Physical, Mechanical, and Biocompatibility Evaluation of Three Different Types of Silicone Rubber

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ABSTRACT: Silicone rubber as a valuable biomaterial is widely used in medical applications, but its surface properties and low wettability make serious problems in long-term implants. This work was undertaken to evaluate the biocompatibility of modified silicone rubber using two different techniques. A blend of poly(acrylamide) and silicone rubber was compared with virgin silicone surfaces as well as with those modified by laser treatment. Physical and mechanical properties of the samples were examined using different techniques. The hydrophilicity of the silicone rubber increased with increasing hydrogel content and decreased as a result of laser treatment. Both fibroblast cell (L929) and platelet behavior in contact with these surfaces were evaluated *in vitro*. The morphology of fibroblast cells that adhered to the blends was similar to the control. In contrast, on the laser-treated surfaces fibroblast cells showed different proliferation. On the other hand, fewer platelets adhered to the laser-treated surface than adhered to the blend and the unmodified PDMS surfaces. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 88: 2522–2529, 2003

Key words: blends; silicones; poly(acrylamide); hydrophilicity; biocompatibility

INTRODUCTION

Polymeric materials have significantly contributed to the development and improvement of devices and systems in artificial organs. Silicone rubbers have been widely used in biomedical applications. They have good mechanical properties and are both nontoxic and nonirritant biomaterials. Medical devices based on silicone rubbers include, for example, cardiac pacemakers, cochlear implants, artificial skins, contact lenses, oxygenators, catheters, and drug delivery systems.[1–4] Further important properties of silicone rubbers are their ease of fabrication, relative inertness, and stability in contact with tissues in the living organisms; however, because of their surface hydrophobicity, serious problems have occurred when silicone devices were implanted for lengthy periods.[3–9]

It is known that cell–polymer interactions are mainly dependent on the physical and chemical properties of the material surface such as surface free energy, microstructure, rigidity, hydrophilicity, and hydrophobic–hydrophilic ratio. The influence of surface hydrophilicity or hydrophobicity on the biocompatibility of polymers has been discussed in many studies.[8–13]

Although substantial amounts of work on the improvement of the biocompatibility of polymeric materials have been carried out, the results are still inconclusive. Therefore the need for the generation of highly biocompatible materials has been increasing. A variety of approaches have been taken to improve the biocompatibility of polymeric materials. One approach involves surface modification by the grafting of hydrophilic hydrogels, such as poly(acrylamide) (PAAm). This approach was based on the concept that hydrogelated polymers have shown biocompatibility.[1,3,4] In our previous studies, a new approach to improved biocompatibility of silicone rubber has been developed using laser irradiation.[14–16] One of the methods to improve hydrophilicity of silicone rubber is blending with a crosslinked hydrogel.[9] Hydrogels are a class of polymers that possess good biocompatibility in many environments and have hydrophilic properties.[1,3,4] The main drawbacks of hydrogels are their very limited fabrication potential and poor mechanical properties in the swollen state.

Blending of silicon rubbers and hydrogels will result in a biomaterial that has suitable properties of both original materials[3,9,17–20] (i.e., good mechanical properties, wettability, and ease of fabrication).

On the other hand, it has been shown that hydrophilicity of silicone rubbers can be changed by laser treatment.[14–16]

In the present work, blends of the crosslinked poly(acrylamide) (PAAm) powder in a matrix of vulcanized poly(dimethylsiloxane) (PDMS) were prepared and their physical, mechanical and biological properties were investigated. In addition, the biocompatibility of PAAm/PDMS blends was compared with the

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laser-treated silicone surfaces as well as the unmodified silicone rubber.

EXPERIMENTAL

Materials

Acrylamide (Aldrich, Milwaukee, WI), *N,N′*-methylenebisacrylamide (Fluka Chemie, Buchs, Switzerland), 2,2′-azobisisobutyronitrile (Merck, Darmstadt, Germany), acetone (Merck), and poly(dimethyl siloxane) (M3090, medical grade; Wacker, Munich, Germany) were used in this study.

Preparation of powdery hydrogel

Powdery PAAm was synthesized and purified according to a method described by Horn et al.[17] Polymerization was performed at 60°C in a reactor equipped with an oil jacket and a U-shaped mixing blade. A solution containing 38 g acrylamide, 1.14 g *N,N′* methylenbisacrylamide as crosslinking agent, and 0.608 g 2,2′-azobisisobutyronitrile (AIBN) as initiator in 550 mL acetone was added to the reactor. The polymerization was performed at 60°C for 4 h. During the reaction the system was purged with purified nitrogen. The PAAm precipitated from the reaction mixture in the form of fine particles. The slurry obtained was filtered off and the powdery PAAm was thoroughly washed with acetone and dried in vacuum at room temperature.

Purification of PAAm was performed by the following method.[17] The powder obtained from the above procedure was suspended in acetone and shaken vigorously for 3 h. The hydrogel was then filtered off and washed three times with dry acetone. The suspending, shaking, and washing procedure was repeated five times and the powder obtained was dried in vacuum at 50°C. The particles of the prepared powder had submicron dimensions.

Blend preparation

Blends were prepared by mixing the highly purified powdery PAAm with PDMS and 0.8 phr dicumylperoxide (DCP) in a two-roll mill. Three compounds containing 6, 12, and 16 wt % PAAm were prepared. Sheets with thickness of 0.3 and 1 mm were obtained from each compound in a press at 150°C for 10 min. The sheets were postcured at 180°C for 2 h and then were Soxhlet extracted with a mixture of toluene/methanol (1 : 1 v/v) for 16 h.

Laser-treating procedure

Laser treatment was carried out using a line-tunable pulsed TEA CO_2 pulsed laser (Lumonics 103-2, Kanata, Canada), which provides laser beams of wavelength from 9 to 11 μm. The strips of vulcanizate PDMS film (cured with 0.8 phr DCP in hot press, 150°C for 10 min) with 0.3 mm thickness were placed on the belt of a step motor. Both sides of the strips were treated by 10 pulses at the wavelength of 9.58 μm, as reported previously.[14,15]

Physical and mechanical property tests

The morphology of the PAAm and silicone–hydrogel blends was investigated by scanning electron microscopy (SEM, Cambridge S-360; Cambridge Biotech, Worcester, MA).

To measure the water uptake of blend films, three weighed pieces of each blend were placed in distilled water and weighed frequently until they reached their equilibrium swollen state (before weighing the swollen specimens, their surfaces were dried by filter paper). The water uptake of each film was calculated by the following equation:

$$\% \text{ Water content} = \frac{\text{Weight of swollen specimen} - \text{weight of dry specimen}}{\text{Weight of dry specimen}} \times 100$$

Advancing (θ_{ad}) and receding (θ_{re}) contact angles were measured at room temperature by the Wilhelmy plate method using a contact angle measurement apparatus (Krüss K12, Germany). Before measurement, the blends were placed in distilled water to swell to equilibrium at room temperature. Three pieces of different regions of each blend were tested and the mean values are reported. The static contact angle of laser-treated and unmodified silicone rubber against water were also measured by the sessile drop method using a Krüss G10 instrument. Five measurements on different parts of the samples were averaged.

Tensile properties were determined on dumbbell-shape test-blend specimens (1 mm thick, 75 mm length, and 5 mm width), at a crosshead speed of 550 mm/min (Instron 6025). Before testing, the dumbbell-shape specimens were placed in distilled water to reach their equilibrium swollen state.

Dynamic mechanical thermal analyses (DMTA) of the blends were measured in bending mode by Polymer Laboratory (Poole, UK) instrument. Both equilibrium-swollen and dry specimens were tested.

Cell culture assays

The mouse L929 fibroblast cells (obtained from Pasteur Institute of Iran) were used as a test model in this study. The cells were maintained in PRMI-1640 growth medium, supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin (Gibco BRL Labora-

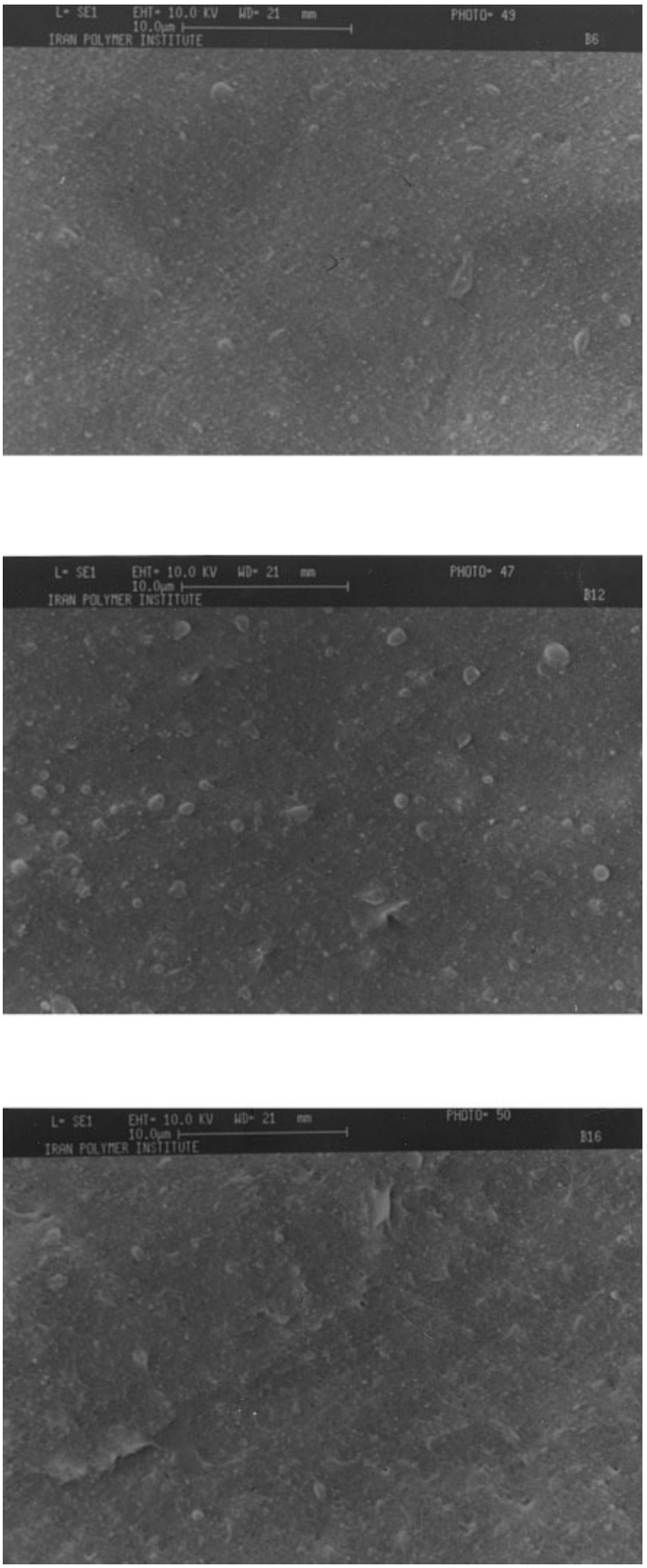

Figure 1 SEM micrograph of blends containing (a) 6%, (b) 12%, and (c) 16% PAAm.

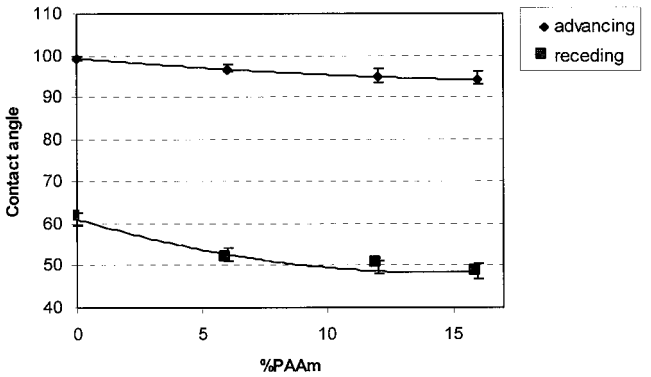

Figure 2 Surface wettability dependency of PDMS/PAAm blends on the amount of PAAm.

tories, Karlsruhe, Germany), and 10% fetal calf serum (FCS; Gibco BRL). A routine subculture was used to maintain the cell line. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C. After a 1-week 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 (except the laser-treated one, which was sterilized by ethanol) and were placed in a multiwell tissue culture polystyrene plate (Nunc, Denmark) with 5 mL cell suspension, with one well kept as a negative control, and then maintained in the incubator for 48 h. After incubation, the samples were removed from the incubator and washed immediately in phosphate-buffered saline (PBS). The cells were fixed in graded alcohol (96, 80, 70, and 60%) and stained with 5% Giemsa for optical microscopic examinations. After microscopic investigations, the total area of the attached cells was determined using an imaging processing system (Image Pro Plus, version 1.33). Ten objective fields of each sample were randomly chosen (except the laser-treated one). Results are expressed as means ± SEM. The unpaired Student's *t*-test was used for all statistical analyses, using Microcal Origin 3.5.

Platelet adhesion test

Platelet adhesion was measured according to a method described by Ikada et al.[21] The PRP (platelet-rich plasma) and PPP (platelet-poor plasma) were prepared from the blood of a healthy human. The platelets were adjusted to 150,000 platelets/mm^3 by adding PPP to PRP. PRP (0.6 mL) was placed on each of the samples in a vial and allowed to stand for 1 h at 37°C. The films were then vigorously washed with PBS and put into 2 mL of 0.1*M* PBS containing 0.5% Triton-X100 to lyse the adhered platelets. Lactic acid dehydrogenase (LDH) activity of the lysate was determined with an enzymatic method to count the adhered platelets with the use of a calibration curve of platelet counts. The experiment of platelet adhesion was repeated three times for the same film using different PRP. All samples were run in triplicate. Results are expressed as means ± SEM.

RESULTS AND DISCUSSION

Physical and mechanical properties

The SEM investigations of powdery PAAm showed that the particles have submicron dimensions (not shown). Attaining to such a fine powder can be related to the method of polymerization, given that in the method of precipitation polymerization the polymer particle size can be controlled by different parameters such as monomer and initiator concentrations and the type of solvent.[22–24] Because the powder was very fine, it could be distributed in the rubber by a two-roll mill. The electron micrograph of a cross section of the blends [Fig. 1(a)–(c)] showed a relatively good distribution of powdery PAAm in the PDMS matrix.

PDMS is a hydrophobic polymer, whereas PAAm is a hydrogel with a high tendency to absorb water. The existence of distributed PAAm in the PDMS matrix caused the product to absorb water. The water-absorbing process of blends was very slow and after 2 weeks the blends reached their equilibrium-swollen state. In this case the water contents of the blends containing 6, 12, and 16 wt % of PAAm were 12.5, 26.1, and 31.2%, respectively.

The contact angle results are shown in Figure 2.

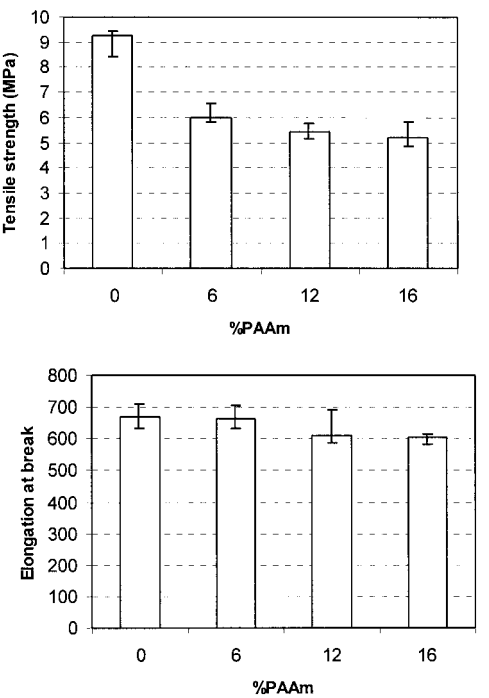

Figure 3 (a) Tensile strength, (b) elongation at break of PDMS/PAAm blends.

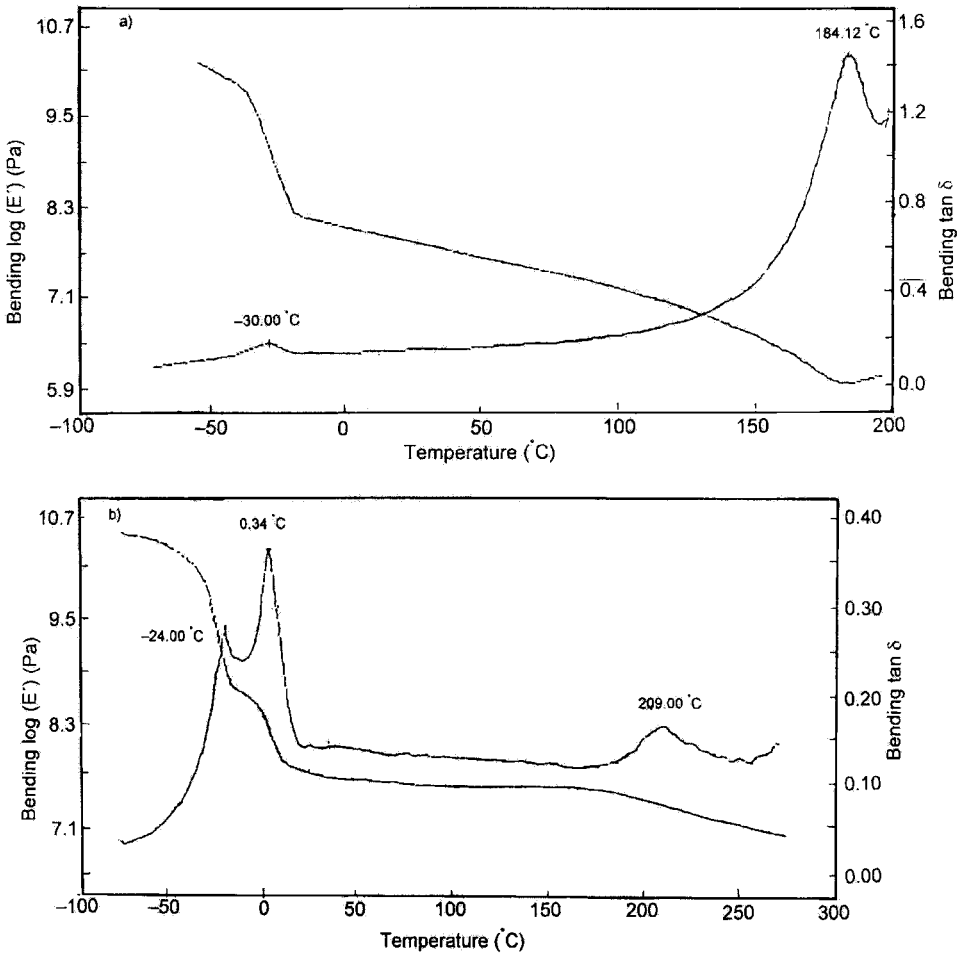

Figure 4 DMTA curves of blends containing 16% PAAm, (a) dry sample, (b) after swelling to equilibrium.

Increasing the amount of PAAm decreased both the advancing (θ_{ad}) and the receding (θ_{re}) angles of the blends, although the slope of θ_{ad} was less than that of θ_{re}. The reason for the observed behavior can be attributed to the structural changes at the solid–water boundary.[25] The hydrophilic groups of PAAm turn toward the aqueous phase within the sample when the blend is exposed to air, but they are able to quickly reorientate in a water environment. In the advancing process when the sample is immersed in water, the water touches a surface with a high density of hydrophobic groups, whereas in the receding process, because of the overturning of hydrophilic groups, the surface is more wettable. On the other hand, the hydrophilicity of a surface has an important role in its biocompatibility, especially in long-term implants.[5–9] In the same way, the increase in hydrophilicity of silicone rubber surface may have a positive effect on the interaction of the surface and tissues and decreases the friction between them.[16]

However, the results of the sessile drop measurements revealed that after laser treatment, the contact angle of PDMS is increased from 98° for the unmodified PDMS to 120° for the laser-treated PDMS. The increase in hydrophobicity of the PDMS surface upon laser treatment was reported previously.[14,15]

Mechanical tests were carried out to study the hydrogel effect on mechanical properties of the rubber. The tensile behavior of the blends after swelling in water is shown in Figure 3. As shown in this figure, the tensile strength of the blends was about 30% lower than that of the unmodified PDMS, but their elongation at break was almost the same. Such a decrease in the tensile strength of blends is attributed to the weak mechanical properties of the swollen PAAm and should be considered in their end-use design, accordingly. However, the blending technique is considerably easier compared with chemical or physical techniques to improve the hydrophilicity of silicone rubber.

DMTA results indicated three peaks in the loss modulus (E') curves of the swollen blends, as shown in Figure 4 for the blend containing 16% PAAm. The peaks consequently are related to the crystal transition of silicone rubber,[26] melting of the frozen absorbed water, and T_g of the hydrogel. Comparison of these curves with the loss modulus curves of the dry blends revealed that after evaporation of water contents in the swollen blends, the glass-transition temperature of

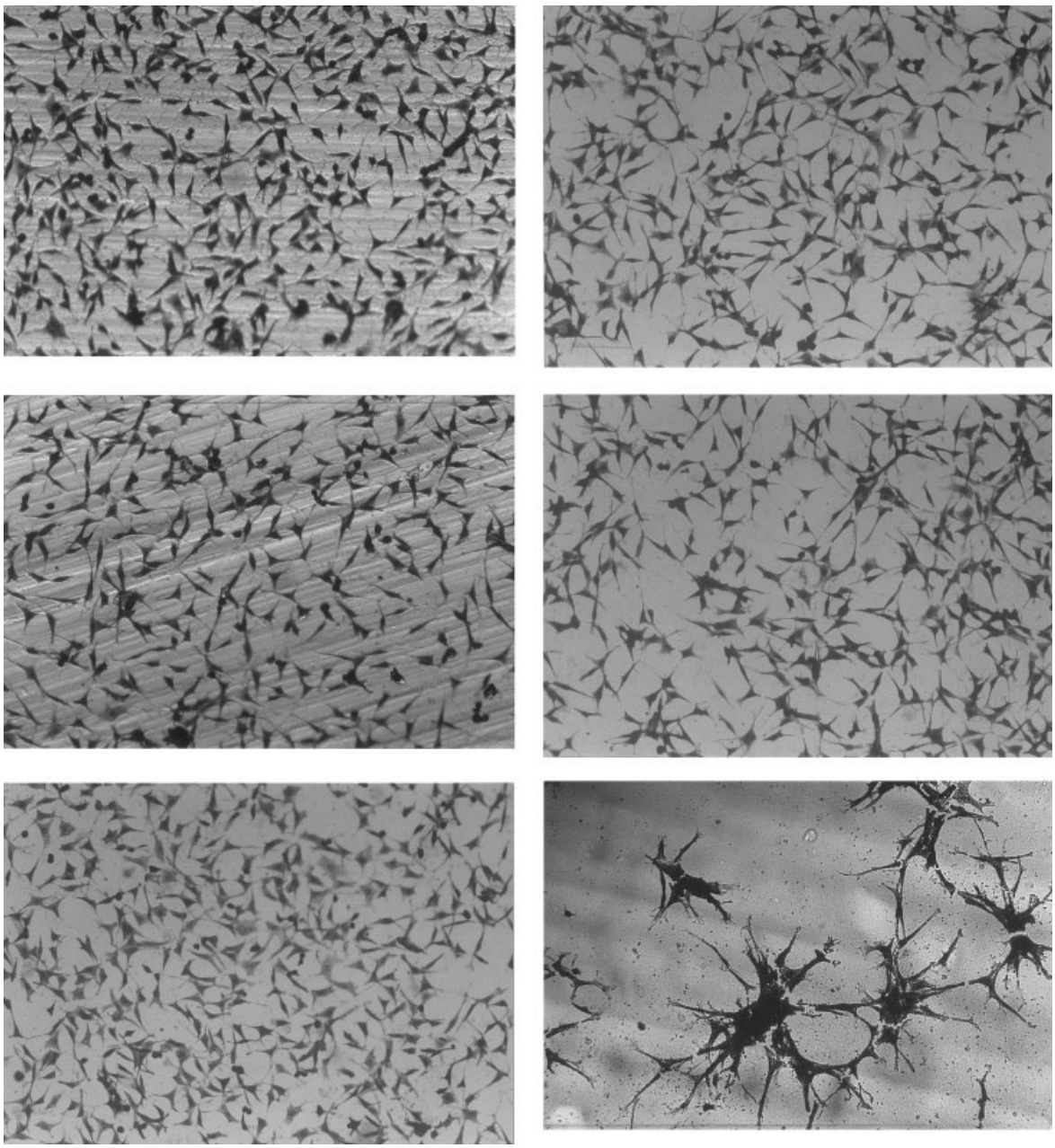

Figure 5 Optical micrographs of L-929 cells cultured for 48 h on the (a) negative control and blends containing (b) 0%, (c) 6%, (d) 12%, (e) 16% PAAm, and (f) laser-treated PDMS (original magnifications ×100).

the hydrogel increased. This behavior is a consequence of the interaction of polymer and solvent. It has been reported that one of the important parameters in polymer behavior is the entanglement density of the molecules. The solution workup (i.e., precipitation of polymer out of dilute or concentrated solutions) alters the entanglement spacing of the polymer as well as the nature of the entanglement coupling, thus leading to changes in the glass-transition temperature of the samples.[27,28] Here, the synthesized poly(acrylamide) used in the blends had precipitated from acetone, which is a nonsolvent for PAAm, whereas water is a good solvent. The difference in the quality of solvents alters the nature of the entanglements as well as the T_g of the polymer.

Cell culture

Cell culture tests were used to evaluate both cytotoxicity and biocompatibility of specimens. The cellular behavior on a biomaterial is an important factor, determining the biocompatibility. The first physiological process that occurs within the initial stages of exposure is the adsorption of biomolecules onto the surface,

TABLE I
Statistical Comparison of Cell Areas on the Samples

Sample	Total area of the attached cells (pixels)
PS (negative control)	18540 ± 1163
Unmodified PDMS	18257 ± 987*
Blend with 6% PAAm	13927 ± 866**,†
Blend with 12% PAAm	14663 ± 974**,†
Blend with 16% PAAm	15075 ± 978**,†

* $p = 0.9$ compared to the negative control.
** $p < 0.03$ compared to the unmodified PDMS.
† $p > 0.3$ compared to the other blends.

and this is usually followed by cellular interactions. The whole process of adhesion and spreading of the cells after contact with biomaterials consists of cell attachment, growth of filopodia, cytoplasmic webbing and flattening of the cell mass, and ruffling of peripheral cytoplasm, which progress in a sequential fashion.[29]

In the cytotoxicity and cell culture method, the growth and proliferation of cells are investigated by comparing with a negative control (polystyrene tissue culture; Nunc) [Fig. 5(a)]. As shown, cell proliferation (i.e., filopodia) and spreading could be observed on both the unmodified [Fig. 5(b)] and the blend samples [Fig. 5(c)–(e)]. The cells were flattened on these samples and there was no change in the morphology. Silicon rubber is a known biomaterial with no cytotoxicity and these results showed the increase of hydrophilicity, caused by the distribution of hydrogel powder in silicon rubber matrix, did not affect its cytotoxicity. On the laser-treated PDMS [Fig. 5(f)], the morphology of cells was changed and they did not flatten on the surface. It seems that the cells preferred to adhere to each other rather than growing on such a surface. Different chemical, physical, and biological parameters present on the surface of synthetic biomaterials may modify the behavior of cells. Wettability is one of the main parameters affecting the interaction of biological species, such as cultured cells with polymeric materials; another main parameter affecting the interaction is surface morphology.[29] It has been reported that laser irradiation of polymeric surfaces led to the formation of different radicals, and hence different peroxides in contact with air. In addition the morphology of the polymeric surface changed with the laser irradiation.[14,15,30] The behavior of the cells on the laser-treated PDMS can be related to both its wettability and surface morphology. As discussed earlier, the hydrophobicity of PDMS increased with the laser irradiation. Because of their wettability and morphology, laser-irradiated PDMS surfaces were not compatible with fibroblast cells.

Cell attachment and spreading were measured using an image processing system, with analysis of optical micrographs after staining the fibroblast cells cultured on the blends and unmodified PDMS. As shown in Table I, the total area of attached cells on the unmodified silicone rubber is similar to that of the negative control ($p = 0.9$). The total area of the attached cells on the blends does not differ significantly with the amount of PAAm ($p > 0.3$), but is less than that with the unmodified PDMS ($p < 0.03$). These results showed that the number of the attached cells or the degree of cell spreading on the blends is less than that of the unmodified PDMS. These observations may be attributed to the change in surface morphology of the rubber. In blends, after swelling of the distributed PAAm particles in contact with water, many hydrated domains were created on the surface, which may have decreased the direct contact of cells with the PDMS surface; thus the cell adhesion and spreading decreased compared with that of unmodified PDMS.

Platelet adhesion

Platelet adhesion onto the films was carried out to learn the extent of interaction of these surfaces with platelets and hence evaluate their blood compatibility. Platelet adhesion on a surface is invariably followed by the appearance of platelet aggregates and platelet spreading and subsequent thrombus formation. In fact, from the standpoint of blood compatibility, a strong interaction between the material and platelets is undesirable. The amount of platelets adhered per unit surface area was calculated under the assumption of a completely smooth surface of the films. The results are shown in Table II, from which it may be seen that the existence of 6% PAAM in blends decreased the number of platelets adhered to the surface, but by increasing the hydrogel, the adhered platelets were increased. In an aqueous solution the distributed hydrogel particles in the blends absorbed water. These hydrated particles altered the interface between the surface and water (given that its contact angle was decreased). In the blend containing 6% PAAm the hydrated particles lowered the direct contact of platelets with PDMS surface; thus, the platelet adhesion was decreased compared with that of the unmodified PDMS. The increase of PAAm in blends increased the surface roughness and chain density, which might affect the platelet trapping attributed to the small dimensions of platelets (2–3 microns) compared with fibroblast cell dimensions (5 microns). Thus increasing the amount of PAAm increased the number of the

TABLE II
Number of Adhered Platelets on the Samples

Sample	Number of platelets/cm^2
Unmodified PDMS	29,000 ± 1141
Blend containing 6% PAAm	21,000 ± 945
Blend containing 12% PAAm	24,000 ± 962
Blend containing 16% PAAm	28,000 ± 1003
Laser-treated PDMS (10 pulse)	14,000 ± 863

adhered platelets attributed to platelet trapping into rough areas. Such behavior was reported by Fujimoto et al.[21] for PAAm grafted polyurethane surfaces. They showed that in low graft levels, the grafted polymer chains may prevent the protein molecules and platelet cells from direct contact with polyurethane surface by the steric hindered effect, although globular protein molecules might be sorbed into the bulk phase of the hydrogel chains if it becomes dense.

On the other hand, the laser-treated PDMS showed the least amount of platelets adhered on the surface. This observation indicated that the platelet adhesion was reduced because of the laser treatment. The effect of laser treatment on the platelet adhesion of the silicone rubber surfaces was reported previously.[16] As a result, the irradiated silicone rubber surface has a lower tendency toward platelet adhesion compared to that of the unmodified silicone rubber and blend samples.

CONCLUSIONS

We found that the blend consisting of PAAm and PDMS showed higher hydrophilicity compared with that of PDMS. The existence of PAAm in PDMS decreased the tensile strength of the rubber but made no changes in its elongation at break. The PDMS–PAAm blends were compatible with fibroblast cells and showed no cytotoxicity. However, the PRP method showed no significant reduction in platelet adhesion onto the blend surface compared to PDMS. On laser-treated PDMS films, because of their hydrophobicity and surface morphology, the cell behavior is changed. A comparison of hydrophilic surfaces (blends) with hydrophobic ones (laser-treated) showed that the silicone rubber–hydrogel blends were more compatible with fibroblast cells, whereas the laser-treated PDMS showed more blood compatibility.

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