Platelet Adhesion on Laser-Induced Acrylic Acid–Grafted Polyethylene Terephthalate

H. Mirzadeh,[1] M. Dadsetan,[2] N. Sharifi-Sanjani[3]

[1]*Amir Kabir University of Technology, Department of Medical Engineering, P. O. Box 15875/4413, Tehran, Iran*
[2]*Laser Research Center, Atomic Energy Organization of Iran, P. O. Box 11365-8486, Tehran, Iran*
[3]*Department of Chemistry, Faculty of Science, University of Tehran, P. O. Box 14155-6455, Tehran, Iran*

Received 20 June 2001; accepted 16 October 2001

ABSTRACT: To improve blood compatibility, acrylic acid (AAc) was grafted onto a polyethylene terephtalate (PET) film surface using lasers. The PET surface was irradiated with a CO_2 pulsed laser, and then graft copolymerization was carried out in an aqueous solution of AAc in the presence of Mohr's salt. Different techniques such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM), and contact angle measurements were used to characterize the modified PET surface. The ATR-FTIR spectra confirmed the creation of new functional groups on the PET surface, and contact angle measurements revealed that the hydrophilicity of the PET surface increased as a result of the AAc graft polymerization. The electron micrographs showed that the grafting changed the surface morphology of the PET film. To evaluate the blood compatibility *in vitro*, the number of platelets adhering to the modified PET surface was determined using lactate dehydrogenase (LDH) activity measurement. The data from LDH method indicated that the extent of platelet adherence on the unmodified PET was much higher than that on the AAc grafted PET. The morphology of adhered platelets on the PET surface was investigated by SEM. The results showed that platelet adhesion and activation onto the PET surface was reduced because of AAc graft polymerization. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 3191–3196, 2002

Key words: CO_2 laser; graft polymerization; platelet adhesion; polyethylene terephthalate; acrylic acid; morphology

INTRODUCTION

Thrombus formation on polymer surfaces in contact with blood is a complicated cellular and molecular process with many mechanistic questions that are still unanswered.[1,2] However, it is generally accepted that plasma protein adsorption will influence subsequent platelet adhesion and activation and does play a major role toward the initiation of thrombus formation at the blood material interface.[3] Therefore, it is essential to understand the mechanism of protein adsorption and to be able to control adsorption onto the polymer surface. With this understanding, the control of subsequent clotting events, such as platelet adhesion and activation, and fibrin formation may be achieved in the design of blood-compatible surfaces.[4]

Surface modification techniques, such as grafting hydrophilic chains onto the surface and coating it with hydrophilic/ hydrophobic microdomain copolymers, have been attempted to control cell adhesion and growth.[5,6] It has been shown that cell behavior is influenced by the physical and chemical properties of the polymer surface, such as wettability, chemistry, charge, roughness, and rigidity.[7–9] Researchers have found that materials that introduce negatively charged groups tend to be antithrombogenic, whereas positively charged surfaces were thrombogenic.[10] Several investigations have introduced sulfonate groups into polymers for the improvement of blood compatibility.[11] In addition to sulfonate groups, many investigators incorporated the carboxylate or carboxylic acid groups into the polymers with the aim of improving their blood compatibility.[12–15]

Graft polymerization can be achieved by many methods, such as ionizing, ultraviolet, and chemical initiators.[12,16] Recently, lasers have been applied to prepare novel polymeric biomaterials.[17–20] The capability of lasers to alter surface physical and chemical properties without affecting the bulk properties of the base material is advantageous in the designing, development, and manufacturing of biocompatible polymers.[21–24] Laser irradiation can induce photochemical reactions to the formation of new chemical species that could, in turn, improve the interactions between the polymer surface and the biological medium.[25–27]

This work has been undertaken to study the graft polymerization of acrylic acid onto the polyethylene terephtalate (PET) surface using a CO_2 pulsed laser to improve blood compatibility. Graft polymerization was carried out by preirradiation of PET surface, fol-

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

Journal of Applied Polymer Science, Vol. 86, 3191–3196 (2002)
© 2002 Wiley Periodicals, Inc.

lowed by heating of irradiated PET in the aqueous solution of monomer. The changes in surface properties of the PET caused by the graft polymerization of acrylic acid (AAc) were investigated using different techniques. Platelet adhesion and activation studies were used to assess the blood compatibility of the acrylic acid–grafted PET.

EXPERIMENTAL

Materials

The sample used was PET film (Dupont Mylar, Wilmington, DE; thickness 70 μm). The surfaces of all samples were ultrasonically cleaned with ethanol and dried at 40°C in a vacuum oven before irradiation. The acrylic acid monomer from Aldrich (Milwaukee, WI) was distilled under reduced pressure to eliminate the stabilizer before use. Mohr's salt (ferrous ammonium sulfate) was purchased from Aldrich and used without further purification.

Laser treatment

Laser-induced graft copolymerization was carried out by preirradiation technique, as reported previously.[27,28] The film samples were irradiated using a line-tunable pulsed TEA CO_2 laser (Lumonics 103-2, Kanta, Ontario, Canada) at the wavelength of 9.25 μm. The laser was operated at a repetition rate of 0.5 Hz and a fluence of 1.5 J/cm^2. The strips of PET film were placed on a belt of a special step motor, and the irradiation was carried out in air with the desired pulses.

Peroxide determination

The concentration of peroxides generated onto the irradiated PET film surfaces was determined spectrophotometrically by means of the iodide method, as described previously.[28]

Graft polymerization

After irradiation with the CO_2 pulsed laser, the PET samples were immersed in the aqueous solution of the monomer in various concentrations and heated under nitrogen atmosphere at 60°C for 3 h to thermally decompose the polymeric peroxides. To minimize homopolymerization, Mohr's salt was added to the monomer solution. The AAc homopolymer formed was removed from the grafted film by extraction with distilled water at 70°C for >15 h under continuous stirring. The density of PAAc grafted was determined gravimetrically using following equation:

$$\text{Graft density } (\mu g/cm^2) = W_g - W_\circ/S,$$

where W_g and $W_\circ$ represent the weights of the grafted and ungrafted film, respectively, and S is the original surface area of the samples.

Surface characterization

To characterize the surface of modified samples, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) with KRS-5 prism and an incident angle of 45° was performed using a FTIR instrument (Brucker model IFS-88, Ettingen, Germany). The surface morphology was observed with an SEM Philips (model-XL30, Eindhoven, The Netherlands) after sputter gold coating the samples. Static contact angles were measured using the sessile drop method with contact angle measurement equipment (Kruss G10, Hamburg, Germany). All water drop contact angles are a mean value of three measurements on different parts of the film plus or minus the standard error of the mean (SE).

In vitro experiment

The experimental procedure employed in the platelet adhesion studies was similar to that used by Tamada et al.[29] Human blood was collected into a 250-mL blood bag containing 35 mL CPDA-1 anticoagulant (100 mL anticoagulant contains 327 mg citric acid monohydrate, 2630 mg sodium citrate dihydrate, 251 mg monobasic sodium phosphate dihydrate, 2900 mg dextrose anhydrate, and 27.5 mg adenine). The blood was centrifuged to obtain platelet-rich plasma (PRP). The PRP concentration was determined by a Cobas Coulter counter (type 4) and adjusted to 300,000 platelets mm^{-3} by adding phosphate-buffered saline (PBS). The PRP was placed on PET films 1 cm in diameter and kept for 1 h at 37°C. Then the films were taken out and dip-rinsed twice with PBS, to remove the platelets that were not attached to the film surfaces, and treated overnight with 2.5% (v/v) glutaraldehyde at 4°C. The samples were washed with saline and subjected to a drying process by passing them through a series of graded aqueous alcohol solutions and dried to the critical point. The dried samples after gold coating were examined by SEM.

The number of adhered platelets was determined by the lactate dehydrogenase (LDH) method.[29] The films were put into 2 mL PBS containing 1% Triton-X100 for 1 h at room temperature to lyse the adhered platelets. The LDH activity of the lysate was measured with an enzymatic method to count the adhered platelets with the use of calibration curve of platelet counts. The change in ultraviolet absorption at 340 nm was measured using a Pharmacia Biotech spectrophotometer (model Novaspec II, Cambridge, England). The experiment of platelet adhesion was repeated three times

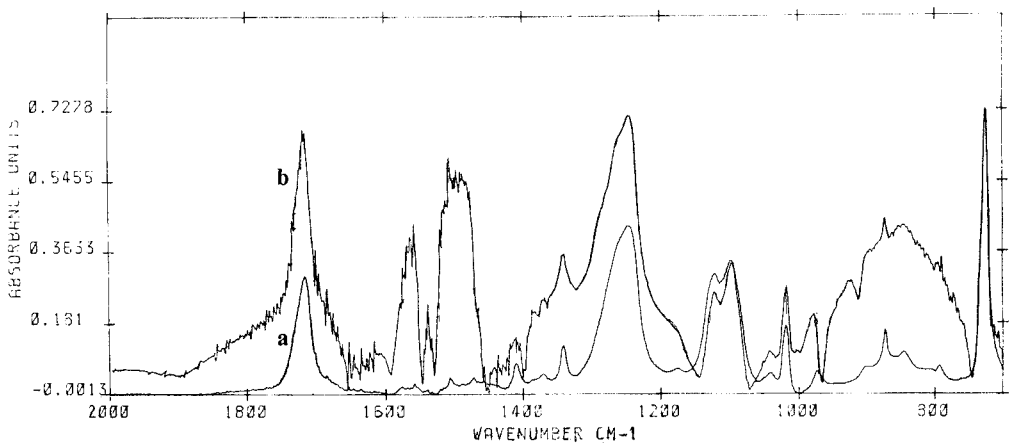

Figure 1 Attenuated total reflectance Fourier transform infrared spectroscopy spectra of the (a) unmodified polyethylene terephtalate (PET) (b) acrylic acid–grafted PET (graft density 76 $\mu g/cm^2$).

using different PRP. Results are the mean value of three determinations ± SE.

Statistical analyses

Analysis of variance and unpaired Student's *t*-tests were performed using Microcal Origin 3.5.

RESULTS AND DISCUSSION

In previous works, we reported that the laser irradiation generated various radicals on the PET film surface.[27,28] The radicals, in contact with air, were rapidly modified into corresponding peroxides. The peroxide density was determined spectrophotometrically by means of the iodide method.[28] The peroxide concentration increased with an increase in the number of laser pulses, and a maximum concentration of 1.8 $\times 10^{-8}$ mol/ cm^2 was obtained when the laser treatment of PET film was performed with 3 pulses. Further treatment with laser light led to less peroxide formation, which was attributed to the photochemical decomposition of the peroxide groups. Although the radiation with higher laser pulses may induce higher radical concentration, simultaneous fragmentation may occur, inducing surface ablation or facilitating the recombination reaction of radicals, both of which are responsible for the reduction of peroxide density. We also showed that the water drop contact angle with irradiated PET surfaces decreased with increases in the laser pulses. The decrease in contact angle was ascribed to the formation of various kinds of oxidized groups such as hydroxyl and carbonyl functions in addition to hydroperoxides.[28]

In this study, the PET surface was irradiated using a CO_2 pulsed laser to introduce peroxide onto the polymer surface. Subsequently, the peroxidized film was immersed in an aqueous monomer solution containing Mohr's salt to prevent homopolymerization[30] and heated to decompose polymer peroxides, which are capable of initiating the monomer polymerization. Graft polymerization was carried out on the irradiated PET surface, and the density of the AAc grafted samples was measured gravimetrically.

Infrared spectra of the AAc-grafted and unmodified PET are shown in Figure 1. The spectrum of AAc-grafted PET revealed a strong absorption band at

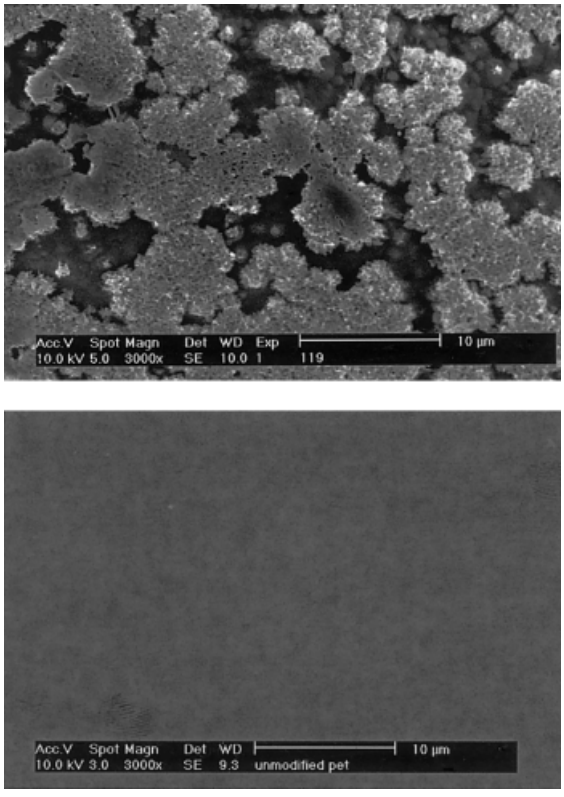

Figure 2 Scanning electron micrographs of the (a) acrylic acid–grafted polyethylene terephtalate (PET) (graft density 102 $\mu g/ cm^2$) and (b) unmodified PET. Original magnifications ×3000.

1680–1730 cm^{-1}, resulting from an overlap between C=O stretching vibrations of PET and acrylic acid. The characteristic adsorption bands of carboxylic acid emerged at 1200–1300 cm^{-1} and 1400–1550 cm^{-1}, corresponding to C–O stretching and O–H bending vibrations, respectively. The broad absorption band was also observed at 870 cm^{-1}, corresponding to O–H out of plane bending vibrations of carboxylic acid. This finding indicated a successful structural modification of acrylic acid onto the PET matrix by the laser preirradiation graft method.

Figure 2 shows SEM micrographs of AAc-grafted PET in comparison with the unmodified PET surfaces. As can be seen, the morphology of the grafted PET surface is highly rough and different from the unmodified PET film surface. Therefore, from the change in surface morphology, increasing the weight of grafted samples as well as the results from IR spectra, we can conclude that the acrylic acid has been graft polymerized onto the PET surface. An SEM micrograph of the cross section of an AAc-grafted PET film has been shown in Figure 3. As shown in this picture, the grafted layer is restricted to the film surface region. The thickness of the grafted layer is estimated from this photograph to be approximately 7 μm.

Figure 4 shows the contact angle as a function of graft density. The water drop contact angle with the unmodified PET surface was 70.2° but decreased to a minimum of 31.2° because of the CO_2 laser–induced graft polymerization of AAc. We reported previously that the CO_2 laser treatment of the PET films at the wavelength of 9.25 μm without any graft polymerization reduced the contact angle to around 60°.[28] However, the contact angle further decreased to 31.2° as a result of AAc graft polymerization. The decrease in contact angle strongly confirms the graft polymerization of acrylic acid on the PET surface. As can be seen in Figure 4, the contact angle of AAc-grafted PET samples decreased with an increase in the graft density up to a definite level, then increased and reached a plateau, probably because of a thick hydrated layer existing at the interface between the grafted surface and water.[31]

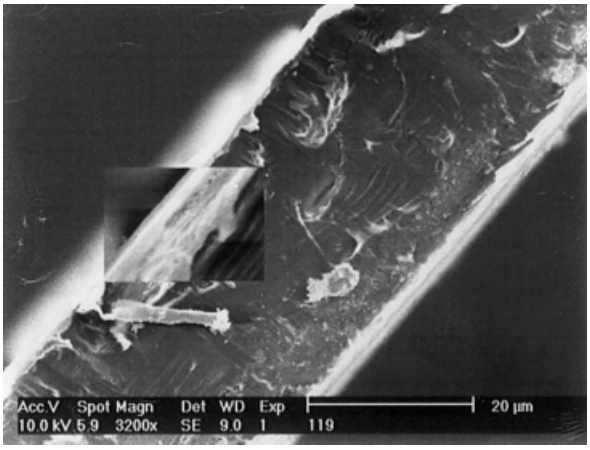

Figure 3 Scanning electron micrograph of the cross section of an acrylic acid–grafted polyethylene terephtalate (graft density 102 $\mu g/cm^2$). Original magnification ×400. Enlargement ×3200.

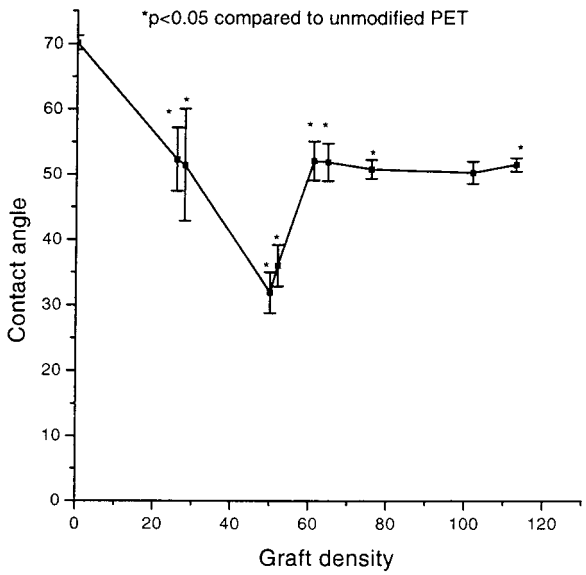

Figure 4 Contact angle of acrylic acid–grafted polyethylene terephtalate as a function of graft density ($\mu g/cm^2$). Results are presented as average $\theta \pm$ standard error of the mean.

Platelet adhesion

Figure 5 shows the typical scanning electron micrographs of the adhered platelets on the AAc-grafted PET compared with the unmodified PET. As shown in this figure, the platelets adhered to the unmodified PET were relatively high in number in comparison with the AAc-grafted PET. The platelets on the unmodified PET surface extend long pseudopods, leading to their complete spreading, whereas the platelets on the grafted PET films retain their discoid shapes. Therefore, graft polymerization of acrylic acid reduced the platelet adhesion and prevented platelet spreading.

Figure 6 shows platelet adhesion as a function of graft density. The data obtained from LDH activity measurements indicated that the platelet adhesion reduced as a result of graft polymerization of AAc. As can be seen, the most optimal graft density required to reduce platelet adhesion is 76 $\mu g/cm^2$. It is likely that excessive grafting takes place, causing platelet trapping in the thick grafted layer region. It is evident from Figures 5 and 6 that platelet adhesion is greatly reduced by the surface graft polymerization of acrylic acid. The grafted polymer chains may prevent the protein molecules and platelet cells from direct contact

with the PET surface owing to their steric hindrance effect.[32] Because cell-surface interaction is a very complicated phenomenon, it is not clear which property is really dominant for cell adhesion on surface. It is recognized that the adhesion and proliferation of different types of cells on polymeric materials depend on surface characteristics such as wettability, chemistry, charge, roughness, and rigidity.[6] Some proteins in serum, like fibronectin and vitronectin, are well known to play an important role in cell attachment onto the substrates.[2] These proteins are adsorbed more on positively charged surfaces than on negatively charged ones.[10] Therefore, the polyacrylic acid–grafted surface that is negatively charged shows poor cell adhesion. It has been also reported that the oxygen-containing groups are involved in making the surface optimal for platelet adhesion.[2] We have observed similar results for fibroblast cells on the AAc-grafted PET, which will be published separately.

A large number of research groups have studied the interaction between different types of cultured cells and various polymers of different wettabilities to correlate the relationship between wettability and blood or tissue compatibility.[10,31] As seen in Figure 4, the water contact angles of the grafted PET decreased compared with those of the unmodified surface, and a minimum contact angle of 50 $\mu g/cm^2$ was obtained at graft density. Figures 4 and 6 indicate that *in vitro* blood compatibility appears to be better when the surface has a low contact angle. However, as can be seen in these figures, the water drop contact angle is not directly correlated to the blood compatibility of AAc-grafted PET. This result is rather reasonable, as hydrophilic surfaces may not always have the same surface texture. The morphology formed on the AAc-grafted PET has a significant effect on the cell behavior. It has been recognized that features of the surface morphology of the implant biomaterials may affect the cell response.[33] Therefore, it can be concluded that the chemical and physical properties of the AAc-grafted PET surface, including charge, chemistry, wettability, and morphology, affect the platelet adhesion and activation.

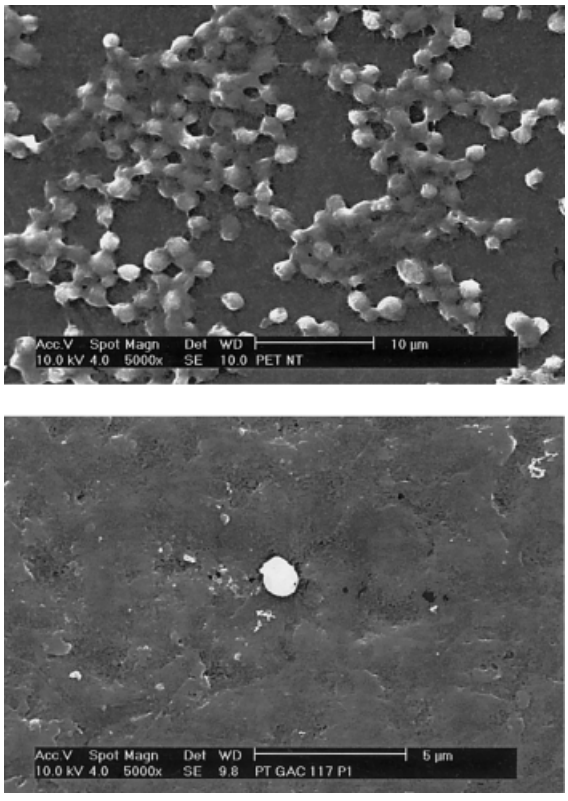

Figure 5 Scanning electron micrographs of adhered platelets on the (a) unmodified polyethylene terephthalate and (b) acrylic acid grafted. Original magnifications ×5000.

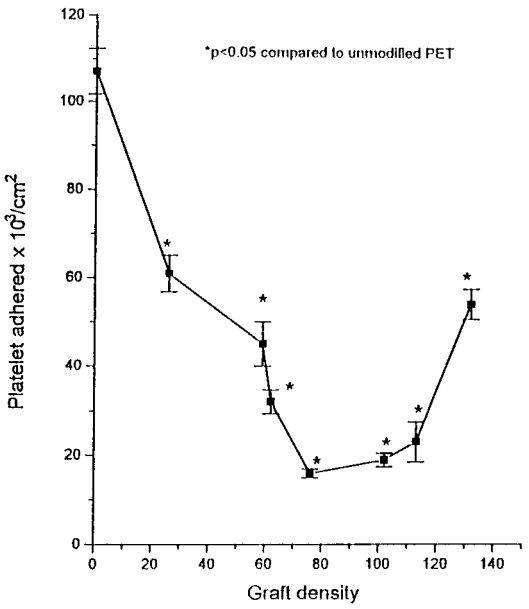

Figure 6 Dependence of the platelet adhesion as a function of graft density ($\mu g/cm^2$). Results are expressed as a mean of three determinations ± standard error of the mean.

In summary, laser irradiation of PET gives peroxides that can be used for surface grafting of acrylic acid. The graft polymerization of AAc changed the morphology and wettability of the PET surface. The contact angle of the AAc-grafted PET surface decreased with increasing the graft density to a definite level and then somewhat increased, probably because of the formation of a hydrated layer at the interface between the grafted surface and water. The results from *in vitro* studies showed that the platelet adhesion and activation onto the PET surface was drastically reduced because of the graft polymerization of AAc. We conclude that the platelet adhesion and activation on the AAc-grafted PET surface is influenced by the surface chemistry, morphology, charge, and wettability.

This work was supported by Faculty of Science, the University of Tehran, Iran, under grant 514/1/363.

References

1. Dawids, S. Polymers: Their Properties and Blood Compatibilityl; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1989; p. 49.
2. Shalaby, S. W.; Hoffman A. S.; Ratner B. D.; Horbett T. A. Polymers as Biomaterials; New York: Plenum, 1984; p. 135.
3. Nojiri, C.; Okano, T.; Jacobs, H. A.; Park, K. D.; Mohammad, S. F.; Olsen, D. B.; Kim, S. W. J Biomed Mater Res 990, 24, 1151.
4. Singh, D. K.; Ray, A. R. J Appl Polym Sci 1994, 53, 1115.
5. Bahuleker, R.; Tamura, N.; Seiko, I.; Kodama, M. Biomaterials 1999, 20, 357.
6. DeFife, K. M.; Colton, E.; Nakayama, Y.; Matsuda, T.; Anderson, J. M. J Biomed Mater Res 1999, 45, 148.
7. Suggs, L. J.; Shire, M. S.; Garcia, C. A.; Anderson, J. M.; Mikos, A. G. J Biomed Mater Res 1999, 46, 22.
8. Jenny, C. R.; Anderson, J. M. J Biomed Mater Res 1999, 44, 206.
9. Chuang, W.Y.; Young, T. H.; Yao, C. H.; Chiu, W.Y. Biomaterials 1999, 20, 1479.
10. Lee, J. H.; Jung, H. W.; Kang, I. K.; Lee, H. B. Biomaterials 1994, 15, 705.
11. Chen, K. Y.; Kuo, J. F.; Chen, C. Y. Biomaterials 2000, 21,161.
12. Ko, T. M.; Cooper S. L. J Appl Polym Sci 1993, 47, 1601.
13. DeFife, K. M.; Shive, M. S.; Hagen, K.M; Clapper, D. L.; Anderson, J. M. J Biomed Mater Res 1999, 44, 298.
14. Olafsen, K.; Stoti, A.; Telefsen, D. A. J Appl Polym Sci 1992, 46, 1673.
15. Dogue, L. J.; Mermilliod, N.; Gandin, A. J Appl Polym Sci 1995, 56, 33.
16. Chapiro, A. Radiat Phys Chem 1977, 9, 55.
17. Hunt, J. A.; Williams, R. L.; Tavakoli, S. M.; Riches, S. T.; J Mater Sci, Mater Med 1995, 6, 813.
18. Mirzadeh, H.; Khorasani, M.; Sammes, P. Iran Polym J 1998, 1, 5.
19. Dadsetan, M.; Mirzadeh, H.; Sharifi-Sanjani, N.; Salehian, P. J Biomed Mater Res 2001, 54, 540.
20. Dadsetan, M.; Mirzadeh, H.; Sharifi, N. Iran Polym J 2000, 9, 203.
21. Mirzadeh, H.; Katbab, A. A.; Khorasani, M. T.; Burford, R.P.; Gorgin, E.; Golestani, A. Biomaterials 1995, 16, 641.
22. Dadsetan, M.; Mirzadeh, H.; Sharifi-Sanjani, N.; Daliri, M. J Biomed Mater Res 2001, 57, 183.
23. Watanabe, H.; Takata , T.; Tsugo, M. Polym Int 1993, 31, 247.
24. Saito, N.; Yamashita, S.; Matsuda, T. J Polym Sci A Polym Chem 1997, 35, 747.
25. Andrew, J. E.; Dyer, P. E.; Foster, D.; Key, P. H. Appl Phys Lett 1983, 43, 717.
26. Dunn, D. S.; Ouderkirk, A. J. Macromolecules 1990, 23, 770.
27. Dadsetan, M.; Mirzadeh, H.; Sharifi, N. Radiat Phy Chem 1999, 56, 597.
28. Dadsetan, M.; Mirzadeh, H.; Sharifi-Sanjani, N. J Appl Polym 2000, 76, 401.
29. Tamada, Y.; Kulik, E. A.; Ikada, Y. Biomaterials 1995, 16, 259.
30. Nho, Y. H.; Jin, J. H. J Appl Polym Sci 1997, 63, 1101.
31. Fujimoto, K.; Tadokoro, H.; Ueda, Y.; Ikada, Y. Biomaterials 1993, 14, 442.
32. Fujimoto, K.; Takebayashi, Y.; Inoie, H.; Ikada, Y. J Polym Sci, Polym Chem Ed 1993, 31, 1035.
33. Wan, H.; Williams, R. L.; Dohetry, P. J.; Williams, D. F. J Mater Sci Mater Med 1997, 8, 45.