Plasma-Induced Graft Polymerization of Acrylic Acid onto Poly(ethylene terephthalate) Films

BHUVANESH GUPTA,[1,*] JONS G. HILBORN,[1] ISABELLE BISSON,[2] PETER FREY[2]

[1] Laboratory of Polymers, Department of Material Sciences, EPFL, Lausanne, Switzerland

[2] Laboratory for Experimental Pediatric Urology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Received 24 February 2000; accepted 14 December 2000

ABSTRACT: The graft polymerization of acrylic acid was carried out onto poly(ethylene terephthalate) films that had been pretreated with argon plasma and subsequently exposed to oxygen to create peroxides. The influence of synthesis conditions, such as plasma treatment time, plasma power, monomer concentration, temperature, and the presence of Mohr's salt, on the degree of grafting was investigated. The observed initial increase in grafting with monomer concentration accelerated at about 20% monomer. The grafting reached a maximum at 40% monomer and subsequently decreased with further increases in monomer concentration. The reaction temperature had a pronounced effect on the degree of grafting. The initial rate of grafting increased with increasing temperature, but the degree of grafting showed a maximum at 50°C. The activation energy of the grafting obtained from an Arrhenius plot was 29.1 kJ/mol. The addition of Mohr's salt to the reaction medium not only led to a homopolymer-free grafting reaction but also diminished the degree of grafting. The degree of grafting increased with increasing plasma power and plasma treatment time. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2993–3001, 2001

Key words: PET; graft polymerization; plasma; acrylic acid

INTRODUCTION

A number of polymers have generated considerable interest for tissue engineering applications involving tissue reinforcement, tissue replacement, and organ transplantation.[1] These polymers, commonly known as *biomaterials,* offer surfaces that may be used as supports for biologically active molecules. Extracellular-matrix proteins such as collagen, laminin, and fibronectin or growth factors immobilized on polymers may provide a structure appropriate to favor cell adhesion, proliferation, and differentiation to mimic the natural cell environment.

The plasma-induced graft polymerization of vinyl monomers has been found to be an extremely attractive method of chemically modifying the surfaces of polymeric materials.[2–6] Polymers may be treated with an inert-gas plasma and then exposed to oxygen to generate hydroperoxide active species that may initiate the grafting of a desired monomer. An interesting aspect of plasma-induced grafting is that the changes are confined to the depth of a few nanometers at the surface without influencing the bulk of the material. As a result, the mechanical properties of the material remain unaffected. This opens up vast possibilities for designing and developing surfaces of scaffolds with tailored chemical function-

Correspondence to: J. G. Hilborn (joens.hilborn@ppfl.ch).
**Permanent address:* Department of Textile Technology, Indian Institute of Technology, New Delhi 110016, India.

Journal of Applied Polymer Science, Vol. 81, 2993–3001 (2001)

ality and morphology suitable for protein and cell interaction. There have been a number of studies on the graft polymerization of monomers on various polymers by plasma exposure with the objective of developing functional interfaces for the immobilization of biomolecules.[7–10] In one of these studies,[10] silicone rubber was grafted with poly(acrylic acid) up to a graft content of more than 400 $\mu g/cm^2$, and this was followed by a carbodiimide-assisted immobilization of 8 $\mu g/cm^2$ of collagen. Corneal epithelial cells exhibited good proliferation on the surface-modified silicone rubber but not on the virgin surfaces or surfaces that had not undergone collagen immobilization.

Poly(ethylene terephthalate) (PET) has excellent mechanical strength, good stability in the presence of body fluids, and high radiation resistance, which make it suitable for sterilization, but its surface is not directly suitable for the immobilization of biomolecules. The PET surface, therefore, needs to be modified so that proteins may be immobilized for the further growth of living cells. Very little has been reported on PET surface modifications and its application as a protein carrier. Piglowski et al.[11] reported that PET can be modified into a biomaterial by plasma exposure under argon (which makes the surface hydrophilic) or perfluorohexane (which leads to a more hydrophobic surface). Both types of surfaces showed very good biocompatibility under both *in vitro* and *in vivo* evaluations. The surface modification of PET films, fibers, and fabrics was performed with plasma- and ozone-induced graft polymerizations of various monomers.[12–14] Kato and Ikada[12] showed that the ozone-induced grafting of acrylic acid led to a PET fiber surface with 0.5 $\mu g/cm^2$ poly(acrylic acid) grafts. This modified surface showed immobilization of the ligand protein at a level of 1 μg/mg of the fiber.

With the ultimate goal of developing suitable scaffolds for the establishment of reproducible human urothelial and smooth muscle cell cultures[15] and engineering *in vitro* functional stratified human urothelium,[16,17] our interest is in developing a PET surface that contains a controlled amount of poly(acrylic acid) graft, which may be used for the coupling of extracellular matrix proteins. In this investigation, we undertook the task of modifying the PET surface through plasma treatment and subsequent graft polymerization of the treated film with acrylic acid under various conditions used to better understand the mechanism of grafting. The influence of the plasma treatment conditions and reaction conditions on the grafting of acrylic acid onto PET was investigated and is explained.

EXPERIMENTAL

Materials

The PET films used in this study were supplied by Goodfellow (England). The films were biaxially oriented with a thickness of 23 μ. Square films (10 $\times$ 10 cm^2) were Soxhlet-extracted with ethanol for 24 h to remove any surface impurities. Clean films were dried under vacuum at ambient temperature (22°C) and stored in a desiccator over dried alumina before use.

Acrylic acid, Mohr's salt ($FeSO_4 \cdot 7H_2O$), acetic acid, and sodium hydroxide were supplied by Fluka (Switzerland). Toluidine Blue O was supplied by Aldrich. Deionized water was used in all experiments. The acrylic acid was purified by distillation under vacuum to remove impurities and stabilizers.

Ultraviolet (UV) spectroscopy (PerkinElmer UV/VIS/NIR spectrometer Lambda 900) was used for the optical density measurements.

Vitrogen 100® solution, a mixture of collagen types I and III, was supplied by Collagen Corp.

Buph 2-[N-morpholino]ethane sulfonic acid (MES)-buffered saline solution (pH 4.7) was provided by PIERCE.

Plasma Treatment

The plasma treatment of the PET films was carried out in a capacitively coupled plasma reactor operating at an RF of 13.6 MHz, as reported earlier.[18] The reactor consisted of two cylindrical electrodes 13 cm in diameter and 4.3 cm apart in a 40-cm^3 vacuum vessel. The upper electrode and the reactor wall were grounded.

The film was placed on the grounded electrode, and the system was evacuated to 5 μbar. Argon was introduced into the chamber at a flow rate of 50 sccm, and the chamber pressure was maintained at 0.4 mbar. Subsequently, plasma was generated at an electric power of 20–100 W for a desired period. After the plasma treatment, the chamber was evacuated to 5 μbar, and oxygen was introduced and maintained at atmospheric pressure for 60 min. The film was then removed for the grafting reaction.

Graft Polymerization

The graft polymerization of acrylic acid onto plasma-treated PET films was carried out in 2 $\times$ 16

cm^2 glass tubes under a nitrogen atmosphere. Care was taken to keep the transfer time of the treated film from the plasma chamber to the grafting reaction tube within 20–25 min. A film was placed in the reaction tube containing an aqueous acrylic acid solution of a required concentration. Nitrogen was bubbled through the solution to remove oxygen. The tube was then placed in a constant-temperature water bath for a specified period. After the grafting reaction, the film was taken out of the tube and Soxhlet-extracted with water overnight to remove any homopolymer adhering to the surface. The film was finally dried under vacuum at 40°C.

Determination of Amount of Grafting

The amount of grafting in PET films was determined by the colorimetric method with Toluidine Blue O staining as reported in the literature.[7] A 0.5 m*M* dye solution was prepared at pH 10, and the grafted film was placed in this solution for 6 h at 30°C. The film was then removed and thoroughly washed with a sodium hydroxide solution of pH 9 to remove any noncomplexed dye adhering to the surface. The dye was desorbed from the film in a 50% acetic acid solution, and the final dye content was obtained by the measurement of the optical density of the solution at 633 nm with an ultraviolet–visible spectrophotometer. The poly(acrylic acid) content (degree of grafting) was obtained from a calibration plot of the optical density versus dye concentration with a 1:1 ratio assumed between the dye and the carboxylic acid groups.

Protein Immobilization and Human Bladder Smooth Muscle Cell Culture

Poly(acrylic acid)-grafted PET ($2 \times 2cm^2$) films were immersed into a 2.5-mL Vitrogen 100® solution of 1.5 mg/mL collagen (diluted with BupH MES-buffered saline solution at pH 4.7) and left for 14 h at room temperature. The supernatant was removed, and the surfaces were dried under a laminar flow for 30 min. Then, 3×10^6 human bladder smooth muscle cells, provided from open kidney or bladder surgery, were seeded onto the film surfaces and on Nunc culture flasks as cell-growth controls. Cell growth and cell morphology were observed over 6 days.

RESULTS AND DISCUSSION

The grafting of acrylic acid onto PET films was carried out to develop a surface that carried a high density of carboxyl groups. The overall process involved the treatment of PET film with argon plasma and subsequent exposure to oxygen. This led to a surface with hydroperoxide groups as the main functionality. The hydroperoxide groups were thermally labile in nature and initiated the graft polymerization of acrylic acid to introduce graft brush layers on the surface. A schematic presentation of the modification of the surface is depicted in Figure 1. The amount of poly(acrylic acid) graft was considerably influenced by the plasma treatment parameters and the reaction conditions. The influence of these conditions on the degree of grafting is discussed next.

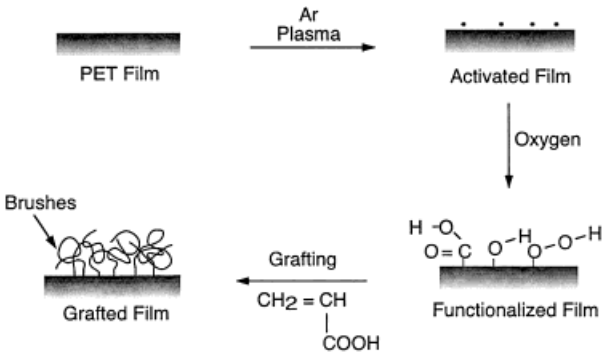


Figure 1 Schematic representation of the surface modification of PET films.

Influence of Monomer Concentration

Grafting was carried out with monomer concentrations of 5–60% for a reaction time of 6 h at 50°C. Results on the variation of grafting with the monomer concentration are presented in Figure 2. The degree of grafting showed a characteristic behavior with increases in the monomer concentration. The initial increase in grafting at low monomer concentrations was relatively slow, but a sharp acceleration was observed at a monomer concentration of 20%. Grafting reached a maximum at 40% monomer and then decreased with further increases in monomer concentration. Films could not be grafted beyond 50% monomer because of gel formation. These observations were quite similar to those of Lee et al.[19] for the plasma-induced grafting of 2-hydroxyethylmethacrylate onto silicone rubber. These authors found a maximum in the degree of grafting at 25% monomer, and the subsequent sharp decrease was attributed to extensive homopolymerization in the system.

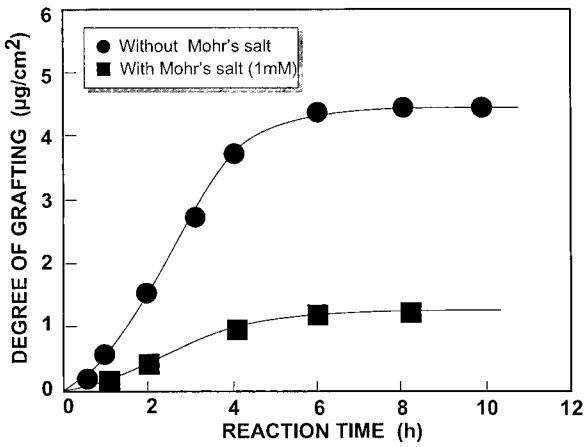


Figure 2 Variation of the degree of grafting with reaction time. Plasma treatment conditions: power = 80 W, time = 60 s. Grafting conditions: monomer concentration = 10%, reaction temperature = 50°C.

It seems that the complex grafting behavior in our system was governed by three independent factors, namely, the availability of monomer at the grafting sites, the extent of homopolymerization, and the viscosity of the reaction medium. The initial linear increase in the degree of grafting with the increase in monomer concentration was caused by the unhindered accessibility of the monomer to the primary radicals, P·, resulting in a smooth initiation step and propagation step :

$$P^{\bullet} + M \xrightarrow{k_i} \text{initiation} \quad (1)$$

$$P\text{—}CHX^{\bullet} + M \xrightarrow{k_p} \text{propagation} \quad (2)$$

Once the homopolymerization started, it changed the viscosity of the reaction medium. The higher the monomer concentration was, the higher the extent of homopolymerization was. The viscosity of the medium may have consequently increased to the extent that the Trommsdorf effect set in. The growing poly(acrylic acid) chains on the surface may be considered as having been solvated in the reaction medium [the acrylic acid/water system acted as a solvent for the poly(acrylic acid) chains]. Because of the high viscosity, the mobility of the chains decreased, and the termination of propagating chains (k_t), described by eq. (3), decreased. Despite some homopolymerization, as long as the propagation step was little affected, the overall result was the observed sharp increase in the degree of grafting with monomer concentration. The decrease in the degree of grafting beyond 40% monomer concentration, however, was caused both by the reduced effective monomer concentration caused by extensive homopolymerization and by the resultant highly viscous levels, which were sufficient to hinder the diffusion of the remaining monomer to the propagating sites. As a result, the rate of propagation (k_p) decreased considerably, and chain transfer (k_{tr}) to another species Q, such as the homopolymer in solution, started to dominate over chain propagation, k_p. The degree of grafting, as a result, showed a decreasing trend. The origin of the gel effect in solution beyond a 50% monomer concentration was so pronounced that it became impossible to separate the gel from the modified films:

$$P\text{—}CHX^{\bullet} + P\text{—}CHX^{\bullet} \xrightarrow{k_t} \text{dead polymer} \quad (3)$$

$$P\text{—}CHX^{\bullet} + Q \xrightarrow{K_{tr}} \text{chain transfer} \quad (4)$$

Influence of Plasma Treatment Time

The influence of the plasma exposure time on the degree of grafting is shown in Figure 3. Similar trends were seen for treatment times of 20, 60, and 100 s. The grafting increased with the increase in reaction time and reached saturation at 6 h in each case. The longer the plasma treatment time was, the higher the degree of grafting was. This is because the number of active species (hydroperoxides) involved in the grafting reaction increased with plasma treatment time. This is in

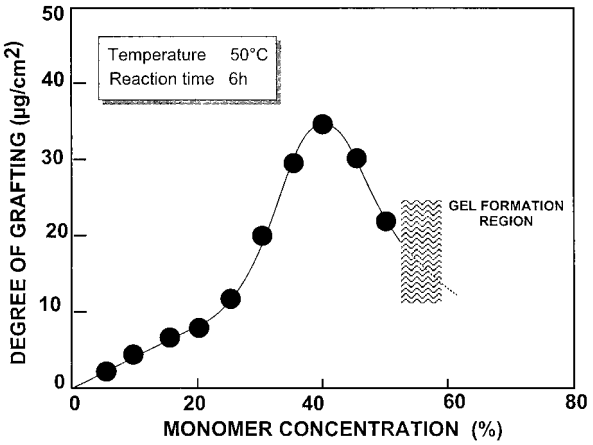


Figure 3 Variation of the degree of grafting with monomer concentration. Plasma treatment conditions: power = 80 W, time = 60 s.

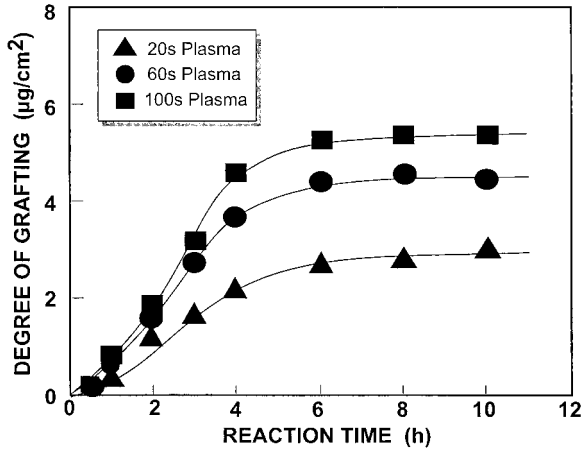


Figure 4 Variation of the degree of grafting with reaction time at different plasma treatment times. Plasma power = 80 W. Grafting conditions: monomer concentration = 10%, reaction temperature = 50°C.

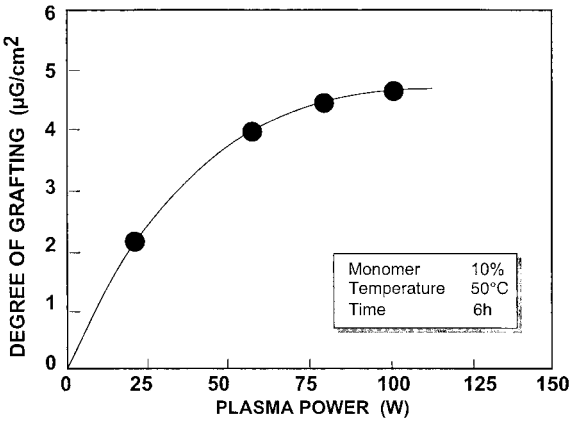


Figure 5 Variation of the degree of grafting with plasma power. Plasma treatment time = 60 s. Grafting conditions: monomer concentration = 10%, reaction temperature = 50°C, reaction time = 6 h.

line with our earlier results,[18] where we observed that the concentration of oxygen-containing species increased with the plasma treatment time from 20 to 100 s.

Influence of Plasma Power

The influence of plasma power on the degree of grafting is shown in Figure 4. The grafting increased with increases in plasma power from 20 to 100 W. The increase in grafting was very rapid up to 60 W and subsequently slowed down. This reflects a steady increase in radical formation by argon plasma at the PET surface with increasing power up to 100 W, but the increase slowed down from 60 to 100 W. A fraction of these radicals probably underwent quick deactivation at higher plasma powers. The hydroperoxide buildup, as a result, would not be proportional to the plasma power and rather would depend on the amount of radical reacting with oxygen. The same was reflected in the leveling off of the grafting at higher plasma powers. Lee et al.[19] showed that an increase in the degree of grafting of hydroxyethyl methacrylate (HEMA) onto silicone rubber was limited to a plasma power up to 80 W, beyond which the grafting decreased sharply.

Influence of Reaction Temperature

The variation of the degree of grafting with reaction time at various temperatures is shown in Figure 5. The grafting was carried out from 30 to 70°C at a 10% monomer concentration. All curves showed an increase in the degree of grafting with reaction time. The initial rates of grafting obtained from the slopes of the plots and the limiting degree of grafting (at 6 h) are shown in Figure 6. The initial rate of grafting increased continuously with the increase in the reaction temperature. However, the limiting degree of grafting increased up to a temperature of 50°C and thereafter tended to decrease.

It is proposed that in the early stages of the reaction, homopolymer formation was very limited and the local stationary concentration of monomer around the growing chain was maintained. This ensured fast chain initiation and

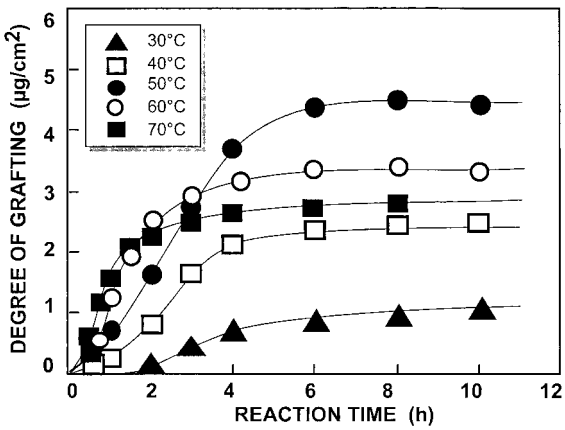


Figure 6 Variation of the degree of grafting with reaction time at different temperatures. Plasma treatment conditions: power = 80 W, time = 60 s. Grafting conditions: monomer concentration = 10%.

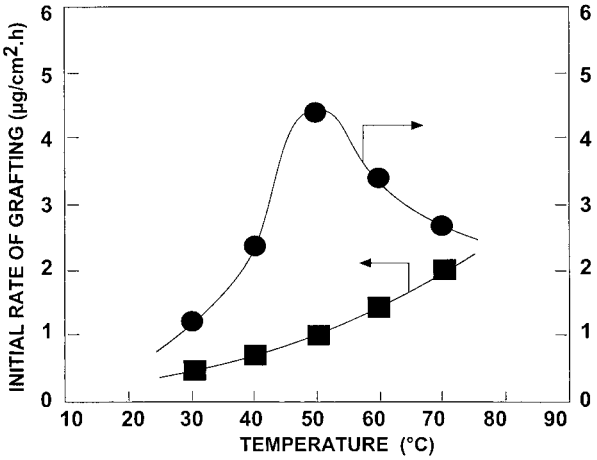


Figure 7 Variation of the initial rate of grafting and limiting degree of grafting with reaction temperature. See Figure 5 for the conditions.

propagation, leading to a high initial rate of grafting in the system. However, with the increasing temperature, the concentration of propagating chains increased as a result of a higher peroxide decomposition rate, and the termination of two growing chains by mutual recombination became a major factor. Once the homopolymer formation was extensive, the monomer depletion favored more chain transfers in the system. It is possible that the chain termination and chain transfer steps given by eqs. (3) and (4) (at temperatures higher than 50°C) dominated to such an extent that the limiting degree of grafting was reduced. It may also be possible that some of the primary radicals (P ·) became deactivated in the reaction medium , contributing to the reduced limiting degree of grafting at higher temperatures:

$$P^{\bullet} + P\text{—}CHX^{\bullet} \rightarrow \text{deactivation} \qquad (5)$$

The Arrhenius plot of the initial rate of grafting versus $1/T$ from 30 to 70°C, as presented in Figure 7, is linear. The activation energy obtained from the slope of the plot was 29.1 kJ/mol. This is of the same order of magnitude as the value of 41.4 kJ/mol reported in the literature for the graft polymerization of acrylic acid by free-radical initiation with benzoyl peroxide.[20] We can, in principle, assume that the grafting of acrylic acid as the homogeneous aqueous polymerization is initiated by free radicals. Because the plasma activation and functionalization are confined to the surface layers, the grafting onto the film takes place by the grafting-from mechanism. Therefore, the activation energy for the diffusion of monomer within the polymer film may be disregarded. A close relationship with the literature value for the activation energy supports the idea that the grafting is initiated by free radicals produced as a result of the decomposition of hydroperoxides. Grafting initiated by trapped radicals would have given an activation energy much lower than that observed in our system.

Influence of Mohr's Salt

The grafting of acrylic acid onto PET films is generally accompanied by the formation of poly(acrylic acid) homopolymer in the reaction medium. This not only leads to large-scale monomer waste but also influences the kinetics of the grafting process. As anticipated, the addition of Mohr's salt to the monomer solution suppressed homopolymer formation. A similar inhibitory effect of Mohr's salt on homopolymerization has been observed for the radiation-induced graft polymerization of acrylic acid onto polyethylene and fluorinated polymer films.[21,22]

The results for the variation of the degree of grafting with and without Mohr's salt are shown in Figure 8. The observed trend was consistent with the presence of surface hydroperoxide groups on PET films. The formation of hydroperoxides during plasma treatment is well known to occur when films are treated with argon and subsequently exposed to an oxygen atmosphere. The initiation of grafting by trapped radicals on the film surface appears unlikely as the argon-treated films were kept under oxygen for 1 h under am-

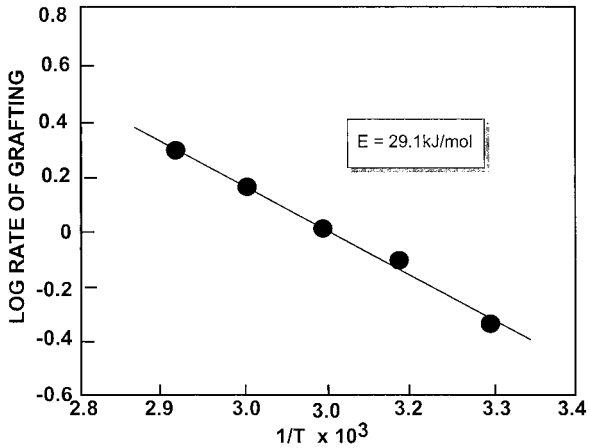


Figure 8 Arrhenius plot of the rate of grafting versus $1/T$. See Figure 5 for the conditions.

bient conditions. Trapped radicals, if any, must have reacted with oxygen under these conditions.

The trend in grafting with and without Mohr's salt was similar. In both cases, grafting increased with time and reached saturation after 6 h. The addition of $1 \times 10^{-3}M$ Mohr's salt to the reaction medium was effective in suppressing homopolymer formation. Mohr's salt, by virtue of its reducing nature, modifies the usual thermal decomposition of hydroperoxides to suppress the formation of hydroxyl radicals; this is partly responsible for homopolymerization during the grafting reaction [eq. (6) overpowered by eq. (7)]. The hydroxyl is transformed to hydroxyl ion, and the primary radical PO · (represented as P · in the preceding text) initiates the grafting reaction:

$$POOH \rightarrow PO^{\bullet} + OH^{\bullet} \quad (6)$$

$$POOH + Fe^{2+} \rightarrow PO^{\bullet} + OH^{-} + Fe^{3+} \quad (7)$$

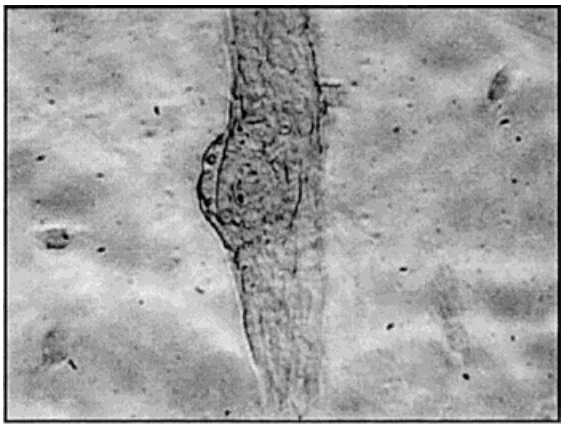
The important observation from these experiments is that the addition of Mohr's salt, although inhibiting to homopolymerization, diminished the degree of grafting as well. This was because some of the propagating poly(acrylic acid) graft chains were also terminated by ferrous ions. It may, therefore, be assumed that the ferrous ions not only enhanced the initiation of grafting by redox reaction with polymeric hydroperoxides but also led to faster chain termination:

$$P\text{—}CHX^{\bullet} + Fe^{2+} + H_2O \rightarrow P\text{—}CH_2X + Fe^{3+} + OH^{-} \quad (8)$$

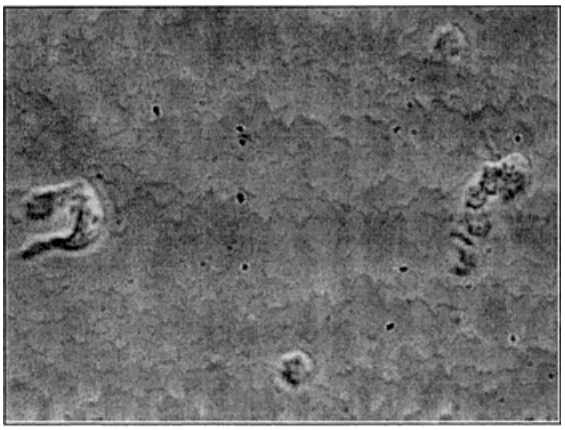
Our observations concerning the addition of Mohr's salt are quite different than those reported in the work of Hsiue and Wang,[23] who observed an increase in grafting with a maximum at 1 mM Mohr's salt. This was further supported by the results of Hirotsu[24] for the plasma grafting of different monomers onto polypropylene films. He found that the presence of metal ions such as Fe^{2+} and Fe^{3+} in the reaction medium increased the degree of grafting. These authors suggested that the metal ions, by virtue of their active role in the redox cleavage of the peroxide bond, offer new sites for the initiation of grafting chains.

Human Bladder Smooth Muscle Cells Seeded on Polymer Surfaces

In a very preliminary study, a bladder smooth muscle cell culture was carried out on a modified PET surface with a polyacrylic acid (PAA) graft density of 0.4 and 5 $\mu g/cm^2$ with or without collagen immobilization. On 5 $\mu g/cm^2$ PAA, smooth muscle cells did not adhere in the absence of immobilized collagen and, consequently, died quickly, as shown in Figure 9. Conversely, on surfaces with 0.4 $\mu g/cm^2$ PAA, bladder smooth muscle cells adhered and grew until confluence, as shown in Figure 10. This suggests that the excess poly(acrylic acid) chains may have been toxic to the cells and that toxicity was dependent on the concentration of grafts. Collagen-immobilized PAA grafted on the PET surface, however, showed cell adherence and growth on surfaces with correct cell morphology. Cell confluence, that is, full-surface coverage, was obtained on all the surfaces onto which collagen was immobilized



(a)



(b)

Figure 9 Observation of human smooth muscle cells seeded on 5 $\mu g/cm^2$ ($n = 3$) of PAA grafted on PET surfaces (a) with and (b) without collagen immobilization after 5 days in culture.

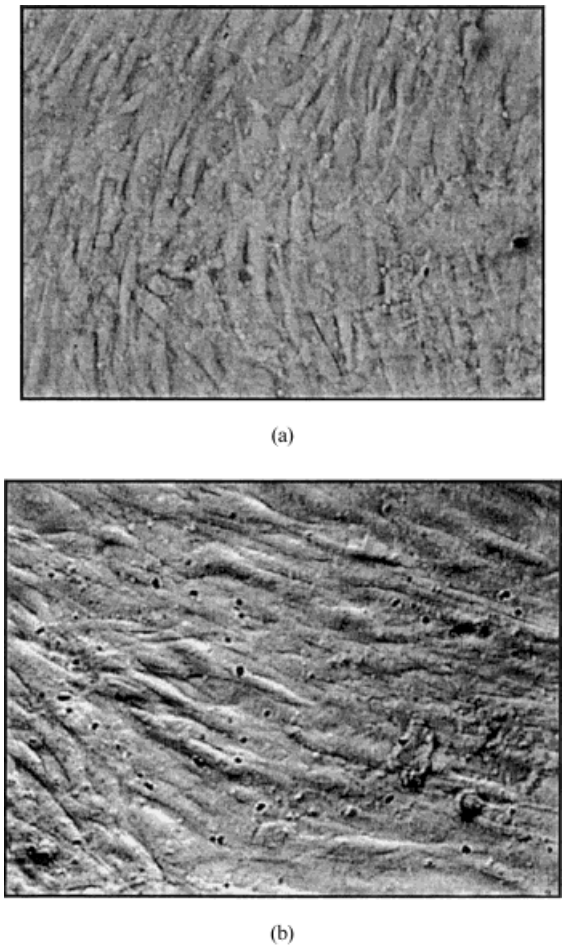


Figure 10 Observation of human smooth muscle cells grown on 0.4 $\mu g/cm^2$ (n = 3) of PAA grafted on PET surfaces (a) with and (b) without collagen immobilization after 5 days in culture.

with 0.4 $\mu g/cm^2$ PAA and partially obtained on 5 $\mu g/cm^2$ PAA-based surfaces after 6 days in culture. The effect of collagen-immobilized surfaces on cell differentiation is currently under investigation in our laboratories and will be communicated later.

CONCLUSIONS

For extracellular protein to be grafted and, therefore, for PET surfaces to be rendered suitable to act as cell scaffolds, they may be modified by the plasma-induced graft polymerization of acrylic acid onto films that are pretreated with argon plasma and subsequently exposed to an oxygen atmosphere. The extent of the modification of the film surface depends on the reaction conditions. Ferrous sulfate is known to cleave hydroperoxide groups in a way that inhibits homopolymerization during the grafting reaction.[21] The mode of action of Mohr's salt in this system indicates a hydroperoxide-initiated grafting mechanism. The overall graft kinetics is determined by two important factors, namely, the accessibility of the monomer to the grafting sites, which is dependent on the extent of competing homopolymerization, and the viscosity of the reaction medium. These factors operate independently and contribute to the kinetics at different stages of the grafting reaction. It may be assumed that the reactivity of primary radicals is an important factor in graft initiation; it is, at last, the viscosity of the grafting medium that dominates the kinetics at higher monomer concentrations and higher temperatures and determines the graft levels. The termination of growing poly(acrylic acid) grafts may follow various routes, as summarized in Figure 11. These routes include the deactivation of primary radicals, bimolecular growing chain termination, and chain transfer to the homopolymer or to an inactive species. The initial evaluation of cell cultures with these surfaces before and after collagen immobilization indicates the possibility of their use as scaffolds.

This study provides information on the construction of PET surfaces with a desired chemical composition by the careful selection of the plasma treatment and the graft reaction conditions and may be extended to complexly shaped objects such as textile fibers and knitted or woven scaffolds. We are currently characterizing the surface structure and morphology of these grafted films for the subsequent immobilization of proteins.

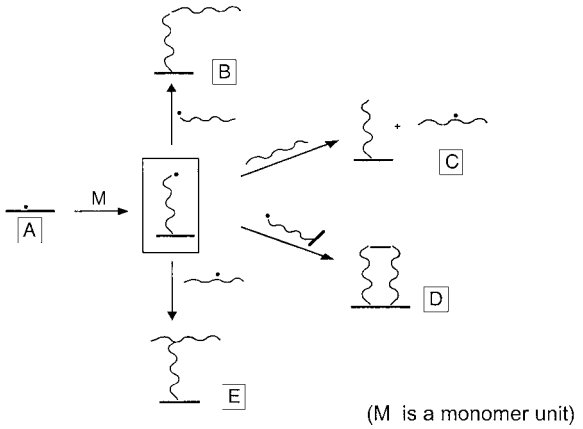


Figure 11 Schematic representation of the termination of growing poly(acrylic acid) graft chains.

REFERENCES

1. Patric, C. W., Jr.; Mikos, A. G.; McIntire, L. V. Frontiers in Tissue Engineering; Pergamon: New York, 1988.
2. Hoffman, A. S. J Appl Polym Sci Appl Polym Symp 1988, 42, 251.
3. Suzuki, M.; Kishida, A.; Iwata, H.; Ikada, Y. Macromolecules 1986, 19, 1804.
4. Johnsen, K.; Kirkhorn, S.; Olasen, K.; Redford, K.; Stori, A. J Appl Polym Sci 1996, 59, 1651.
5. Poncin-Epaillard, F.; Chevet, B.; Brosse, J. J Appl Polym Sci 1994, 53, 1291.
6. Qiu, Y. X.; Klee, D.; Pluester, W.; Severich, B.; Hoecker, H. J Appl Polym Sci 1996, 61, 2373.
7. Sano, S.; Kato, K.; Ikada, Y. Biomaterials 1993, 14, 817.
8. Kang, I.; Kwon, B. K.; Lee, J. H.; Lee, H. B. Biomaterials 1993, 14, 787.
9. Bae, J.; Seo, E.; Kang, I. Biomaterials 1999, 20, 529.
10. Lee, S.; Hsiue, G.; Chang, P. C.; Kao, C. Biomaterials 1996, 17, 1599.
11. Piglowski, J.; Gancarz, I.; Staniszewska-Kus, J.; Paluch, D.; Szymonowicz, M.; Konieczny, A. Biomaterials 1994, 11, 909.
12. Kato, K.; Ikada, Y. Biotechnol Bioeng 1995, 51, 581.
13. Kulik, E. A.; Kato, K.; Ivanchenko, M. I.; Ikada, Y. Biomaterials 1993, 14, 763.
14. Seto, S. F.; Muraoka, Y.; Sakamoto, N.; Kishida, A.; Akashi, M. Angew Makromol Chem 1999., 266, 56.
15. Ludwikowski, B.; Zhang, Y. Y.; Frey, P. Br J Urol 1999, 84, 507.
16. Sugasi, S.; Lesbros, Y.; Bisson, I.; Zhang, Y. Y.; Kucera, P.; Frey, P. J Urol 2000, 164, 951.
17. Hilborn, J.; Frey, P. Brit. Pat. Appl. 9824562.
18. Gupta, B.; Hilborn, J.; Hollenstein, C.; Plummer, C. J. G.; Houriet, R.; Xanthopoulos, N. Personal communication, 1999.
19. Lee, S.; Hsiue, G.; Wang, C. J Appl Polym Sci 1994, 54, 1279.
20. Sacak, M.; Oflaz, F. J Appl Polym Sci 1993, 50, 1909.
21. Gupta, B.; Chapiro, A. Eur Polym J 1989, 25, 1137.
22. Bozzi, A.; Chapiro, A. Radiat Phys Chem 1988, 32, 193.
23. Hsiue, G.; Wang, C. J Polym Sci Part A: Polym Chem 1993 , 31, 3327.
24. Hirotsu, T. J Macromol Sci Pure Appl Chem 1996, 33, 1663.