

Biological Affinity and Biodegradability of Poly(propylene carbonate) Prepared from Copolymerization of Carbon Dioxide with Propylene Oxide

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Abstract: In this study we investigated bacterial and cell adhesion to poly(propylene carbonate) (PPC) films, that had been synthesized by the copolymerization of carbon dioxide (a global warming chemical) with propylene oxide. We also assessed the biocompatibility and biodegradability of the films *in vivo*, and their oxidative degradation *in vitro*. The bacteria adhered to the smooth, hydrophobic PPC surface after 4 h incubation. *Pseudomonas aeruginosa* and *Enterococcus faecalis* had the highest levels of adhesion, *Escherichia coli* and *Staphylococcus aureus* had the lowest levels, and *Staphylococcus epidermidis* was intermediate. In contrast, there was no adhesion of human cells (cell line HEp-2) to the PPC films, due to the hydrophobicity and dimensional instability of the surface. On the other hand, the PPC films exhibited good biocompatibility in the mouse subcutaneous environment. Moreover, contrary to expectation the PPC films degraded in the mouse subcutaneous environment. This is the first experimental confirmation that PPC can undergo surface erosion biodegradation *in vivo*. The observed biodegradability of PPC may have resulted from enzymatic hydrolysis and oxidative degradation processes. In contrast, the PPC films showed resistance to oxidative degradation *in vitro*. Overall, PPC revealed high affinity to bioorganisms and also good biodegradability.

Keywords: poly(propylene carbonate), bacterial adhesion, cell adhesion, biocompatibility, biodegradability.

Introduction

Carbon dioxide (CO₂) is the cheapest and most abundant carbon source raw material, but is considered the main greenhouse gas responsible for global warming, a process which may result in climate change.¹ Thus reduction of CO₂ emissions is a high priority, and methods for CO₂ capture and utilization (or disposal) are currently under active investigation worldwide. Conversion into polymeric materials is one possible form of CO₂ utilization. Poly(alkylene carbonate), for example, can be produced by the copolymerization of CO₂ with alkylene oxide.²⁻¹⁸ We previously reported a very efficient process for the copolymerization of CO₂ and

propylene oxide (PO; a representative alkylene oxide) using zinc glutarate as a catalyst, producing a high yield of poly(propylene carbonate) (PPC).¹⁴ Because PO is used in this process as a reaction medium as well as a comonomer, no other organic solvent is involved in copolymerization and no organic solvent waste results.¹⁴ Therefore, this can be considered a green polymerization process.

Aliphatic polyesters are an attractive class of artificial polymers from an environmental standpoint, as they degrade in contact with living tissues as well as in natural environments.^{19,20} In particular, poly(ϵ -caprolactone) (PCL) is biodegradable and of commercial interest because of its remarkable miscibility with a number of polymers.^{19,20} In view of this, investigation of PPC was warranted as its aliphatic backbone is similar to that of PCL. To assess the enzymatic degradability of PPC, films of the polymer were

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treated with a variety of enzymes (a total of eighteen enzymes representing four different enzyme families) in a phosphate buffer. Positive degradability was found for *Rhizopus arrhizus* lipase, esterase/lipase ColoneZyme A and Proteinase K.²¹ These results indicate that the PPC copolymer is susceptible to these enzymes even though it contains methyl side groups, which are responsible for its relatively high glass transition temperature and high modulus. The enzymatic degradation of PPC appeared to occur via an erosion process. However, the biodegradability of PPC has not yet been investigated so far.

In the present study we investigated bacterial and cell adhesion to PPC *in vitro*, and biocompatibility and biodegradability in mice. Adhesion of human cells (cell line HEp-2) and five bacterial species [*Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecalis* (*E. faecalis*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*)] to PPC films was examined. PPC was also implanted subcutaneously in mice to assess biocompatibility by examining tissue reactions around implant sites and to assess biodegradation causing morphologic changes to the film surface. In addition, *in vitro* oxidative degradation tests were carried out to understand the mechanism of PPC biodegradation in mice.

Experimental

Materials. PPC (weight-average molecular weight 673,000; polydispersity 2.97) was synthesized from CO₂ and PO using zinc glutarate as catalyst, as previously described (Figure 1).¹³⁻¹⁶ The PPC polymer was fabricated into films by compression molding under nitrogen at 120-130 °C for 10 min at a pressure of 100 kg/cm², followed by cooling in liquid nitrogen. The resulting films (0.20 ± 0.01 mm thick) were cut into appropriately-sized pieces (disk, square or rectangular shape) and dried under vacuum at room temperature for 2 days before use. Special care during the film fabrication was provided to minimize the microbial contamination on the film surface.

S. epidermidis (ATCC No. 12228), *S. aureus* (ATCC No. 6538), *E. faecalis* (ATCC No. 29212), *P. aeruginosa* (ATCC No. 15442) and *E. coli* (ATCC No. 25922) were obtained from the Korean Culture Center of Microorganisms (Seoul, Korea), and the HEp-2 cell line was obtained from the Korea Cell Line Bank (Seoul, Korea). Here it is noted that *S. epidermidis*, *S. aureus* and *E. faecalis* are Gram-positive bacteria while *P. aeruginosa* and *E. coli* are Gram-negative bacteria. ICR mice were purchased from Korea Research Institute of Bioscience & Biotechnology (Daejeon, Korea).

Bacterial culture media were obtained from Difco Laboratories (Detroit, MI, USA). Cell culture media and reagents including fetal bovine serum (FBS), advanced minimum essential medium (AMEM), L-glutamax and trypsin with

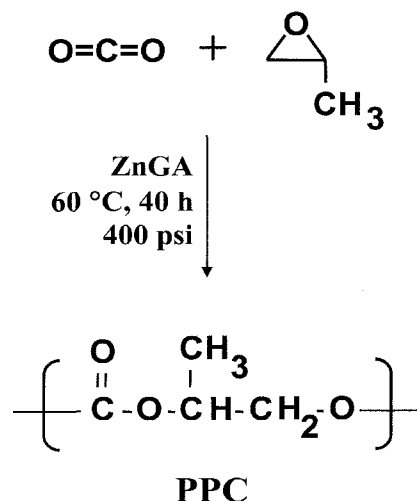


Figure 1. Synthetic scheme and chemical structure of poly(propylene carbonate) (PPC).

ethylenediaminetetraacetic acid (EDTA) were purchased from Invitrogen (Grand Island, NY, USA). Other cell culture grade chemicals were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Phosphate buffered saline (PBS; pH = 7.4) for bacterial culture contained 0.20 g potassium chloride, 8.00 g sodium chloride, 1.36 g potassium dihydrogen phosphate and 1.42 g sodium hydrogen phosphate in 1.00 L deionized distilled water. Dulbecco's phosphate buffered saline (DPBS; pH = 7.4) for cell culture contained 0.20 g potassium chloride, 8.00 g sodium chloride, 0.20 g potassium dihydrogen phosphate and 1.15 g sodium hydrogen phosphate in 1.00 L deionized distilled water. Disposable laboratory supplies were obtained from Falcon (Franklin Lakes, NJ, USA).

Surface Characteristics and Water Sorption. The surface of PPC films was examined by atomic force microscopy (AFM). AFM measurements were carried out in tapping mode using a digital microscope (Multimode Nanoscope IIIa) equipped with a J-scanner. Noncoated silicon etched probes (TESP, Veeco, Santa Barbara, CA, USA) were used with a resonance frequency of 300 kHz; the spring constant of the probe tip and the scan rate were 40 Nm⁻¹ and 0.7 Hz, respectively. Optical microscopy (OM) images were taken using an Olympus microscope (BX51, Tokyo, Japan) equipped with a digital camera. Scanning electron microscopy (SEM) images were obtained using a field emission scanning electron microscope (S-4200, Hitachi, Tokyo, Japan) after platinum coating of the film surface. Contact angle measurements were conducted at 25 °C with a contact angle meter (CA-D, Face, Japan) using two solvents as standards: water with a surface energy γ of 72.2 mJ/m² and diiodomethane with a surface energy of $\gamma = 50.8$ mJ/m².²² From the measured contact angles, surface energies of the PPC films were calculated according to the Owens-Wendt

geometric mean formula.²³ In addition, swelling measurements were conducted in water at room temperature and in PBS at 37 °C using a high precision chemical balance.

Bacterial Adhesion. The bacteria were routinely grown at 37 °C on nutrient agar plates (NAP), or in nutrient broth (NB) with shaking (250 rpm). An overnight culture of each bacterium was diluted 100-fold in NB and incubated until mid-logarithmic phase was reached. The bacterial cells were centrifuged, resuspended and serially diluted in PBS, and counted following plating and colony growth on NAP. The bacterial concentration used in adhesion experiments ranged from 6×10^7 - 9×10^8 colony forming units (CFU) per mL. Pieces of PPC film (1 cm \times 1 cm) were sterilized by immersion in 70% ethyl alcohol and dried aseptically in air. Film sterility was confirmed by incubating the ethanol-treated films in NB at 37 °C overnight. Individual film pieces were immersed in 10 mL PBS in a 50 mL conical tube. Bacterial suspension (100 μ L; approximately 10^7 CFU) was added to the tube, which was incubated at 37 °C for 4 h with gentle shaking (200 rpm). After incubation the film was rinsed several times in PBS to remove nonadherent bacteria, transferred to a new tube with 5 mL PBS containing 0.05 wt% Tween-20 (polyoxyethylene(20) sorbitan monostearate), and sonicated twice for 5 s at 37 °C to detach the adherent bacteria from the film surface. OM of the films treated by Gram staining demonstrated that this process removed all adherent bacteria. The detached bacterial cells were counted as described above.

Cell Adhesion. HEp-2 cells were routinely cultured in a humidified 5% CO₂ atmosphere at 37 °C in T-75 culture flasks containing 10-12 mL AMEM, 10% (v/v) FBS and 0.5% (v/v) L-glutamax, as described previously.^{24,25} Cell growth was observed using an inverted microscope (Nikon Eclipse TS-100 with Coolpix5400 camera, Tokyo, Japan) at 400 \times magnification. PPC film specimens were cut into squares (1 \times 1 cm²), sterilized by immersion in 70% ethyl alcohol for 20 min, washed for 2 h with DPBS, and placed in the wells of a T-25 culture tray. HEp-2 cells were seeded onto the films at a density of 0.5×10^6 cells/mL ($A_{660} = 0.15$) and incubated for 10 days, and the medium was changed every second day.^{26,27} A PPC specimen was removed carefully from the trays at 6 h, 1, 3, 7, and 10 days to check the cell growth on the film surface using an inverted microscope.

Implantation. The mouse experiment was reviewed and approved by Animal Care/Ethics Committee of Dongguk University College of Medicine. Healthy 8 week-old male ICR mice (body weight 28-32 g) for implantation were kept at an animal facility and maintained for 1 week according to University guidelines for the care and use of animals. PPC films (0.7 cm diameter, 0.2 mm thickness) were sterilized by immersion in 100% ethyl alcohol for 20 min then 70% ethyl alcohol for 20 min, and rinsed with normal saline (0.9%) for 1 h. Mice were anesthetized by intraperitoneal

injection with a mixture of Rompun[®] (xylazine, 10 mg/kg body weight, Virbac, France) and Zoletil (tiletamine and zolazepam, 50 mg/kg body weight, Bayer, Germany). The mouse dorsal area was shaved and disinfected using beta-dine solution (10%) and then ethyl alcohol (70%). Two incisions (approx. 1 cm long) were made laterally at the midline of the back. After the PPC film specimens were inserted into the subcutaneous pockets formed by blunt dissection, the incision sites were closed by wound clips (Reflex 7, CellPoint Scientific INC, Gaithersburg, MD, USA) and cleaned again with 70% ethanol.

Mice were euthanized by ether inhalation and cervical dislocation at 1, 2, 4, 8 and 12 weeks postimplantation. The PPC films and surrounding tissues were removed for morphological examination for film degradation, and histological analyses to assess tissue reactions. For morphological analysis the films were rinsed with PBS, fixed in methanol for 15 min, and examined by OM before and after drying. The film specimens were freeze dried, then platinum-coated and examined by SEM. To assess tissue reactions, PPC films and surrounding tissues were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned (4 μ m), and the sections stained with hematoxylin and eosin (H&E) for OM analysis.²⁸

In vitro Oxidation. To investigate the mechanism of PPC biodegradation in mice, *in vitro* oxidative degradation measurements were carried out according to a published method.²⁹ The PPC films were treated for up to 21 days in an oxidative solution of 20% hydrogen peroxide (H₂O₂) in aqueous 0.1 M cobalt chloride (CoCl₂). The solution was changed twice each week to maintain a constant concentration of oxygen radicals during the degradation process. Representative films were removed every 7 days, platinum coated, and examined by SEM.

Results and Discussion

Surface Characteristics and Water Sorption. The obtained PPC was an amorphous polymer with a glass transition over the temperature range 17-37 °C (glass transition temperature $T_g = 28$ °C), a degradation temperature T_d of 252 °C, and a density of 1.27 g/cm³. The PPC polymer was easily molded into films or sheets. The surfaces of the PPC films were examined by OM, SEM, and AFM. A root mean square surface roughness of 3.5 nm over a film surface area of 2 \times 2 μ m² was determined by AFM examination (AFM image not shown). These microscopy analyses confirmed that the PPC films have a very smooth surface.

Water absorption by the PPC films, measured in water at room temperature and in PBS at 37 °C, was very low. The films had no water sorption within 10 min of immersion in either solution, and reached only 1.5 wt% in water and 1.8 wt% in the PBS solution after immersion for 4 h or longer.

The dried PPC films had a water contact angle of 76.6°, which corresponded to a surface energy of 42.9 mJ/m². The water contact angle barely changed even after immersion in PBS for 4 h at 37 °C. These results indicate that the PPC film had a hydrophobic surface characteristic even though the polymer has hydrophilic carbonate units in the backbone.

Bacterial Adhesion. We tested adhesion to PPC films by five bacterial species. All the tested strains revealed various levels of adhesion to the PPC surface after 4 h incubation (Table I). The mechanisms of bacterial adhesion and colonization on biomaterial surfaces are not fully understood because of the complexity of the processes involved. Initial bacterial adhesion to surfaces depends on physicochemical properties (hydrophobicity, hydrophilicity, degree of water swelling, surface energy), the roughness of the material surface, and hydrodynamics and other characteristics (e.g. pH, nutrient concentrations, ionic strength and temperature) of the aqueous medium.³⁰⁻³² Most bacteria are negatively charged but contain hydrophobic and hydrophilic surface components.³³ Thus, the further rate and extent of bacterial adhesion depends on bacterial surface hydrophobicity, the presence of surface appendages (fimbriae, pili, fibrils and flagella), the presence of other surface proteins (adhesins) and lipopolysaccharide (LPS), and the production of extracellular polymeric substances (EPS).³⁰⁻³⁴ Fimbriae, other surface appendages and adhesin proteins appear to dominate adhesion to the hydrophobic substrata, while LPS and EPS are more important in adhesion to hydrophilic materials.^{33,34} The water contact angles for *P. aeruginosa* and *S. aureus* are 84-132° and 20-36°, respectively,³⁵ and that for *S. epidermidis* is 26-42°.³⁶ These indicate that the cell surface of *P. aeruginosa* is hydrophobic, whereas the surfaces of *S. aureus* and *S. epidermidis* cells are hydrophilic.

During the 4 hours of the adhesion experiment the initial deposition rate would have been primarily PPC substratum-dependent, and subsequent bacterial adhesion would have been more bacteria-dependent, resulting in differences in the numbers of adhering bacteria. *P. aeruginosa* and *E. faecalis* had the highest levels of adhesion, *E. coli* and *S. aureus* had lowest levels, and *S. epidermidis* was intermediate (Table I). The exceptional adhesion observed for *P. aeruginosa* is predictable, considering its ability to colonize and form biofilms in low nutrient environments in nature as

well as in niches in the human body, facilitated by a variety of adhesive tools including fimbriae, an alginate slime layer, and the presence of protease enzymes. *E. faecalis* is also known to cause serious infections associated with indwelling foreign bodies, and expresses aggregation substances

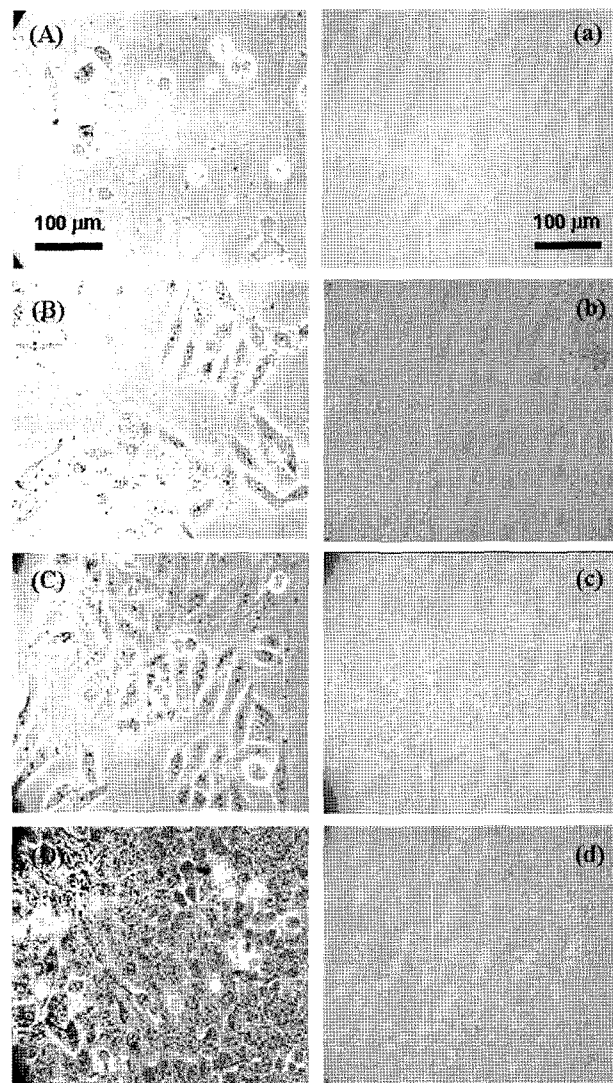


Figure 2. HEp-2 cell adhesion to PPC films. Normal cell adhesion and growth were exhibited on the bottom floor of polystyrene culture tray (A) after 6 h; (B) after 1 day; (C) after 3 days; (D) after 7 days. No cell adhesion was observed on the PPC film surface (a) after 6 h; (b) after 1 day; (c) after 3 days; (d) after 7 days.

Table I. Bacterial Adhesion to PPC Film

Bacterium	Inoculum ^a (CFU)	Bacterial Adhesion ^{a,b} (CFU)	Bacterial Adhesion per Unit Area (mm ²)
<i>E. faecalis</i>	9.2(±2.8)×10 ⁷	3.8(±1.5)×10 ⁵	1.7(±0.7)×10 ³
<i>P. aeruginosa</i>	5.3(±2.6)×10 ⁷	3.6(±1.8)×10 ⁵	1.6(±0.8)×10 ³
<i>S. epidermidis</i>	2.1(±1.4)×10 ⁷	2.8(±0.4)×10 ⁴	1.3(±0.2)×10 ²
<i>E. coli</i>	2.9(±1.6)×10 ⁷	1.0(±0.3)×10 ⁴	4.5(±1.4)×10 ¹
<i>S. aureus</i>	6.3(±2.0)×10 ⁶	5.0(±6.1)×10 ³	2.2(±0.8)×10 ¹

^aAveraged from triplicated experiments. ^bMeasured after incubation with bacterial suspensions in PBS for 4 h at 37 °C.

(Agg) and a surface protein (Esp) aiding adhesion to various surfaces.³⁷ *S. epidermidis*, a skin flora bacterium with very low pathogenicity compared to *S. aureus*, is one of the most frequently encountered causative agents of implant-derived infections, and expresses EPS slime to adhere to medical devices.

Cell Adhesion. After seeding the PPC films with HEp-2 cells, we monitored cell growth for 10 days. As seen in Figures 2(A-D), HEp-2 cells adhered, grew, and formed a confluent monolayer on the bottom floor of the T-25 culture tray which was made of cell culture-grade polystyrene, indicating the normal growth of the cells. Surprisingly, no cells were observed during the test period on the surface of PPC films which had been laid on the bottom of the culture trays before the cell seeding, indicating the cell adhesion was prohibited on the PPC films (Figures 2(a-d)).

In general, cell adhesion to substrata depends on substratum surface solidity, surface charge density, and hydrophi-

licity or water swellability.³⁸ As discussed above, the PPC film surface is rather hydrophobic and exhibits a very low degree of water swelling. The cell culture was conducted at 37 °C, which is the upper temperature limit of the glass transition ($T_g=28$ °C) of the amorphous PPC films. Thus, the PPC film was sufficiently softened at the incubation temperature to result in some surface mobility and undulation reflecting a degree of dimensional instability. Therefore, it may be that factors including hydrophobicity, low water swelling, and low T_g combine to discourage the adhesion and stable growth of HEp-2 cells on the PPC films.

Biocompatibility and Biodegradability. The importance of biocompatibility in determining the therapeutic value of biomaterials, and ensuring the efficacy and safety of implanted devices, is well recognized.³⁹ Therefore, we carried out subcutaneous implantation of PPC films in mice to test biocompatibility of the PPC films. The implanted films were removed with surrounding tissues at 1, 4, 8 and 12 weeks postimplan-

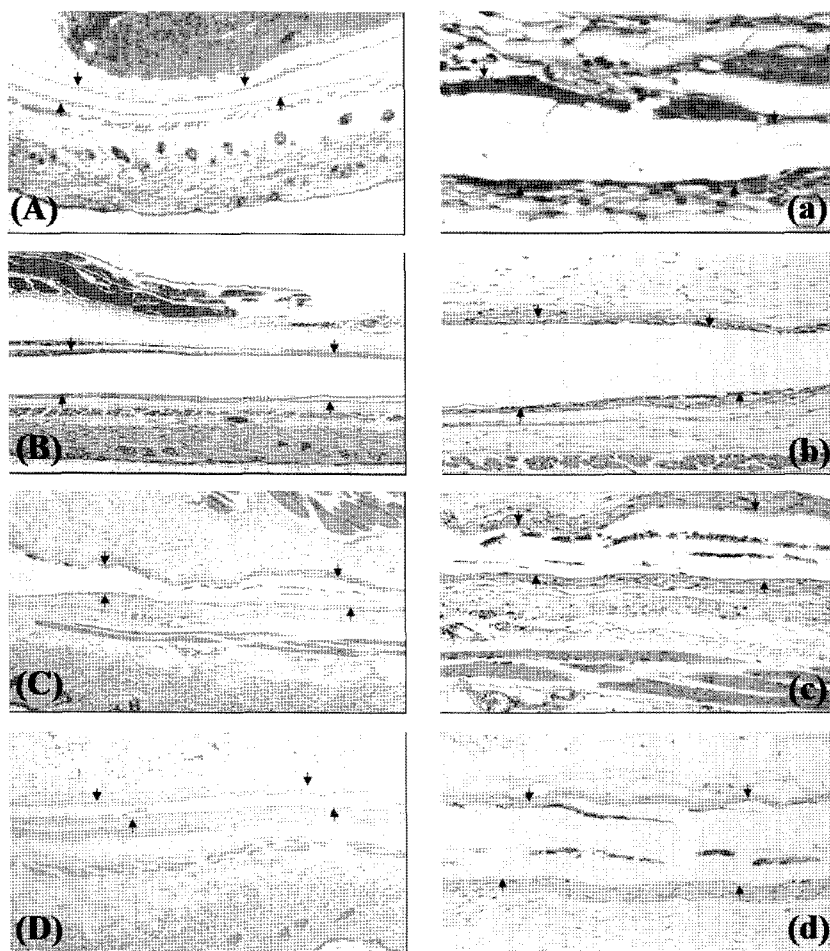


Figure 3. Tissue reactions at various stages after implantation: (A) and (a), after 1 week multinucleated giant cells have adhered to the PPC film and persist throughout the experiment; (B) and (b), after 4 weeks active inflammation has completely subsided, and a thin and delicate capsule has formed around the implant; (C) and (c), after 8 weeks the capsule has thickened; (D) and (d), after 12 weeks the giant cell reaction is still present. PPC films were dislodged during the staining process, leaving the empty spaces. Original magnifications: (A)-(D), 100 \times ; (a)-(d), 40 \times .

tation. Figure 3 shows optical micrographs of the subcutaneous tissue responses to the films at various times after implantation.

As seen in Figures 3(A) and 3(a), multinucleated giant cells were observed near the PPC film after 1 week, and the recruitment of some inflammatory cells (macrophages, lymphocytes and neutrophils) was evident following tissue wounding and implantation of the foreign PPC film. The inflammatory reactions around the implanted films appeared to be a normal host defense mechanism typical in surgical implantation.³⁹ The acute inflammatory response was almost completely resolved by 4 weeks, but many foreign-body giant cells (FBGCs) were still located near the dense fibrous capsule (Figures 3(B) and 3(b)). At 6 weeks the fibrous capsule appeared thin and loosely assembled (Figures 3(C) and 3(c)). By 12 weeks the inflammatory reactions had decreased further with the exception of FBGCs, and the tissues surrounding the implants, comprising thick fibrous capsule, were stably retained (Figures 3(D) and 3(d)). These results indicate that the PPC implants elicited relatively low levels of acute inflammatory reaction and that there was no extended chronic inflammation and tissue necrosis. Furthermore, no visible signs of physical impediment or systemic and neurological toxicity were observed for 12 weeks. Overall, the tissue response in the mouse subcutaneous model suggests that PPC has good biocompatibility.

The PPC film samples explanted at various stages after implantation in mice were examined by OM and SEM. To our knowledge, PPC has not been reported to be degradable *in vivo*. Surprisingly, representative SEM surface images (Figure 4) showed surface PPC degradation. After only 4 weeks of implantation, the film had a much rougher surface (Figure 4(a)) than before implantation. At 6 weeks, the PPC surface was irregular and rougher (Figure 4(b)) than at 4

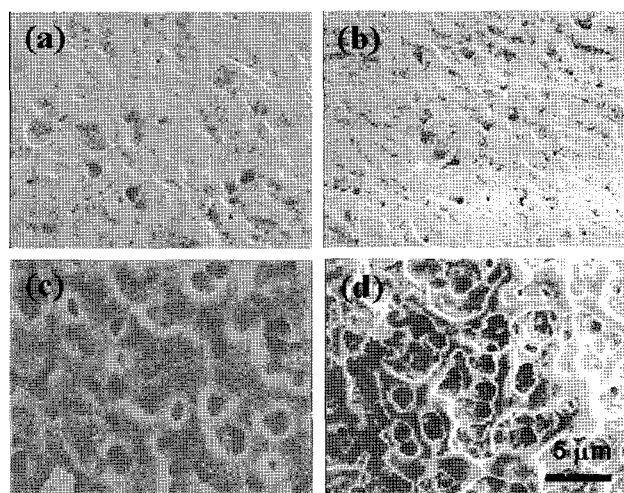


Figure 4. SEM surface images of PPC film samples explanted at various stages after implantation: (a) after 4 weeks; (b) after 6 weeks; (c) after 8 weeks; (d) after 12 weeks.

weeks. At 8 weeks there were numerous pits and holes (diameter of 2-5 μm) of considerable depth (Figure 4(c)), indicating a high degree of degradation of the PPC film surface, which continued up to 12 weeks from implantation (Figure 4(d)). These results demonstrated for the first time that PPC films implanted in mice undergo surface erosion biodegradation.

***In vitro* Oxidation Analysis and the Mechanism of Biodegradation.** There are three likely major mechanisms of PPC biodegradation *in vivo*: (i) oxidative degradation, (ii) enzymatic degradation, and (iii) hydrolytic degradation. We treated the PPC films for various times with an oxidative ($\text{H}_2\text{O}_2/\text{CoCl}_2$) solution to mimic at an accelerated rate of *in vivo* oxidative degradation²⁹ and made SEM observations for degradation. There was no evidence of degradation in films treated for 1 or 2 weeks (Figures 5(a) and 5(b)), but samples treated for 3 weeks had some damage to the surface (Figure 5(c)). These results suggest that PPC shows resistance to oxidative degradation, particularly because the oxidative solution, $\text{H}_2\text{O}_2/\text{CoCl}_2$ mimics the effect of the *in vivo*

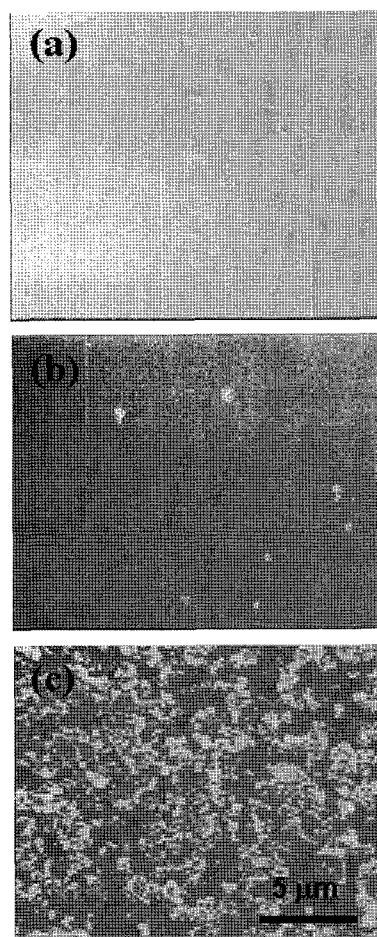


Figure 5. SEM surface images of PPC film samples treated in an oxidative ($\text{H}_2\text{O}_2/\text{CoCl}_2$) solution for various times: (a) after 1 week; (b) after 2 weeks; (c) after 3 weeks.

microenvironment at the adherent cell-material interface.⁴⁰ Thus, *in vivo* oxidation may have been partially involved in PPC biodegradation in the mice, but its contribution was unlikely to have been responsible for the observed biodegradation.

In a previous investigation of hydrolytic degradation of PPC in tetrahydrofuran solutions containing 10 wt% acidic or basic aqueous solutions of varying pH at 30 °C,⁴¹ we found evidence of degradation in strongly acidic (pH < 5.0) and basic (pH > 9.0) solutions. However, PPC was very stable for 20 days in solutions of pH 5.0-9.0. Since the physiological pH of mice is 6.8-7.4, hydrolytic processes may not be involved in PPC biodegradation. However, the PPC films were subject to degradation by enzymes purified from several microbes.²¹

The mechanism of PPC degradation in mice remains to be established, but the results of this study indicate that the *in vivo* degradation of PPC specimens observed in this study may have resulted from enzymatic hydrolysis and oxidative degradation processes. It may be that the FBGCs, which occurred around the implanted films throughout the experiment, may be the source of the oxidizing radicals and the degrading enzymes. Further investigation is planned to involve C¹⁴-labeled PPC to follow the degradation process, and to assess any adverse effects of the PPC or the degradation products.

Conclusions

Films of a PPC polymer synthesized by polymerization of carbon dioxide with propylene oxide were studied with respect to bacterial and cell adhesion as well as biocompatibility and biodegradability. Important biological characteristics of the hydrophobic PPC films were observed as follows.

Firstly, all the tested bacteria (*S. epidermidis*, *S. aureus*, *E. faecalis*, *P. aeruginosa* and *E. coli*) adhered to the PPC film over 4 h at various levels. The bacterial adhesion can be attributed to the surface structure and characteristics of the bacteria, and their interactions with the hydrophobic PPC film surface.

Secondly, adhesion of human HEp-2 cells was not observed on the PPC surface. This result might be probably due to the combined effect of three characteristics of the PPC films – hydrophobicity, very low water swelling, and dimensional instability – which serve to discourage adhesion and stable growth of the HEp-2 cells on the film surface.

Thirdly, PPC exhibited good biocompatibility in mice. The PPC implants elicited relatively low levels of acute inflammatory reaction and there was an absence of extended chronic inflammation and tissue necrosis at the site of implantation.

Finally, PPC was biodegradable in mice by surface erosion, which might probably result from enzymatic hydrolysis and oxidative degradation processes.

In summary, PPC reveals high affinity to bacteria and furthermore good biodegradability.

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References

- (1) J. Paul and C. M. Pradier, Editors, *Carbon Dioxide Chemistry: Environmental Issues*, Royal Soc. Chem., Cambridge, 1994.
- (2) J. H. Jung, M. Ree, and T. Chang, *J. Polym. Sci. Part A: Polym. Chem.*, **37**, 3329 (1999).
- (3) J. S. Kim, H. Kim, and M. Ree, *Chem. Mater.*, **16**, 2981 (2004).
- (4) S. Inoue, H. Koinuma, and T. Tsuruta, *Makromol. Chem.*, **130**, 210 (1969).
- (5) K. Soga, E. Imai, and I. Hattori, *Polymer J.*, **13**, 407 (1981).
- (6) D. J. Darensbourg, J. R. Wildeson, J. C. Yarbrough, and J. H. Reibenspies, *J. Am. Chem. Soc.*, **122**, 12487 (2000).
- (7) M. Super, E. Berluce, C. Costello, and E. Beckman, *Macromolecules*, **30**, 368 (1997).
- (8) C. S. Tan and T. J. Hsu, *Macromolecules*, **30**, 3147 (1997).
- (9) M. Cheng, E. B. Lobkovsky, and G. W. Coates, *J. Am. Chem. Soc.*, **120**, 11018 (1998).
- (10) M. Ree, J. Y. Bae, J. H. Jung, and T. J. Shin, *Korea Polym. J.*, **7**, 333 (1999).
- (11) M. Ree, J. Y. Bae, J. H. Jung, T. J. Shin, Y. T. Hwang, and T. Chang, *Polym. Eng. Sci.*, **40**, 1542 (2000).
- (12) J. S. Kim, M. Ree, T. J. Shin, O. H. Han, S. J. Cho, Y. T. Hwang, J. Y. Bae, J. M. Lee, R. Ryoo, and H. Kim, *J. Catalysis*, **218**, 209 (2003).
- (13) J. S. Kim, M. Ree, S. W. Lee, W. Oh, S. Baek, B. Lee, T. J. Shin, K. J. Kim, B. Kim, and J. Luning, *J. Catalysis*, **218**, 386 (2003).
- (14) M. Ree, J. Y. Bae, J. H. Jung, and T. J. Shin, *J. Polym. Sci. Part A: Polym. Chem.*, **37**, 1863 (1999).
- (15) Y. T. Hwang, H. Kim, and M. Ree, *Macromol. Symp.*, **224**, 227 (2005).
- (16) M. Ree, J. Y. Bae, J. H. Jung, T. J. Shin, Y. T. Hwang, and T. Chang, *Polym. Eng. Sci.*, **40**, 1542 (2000).
- (17) B. Lee, J. H. Jung, and M. Ree, *Macromol. Chem. Phys.*, **201**, 831 (2000).
- (18) Y. Hwang, J. Jung, M. Ree, and H. Kim, *Macromolecules*, **36**, 8210 (2003).
- (19) I. Arvanitoyannis, *Rev. Macromol. Chem. Phys.*, **C39**, 205 (1999).
- (20) G. Scott and D. Gilead, *Degradable Polymer*, Chapman Hall, London, 1995.
- (21) Y. Hwang, M. Ree, and H. Kim, *Catalysis Today*, **115**, 288 (2006).
- (22) A. J. Kinloch, *Adhesion and Adhesives: Science and Technology*, Chapman Hall, New York, 1987, p 30.
- (23) D. K. Owens and R. C. Wendt, *J. Appl. Polym. Sci.*, **13**, 1740

- (1969).
- (24) J. H. Chung, K. H. Park, B. M. Seo, E. S. Kim, J. R. Hong, I. H. Chung, N. Kang, B. M. Min, Y. H. Choung, T. Akaike, and P. H. Choung, *J. Biomed. Mater. Res.*, **67A**, 1055 (2003).
- (25) J. Watanabe and K. Ishihara, *Artif. Organs*, **27**, 242 (2003).
- (26) E. Imbert, A. A. Poot, C. G. Figdor, and J. Feijen, *J. Biomed. Mater. Res.*, **56**, 376 (2001).
- (27) F. E. Khadali, G. Helary, G. Pavon-Djavid, and V. Migonney, *Biomacromolecules*, **3**, 51 (2002).
- (28) R. S. Labow, E. Meek, L. A. Matheson, and J. P. Santerre, *Biomaterials*, **23**, 3936 (2002).
- (29) E. Christenson, J. Anderson, and A. Hiltner, *J. Biomed. Mater. Res.*, **70A**, 245 (2004).
- (30) W. G. Characklis and K. C. Marshall, Edtors, *Biofilms*, Wiley, New York, 1990.
- (31) M. Fletcher, Editor, *Bacterial Adhesion: Molecular and Ecological Diversity*, Wiley-Liss, New York, 1996.
- (32) B. Bendinger, H. H. M. Rijnaarts, K. Altendorf, and A. J. B. Zehnder, *Appl. Environ. Microbiol.*, **59**, 3973 (1993).
- (33) M. Rosenber and S. Kjelleberg, *Adv. Microbial Ecology*, **9**, 353 (1986).
- (34) R. Bullitt and L. Makowski, *Nature*, **373**, 164 (1995).
- (35) G. M. Bruinsma, H. C. van der Mei, and H. J. Busscher, *Biomaterials*, **22**, 3217 (2001).
- (36) H. C. van der Mei, B. van de Belt-Gritter, G. Reid, H. Bialkowska-Hobrzanska, and H. J. Busscher, *Microbiology*, **143**, 3861 (1997).
- (37) A. E. van Merode, H. C. van der Mei, H. J. Busscher, K. Waar, and B. P. Krom, *Microbiology*, **152**, 807 (2006).
- (38) M. A. Hjortso, *Cell adhesion: Fundamentals and Biotechnological Applications*, Dekker, New York, 1995.
- (39) S. V. Fulzele, P. M. Satturwar, and A. K. Dorle, *Eur. J. Pharm. Sci.*, **20**, 53 (2003).
- (40) M. A. Schubert, M. J. Wiggins, M. P. Schaefer, A. Hiltner, and J. M. Anderson, *J. Biomed. Mater. Res.*, **29**, 337 (1995).
- (41) J. H. Jung, M. Ree, and H. Kim, *Catalysis Today*, **115**, 283 (2006).