then washed with PBS and mounted for actin examination with a confocal laser scanning microscope (CLSM, LSM-MRC1024, Bio-Rad).

Scanning electron microscopy

The cells were rinsed with PBS and fixed with 2% paraformaldehyde at 4°C for at least 1 hour, followed by dehydration through an ethanol series. The samples were then freeze-dried and sputter coated with a thin layer of Au-Pd alloy. Specimens were observed by a scanning electron microscope (JSM-6340F; JEOL, Japan).

Fibronectin and albumin adsorption assay

Since fibronectin (cell adhesion protein) and albumin (cell adhesion inhibiting protein) are included in the FBS used for cell culture, adsorption behavior of these proteins on the HMDSO surfaces were quantitatively analyzed by the immunofluorescence technique using mixed solution of these proteins. Bovine plasma fibronectin (Fn, Wako pure chemical, Japan) and bovine serum albumin (Alb, Wako pure chemical, Japan) were dissolved in PBS solution at a concentration of 100mg/L and 400mg/L, respectively, with pH of 7.2. Subsequently, 1.0mL of

an equal mixture of each solution was drop onto four kinds of surfaces that had a contact angle of 0, 40, 80 and 106 degree, respectively, and incubated for 20 minutes. These specimens then washed with PBS gently twice for 5 minutes. This was followed by the use of primary antibody solution of rabbit anti bovine Fn (LSL Co. Ltd., Japan) at a dilution of 1:200. After one hour's incubation the samples were rinsed with PBS twice for 5 minutes each. Then the mixed solution of sheep anti bovine Albumin (FITC labeled, 1:500, Bethyl Laboratories, USA), and goat anti rabbit IgG (RITC labeled, 1:200, ICN Pharmaceuticals, USA) was prepared. After incubation in the dark for one hour the samples were rinsed again and then mounted for observation. The samples were then studied by a CLSM (LSM-MRC1024, Bio-Rad). The samples were observed just after the mounting to ensure the validity of the fluorescence intensity. The two channel fluorescence images were obtained simultaneously (red channel for Rodamine and green channel for FITC). 4 parallel samples were employed for the same observation. The images of red (RITC, Rhodamine) and green (FITC) channel of each observation were stored.

For quantitative measurement of the protein adsorbed, the images of red (RITC, Rhodamine) and green (FITC) channel of each observation were opened with Photoshop Software and the L*a*b* value was calculated in four random selected areas (5*5 dot for each) with a color sampling tool and averaged as the L*a*b* value of the channel. The red channel of albumin-only sample and the green channel of Fn-only sample were served as negative control (L_0, a_0, b_0) in calculating the fluorescence intensity (? E*ab) with the formula as: ? E*ab = $[(L_x-L_0) +(a_x-a_0)+ (b_X-b_0)]^{1/2}$. L_x, A_x, B_x represent the L*a*b* value of the red or green channel of a sample.

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Statistical Analysis

A statistical analysis was done by ANOVA, followed by Fisher's PLSD (protected least significant difference) method for multiple comparisons between pairs at p=0.05.

RESULTS

Influence of O_2- plasma Treatment on Surface Wettability

The contact angle of HMDSO surface decreased gradually with the increasing duration of the O_2 plasma treatment (Figure 1). It was reached almost 0 degree, i.e. super-hydrophilicity, when the plasma duration exceeded 80 seconds. The contact angle of this condition was represented by 0 degrees.

The surface contact angle was controllable and repeatable by controlling the duration of the plasma-treatment. In air, the O_2 plasma surface showed an increase of contact angle over time. For the 0-degree samples, it reached about 40 degrees after 24 hours in air. However, when stored in distilled water, it changed very slowly with a slight increase in the contact angle of less than 5 degrees after 24 hours. Accordingly, all the samples were prepared just before the experiment including the cell culture.

Surface Roughness and Microstructure

HMDSO polymerization resulted in a slightly rougher surface on the coverslip surface with Ra from 0.03 to 0.06 um. However, O_2 plasma treatment did not show any significant change in the roughness of the HMDSO surface (both Ra less than 0.08 μm, $p>0.05$). The microstructure of the as-coated surface (contact angle: 106 degrees) and the O_2 plasma surface (contact angle: 0 degree) were both mirror-like surfaces.

XPS Analysis

The relationship between the intensity ratio of O/C, O/Si, C/Si and the contact angle is shown in Figure 2. The intensity ratio of O/C and O/Si increased with decreasing contact angle. On the other hand, the intensity ratios of C/Si decreased with decreasing contact angle. These

results showed that O_2 plasma treatment introduced the hydrophilic groups such as –COOH and decreased the hydrophobic groups such as $–CH_3$ on the surfaces.

Protein adsorption by CLSM

The fluorescence intensity of the proteins (ΔE) was shown in Fig. 4. There was a competitive effect between fibronectin and albumin when they were mixed together, the fibronectin adsorbed much more on a hydrophilic surface while albumin dominated on a hydrophobic surface in a competing mode.

Cell Attachment

The results of the cell attachment protocol are demonstrated in Figure 3. The typical images of the SEM and CLSM results are shown in Figures 4 and 5. L929 cells showed different speeds of attachment with different surface wettability. The hydrophilic surfaces showed more cell attachment during the initial stages (6 and 12 hour, $p<0.05$). After 6 hours of incubation, cells attached very poorly on the 106-degrees surface. Most cells remained round. The hydrophilic surface attracted more cells and the cells seemed to begin spreading. The cell numbers demonstrated a proportional relationship with surface wettability during the initial stage. After 24 hours, all surfaces except the 106-degrees one showed almost the same number of attached cells ($p>0.05$). However, SEM observation showed that the cell morphology on the hydrophilic surface spread much farther than it did on the hydrophobic surfaces. On the 106-degrees surface, most of the cells were round with some very slim cell extending while the 0-degree surface showed patch-like spreading. The number of attached cells of the as-coated surface was only one fourth that of the O_2 plasma ones after 24 hours ($p<0.05$). The results of CLSM observation were comparable to those of the SEM observation. Actin stress fibers were evident on the hydrophilic surface but very little in evidence on the hydrophobic surfaces.

Cell Proliferation

As for cell proliferation (Figure 6), there was no significant difference among the O_2 treated surfaces although an increasing tendency of cell numbers was observed with decreasing the contact angle ($p>0.05$). On the as-coated (106-degrees) surfaces, however, the cell number was low ($p<0.05$). SEM observation of L929 cells cultured after 3 days of incubation are shown in Figure 7. Interestingly, the cells tended to agglomerate on the as-coated surface (106-degrees) while the cells on the O_2-treated surface (80 and 0-degrees) scattered and spread. Actin stress fibers were more evident on the hydrophilic surface under CLSM observation (Figure 5, lower).

DISCUSSION

Many biological applications of material surfaces require improving or preventing cell adhesion for different purposes. A great amount of research has been done on the effects of surface topography, composition, surface charge, and wettability on cell function[1-4, 11, 12, 17]. In the present experiments, we focused on the effect of surface wettability on fibroblast adhesion over a wide range of wettability. The results showed that the initial attachment of cells could be greatly altered due to a change in surface wettability.

Surface wettability can be altered in many ways such as plasma treatment, surface coating, introduction of hydrophilic or hydrophobic groups by self-assembled monolayers (SAMs)[11, 12, 14, 22-26]. Among these methods, plasma treatment (radio frequency glow discharge) has a long history of modifying surface wettability.

Many studies using plasma-treated polymer surfaces with Ar, O_2, NH_3, or N_2 reported increasing the surface wettability and improving the cell adhesion or spreading [9, 10]. In most studies, however, the ranges of surface wettability employed were narrow; 46-67degrees[7], 27-80 degrees[17], 30-92 degrees [25], 15-58 degrees [27]. In contrast to those studies, in the present study, specimens were employed in wide ranges of surface wettability with a water contact

angle from 106 degrees (hydrophobicity) to almost 0 degrees (super-hydrophilicity).

It should be mentioned that in some studies, many other factors, such as surface topography or substrate, were quite different [14-17], which made it difficult to explain the results only by surface wettability. For example, Groth T and Altankov G used five kinds of materials; clean glass, aminopropylsilane, octadecylsilane, polylactate, and silicone (represents contact angles from 25 to 111 degrees) in their studies [6, 8]. Research by Hao et al. showed that CO_2 laser treatment could alter both surface wettability and surface roughness of magnesia partially stabilized zirconia surfaces, and that the combination of surface wettability and roughness affected protein adsorption as well as cell attachment [16]. In the present study, we used the HMDSO coatings as a substrate throughout the experiment, and a low-energy O_2 plasma treatment did not show any significant change in the surface roughness. Consequently, plasma polymerization with HMDSO followed by O_2 plasma treatment enables the controlling a wide range of surface wettability without change in surface roughness.

In our study, XPS analysis revealed that the intensity ratio of O/C and O/Si increased with increasing O_2-plasma treatment duration on the HMDSO surfaces, suggesting the introduction of O_2 functional groups such as carboxyl group with increasing in plasma duration. Accordingly, the increase of surface wettability by O_2 plasma treatment was considered due to the introduction of hydrophilic groups such as COOH and OH [14, 17, 24-26]. The change of the HMDSO monomer structure caused by plasma polymerization and the O_2-plasma treatment that followed can be presumed to be as illustrated in Figure 8. That is, the fragmentation of the Si–C and C–H bonds of the HMDSO monomers were caused by plasma polymerization and changes in the hydrophilic surfaces were concomitant with introduction of O_2-functional groups during O_2-plasma treatment [21].

Although most studies indicated that cells tended to attach onto hydrophilic surfaces than onto hydrophobic surfaces [6-10, 17, 25, 27], some different results have also been reported. Tamada

and Ikada[13] employed various polymer substrates (with contact angles from 18 to 116 degrees) in their study. Mouse fibroblast cell adhesion, cell growth, and cell morphology were observed among the substrates. Fibroblasts adhered and proliferated at the highest rate and showed the highest-ordered morphology when cultured on a substrate with a contact angle of around 70 degrees. On the contrary, both rates were lower on more hydrophilic or more hydrophobic surfaces. They thought that this result might stem from the differences in protein adsorption onto the substrates or from the differences in the amount of proteins synthesized by the cells on the substrates during proliferation. Swart et al. used Ar plasma cleaning on Ti to produce hydrophilic surfaces (contact angle: Ti 62.3 degrees and lower than 20 degrees when plasma duration was more than 1 minute)[14]. Osteoblasts attached lesser when a longer duration was employed, suggesting that the inorganic contaminants generated during the long plasma cleaning reduced the degree of cell attachment. Bumgardner et al. employed a chitosan-coated surface (contact angle 76.4 degrees) and a Ti surface (contact angle 32.2 degrees), resulting that cell attachment on the former surface was better than that on pure Ti[15]. Wang et al. treated the polyhydroxybutyrate surface with lipase or HA coating and a surface contact angle from 40 to 85 degrees, and reported that L929 cells proliferated well on the lipase-treated surface (hydrophobic surface) but very poor on a hydrophilic surface treated by a combination of lipase and HA[11]. These confusing results on between hydrophilic and hydrophobic surfaces were considered to be based on the different materials used, different surface topographies employed, or the different plasma procedures as mentioned above. In contrast to these studies, the present study revealed that a wide range of wettability from hydrophobic to super-hydrophilic was obtained on the same HMDSO polymer surfaces by controlling the plasma treatment duration. A wide range of wettability helps give a full understanding of this property with mirror-like surfaces of all specimens. Only the surface composition, i.e., the ratio of O and C were changed. These groups made it possible to study wettability with fewer confusing factors.

The cell attachment in this study showed that the more hydrophilic the surface, the more fibroblasts adhered in the initial stage. Cells spread much more widely on the hydrophilic surfaces than on the hydrophobic surfaces. There was no significant difference in fibroblast proliferation on the O_2 plasma surfaces, but cell spreading was much greater on the hydrophilic surfaces. These results agreed with many other studies even if a wide range of wettability was employed in this study compared to other studies. In addition, our results indicated that super-hydrophilic surfaces (water contact angle approximately equal to 0 degrees) were more favorable for cell attachment and spreading that were not revealed before.

However, high cell adhesion on super-hydrophilic surface demonstrated in this study contradicts many reports that the super-hydrophilic surface, i.e. non-fouling surface, does not promote protein and cell adhesion [28-32]. The reason for this discrepancy is still unclear. A possible explanation for the differences is considered due to a surface morphology, that is, fluid-like character or rigid character of the surfaces. Many non-fouling surfaces that resist to cell adhesion had a fluid-like character[32]. On the other hand, super-hydrophilic surface of titanium dioxide films[33] or O_2-treated HMDSO films that showed a high rate of cell attachment had a rigid surface. It is also necessary to investigate the protein conformation that influences cell adhesion by using a TOF-SIMS system[29, 30, 34] for better understanding of these phenomena.

Even though almost the same number of attached cells after 24 hours all surfaces between 0 and 80 degrees, cells more spread and had more micro spikes with increasing the wettability. These changes in cell morphology would be expected that they also alter gene expression including the secretion of particular macromolecule such as fibronectin or vitronectin[35].

It is obvious that protein adsorption plays an important role before cell adhesion. Within cell-material interactions, protein adsorption is one of the first occurrences at the solid/liquid interface when it is exposed to a body fluid or a culture media. The nature of the surface

influences the composition and also the conformation of the protein layer and, consequently, subsequent cellular adhesion and spreading. Some types of proteins such as fibronectin and vitronectin have been known to be the main adhesive proteins that promote cell adhesion. The amount and proper conformation of these adhesive matrix molecules influences cellular adhesion and spreading[36, 37]. Some studies showed that the adhesive protein fibronectin tended to adhere onto a hydrophilic surface, which could explain the results of the high initial rate of cell attachment[16, 24]. Recently, the results published by Yoshinari et al.[21] demonstrated the largest amounts of fibronectin adsorption were both at the hydrophobic surface and hydrophilic surface, while less was at moderate hydrophilic surface. That was explained by the hydrophobic interaction on a hydrophobic surface and ionic bonds to hydrophilic surfaces. Besides the amount of adhesive molecules attached, it is known that the conformation of a protein affects its function. Adsorbed fibronectin showed two different conformations according to surface wettability. With a more active conformation on a hydrophilic surface, antibodies tend to bind to the proteins on a hydrophilic surface to a much greater degree than they do on a hydrophobic surface. It is supposed that an adhesive sequence such as RGD is in an active state on a hydrophilic surface[36]. Furthermore, adhesive protein represents only a very little part in the whole proteins of serum. The result of in vitro adsorption tested by only one protein carries with it the risk of biasing the true procedure within the physical condition. Further studies are necessary to clarify the competitive adsorption of adhesive molecules in a multi-protein solution as well as in the protein conformation against wettability.

In summary, surface wettability of HMDSO could be controlled precisely by altering the duration of O_2-plasma treatment without change in the surface morphology. Surfaces with a wide range of wettability of which contact angles were varied from 106 degrees (hydrophobicity) to almost 0 degree (super-hydrophilicity) were obtained in this study. The production of O_2-functional group is one of the reasons for the increase in surface wettability.

Tests of in vitro cell attachment showed that the hydrophilic surface is important for the rapid attachment and spreading of L929 cells. These findings are significant for the assessment and design of new surfaces for biological applications. It is suggested that surface wettability be taken into account in the design of new biomaterial surfaces, especially in orthopedic implants and tissue engineering scaffolds.

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Legends for Figures

Figure 1 Surface contact angle of HMDSO surface with different O_2 plasma durations.

Figure 2 Intensity ratio of O/C O/Si and C/Si on surfaces with series wettability under XPS analysis.

Figure 3 Initial cell attachment of L929 within 24 hours' incubation.

Figure 4 SEM of L929 attached to surfaces with different wettability in 6 and 24 hours in low magnification (original: × 500) and high magnification (original: × 3000).

Figure 5 CLSM images of L929 in 6 hours (upper) and 3 days (lower) of incubation.

Figure 6 L929 proliferations after 3 days (upper) and 7 days (lower) of incubation.

Figure 7 SEM of L929 cells cultured after 3 days of incubation in low magnification (original: x 500) and high magnification (original: x 2000)

Figure 8 Possible structure changes of HMDSO monomers by plasma polymerization and following O_2-plasma treatment.

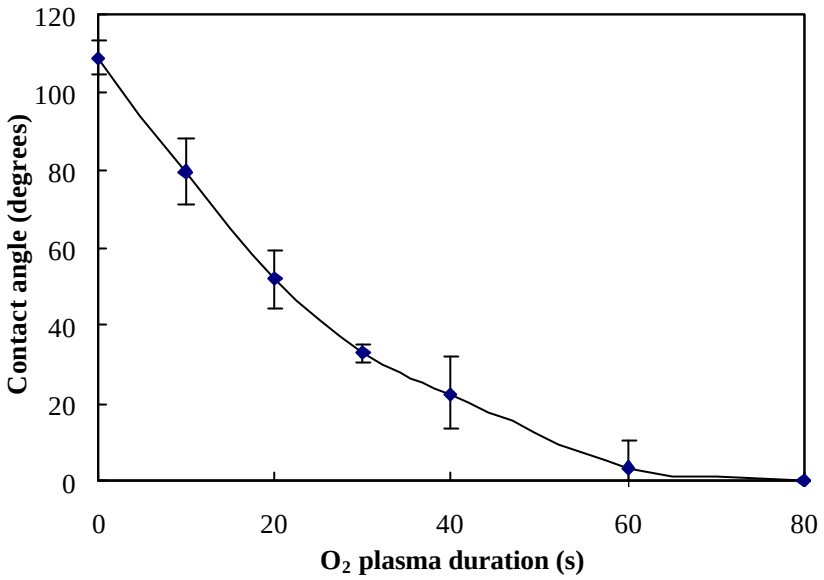

120
100
80
60
40
20
0
Contact angle (degrees)
0
20
40
60
80
O2 plasma duration (s)

Fig. 1

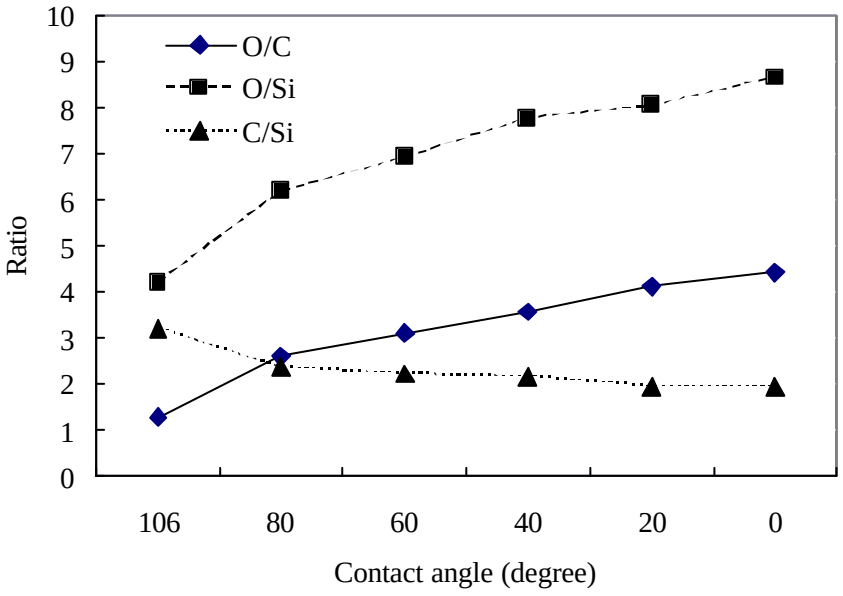

10
9
8
7
6
5
4
3
2
1
0
Ratio
O/C
O/Si
C/Si
106
80
60
40
20
0
Contact angle (degree)

Fig. 2

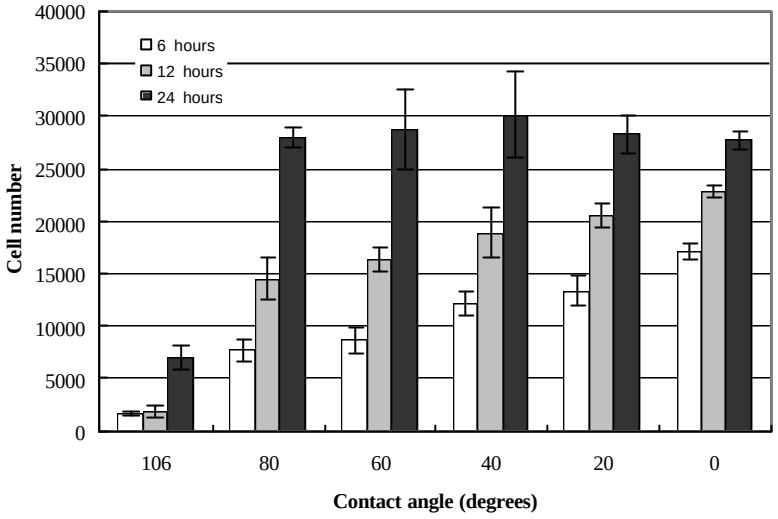
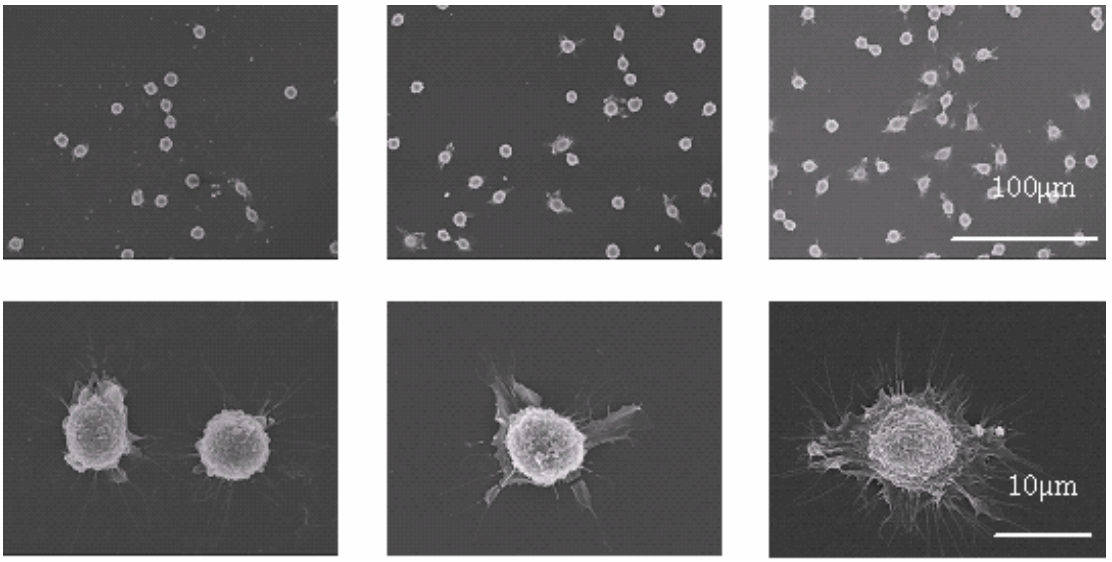
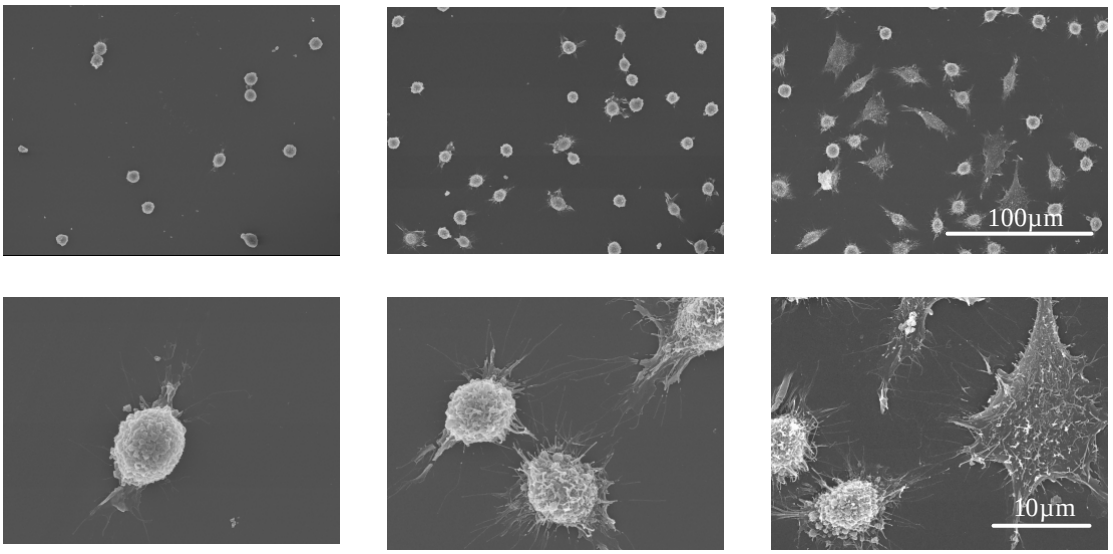

40000
35000
30000
25000
20000
15000
10000
5000
0
Cell number
6 hours
12 hours
24 hours
106
80
60
40
20
0
Contact angle (degrees)

Fig. 3

6 hours



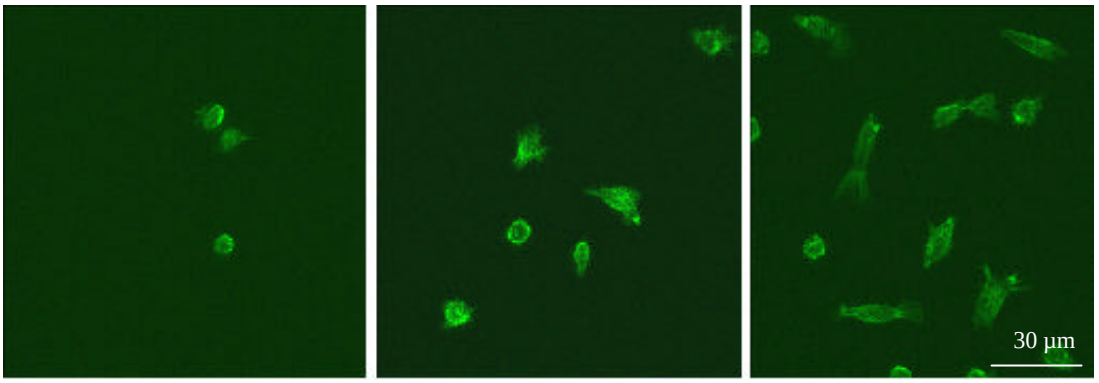
24 hours



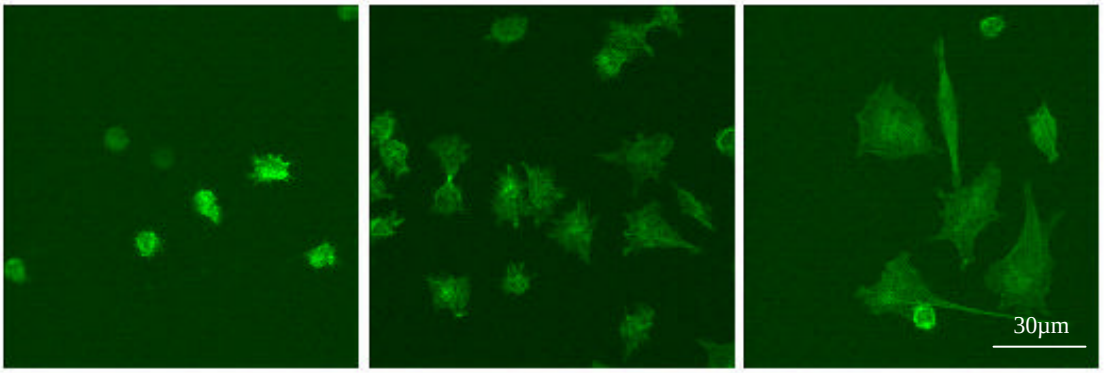
106 degrees **80 degrees** **0 degrees**

Fig. 4

6 hours

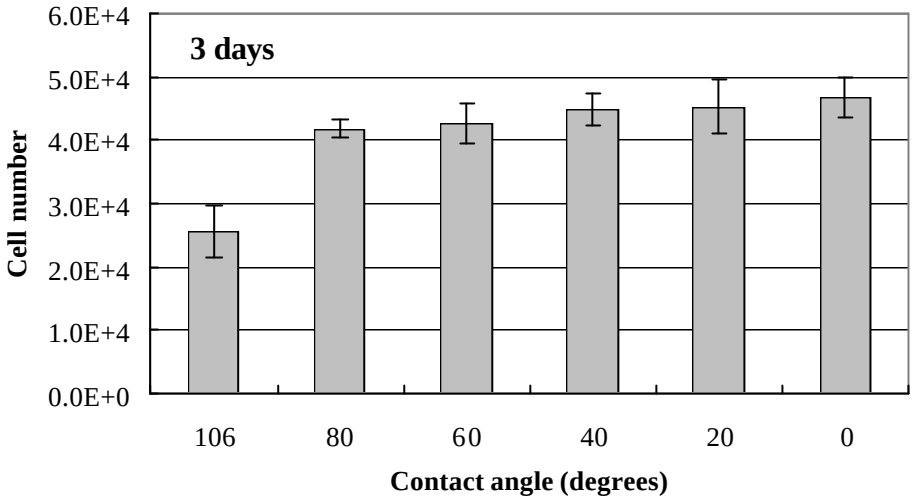


3 days



106 degrees **80 degrees** **0 degrees**

Fig. 5


3 days
6.0E+4
5.0E+4
4.0E+4
3.0E+4
2.0E+4
1.0E+4
0.0E+0
Cell number
106
80
60
40
20
0
Contact angle (degrees)

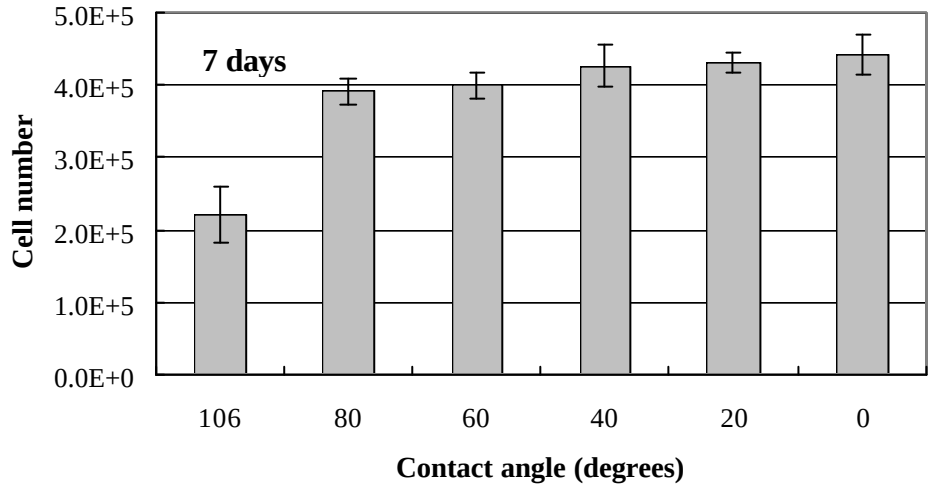
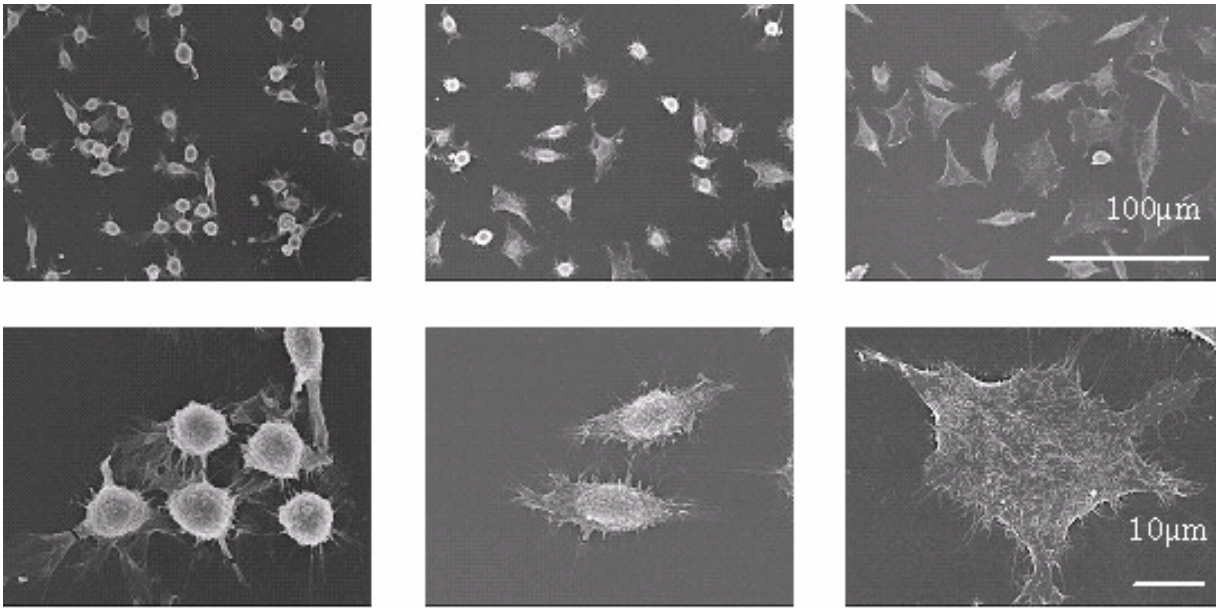
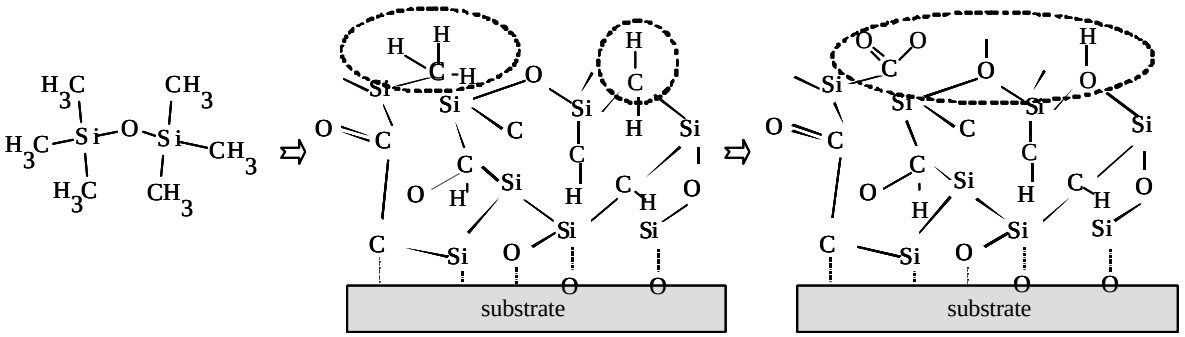

7 days
5.0E+5
4.0E+5
3.0E+5
2.0E+5
1.0E+5
0.0E+0
Cell number
106
80
60
40
20
0
Contact angle (degrees)

Fig.6



106 degrees **80 degrees** **0 degrees**

Fig.7



HMDSO monomer Plasma polymerization O_2-plasma treatment

Fig.8