Physicochemical and Biological Evaluation of Plasma-Induced Graft Polymerization of Acrylamide onto Polydimethylsiloxane

A. Parvin, H. Mirzadeh, M. T. Khorasani

Polymeric Biomaterials Department, Iran Polymer and Petrochemical Institute (IPPI), Tehran, Iran

Received 11 March 2006; accepted 4 September 2007
DOI 10.1002/app.27281
Published online 6 November 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Polydimethylsiloxane (PDMS) rubbers exhibit good mechanical properties for biomedical and industrial applications, but their inherently high hydrophobicity limits biomedical applications of this material despite its favorable mechanical properties. In this work, surface modification of PDMS by radio-frequency glow discharge and subsequently graft polymerization of acrylamide was studied. PAAm-grafted, oxygen plasma-treated, and control (untreated) PDMS rubbers were characterized using attenuated total reflectance Fourier transform infrared, scanning electron microscopy, dynamic mechanical thermal analyses, zeta potential, and contact angle techniques. Fibroblast (L929) cell attachment and growth onto these surfaces were examined by optical microscopy. The data from *in vitro* assays showed that cell attachment onto control surface was very negligible while significant cell attachment and growth was observed onto oxygen plasma-treated and PAAm-grafted PDMS surfaces. The method developed in this work offers a convenient way of surface modifications of biomaterials to improve attachment of cells onto substrates. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 2343–2349, 2008

Key words: polydimethylsiloxane; radio-frequency glow discharge; acrylamide; graft polymerization; biocompatibility

INTRODUCTION

Since the end of the 1950s, silicone has been widely used for medical devices such as breast prosthesis, shunt valve for hydrocephalus, cardiac pacemakers, cochlear implants, artificial skins, temporomandibular joint, contact lenses, catheters, membrane for oxygenator, adhesives, drug delivery systems, drainage implants in glaucoma, maxillofacial reconstruction, replacement esophagus, finger joints, and denture liners.[1–3]

Although silicone has been used mostly as soft tissue substitutes because of its excellent softness, stability, and bioinertness, serious problems have occurred when the silicone devices were implanted for a long time. They include damages to the tissues in direct contact through mechanical friction and dense fibrous tissue formation around the silicone. One possible method to overcome these problems is to modify the silicone surface without bulk deterioration.[1,2]

It is the surface of a biomaterial that first comes into contact with the living body when the biomaterial is placed in the body or fresh blood. Therefore, the initial response of the living body to the biomaterial must depend on its surface properties. Any biomaterial that is to be clinically used for next generation should have excellent properties both in bulk and surface, but it is very rare that a material with good bulk properties also possesses the surface characteristics required for the biomaterial. This is the reason why surface modification is in many cases essential for a material to be applied in medicine.[4]

The low-pressure plasma method provided advantages, such as (a) economic feasibility (small-size instruments, commercially available product, and low energy power); (b) environmental compatibility (dry process, lack of harmful waste); (c) high efficiency (a short treatment time of only a few seconds or minutes); (d) the design of a wide range of surface properties using active gas or active monomers to allow for a particular application; and (e) a wide range of materials such as inorganic materials and organic materials, which are utilized under different treatment conditions.[5,6]

At least two methods are available for the grafting of a polymer surface: coupling reaction of existing polymer chains and graft polymerization of monomers. A terminal group which is reactive to the functional groups present on the substrate polymer surface is required for the polymer chains to be used for the coupling reaction, while the graft polymerization method needs active species on the substrate polymer to initiate radical polymerization.[4,6]

Correspondence to: H. Mirzadeh (h.mirzadeh@ippi.ac.ir).

In this study, plasma-induced graft polymerization is utilized to modify the surface properties of Polydimethylsiloxane (PDMS) with acrylamide (AAm) monomer. PDMS is pretreated with oxygen plasma, generated by radio-frequency glow discharge to introduce peroxide groups onto the surface of PDMS which initiate graft polymerization of acrylamide. The method developed in this work offers a convenient way of surface modifications of biomaterials to enhance cell attachment on the substrates, and enables culturing of cells for a number of biological research applications where cells need to be exposed to well-controlled fluidic microenvironment.

EXPERIMENTAL

Materials

The substrate polymer for plasma-induced graft polymerization was PDMS, M3090 medical grade, purchased from Wacker. The acrylamide monomer (MERCK, Munich, Germany) was recrystallized from chloroform prior to use.

Vulcanization of PDMS

PDMS was milled with 1 phr dicumylperoxide (98%) as curing agent at 60–70°C. The PDMS films were prepared by hot compression molding (150 kg/cm^2, 160°C, 5 min). The vulcanized films with 0.4-mm thickness and 4 cm × 2 cm dimensions were purified by Soxhelt extraction with toluene/methanol (50/50, v/v) for 24 h and then dried in a vacuum oven at ambient temperature.

Glow discharge treatment

Plasma treatment was performed in a vacuum reactor (EMITECH, K1050X) comprising a glass chamber 11 cm in diameter and a pair of electrodes. Power was supplied to the electrodes by a RF generator with a frequency 13.56 MHz and out put of 0 to 100 W.

The PDMS film was located in the center of two electrodes and the chamber was evacuated to a pressure of 0.0001 mbar. Then, by oxygen introduction into the chamber for 3 min with the power plasma of 50 W, the pressure inside the chamber increased to 0.6 mbar. The flow rate of gas into the chamber was 15 mL/min. The plasma-treated films were exposed to air to introduce peroxide groups onto the film surfaces.

Graft polymerization

The plasma-treated PDMS films were removed from the plasma chamber and transferred into a reactor containing aqueous AAm monomer solution (30% w/w). The reactor first was purged by N_2 gas for 20 min, then sealed and kept at 65°C for 24 h for graft polymerization. The residual monomers and homopolymers onto the surface films were removed by Soxhlet extraction in distilled water for 72 h.[6] The amount of grafted PAAm was determined according to the following Eq. (1):

$$\text{Graft level } (\mu g/cm^2) = \frac{w_1 - w_0}{A} \quad (1)$$

where w_1 is the dry weight of the grafted film, w_0 is the dry weight of the initial film and A is the surface area of the film.[7,8]

ATR-FTIR spectroscopy

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy (BRUKER IFS 48) with a KRS-5 prism and an incident angle of 45° was used. Scanning was carried out in the range of 4000–500 cm^{-1} to confirm the formation of grafted PAAm chains onto PDMS surface.

Scanning electron microscopy

The surface morphology was observed with a scanning electron microscope (SEM-Cambridge S-360) operating at 1 kV, after gold coating of the samples using a Polaron sputter coater (BIORAD, E-5200).

Measurement of contact angle

Static contact angles were measured using the sessile drop method by contact angle measurement equipment (Kruss G10). The contact angle was read 1 min after the droplet of the double distilled water (5 μL) was applied. Five measurements on different surface sites were averaged.

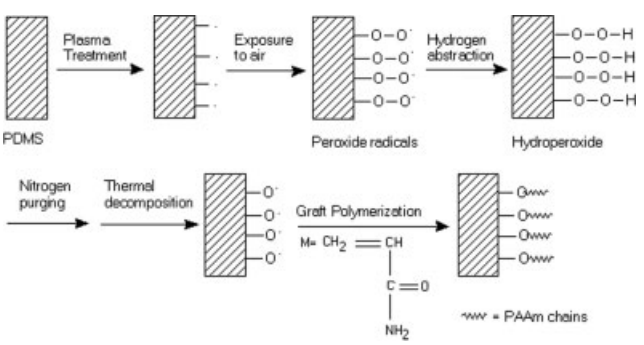

Figure 1 Schematic illustration of plasma-induced graft polymerization (• indicates radical).

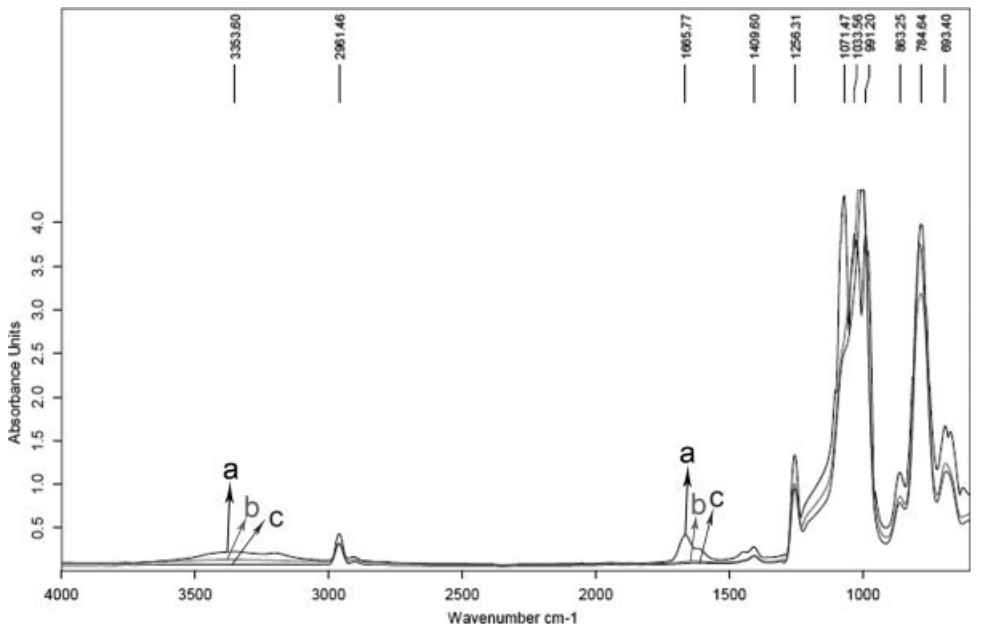

Figure 2 ATR-FTIR spectra of (a) PAAm-grafted, (b) oxygen plasma-treated (50 W, 3 min), (c) control PDMS films.

Zeta potential measurement

The zeta potential measurements were carried out at 25°C and pH 6.5–7 with an Anton Paar electro kinetic analyzer. The sample of PDMS film with dimensions of 40 mm × 20 mm × 0.4 mm was prepared and used from clamping cell. The zeta potential was calculated from streaming potential and four measurements were taken and averaged. KCl solution ($10^{-3}M$) was used as electrolyte.

Dynamic mechanical thermal analyses

The viscoelastic properties of unmodified and modified samples were studied using a dynamic mechanical thermal analyses (DMTA) analyzer; model PL (polymer laboratory). The samples were vibrated in bending mode (single cantilever) at 1 Hz and temperature range from −100 to 50°C.

In vitro test

Cell culture assay

The mouse L929 fibroblast cells (obtained from *in vitro* laboratory of Iran Polymer and Petrochemical Institute) were used in this study. The cells were maintained in PRMI-1640 growth medium, supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin (Gibco BRL laboratories), and 10% fetal calf serum (FCS, Gibco BRL). The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

After 48 h incubation, the monolayer was then harvested by trypsinization. The cell suspension of 4×10^5 cells/mL was prepared before seeding. The samples were sterilized in an autoclave and two samples of each film was placed in a multiwell tissue culture polystyrene (TCPS) plate (Nunc, Denmark) with 5 mL cell suspension and one well kept as a negative control and all maintained in the incubator for 48 h. After incubation, the samples were removed from the incubator and washed immediately in phosphate buffered saline. The cells were fixed in graded ethanol (60, 70, 80, and 95%) for optical microscopic observation using an optical microscope model Nikon, T-B2.5x, Japan.

RESULTS AND DISCUSSION

The schematic reactions of the plasma-treated and AAm grafted films is shown in Figure 1. First, radicals

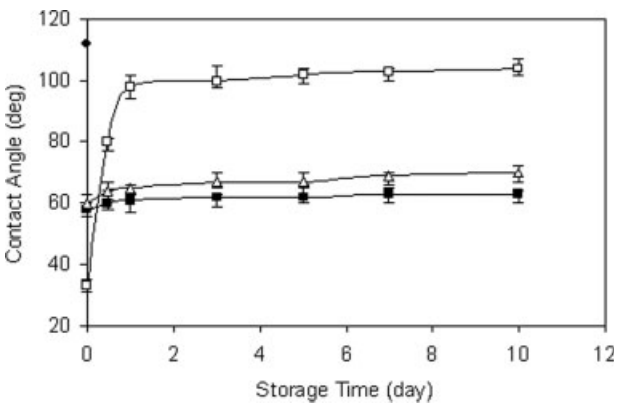

Figure 3 Effect of storage time on contact angle: (●) control, oxygen plasma-treated (50 W, 3 min) films: (□) stored in air, (■) stored in water, and (△) PAAm-grafted (40 μg/cm^2) film stored in air.

are formed on the PDMS films surface. When the films are exposed to air, the produced radicals immediately reacted with oxygen and peroxide radicals were formed. Then hydrogen from neighbor PDMS chains was abstracted to form hydroperoxide groups.[9,10]

It should be emphasized that it is critical to exclude oxygen from the silicone interior as much as possible prior to the graft polymerization process. When degassing of the treated silicone is insufficient, graft polymerization of AAm onto the silicone surface will not occur due to the oxygen inhibition.[1] This implies that silicone contains plenty of dissolved oxygen which inhibits radical graft polymerization. No graft polymerization took place in the presence of monomer when the plasma-treated silicone was not heated to higher than 50°C, indicating that the thermal decomposition of peroxides is essential for the initiation of graft polymerization. No graft polymerization was also observed with the untreated silicone in the same conditions.[1,9]

The ATR-FTIR spectra of the control, oxygen plasma-treated, and PAAm-grafted PDMS films are

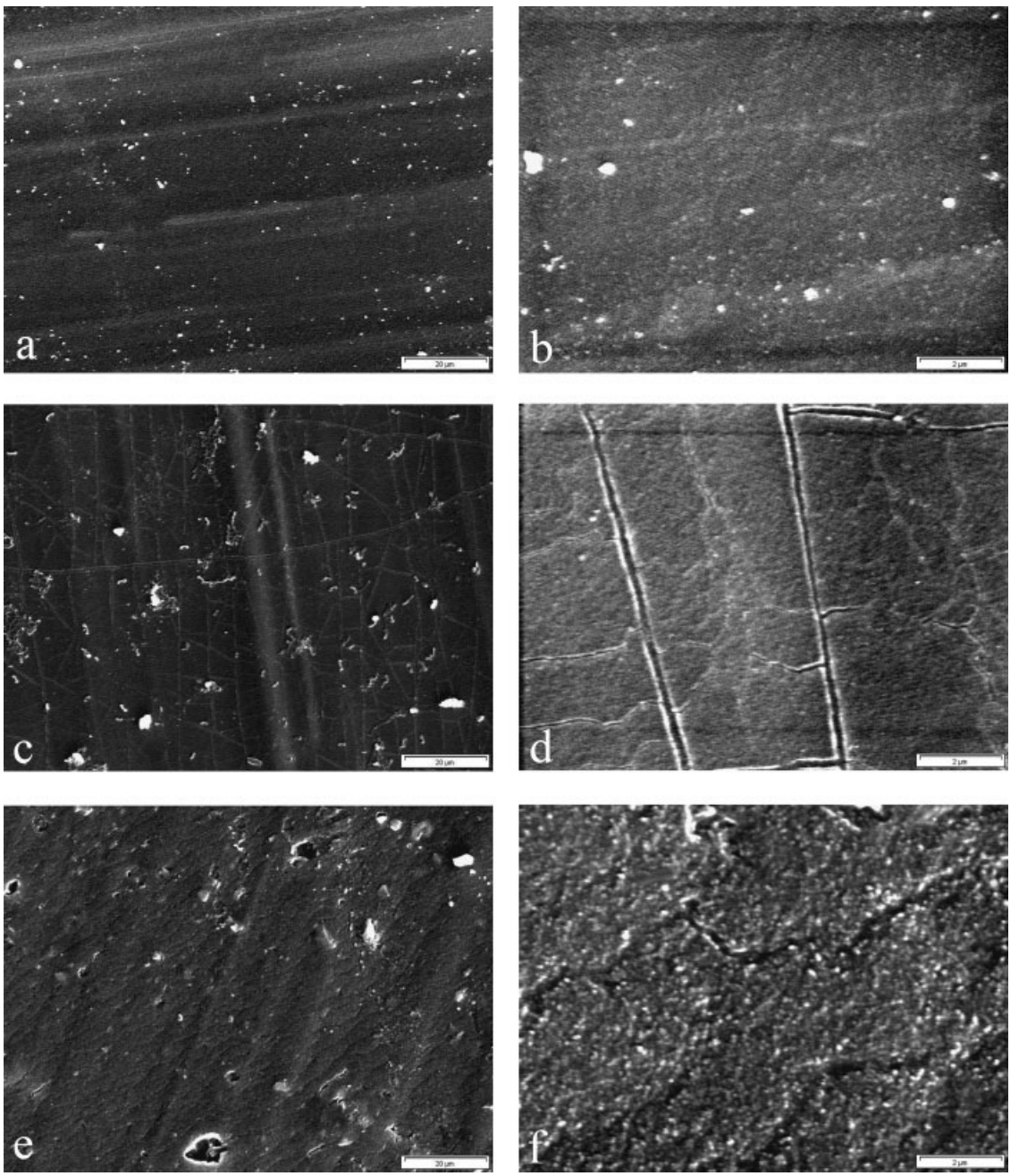

Figure 4 SEM micrographs of (a, b) control, (c, d) oxygen plasma-treated (50 W, 3 min), and (e, f) PAAm-grafted (40 μg/cm^2) films. (a, c, e) ×1000 and (b, d, f) ×10,000.

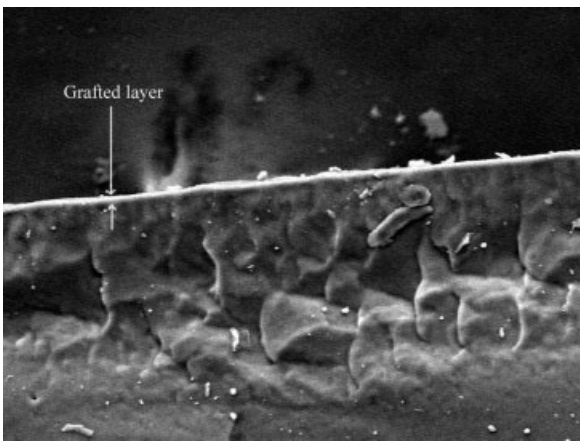

Figure 5 PAAm-grafted PDMS film cross section (×300). Note that the thickness of the grafted layer is about 8 μm.

shown in Figure 2. The vibrations in the range 1630–1670 cm^{-1} and 3200–3500 cm^{-1} correspond to C=O stretching and N—H stretching, respectively.[9,11,12] These groups are absent in the spectra of control and oxygen plasma-treated samples.

Figure 3 shows the static contact angles of water on control, oxygen plasma-treated, and PAAm-grafted PDMS films. The polymeric films without plasma treatment (control) exhibit a water contact angle of 112°, indicating that hydrophobicity of the PDMS films is drastically decreased by oxygen plasma treatment. The film treated with plasma (nongrafted) show a gradual increase in contact angle with time, when stored in air at room temperature.[8,13] Graft polymerization of a water-soluble monomer (AAm) produces permanently hydrophilic macromolecular chains onto the surface of hydrophobic substrates which is entirely different from the oxidation by oxygen plasma discharge. On the other hand, the oxidized surface has only oxygen-containing polar groups that owing to their small size, in comparison with PAAm chains, would become buried in the bulk phase with time.[8] However, the PAAm-grafted films show a stable constant contact angle with time when stored in air.

The SEM micrographs of control, oxygen plasma-treated, and PAAm-grafted PDMS films are shown in Figure 4. The surface of control is smooth with the occasional fragment deposited. However, the plasma-treated surfaces has considerable cracks, also plasma clears the contamination of surface. The hypothesis is that the outermost layers of the film surfaces, which receive the highest plasma irradiation, oxidize to a thin, wettable, and brittle layer. These cracks are corresponding to the silicalike layer.[14] The surfaces of PAAm-grafted films are rougher than the surface of control and oxygen plasma-treated films, as shown in Figure 4.

A cross-sectional view of the PAAm-grafted PDMS film is shown in Figure 5. This figure clearly indicated that the grafted layer was restricted to the surface region of the film with a thickness of about 8 μm.

In Figures 6 and 7 the storage modulus and loss tangent (tan δ) versus temperature are shown in comparison with the control, plasma-treated, and PAAm-grafted PDMS films. Over the temperature range of −100 to +50°C, the plasma-treated and PAAm-grafted PDMS films have lower storage modulus than the control PDMS film. Figure 7 shows that the glass transition peak[15] for the plasma-treated and PAAm-grafted PDMS films appears at −45°C, but this peak appears at −25°C for control PDMS film. This indicates that these conditions of treatment (50 W, 3 min) can affect the mechanical properties. PAAm was grafted on sheet surface of PDMS. They are not blended and because the graft quantity is

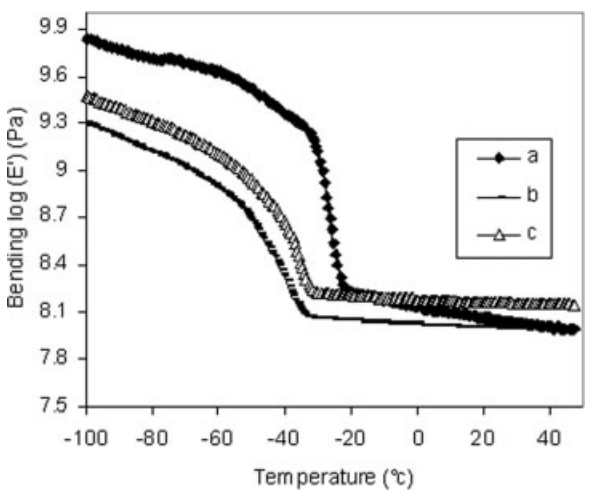

Figure 6 Storage modulus of (a) control, (b) PAAm-grafted, and (c) oxygen plasma-treated (50 W, 3 min) films.

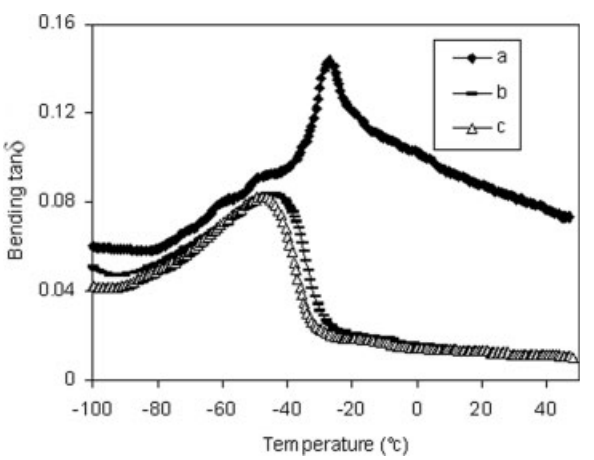

Figure 7 Tan δ for (a) control, (b) PAAm-grafted, and (c) oxygen plasma-treated (50 W, 3 min) films.

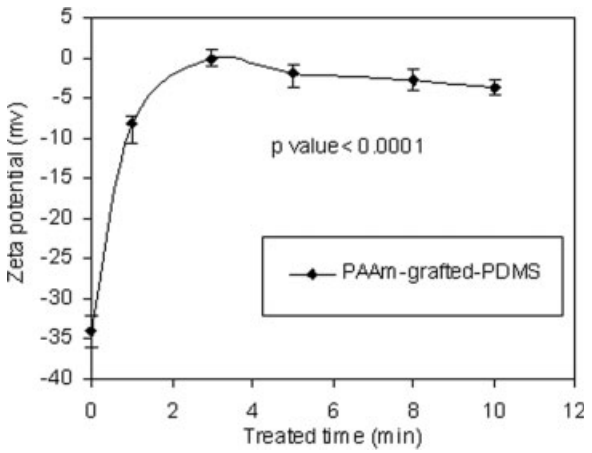

Figure 8 Effect of plasma treatment time on the amounts of zeta potential (ξ) for the PAAm-grafted PDMS film.

very low (40 μg/cm^2), DMTA results show just one T_g.

Figure 8 shows the zeta potential of control and PAAm-grafted PDMS films. It can be seen from this figure that the zeta potential for grafted films approaches to zero with increasing plasma treatment time, but decreases after 3 min. This implies that the graft density increases with increasing plasma treatment time, and therefore, the PDMS surface would be more widely covered with the nonionic, hydrophilic grafted layer. A surface grafted with nonionic soluble chains shows virtually no zeta potential.[16,17] The decrease in the zeta potential is due to the decomposition of peroxide groups with increasing plasma treatment time that causes the amount of PAAm-grafted decreases.[18] The statistical comparison of the zeta potential of control with PAAm-grafted PDMS films was carried out (P value $<$ 0.0001) showing the differences were extremely significant.

The attachment and growth of fibroblast cells onto the control, oxygen plasma-treated, PAAm-grafted PDMS films, and negative control are shown in Figure 9. The cellular behavior on a biomaterial is an important factor to determine biocompatibility. The whole process of adhesion and spreading of cells after contact with biomaterials consists of cell attachment, growth of filopodia, cytoplasmic webbing and flattening of the cell mass, and the ruffling of peripheral cytoplasm, which progress in a sequential fashion.[2,19]

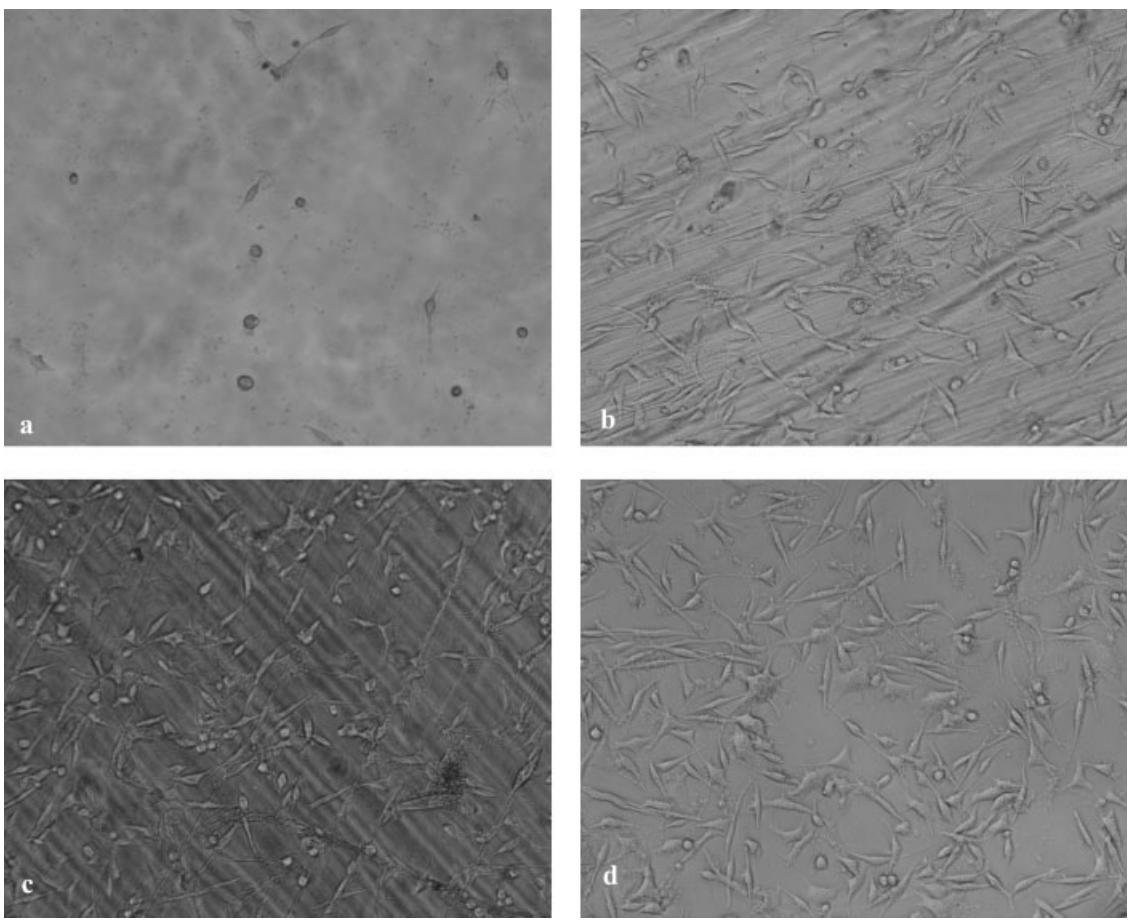

Figure 9 Optical micrographs (×100) of L-929 fibroblast cells cultured on the (a) control, (b) oxygen plasma-treated (50 W, 3 min), (c) PAAm-grafted (40 μg/cm^2) PDMS films, and (d) negative control (TCPS).

Cell attachment onto the control surface [Fig. 9(a)] is very negligible, while improved attachment and growth of fibroblast cells were observed onto oxygen plasma-treated and PAAm-grafted PDMS surfaces [Fig. 9(b,c)] in comparison with negative control (TCPS) [Fig. 9(d)].

As reported in the literature, cell adhesion onto biomaterial surfaces depends upon the wettability of the surface.[19,20] Such observation correlates to oxygen plasma-treated and PAAm-grafted PDMS surfaces, which show better cell adhesion and growth than the PDMS as control which has hydrophobic surface property. However, the electrostatic interaction between the cell and surface has an important role in cell contact. The cells have a negative charge, and therefore, the positively charged surfaces improve cell adhesion and negatively charged surfaces have shown to repel cells.[17,20] Plasma treatment render surfaces positive due to formation of radicals which provide the grafting of AAm monomers onto PDMS surface.[20] Here, the oxygen plasma-treated [$\xi = -9.6$ mV] and PAAm-grafted [$\xi = +0.3$ mV] PDMS films showed more cell adhesion and proliferation than control surface [$\xi = -34$ mV]. Studies have also shown that the surface morphology has a strong effect on cell growth and proliferation.[2,19,20] Hence, it can be concluded that the oxygen plasma-treated and PAAm-grafted PDMS films with submicrometer or nanometer scales roughness will provide an environment for better cell attachment and growth.

CONCLUSIONS

Our results show that the surface of hydrophobic PDMS can be grafted with acrylamide when pretreated with a plasma glow discharge. The graft polymerization is initiated by peroxides formed in the surface region of polymer exposed to glow discharge providing peroxide decomposition by heating in the absence of oxygen.

The ATR-FTIR spectra showed the characteristic absorption bonds correspond to PAAm-grafted onto the surface of PDMS film.

The DMTA results showed that the oxygen plasma can affect mechanical properties of PDMS films.

The enhancement of fibroblast cell adhesion and growth onto the oxygen plasma-treated and PAAm-grafted PDMS films are due to the improved wettability, alteration of the electrostatic charge distribution (zeta potential), and change morphology of the surfaces. This method offers a convenient way of surface modifications to create a new surface to improve biocompatibility with promising biomedical applications.

References

1. Okada, T.; Ikada, Y. Macromol Chem 1991, 192, 1705.
2. Fallahi, D.; Mirzadeh, H.; Khorasani, M. T. J Appl Polym Sci 2003, 88, 2522.
3. Abbasi, F.; Mirzadeh, H.; Katbab, A. A. Polym Int 2001, 50, 1279.
4. Ikada, Y. Biomaterials 1994, 15, 725.
5. Lee, S. D.; Hsiue, G. H.; Kao, C. Y. J Polym Sci Part A: Polym Chem 1996, 34, 141.
6. Karkhaneh, A.; Mirzadeh, H.; Ghaffariyeh, A. R. J Appl Polym Sci 2007, 105, 2208.
7. Lee, S. D.; Hsiue, G. H.; Wang, C. C. J Appl Polym Sci 1994, 54, 1279.
8. Hsiue, G. H.; Lee, S. D.; Wang, C. C.; Shiue, H. I.; Chang, P. C. T. Biomaterials 1994, 15, 163.
9. Khorasani, M. T.; Mirzadeh, H. J Biomater Sci Polym Ed 2004, 15, 59.
10. Khorasani, M. T.; Mirzadeh, H.; Sammes, P. G. Radiat Phys Chem 1999, 55, 685.
11. Zang, J. J Appl Polym Sci 1997, 64, 1713.
12. Suh, T. S.; Joo, C.; Kim, Y. C.; Lee, M. S.; Lee, H. K.; Choe, B. Y.; Chun, H. J. J Appl Polym Sci 2002, 85, 2361.
13. Hsiue, G. H.; Lee, S. D.; Wang, C. C.; Shiue, H. I.; Chang, P. C. T. Biomaterials 1993, 14, 591.
14. Owen, M. J.; Smith, P. J. J Adhes Sci Technol 1994, 8, 1063.
15. Falender, J. R.; Lindsey, S. E.; Saam, J. C. Polym Eng Sci 1976, 16, 54.
16. Fujimoto, K.; Tadokoro, H.; Ueda, Y.; Ikada, Y. Biomaterials 1993, 14, 442.
17. Ikada, Y.; Iwata, H.; Horii, F.; Matsunaga, T.; Tamiguchi, M.; Suzuki, M.; Taki, W.; Yamagata, S.; Yonekawa, Y.; Handa, H. J Biomed Mater Res 1981, 15, 697.
18. Dadsetan, M.; Mirzadeh, H.; Sharifi-Sanjani, N. J Appl Polym Sci 2000, 76, 401.
19. Dadsetan, M.; Mirzadeh, H.; Sharifi-Sanjani, N.; Daliri, M. J Biomed Mater Res 2001, 57, 183.
20. De, S.; Sharma, R.; Trigwell, S.; Laska, B.; Ali, N.; Mazunder, M. K.; Mehta, J. L. J Biomater Sci Polym Ed 2005, 16, 973.