Effects of Polydimethylsiloxane Grafting on the Calcification, Physical Properties, and Biocompatibility of Polyurethane in a Heart Valve

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ABSTRACT: Segmented polyurethane (PU) has proven to be the best biomaterial for artificial heart valves, but the calcification of polyurethane surfaces causes serious problems in long-term implants. This work was undertaken to evaluate the effects of polydimethylsiloxane (PDMS) grafting on the calcification, biocompatibility, and blood compatibility of polyurethane. A grafted polyurethane film was compared with virgin polyurethane surfaces. Physical properties of the samples were examined using different techniques. The hydrophobicity of the polyurethane films increased as a result of silicone modification. The effects of surface modification of polyurethane films on their calcification and fibroblast cell (L 929) and platelet behavior were evaluated *in vitro*. Fourier transform infrared spectroscopy indicated the direct involvement of the polyether soft segments of the polymer in the calcification process. Scanning electron microscopy of films indicated that grafting of silicone rubber to the surface of polyurethane successfully prevented the calcification process. The morphology of fibroblast cells that adhered to the PU films and modified films was similar to that of controls and showed the same proliferation. On the other hand, grafting PDMS onto PU did not affect the amount of platelets that adhered to the polyurethane surfaces. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 758–766, 2005

Key words: polyurethanes; modification; calcification; heart valve; siloxanes

INTRODUCTION

The second most common major heart operation in the world is valve replacement. An artificial valve is implanted when hemodynamically significant vascular disease leading to incompetence or stenosis is diagnosed. Such a prosthetic valve can be purely mechanical or made of biological tissue. Mechanical valves have been routinely implanted over the past 40 years and they are relatively durable, but the patient should be on continuous anticoagulation therapy. They fail rarely but failure is often catastrophic to the patient. The bioprosthetic valves tend to be less thrombogenic, but they tend to be less reliable.[1–5]

The search for a durable, nonthrombogenic valve has led to the consideration of synthetic trileaflet valves. Synthetic polymer materials have the advantage over biological materials in being easily engineered, both physically and chemically, able to overcome design features leading to high stresses and early failure, and able to combat specific material interactions with the surrounding environment.[6–12] So far, segmented polyurethane appears to be one of the best compromise materials available in terms of biocompatibility and mechanical flexibility and strength. Therefore, recent attention has been directed toward development of trileaflet polyurethane valves. Such valves have improved hydrodynamic function compared to mechanical valves and are less thrombogenic; however, these same materials have shown severe calcification in physiological environments and may not be suitable for long-term implantation.[3,13–19]

Calcification occurring in polyurethane valves may be an intrinsic property of the polymer. Implant factors affecting the rate of calcification, which have received the most attention, are the effects of calcium complexation absorption, the presence of surface defects, and local stress concentrations. The influence of surface calcium complexation and surface cracks on the calcification has been discussed in many studies.[19–25]

Although many researchers have offered explanations for calcification of polyurethane, there have been very few suggestions on how to stop this phenomenon. One of them involves surface modification because in heart valves the surface properties are especially important, and it is therefore of interest to surface-modify polyurethane, increasing its blood

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compatibility, and at the same time retain its excellent mechanical properties.[26–29]

A number of modification methods have been published, including heparinization and polyethylene oxide grafting,[30] sulfonation,[31] and reaction with prostaglandin derivatives.[32]

In the present work, films of grafting polydimethylsiloxane onto polyurethane were prepared to evaluate calcifical, physical, and biological properties. In addition, the calcification and biocompatibility of silicone-grafted polyurethane were compared to the unmodified polyurethane.

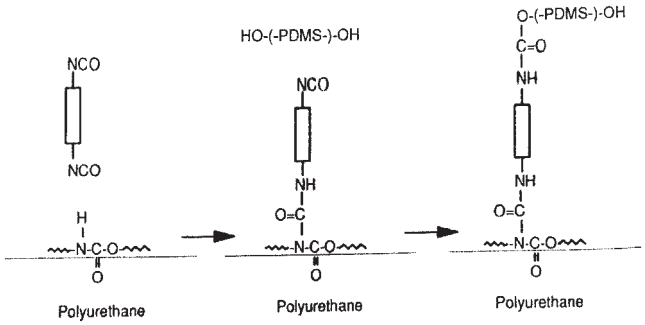

Figure 1 Surface grafting of extracted PU with PDMS. In the first grafting step triethylamine was used as a catalyst. The grafting reactions were carried out in toluene.

EXPERIMENTAL

Materials

Polyurethane (Estane 58,315, BF Goodrich, Westerlo-Oevel, Belgium), dimethyl formamide (DMF, Merck, Darmstadt, Germany), polydimethylsiloxane (Q7 2213 from Dow Corning), toluene (Merck), hexamethylene diisocyanate (HDI, Merck), triethylamine (Jensen Chimera), streptomycin (Gibco BRL Laboratories, Karlsruhe, Germany), L929 fibroblast cells (obtained from Pasteur Institute of Iran), and fetal calf serum (Gibco BRL) were used in this study.

Polyurethane film preparation

Pellets of polyurethane that have a polyether soft segment composed of polytetramethylene glycol and a hard segment of 4,4′-diphenylmethane diisocyanate, chain-extended with butanediol were Soxhlet-extracted for 24 h with methanol to remove processing agents and low-molecular-weight components. Recent evidence suggests that development of polyurethane calcification can be retarded through extraction of low-molecular-weight polymer fraction.[15,20]

The pellets were dried thoroughly in a vacuum oven at 40 °C and then dissolved in DMF to 35% (w/v) solutions. Films with thicknesses from 250 to 300 μm were obtained from this solution in a single dip process onto a plate;[15,23] the solvent evaporation technique was used under positive air pressure in a vacuum oven for 24 h at 70–80 °C.

Grafting of polydimethylsiloxane onto polyurethane surfaces

Polydimethylsiloxane was grafted onto polyurethane surfaces in two steps (Fig. 1). First, polyurethane films were immersed in a toluene solution containing 7.5% (w/v) HDI, 2.5%(w/v) triethylamine as a catalyst. Diisocyanates have been used for activating polyurethane surfaces for a subsequent grafting operation. Formation of allophantes from urethane and isocyanate groups generally does not occur below 100 °C; but in the presence of a catalyst the reaction can take place at lower temperatures. Triethylamine is used as a mild catalyst for the reaction of HDI with polyurethane films at 50 °C for 120 min under N_2 atmosphere. The reaction was relatively slow compared to the rate obtained using more active catalysts, for example, cobalt salts, but an active catalyst made the reaction more difficult to control. If the reaction was run too far, crosslinked brittle films were obtained, and the number of free NCO groups decreased.[33,34]

In the second reaction step, the reacted PU films with HDI were immersed in a toluene solution containing 15% (w/v) PDMS for 24 h at 40 °C. After the reaction the films were rinsed with toluene to remove unreacted PDMS and HDI from the surfaces. The films were characterized after being dried under vacuum at 20 °C for 24 h. To examine the hydrolytic stability of the grafted layers, as well as the leaching out of entrapped PDMS, grafted films were immersed in a Soxhlet extractor with toluene. The leaching of entrapped polydimethylsiloxane from the films was registered as a weight loss after different leaching times in toluene and subsequent drying. The resultant films were dried under vacuum at 40 °C for 24 h.

Physical property tests

Contact angle measurements

The static contact angle of grafted and unmodified polyurethane against water was also measured by the sessile drop method using a Krüss G10 instrument. Five measurements on different parts of the samples were averaged. 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 films were placed in distilled water to swell to equilibrium at room temperature. Five points of different regions of each film were tested and the mean values are reported. Two immersion cycles were made for each film.

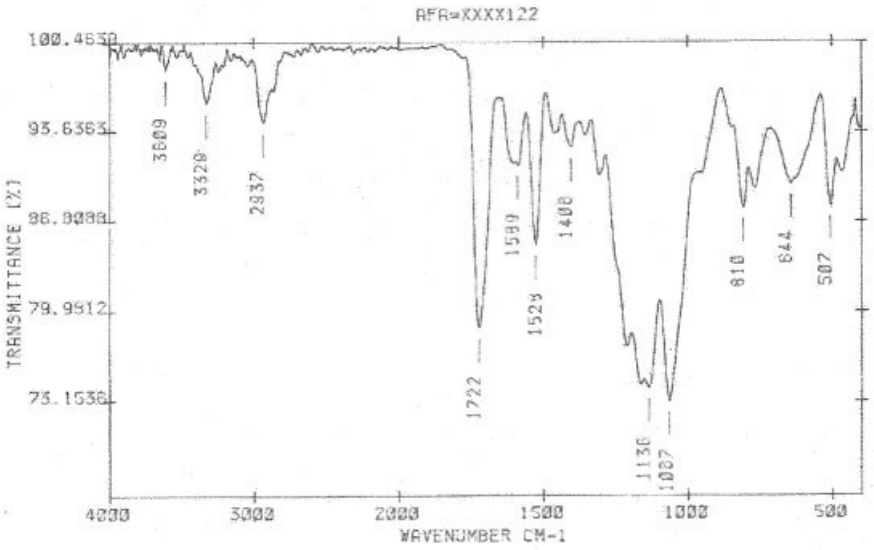

Figure 2 FTIR spectra of an untreated PU surface.

In vitro calcification

The concentration product of calcium ($CaCl_2 \cdot 2H_2O$) and phosphate (K_2HPO_4) in the incubation solutions was 9 mM^2, 3.87 mM calcium, and 2.32 mM phosphate, yielding a ratio of Ca/PO_4 = 1.67, as in hydroxyapatite. The concentration product of 9 mM^2 was chosen since measurable and reproducible calcification levels of polyurethane were produced after a short duration, which also provided practical working conditions. Each salt solution was prepared in 0.05M Tris buffer, pH 7.4. The vials containing the polymer film mounted by surgical silk suture thread were placed in a shaker (100 rev min^{-1} at 37 °C;[33] the total incubation time was 30 days. The resultants were rinsed three times with water to remove excess solution and the loosely attached deposits.

For Fourier transform infrared (FTIR, Unicam, Cambridge, UK) spectroscopy and scanning electron microscopy (SEM, Cambridge *S*-360; Cambridge Biotech, Worcester, MA) examinations, representative films were rinsed with double-distilled water and oven dried (2 h, 110 °C).

Cell culture assays

The mouse L929 fibroblast cells were used as a test model in this study. The cells were maintained in Roswell Park Memorial Institute (RPMI)-164° growth

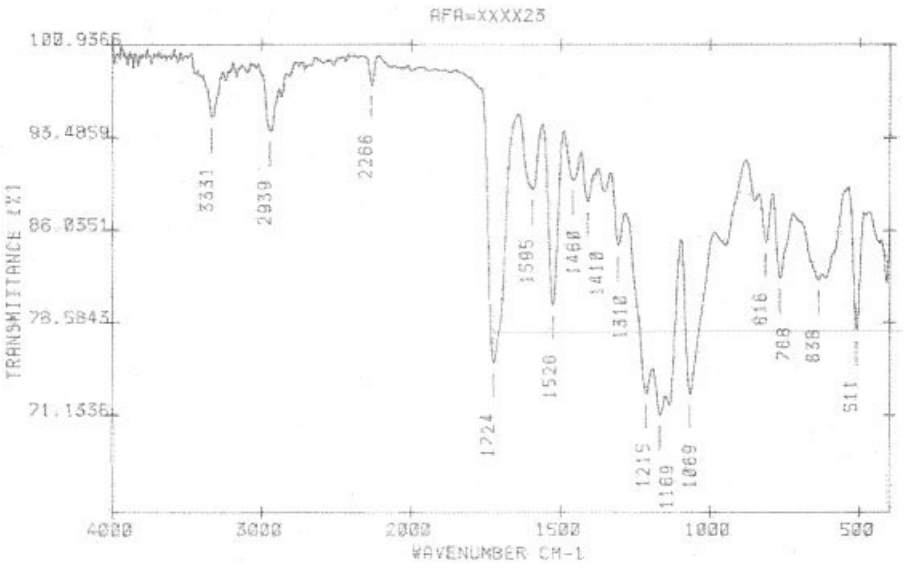

Figure 3 FTIR spectra of an HDI-grafted PU surface.

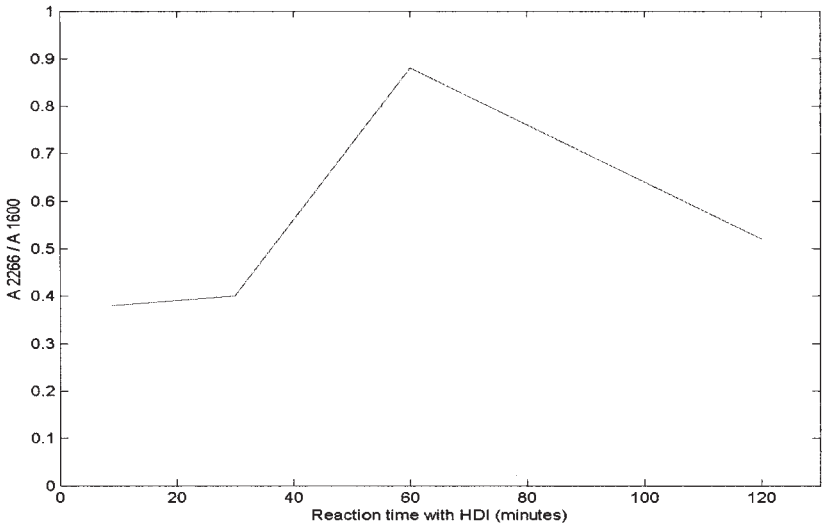

Figure 4 Extent of reaction of HDI with PU surfaces, measured from FTIR spectra as the ratio of the NCO absorption at 226 cm^{1} and phenyl group absorption at 1600 cm^{1}.

medium, supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, and 10% fetal calf serum. 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 and 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.

Platelet adhesion test

Platelet adhesion was measured according to a method described by Ikada et al.[36] 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

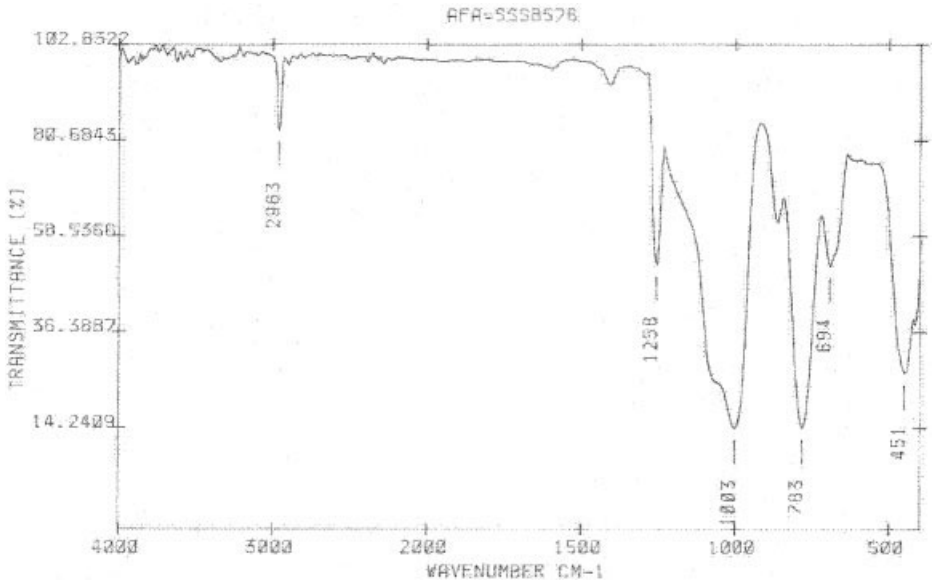

Figure 5 FTIR spectra of a polydimethylsiloxane-grafted PU surface.

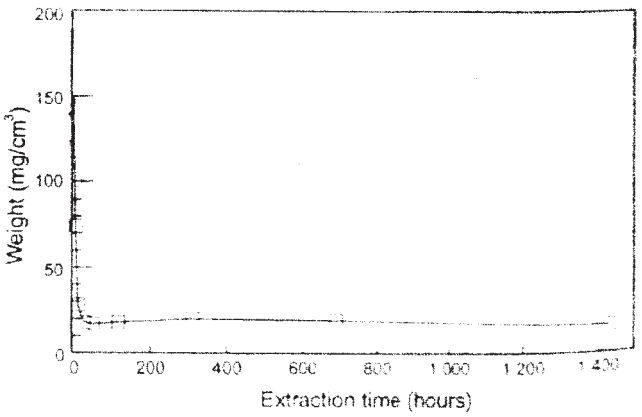

Figure 6 Extraction of PDMS-grafted PU films in toluene.

put into 2 mL of 0.1*M* PBS containing 0.5% Triton X100 to lyse the adhered platelets. The experiment of platelet adhesion was repeated three times for the same film using different PRP. All samples were run in triplicate.

RESULTS AND DISCUSSION

Grafting of polydimethylsiloxane onto a polyurethane surface

The extent of the reaction was followed by FTIR spectroscopy analysis. Figures 2 and 3 show FTIR spectra of untreated PU and HDI-grafted PU. After reaction with HDI a sharp band at 2266 cm^{-1}, characteristic of NCO groups, appeared in the PU spectrum (Fig. 3). The number of free NCO groups changed with the HDI reaction time. From the FTIR spectra the ratio between the absorption band at 2266 cm^{-1} (NCO) and the band at 1600 cm^{-1} (aromatic band) in the spectrum was calculated and is reported in Figure 4 as a function of the reaction time with HDI. The presented results indicate that a maximum number of free NCO groups were obtained after a reaction time of 60 min. A decrease in the number of free NCO groups at longer reaction times may be a result of ring formation through dimerization of NCO groups and/or allophanate reactions.

In the second reaction step, hydroxylic groups of polydimethylsiloxane were allowed to react with the free NCO groups at the surface (Fig. 1). The presence of polydimethylsiloxane at the surface after the reaction was evident from the FTIR spectra and the characteristic peak of Si-O-Si at 1003 cm^{-1} is shown in Figure 5. However, the PU absorbs about 80% by the weight of toluene during the grafting procedure. HDI as well as polydimethylsiloxane can diffuse into the swelled material, allowing NCO groups and polydimethylsiloxane to react in the bulk in the same way as at the surface. Consequently, the grafting will not be located entirely at the surface. Furthermore, an excess of polydimethylsiloxane that has not reacted with isocyanate groups will be physically entrapped in the material upon drying when the swelled gel-like structure collapses. After drying, the glass transition temperature of the PU is substantially lower than 0 °C. The entrapped polydimethylsiloxane chains migrates to the film surface and crystallizes, giving rise to a characteristic spherulitic structure that makes the surface opaque and rough. This migration of PDMS chains is due to minimization of surface energy

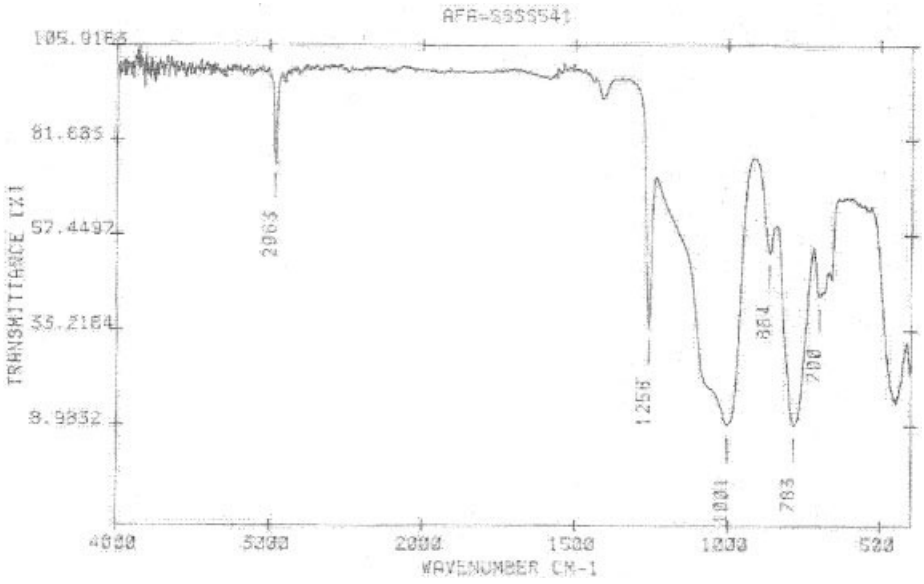

Figure 7 FTIR spectra of a polydimethylsiloxane-grafted PU surface, leached in toluene for 50 h.

TABLE I
Dynamic Contact Angle Results

	Dynamic contact angles			
	First cycle angles		Second cycle angles	
Sample	Advancing	Receding	Advancing	Receding
PU	85 ± 2	69 ± 2	81 ± 2	68.5 ± 2
Grafted PU	97 ± 2	80 ± 2	94.5 ± 2	78.5 ± 2

TABLE II
Static Contact Angle Results

Sample	Static contact angles
PU	78 ± 2
Grafted PU	110 ± 2

according to the second law of thermodynamics. On the other hand, PDMS chains are hydrophobic and tend to relay into air, which is hydrophobic as well. The entrapped polydimethylsiloxane consequently has a major effect on the surface properties, as well as on the mechanical behavior.

To investigate the stability of grafting process and influence of the covalently bonded polydimethylsiloxane on the properties of the PU surfaces, the entrapped polydimethylsiloxane in the grafted films had to be removed. To evaluate the leaching of polydimethylsiloxane from grafted films, samples were immersed in toluene and into a Soxhlet extractor, respectively, and the weights of the samples were registered at different times. As seen in Figure 6, the rate of leaching is quite high in toluene, which is a swelling agent for PU. After 50 h the weights of the films reached a steady-state value, and the surfaces changed to be clear and the surface properties became stable. After removal of the entrapped PDMS by leaching in toluene, the FTIR spectra were similar to that for the original PU (Fig. 7).

Wettability

Table I shows the results of the dynamic contact angle measurements. The advancing and receding angles are listed for the first and second cycles. Grafting PDMS onto PU surfaces increases both the advancing (θ_{ad}) and the receding (θ_{re}) angles. The reason for this behavior can be attributed to the structural changes at the solid–water boundary.[37] When PU surfaces are grafted with polydimethylsiloxane they become strongly hydrophobic, and the water contact angle increased considerably compared to contact angles for an unmodified surface. 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. The advancing angles for all polymers are slightly higher in the first cycle. This may be due to the hydration of the grafted chains.

The general trends of the dynamic receding contact angles agree with the static underwater contact angles. The results of the sessile drop measurements revealed that after grafting the contact angle of PU is increased from 78° for the unmodified PU to 110° for the grafted PU (Table II).

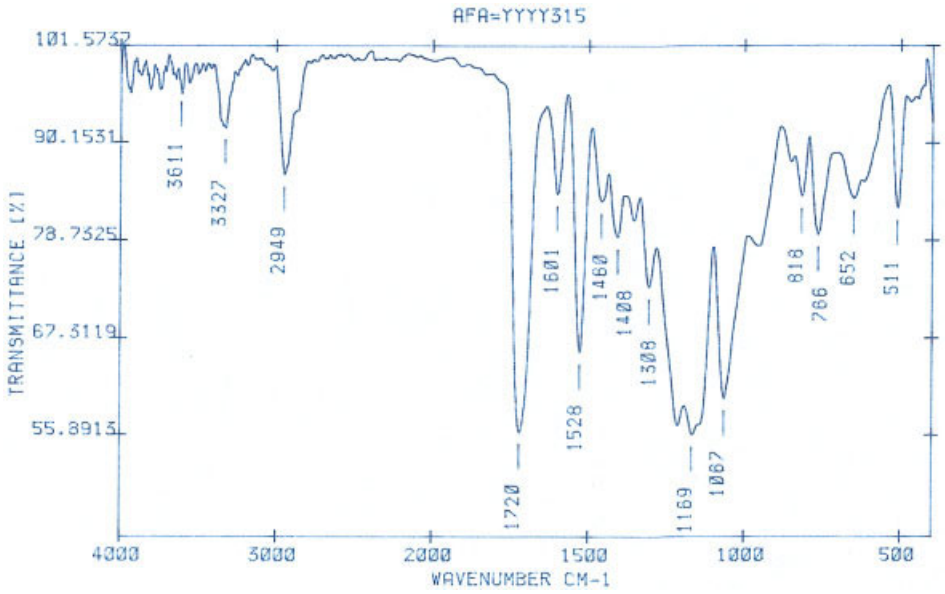

Figure 8 FTIR spectra of the surface of calcified polyurethane. Note the appearance of a new peak at 1169 $cm^{\bar{1}}$ in the calcified surface spectrum.

Figure 9 SEM image of the surface of calcified unmodified PU.

Figure 10 SEM image of the surface of calcified modified PU.

Calcification

In vitro calcification tests were applied to study calcification processes in biomaterials. Knowing the mechanism of calcification is an essential factor to the development of rational approaches to the prevention of calcification and enables the isolation of specific factors involved in the process. Calcification occurring in polyurethane is an intrinsic property of the polymer. Polyurethane is susceptible to calcification via a process of cation complexation by the polyether segment of polyurethane. Cyclic polyethers containing 5–10 oxygen atoms do from complexes with metal ions, including calcium.[20,38] In fact, to prevent the calcification of PU, a process between the polyurethane and calcium is undesirable.

FTIR spectroscopic analysis of calcified films indicated the appearance of a new peak on the calcified film surfaces at 1169 cm^{-1}. Thoma[39] reported the appearance of a peak of 1173 cm^{-1} in complexed polyether model compounds, which he attributed to the stretching frequency of a complexed ether functional group. This peak is very close to that found in our films (Fig. 8) and suggests a process of intrinsic calcification involving, at least, the ether functional groups of the polyurethane.

SEM micrographs of PU and grafted PU are shown in Figures 9 and 10. PU films after a 1-month incubation at the calcium phosphate solution demonstrate severe calcification but grafted PU did not demonstrate calcification and spectroscopic analysis of modified films did not indicate any new peak, while as shown in Figure 8 there is a new peak at 1169 cm^{-1} in the spectroscopic analysis of calcified PU. This new peak confirms the process of intrinsic calcification in the surface of PU. In fact, covalent bonding of polydimethylsiloxane to the surface of the urethane successfully inhibited the biodegradation and calcification process. The formation of graft polymers of polyurethane with micrometer-thick layers of polydimethylsiloxane polymer takes advantage of the excellent physical properties and manufacturability of urethanes and the biological inertness and similar properties of silicon rubber.

Cell culture

Cell culture tests were used to evaluate both cytotoxicity and biocompatibility of the specimens. The cellular behavior on a biomaterial is an important factor in determining the biocompatibility. The first physiological process that occurs within the initial stages of exposure is the adsorption of biomolecules onto the surface; 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.[40]

In the cytotoxicity and cell culture method, the growth and proliferation of cells are investigated by comparison with a negative control (polystyrene tis-

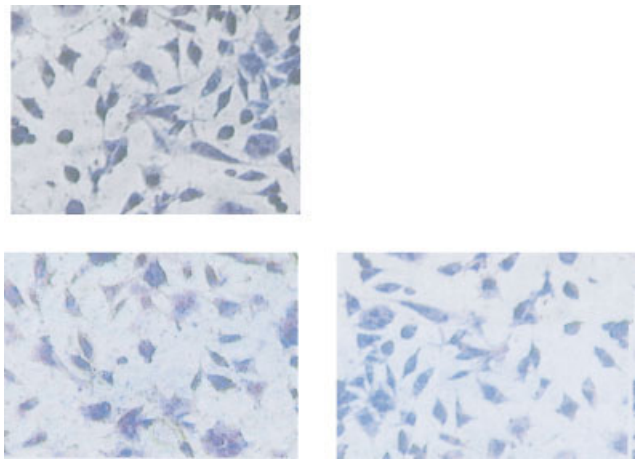

Figure 11 Optical micrographs of L-929 cells cultured for 48 h on the (a) negative control, (b), PU, and (c) modified PU.

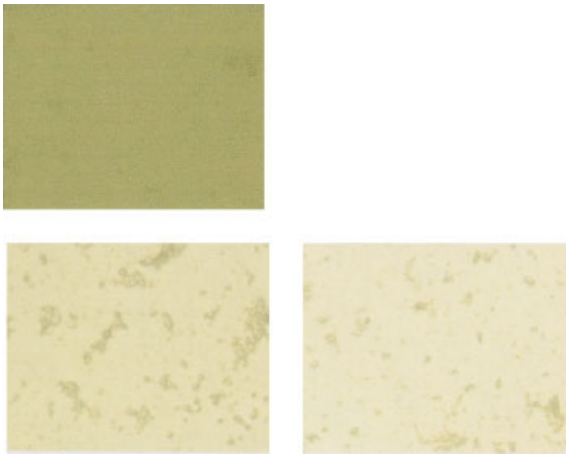

Figure 12 Optical micrographs of platelet adhesion for 1 h on the (a) positive control, (b) PU, and (c) modified PU.

sue culture; Nunc) [Fig. 11(a)]. As shown, cell proliferation (i.e., filopodia) and spreading could be observed on both the unmodified [Fig. 11(b)] and the grafted sample [Fig. 11(c)]. The cells were flattened on these samples and there was no change in morphology. Polyurethane and polydimethylsiloxane are two known biomaterial with no cytotoxicity and these results showed that the increase of hydrophobicity caused by the grafting of PDMS on the PU surface did not affect its cytotoxicity.

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. The appearance of platelet aggregates and platelet spreading and subsequent thrombus formation invariably follow platelet adhesion on a surface. In fact, from the standpoint of blood compatibility, a strong interaction between the material and platelets is undesirable.

The results of platelet spreading on PU and grafted PU are shown in Figure 12(a,b). Because both PU and PDMS have excellent blood compatibility properties, grafting of PDMS onto PU surface did not affect the amount of platelet adhered per unit surface of PU.

CONCLUSIONS

Upon grafting of polyurethane surfaces with polydimethylsiloxane in toluene, PDMS is covalently bonded to the surface. We found that surface modification of polyurethane films prevents the calcification of PU in *in vitro* calcification tests in 30 days. Polydimethylsiloxane grafted films were compatible with fibroblast cells and showed no cytotoxicity. However, the PRP method showed no change in platelet adhesion onto the grafted surface compared to PU. Graft polymers of polydimethylsiloxane on to polyurethane combine excellent physical properties and manufacturability of polyurethane with the biological inertness of polydimethylsiloxane. Potential applications for these material include heart valve leaflets, vascular grafts, blood pump diaphragms, and pacemaker lead insulators.

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