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Evaluation of biodegradable synthetic scaffold coated on arterial prostheses implanted in rat subcutaneous tissue

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Abstract

Polyester arterial prostheses impregnated with various synthetic biodegradable materials and with gelatin were implanted subcutaneously in rats for 3–180 days. The inflammation was assessed by quantifying the activity of alkaline phosphatase and by histology. The degradation of the scaffold materials was determined by scanning electron microscopy (SEM), size exclusion chromatography (SEC), and differential scanning calorimetry (DSC). The alkaline phosphatase activity induced by the polymer-impregnated grafts was similar to that induced by the non-impregnated controls during most of the post-implantation periods. Histological studies revealed that the acute inflammatory response was moderate to mild and was similar for all types of specimens, except for the gelatin-impregnated grafts that induced a severe acute inflammation during the first 2 weeks post-implantation. At 4 and 6 months, significant disintegration of the scaffold was observed, accompanied by enhanced tissue infiltration and a reactivation of the acute inflammatory phase. Linear and exponential degradation rates of the synthetic polymers were described. The relative degradation rates of the biodegradable polymers were ranked as following: PLLACL > PDLLA > PLLA > PCEL. In conclusion, biodegradable polymers may provide an option as sealant/scaffolding materials for vascular prosthesis. It is suggested that the degradation rate of the polymer scaffolding materials should be higher to achieve early healing while without inducing strong inflammation.

Keywords: Biodegradable polymers; Vascular prostheses; Scaffold; Sealant; Implant

1. Introduction

The concept of impregnated grafts was proposed as a way to avoid the risks of hemorrhage at implantation of the knitted poly(ethylene terephthalate) (PET) grafts [1,2], which were manufactured more and more porous to promote healing [3]. Early results of grafts made impervious with bioerodible polymers were not successful [4]. Nevertheless, the issue of blood percolating through the textile wall was considered as a persisting problem and was addressed periodically [5,6] because the mandatory on-site preclotting was raising question marks [7]. Domurado et al. developed the concept of proteic polymerization to manufacture films likely to coat or impregnate cardiovascular devices [8,9]. This opens the way to the broad acceptance of compound grafts, i.e., polyester grafts compounded with albumin, collagen, and gelatin [10–15], which are clinically accepted. However, the clinical benefits are still far from being well established. Today most surgeons use gelatin- or collagen-impregnated PET grafts, or use the slightly more rigid low-porosity PET grafts, as no preclotting is required.

A new approach is to consider the coating material or sealant as a temporary scaffold. This bioerodible

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scaffold may serve at least two purposes, i.e., a sealant to prevent significant oozing of blood at implantation and a carrier of healing-enhancing growth factors or chemicals. Indeed, biodegradable polymers have been studied to release bioactive factors such as TGF-β1 [16] or other inductive proteins [17]. Compared with naturally occurred polymers such as gelatin and collagen, the major advantages of using synthetic biodegradable polymers are that they do not display the xenogenetic nature of proteins and therefore do not transmit disease between species, and their properties between batches and manufacturers are relatively constant. The degradation rate of synthetic polymers can be easily tailored as well. But the in vivo degradation rate of biodegradable scaffold remains largely unclear.

Polylactide (PLA) and its copolymers with glycolide and ε-caprolactone are synthetic biocompatible and bioresorbable materials. Following the pioneering research of Kulkarni [18] on biodegradable implant, Gilding and Reed on the synthesis and in vitro degradation of PLA, PGA and their copolymer PGA/LA [19,20], biodegradable polymers have been widely studied for various biomedical applications such as the repair material for bones [21,22], peripheral nerve guidance channels [23,24], vascular devices [25,26], and drug delivery [27–30]. These works have been nicely reviewed recently [31,32]. Compared to PLA, poly(ε-caprolactone) (PCL) is more flexible, permeable to small molecules, and has also been widely investigated as a biocompatible and biodegradable material [33–36]. PCL is particularly useful in forming copolymers with PLA to render the copolymers soft and flexible [37–39]. In recent years, poly(ethylene glycol) (PEG) has also been copolymerized with either PCL or PLA to form amphiphilic block copolymers [40–43]. This type of amphiphilic copolymer is particularly interesting as a potential scaffold material for vascular prostheses because of its conformability and hydrophilicity. To date, however, no study has been done on this subject.

The objectives of the present study were to investigate the in vivo biodegradation of four types of synthetic biodegradable polymers within the prosthetic structure of PET vascular prostheses and to study the tissue regeneration in these polymer-impregnated prostheses. Instead of replacing or bypassing a segment of blood vessel, the specimens were implanted subcutaneously in rats. The rat subcutaneous model has been widely used to evaluate biocompatibility and biodegradation of synthetic polymers that will ultimately used in other locations such as nerve guidance channel [44] or blood contacting heart valve [45]. The results obtained in this experiment were expected to be useful as the initial step of an in vivo evaluation of synthetic scaffold within a vascular prosthetic structure.

2. Materials and methods

2.1. Biodegradable synthetic sealants

Four types of biodegradable synthetic polymers were used as sealants in this study, namely, poly(D,L-lactide) (PDLLA, Mn = 90k, PDI = 1.39), poly(L-lactide) (PLLA, Mn = 126k, PDI = 1.66), random copolymer of L-lactide and ε-caprolactone (PLLACL, Mn = 137k, PDI = 1.68) at 70/30 mole ratio, and random terpolymer of copoly(caprolactone/L-lactide/PEG (PCEL, Mn = 90k, PDI = 2.21) at 1/5.2/0.67 mole ratio. These materials were synthesized at the Institute of Chemistry of the Chinese Academy of Sciences. To synthesize the terpolymer, pre-calculated amount of PEG, L-lactide, ε-caprolactone and stannous octoate were added into a polymerization tube. The tube was sealed under vacuum after de-oxygen and heated at 140 °C for prolonged period of time. The product was purified through dissolution in chloroform and precipitation in petroleum ether. Details on the synthesis and characterizations of the PCEL terpolymer were published elsewhere [40,41]. The PDLLA, PLLA and PLLACL were synthesized according to references [46,47], [41,48] and [49], respectively.

2.2. Vascular grafts and impregnation

The above-mentioned biodegradable polymers were used to impregnate VP1200K ESR PET arterial vascular grafts (Vascutek Ltd., Inchinnan, Scotland). Virgin grafts were first soaked in 10% (w/v) polymer chloroform solution, then air-dried at room temperature for 3 days, followed by further drying in a vacuum oven at room temperature for an additional 3 weeks. Non-impregnated and gelatin-impregnated grafts (Gelsoft Plus, Vascutek Ltd.) were used as references. The experimental and reference implants were cut into 2-cm segments and sterilized with ethylene oxide (EO) gas at 37 °C under standard industrial procedures, which include a 24 h-aeration. The average amount of impregnated polymer on each segment of graft was determined by dividing the total weight increase of the entire graft before and after impregnation with the number of segments cut from that graft. The average thickness of the impregnated polymer is comparable with the thickness of the graft wall.

2.3. Subcutaneous implantation

One hundred and sixty-eight female Sprague-Dawley rats, each weighing 200–250 g (Charles River Inc., St-Constant, QC, Canada) were divided into six groups, namely, non-impregnated, gelatin-impregnated, and four types of polymer-impregnated grafts. For each group, four rats were used for each of the following periods of implantation: 3, 7, 14, 30, 60, 120, and 180 days.

These time points were selected to investigate the tissue reaction at acute (3 and 7 days), transition (7 and 14 days), and chronic phases (30 days or longer). The 120 and 180 days were designed to study the material degradation at longer terms. The number of four animals for each time point was determined by experience in such a way that there would be enough specimens for triplicate tests in each chemical analysis

(differential scanning calorimetry (DSC), size exclusion chromatography (SEC), and scanning electron microscopy (SEM)), and duplicate tests for each embedding medium in histology analysis.

Following anesthesia using isoflurane (Forane®, Anaquest Inc., Pointe-Claire, QC, Canada), the back of each rat was shaved and skin disinfected first with gluconate chlorexidine (Novopharm, Toronto, ON, Canada), then with Proviodine® solution (Rougier Inc., Chambly, QC, Canada) and finally with 70% ethanol. A 2-cm-long incision was then performed longitudinally on the skin, and a segment of graft was implanted subcutaneously. The incision was then closed with a disposable skin stapler (APPOSE®ULC, American Cyanamid Company, Wayne, NJ, USA) and disinfected again. After awakening, each animal was returned to the housing facility, fed an unrestricted diet, and treated according to the Canadian Council on Animal Care Regulations. The experimental protocol was approved by the Animal Protection Committee at CHUQ.

2.4. Graft harvesting

The animals were sacrificed at pre-scheduled times, and the implants along with the surrounding tissue were removed and divided into two parts for histological and chemical analysis.

2.5. Quantification of alkaline phosphatase activity

Immediately after harvesting, the tissue surrounding each implant was separated, weighed, and homogenized in cold glycine buffer (50 mM, pH 9.8). To remove the unground tissue, the homogenized tissue was centrifuged at 5000 rpm at 0 °C for 15 min. The supernatant was then removed and immediately tested for alkaline phosphatase activity. At 37 °C, a 1 ml substrate solution containing *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO, USA) at a concentration of 0.4 mg/ml in glycine buffer was mixed with 200 µl of supernatant to start the reaction. After 30 min, 1 ml of 0.02 N NaOH solution was added to stop the reaction. The optical density of the solution was read at 400 nm using a Spectronic 21 spectrophotometer (Milton Roy Inc., Rochester, NY, USA). The enzyme activity was determined by comparing the optical reading against a standard curve which was established by using porcine alkaline phosphatase and the same substrate (Sigma Chemical Co.). Each specimen was measured in triplicate. The alkaline phosphatase secretion was expressed as micrograms per gram of tissue.

2.6. Histology study

Specimens fixed in 10% formalin were rinsed and dehydrated in a series of ethanol solutions of increasing concentration. The dried specimens were embedded in paraffin and cut into 5-µm-thick sections which were mounted on glass slides and stained separately with hematoxylin–phloxine–safran (HPS) for cells and Masson's trichrome for total collagenous tissue. The prepared slides were finally viewed under an Axiophot light microscope (Zeiss, Oberkochen, Germany). Another set of specimens were embedded in L.R White resin using a procedure that has been described previously [50]. Five-micrometer-thick sections were cut from the resin-embedded specimens and stained with Azure 2 and toluidine blue.

The degree of inflammation was measured by counting the number of inflammatory cells per field under a microscope (10×40), in comparison with the number of cells in adjacent normal tissue. Five fields were counted for each sample and the numbers averaged. Based on the average number of inflammatory cells, four degrees of tissue reaction were categorized as follows: minimal ($<$ 20 cells), mild (20–50 cells), moderate (50–100 cells), and severe ($>$100 cells). The amount of collagen deposition, as revealed by the Masson's blue stain, was expressed as follows: onset (+), intermediate (++), and abundant (+++).

These methods have been used elsewhere [51]. The inflammatory cells, such as polymorphonulear (PMN) cells, lymphocytes, and foreign-body giant cells (FBGC), were identified morphologically.

2.7. Scanning electron microscopy (SEM)

In order to observe the morphology of the impregnated biodegradable polymers, the tissue on each explant was removed. This was achieved by incubating the non-fixed specimens in a collagenase Tris–HCl buffer solution at 37 °C overnight, followed by a thorough wash in distilled water [52]. The cleaned specimens were dried in a vacuum oven at room temperature, then sputter-coated with gold for SEM analysis. The morphology on the surface and at the cross-section of the specimens was investigated using a Jeol JSM 35CF SEM (Soquelec Inc., Montréal, QC, Canada) at an accelerating voltage of 15 kV.

2.8. Differential scanning calorimetry (DSC)

Fresh tissue was carefully separated from each explanted graft. The grafts were then rinsed in distilled water and dried in a vacuum oven at room temperature. A DSC-7 thermal analyzer (Perkin Elmer, Montréal, QC, Canada) was used to measure the glass transition (T_g) and melting temperatures (T_m) and the heat of fusion (ΔH). Specimens weighing approximately 10 mg were cut from each explant and sealed in standard aluminium sample pans. The specimens were heated from −40 to 180 °C at a scanning rate of 10 °C/min using liquid nitrogen as the coolant and helium as the protective atmosphere. The instrument was calibrated with indium standard and pure water. Three measurements were performed for each explant and the averages of T_g, T_m and ΔH were calculated.

2.9. Size exclusion chromatography (SEC)

The changes in molecular weight of the impregnated biodegradable polymers were determined by SEC. To collect biodegradable polymers from the explants, explant with tissue attached was stirred in chloroform for 4 h and extracted with a solution of methanol (50%): chloroform (1:3) at room temperature [50]. The polymer-containing chloroform phase was then separated from the tissue-containing water phase with the addition of methanol solution. The

polymer-containing chloroform phase was collected and dried at room temperature for 3 weeks prior to use. The collected biodegradable polymers were dissolved in chromatograph-grade tetrahydrofuran (THF) which was previously filtered with a 0.45 μm nylon filter (Vanden Abeele Inc., Montréal, QC, Canada) at a concentration of 0.5% (w/v). The sample solution was filtered again with a 0.45 μm nylon filter (Vanden Abeele Inc.). One hundred microliters of polymer solution were injected through a syringe sample loading injector into a Waters Millipore® Model 590 pump (Millipore Canada Ltd., Waters, Ville St-Laurent, QC, Canada) operating at a flow rate of 1.0 ml/min. An Ultrastyragel linear column (2000-4m, Millipore Canada Ltd.) and a Shodex® KF804 column (exclusion limit 400,000, Showa Denko KK, Millipore Canada Ltd.) were used to separate the molecules. A Wyatt/Optilab 903 interferometric refractometer (Wyatt Technology Corporation, Santa Barbara, CA, USA) was connected to the downstream of the columns to measure the refractive index of the solutes, which was then used to calculate the relative molecular weight by using monodisperse polystyrene standards.

3. Results

3.1. Visual observations at harvesting

At 3 days, all of the implants were surrounded by light red exudates (a few millilitres). At 7 days, the exudates were found in only a few rats. At 14 days, no exudates were observed surrounding the implants which were encapsulated by connective tissue. At 1 month, capillaries were found indiscriminately on all of the capsules.

3.2. Histological study

Table 1 summarizes the histological observations. In general, every implant (except the gelatin-impregnated implant) induced a minimal to mild acute inflammation at 3 and 7 days, which was characterized by the infiltration of a large amount of inflammatory cells including mostly PMNs and lymphocytes. A newly formed fragile capsule was present at day 7, which was easily separated from the prosthesis. A moderate transitional inflammation appeared at 14 days, featured with an increased number of macrophages and a decrease in PMNs and lymphocytes. The capsule remained thick and became better organized. A mild chronic inflammatory phase began after 1 month, identified by an increased number of fibroblasts, a decrease in inflammatory cells and the formation of a well-defined thin capsule. In contrast, the gelatin-impregnated implants induced strong, acute and transitional inflammation in the first 14 days, which gradually diminished and became mild at 30 days. During the acute phase (particularly at day 3), the number of inflammatory cells adjacent to implant was noticeably lower than what was expected because of the lack of adhesion between tissue and implant.

Fig. 1 shows the representative acute tissue reactions at day 7. Surrounding the implant, inflammatory cells, primarily PMNs and lymphocytes were present in the fibrin network. For the biodegradable polymer-impregnated implants (Fig. 1C–F), the inflammation was mild, with the inflammatory cells remaining at the interface between the grafts and tissue. In the case of the gelatin-impregnated implants, however, many inflammatory

Table 1
Histological observations of the tissue reaction surrounding the implants

Type of sealants	Phases of tissue reaction	Time of implantation	Total inflammatory cells	Collagen generation	Remaining of sealant
Non-impregnated	Acute	Days 3 and 7	Discrete	No	—
	Transition	Day 14	Moderate	Collagen +	—
	Chronic	1 month to 6 months	Mild	Collagen ++ to +++	—
PDLLA	Acute	Days 3 and 7	Discrete to mild	No	Yes
	Transition	Day 14	Moderate	Collagen +to +++	Yes
	Chronic	1 month to 6 months	Mild	Collagen ++ to +++	Yes
PLLACL	Acute	Days 3 and 7	Discrete to mild	No	Yes
	Transition	Day 14	Moderate	Collagen +to +++	Yes
	Chronic	1 month to 6 months	Mild	Collagen ++ to +++	Yes
PLLA	Acute	Days 3 and 7	Discrete to mild	No	Yes
	Transition	Day 14	Moderate	Collagen + to +++	Yes
	Chronic	1 month to 6 months	Mild	Collagen ++ to +++	Yes
PECL	Acute	Days 3 and 7	Discrete to mild	No	Yes
	Transition	Day 14	Moderate	Collagen + to +++	Yes
	Chronic	1 month to 6 months	Mild	Collagen ++ to +++	Yes
Gelatin	Acute	Days 3 and 7	Severe	No	Yes
	Transition	Day 14	Severe to moderate	Collagen +	Yes
	Chronic	1 month to 6 months	Mild	Collagen +++	No

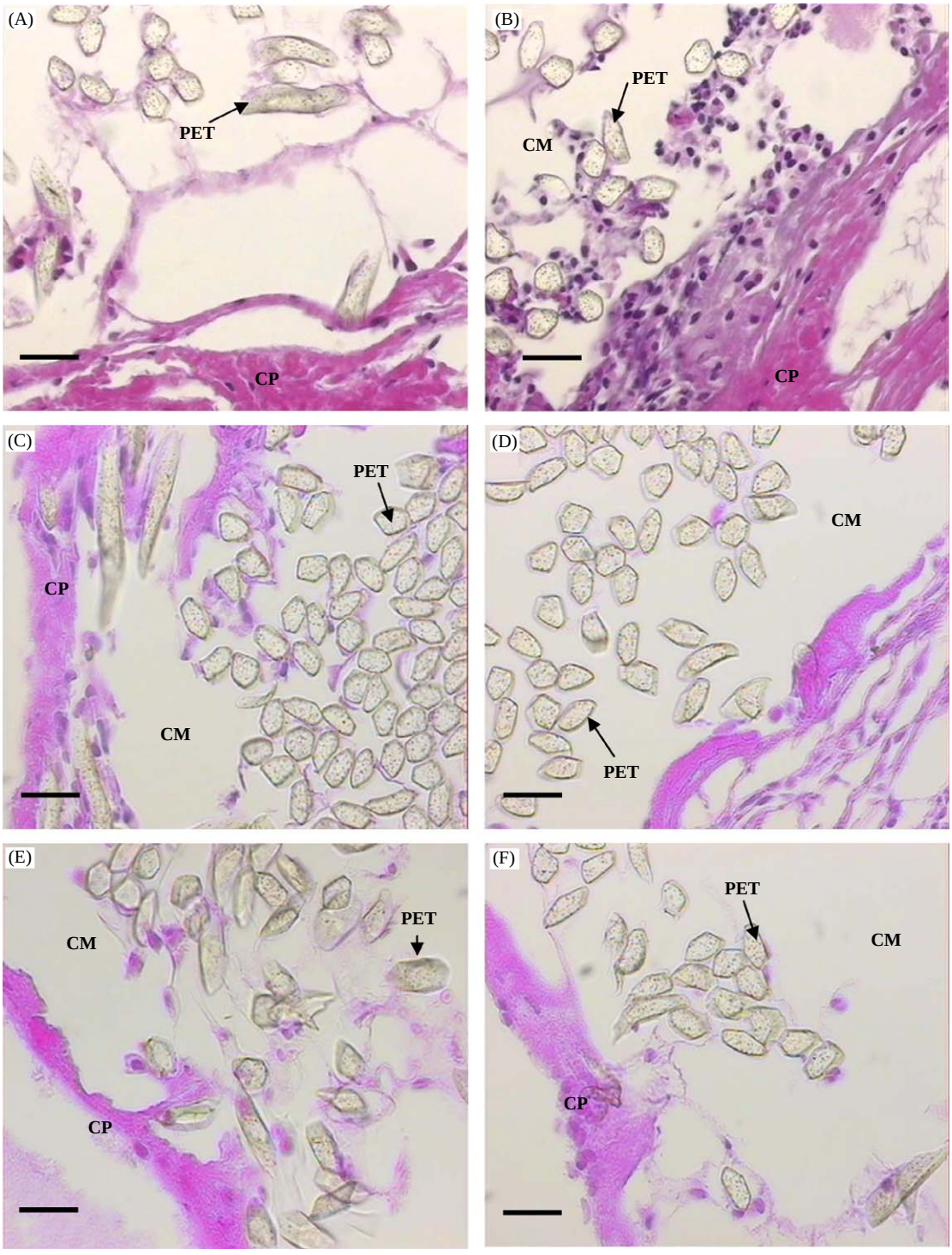

Fig. 1. HPS stain at 7 days, showing typical acute tissue reaction to the implants. (A) Non-impregnated, (B–F) gelatin-, PDLLA-, PLLACL-, PLLA-, and PCEL-impregnated, respectively. Bars = 30 μm.

cells were present at the tissue/microfibre interface and had already migrated into the interspace of the microfibres (Fig. 1B). Similar to the polymer-impregnated implants, the non-impregnated implants showed very little cellular infiltration between the microfibres (Fig. 1A).

Among the polymer-impregnated and non-impregnated implants, no statistical difference in the number of inflammatory cells was observed. At 14 days, FBGCs were found in some samples and particularly in the gelatin-impregnated implants. Collagen deposition became significant and newly generated blood vessels were

observed in the proximity of the implants. Impregnated materials were identified in each polymer-impregnated implant. At 1 month, the thickness of the external capsule had significantly decreased and tissue infiltration had become more evident, particularly in the gelatin-impregnated and non-impregnated implants (Fig. 2). Among the sealants, gelatin had for the most part disappeared from the inter-microfibre space, provoking a severe chronic inflammation characterized by the increased presence of macrophages and FBGCs. At 2 and 4 months, with further degradation of the impregnated polymers, extended healing was evidenced by increased tissue infiltration into the wall of the prosthesis and by a lower number of inflammatory cells. The thickness of the capsules was similar to that observed at 1 month (Fig. 3). At 6 months, both the non-impregnated and gelatin-impregnated implants displayed minimal chronic inflammation with the significant presence of collagenous tissue within the microfibres. The other implants displayed a mild chronic inflammation, less tissue infiltration and stable capsule thickness, which was similar to that observed at 4 months. Surprisingly, elevated numbers of PMNs were found again in two out of the four PDLLA-impregnated implants. Fig. 4 shows the development of polymer degradation and tissue infiltration in the PDLLA-impregnated implants.

3.3. Alkaline phosphatase activity

As shown in Fig. 5, the alkaline phosphatase activity induced by the polymer-impregnated implants was similar to that induced by the non-impregnated implants during the first 2 weeks post-implantation. At 1 and 2 months, we observed reactivations of alkaline phosphatase for the PCEL and PLLA, respectively. The peak activity of the alkaline phosphatase induced by the gelatin-impregnated implants appeared as early as the second week.

3.4. SEM results

Figs. 6 and 7 show the progressive degradation of the four types of sealants. Prior to implantation, the surfaces were smooth, with no pits or cracks. At 2 or 4 weeks, the evidence of degradation appeared as small pits or caves on the surfaces. With the increased implantation time, these surface defects became more noticeable and, at 2 months, had formed cracks in most of the implants. These cracks became both more frequent and larger in size, and eventually caused a significant loss of coating material at 4 and 6 months. Among the synthetic sealants, the degradation of the PLLACL was the most severe. Similar morphological changes, i.e., small holes appeared at 5 weeks and then changed to more porous and then to large cracks, were reported on the in vitro degradation of poly(DL-lactide-ε-caprolactone) nerve guide [53].

3.5. DSC results

Prior to implantation, the melting temperature (T_m) of the PLLA was 138.7 °C. As shown in Fig. 8A, the T_m of the PLLA was almost constant during most of the implantation times but increased to 144.4 °C at 6 months. It suggests that crystallinity of the remained PLLA increased with degradation. The melting temperature of the PCEL, which was associated with the caprolactone component in the copolymer, was at 43.3 °C before implantation and stayed fairly stable during entire implantation except at 6 months when it split into two peaks (Fig. 8B) which normally means two distinct features in its crystalline structure. The glass transition temperature (T_g) for the PLLA, which was at 57.4 °C before implantation, increased to 66.9 °C (first scan) after 4 months implantation, reflecting a more closely compacted molecular structure formed during implantation. The T_g of the PCEL was not detectable during the first run of the DSC heating sequence, possibly because the overlap with the tail of the melting endotherm. For the PDLLA and PLLACL, no T_m was recorded because of their amorphous nature.

3.6. Molecular weight

Fig. 9 shows the percentage changes of molecular weight (Mn) of the four synthetic polymers during implantation. It is evident that except for the PLLACL, the other three polymers recorded an almost linear degradation. The three experimental curves fitted with linear regression lines very well, with which the half-life time, i.e., where the molecular weight reached 50% of its original value, was calculated to be 104, 139, and 192 days for the PDLLA, PLLA and PCEL, respectively. The PLLACL, on the other hand, fitted very well with an exponential decay with a half-life time calculated at 20 days and molecular weight dropped to only 9% at 120 days.

A three-parameter instead of two-parameter exponential model was chosen primary because it fits the PLLACL curve well. The Y_0 in the formula represents the molecular weight of the residual polymer that can never be zero as long as sampling is possible. Theoretically, Y_0 should be zero when implantation is long enough and a two-parameter model should be applicable.

4. Discussion

Gelatin is a protein of animal origin. Fever caused by gelatin has been reported in clinics where it was used as a plasma expander [54,55]. Post-operation fever induced

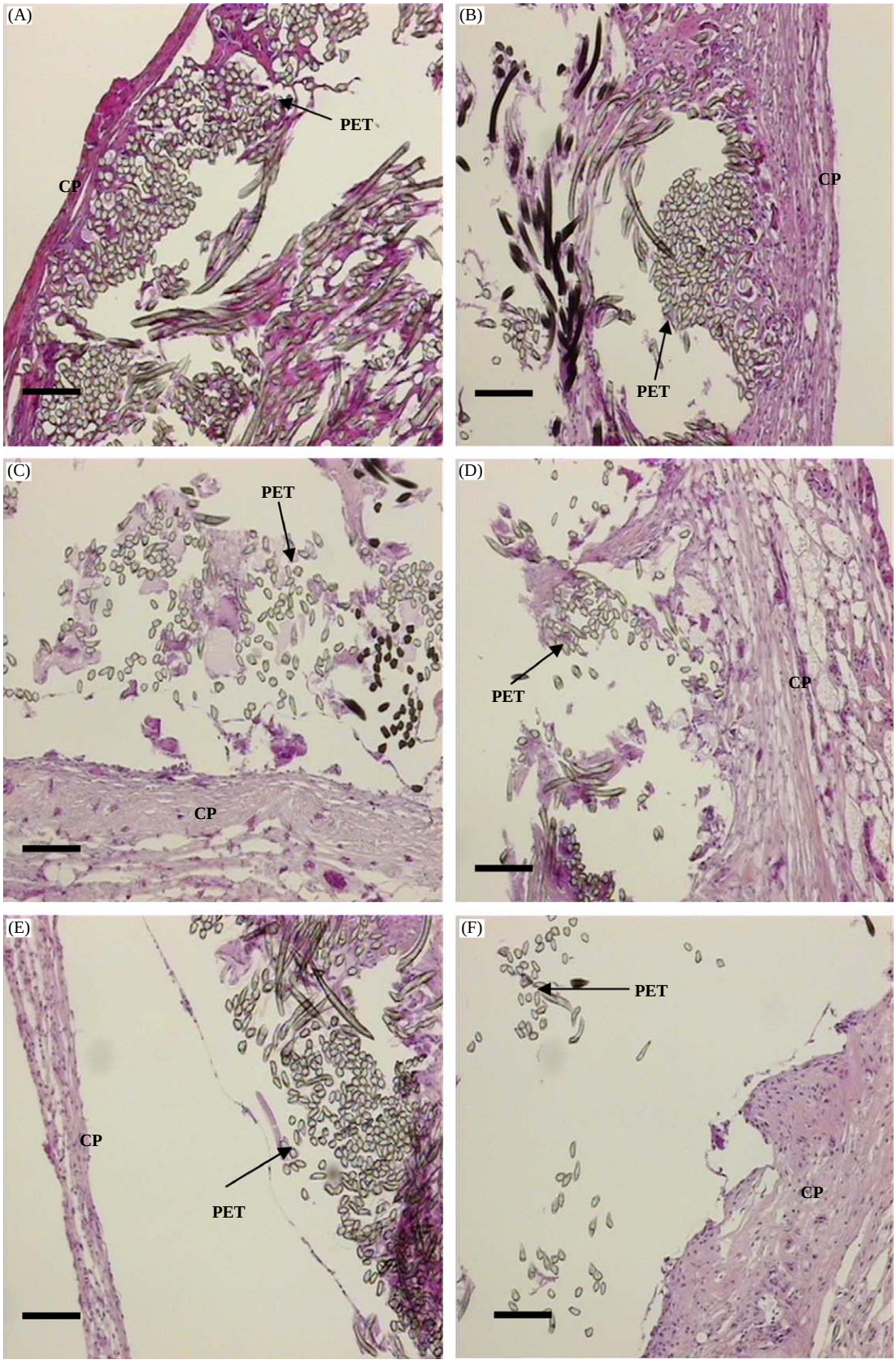

Fig. 2. HPS stain at 1 month. (A) Non-impregnated, (B–F) gelatin-, PDLLA-, PLLACL-, PLLA-, and PCEL-impregnated, respectively. Bars = 100 μm.

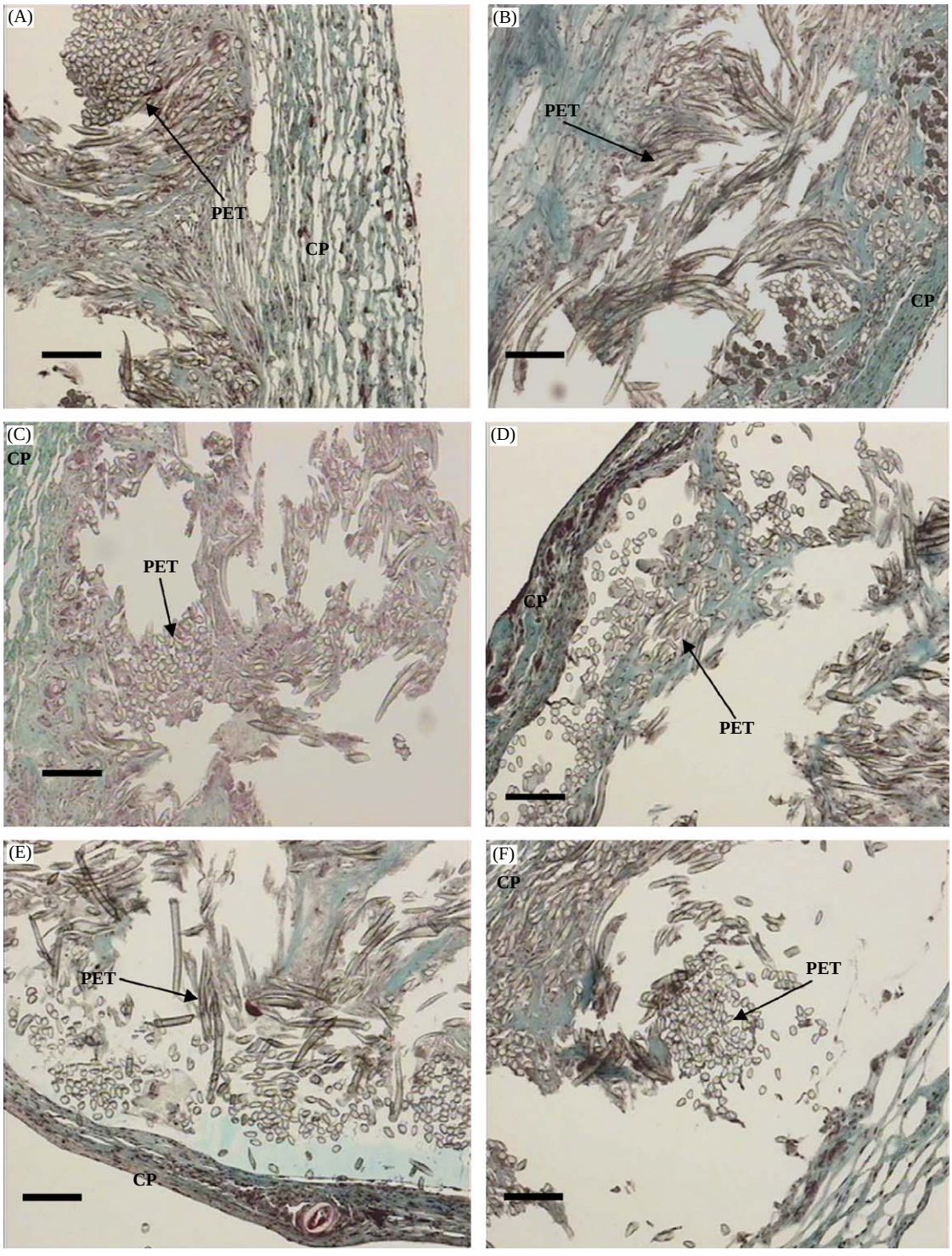

Fig. 3. Masson's trichrom stain at 2 months. (A) Non-impregnated, (B–F) gelatin-, PDLLA-, PLLACL-, PLLA-, and PCEL-impregnated, respectively. Bars = 100 μm.

by a gelatin-sealed graft has also been reported [56]. In this study, the gelatin-sealed prosthetic implants induced severe acute inflammation at 7 and 14 days, as was confirmed by both histology and alkaline phosphatase activity. This severe acute inflammation was likely provoked by the fast degradation of the gelatin sealant. After 14 days, this sealant disappeared and the inflammation induced by the remaining implant was similar to that induced by the non-impregnated implants. Compared to the gelatin-sealed implants, the four types of polymer-impregnated implants induced much less inflammation at both the acute and transition

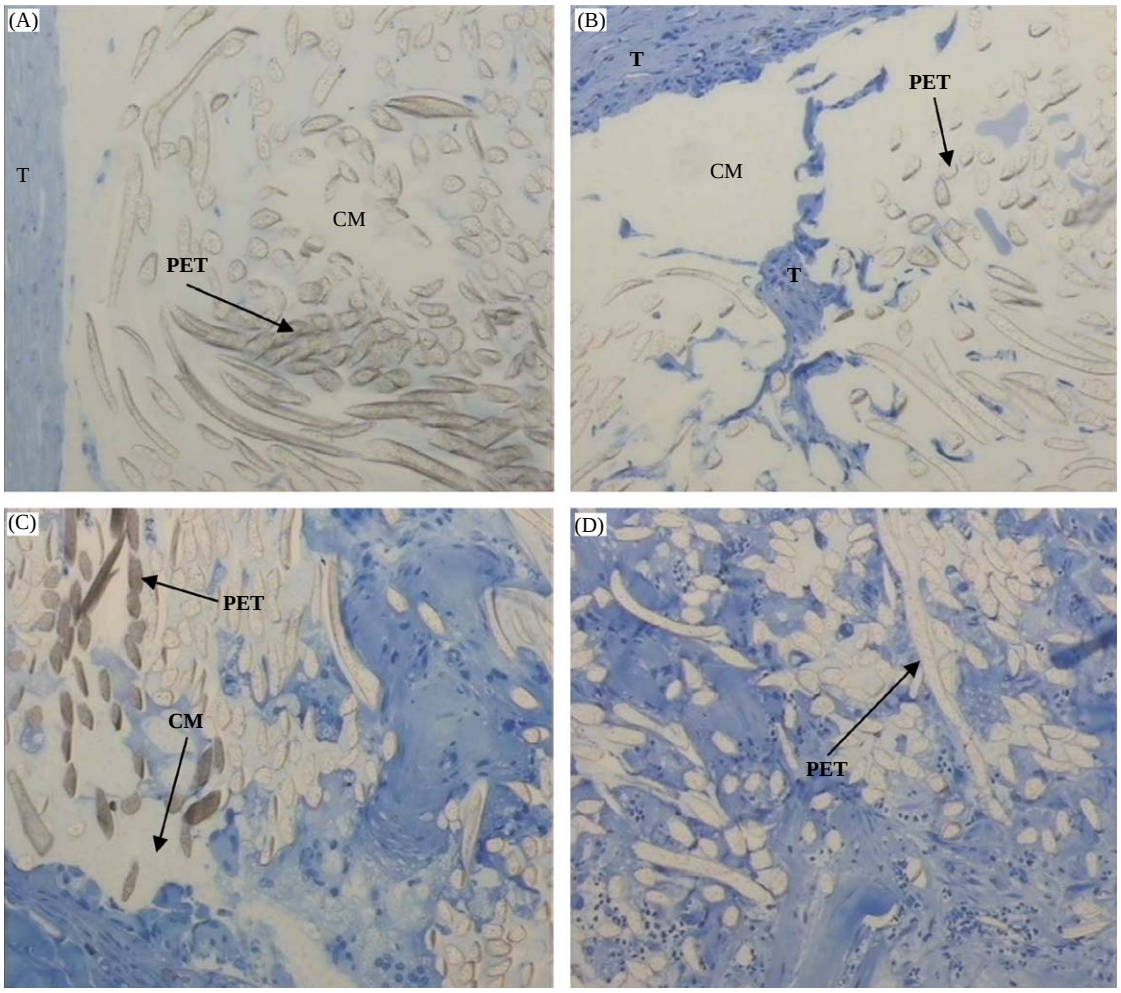

Fig. 4. Light photomicrographs of the PDLLA-impregnated graft at various implantation times, showing the progressive degradation of the PDLLA, which was accompanied by a progressive tissue ingrowth, and the reactivation of an acute tissue reaction at 6 months. (A) 14 days; (B) 4 weeks; (C) 4 months; (D) 6 months (L.R. White-embedded samples, Azure 2 and toluidine blue stain).

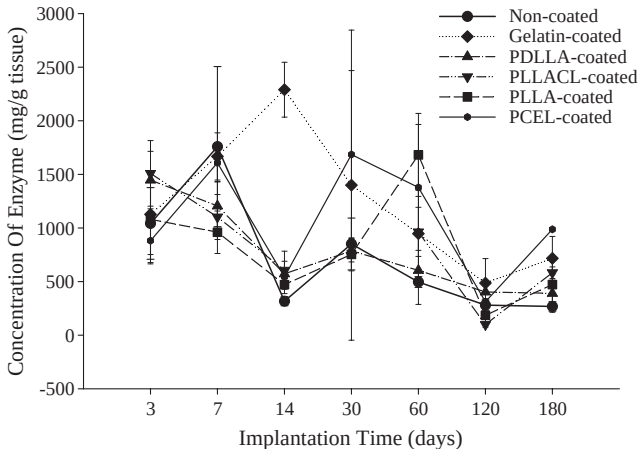

Fig. 5. Alkaline phosphatase activity as function of implantation time.

phases. In the chronic phase, the inflammation caused by the polymer-impregnated implants was similar or stronger than that caused by the non-impregnated implants. In contrast to the gelatin-sealed implants, the biodegradable polymers used in this experiment recorded a prolonged mild inflammation.

An elevated level of alkaline phosphatase, which is largely located in the PMN granules, indicates the cellular activation in the acute phase of inflammation. The high alkaline phosphatase activity at 3 days remained largely due to the surgical trauma. Nevertheless, it was apparently very sensitive to the degradation of the gelatin, and was also probably sensitive to the degradation of the polymers, as was evidenced by the highest enzyme activity at 14 days caused by the gelatin-impregnated grafts, and by the reactivation at 30 and 60 days, most likely caused by the increased degradation of the PCEL and PLLA, respectively. The non-impregnated implants, which released no degradation products, recorded the lowest enzyme activity at day 14. These findings emphasize that PMN activation at the tissue/implant interface was sensitive enough to the degradation products released from the biodegradable scaffold.

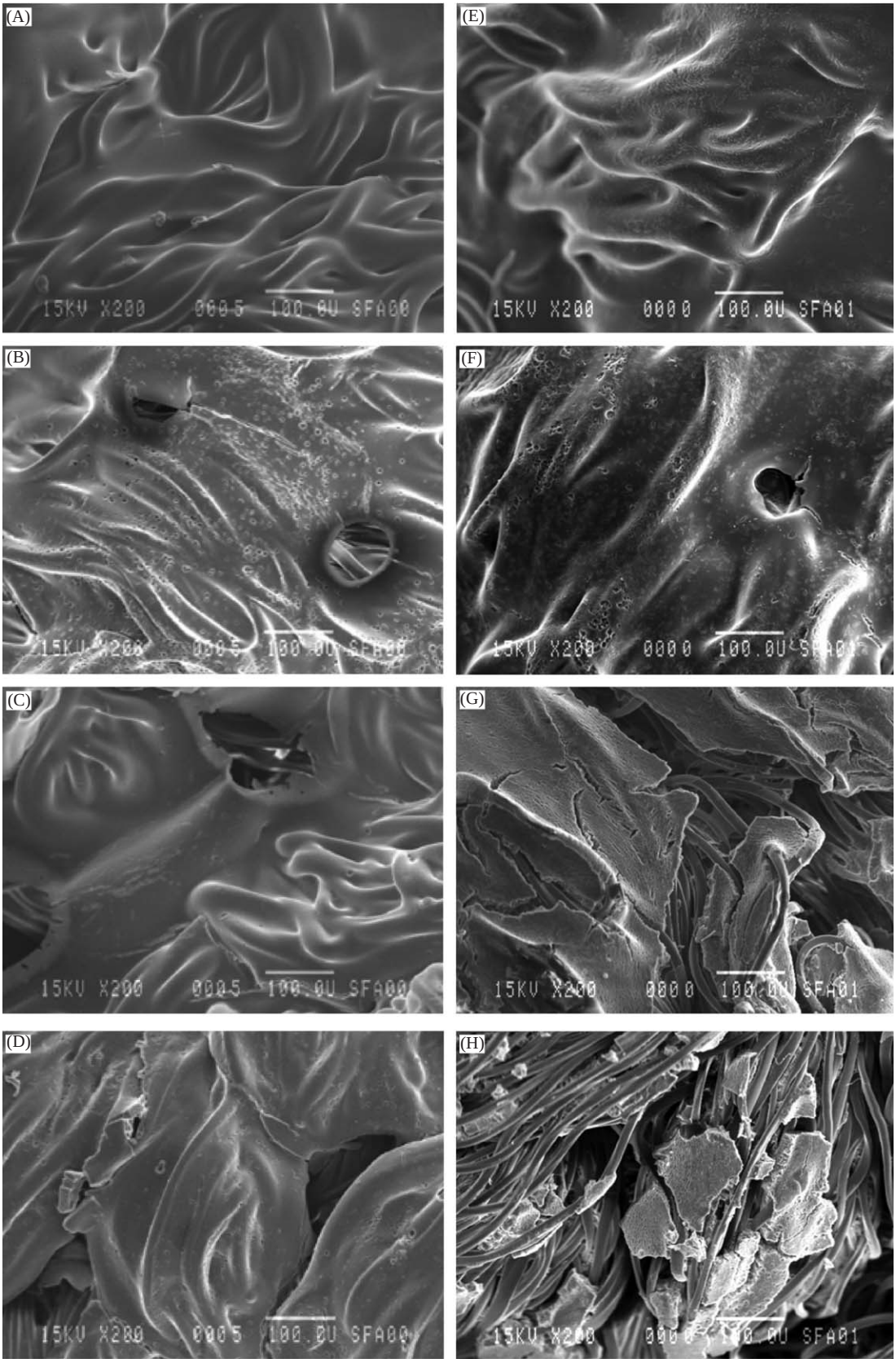

Fig. 6. Scanning electron photomicrographs of the PDLLA (A,B,C,D) and PLLACL (E,F,G,H) impregnated grafts implanted in rats at various times. (A,E) Before implantation; (B,F) 1 month; (C,G) 4 months; (D,H) 6 months.

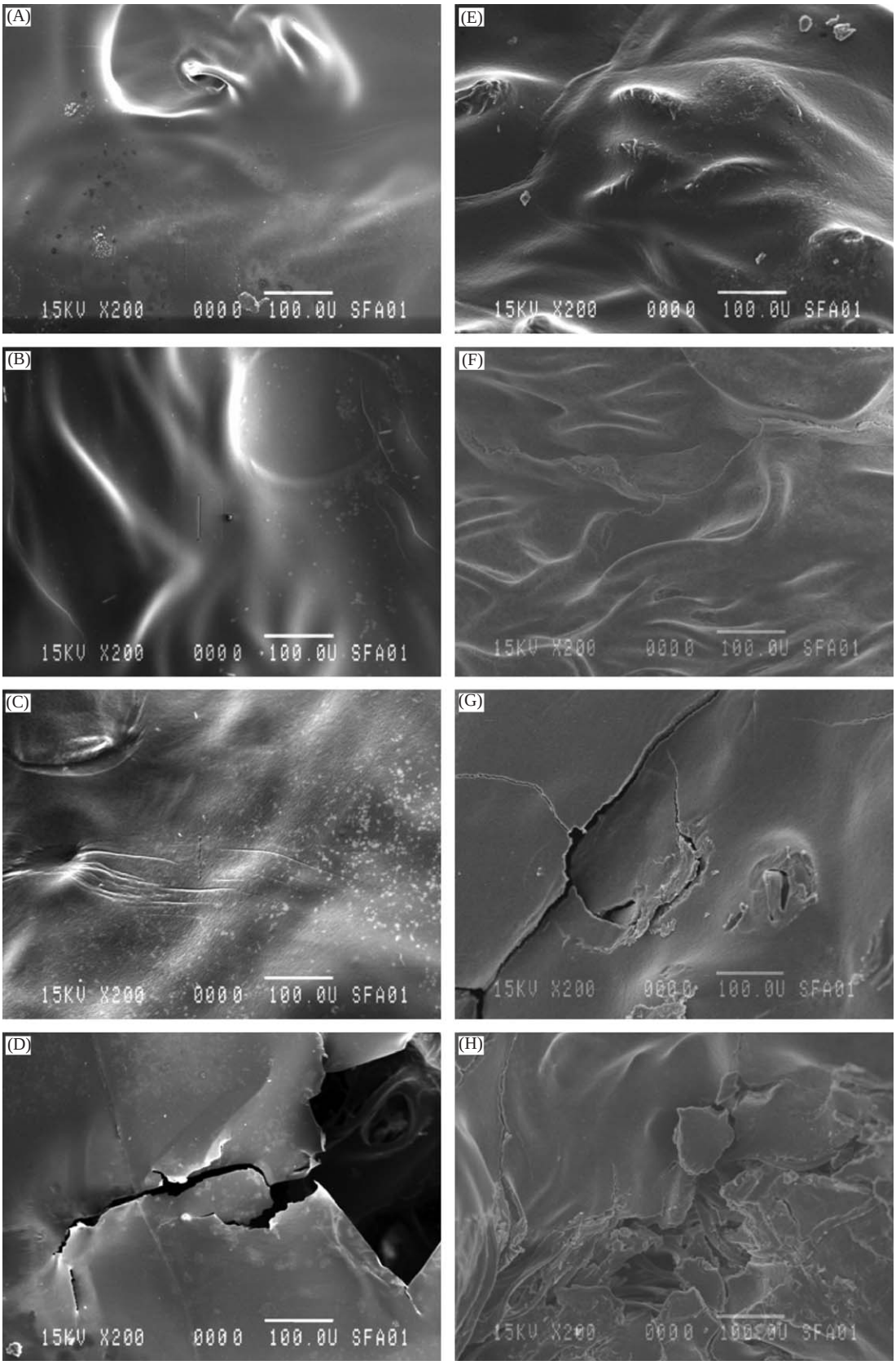

Fig. 7. Scanning electron photomicrographs of the PLLA (A,B,C,D) and PCEL (E,F,G,H) impregnated grafts implanted in rats at various times. (A,E) Before implantation; (B,F) 1 month; (C,G) 4 months; (D,H) 6 months.

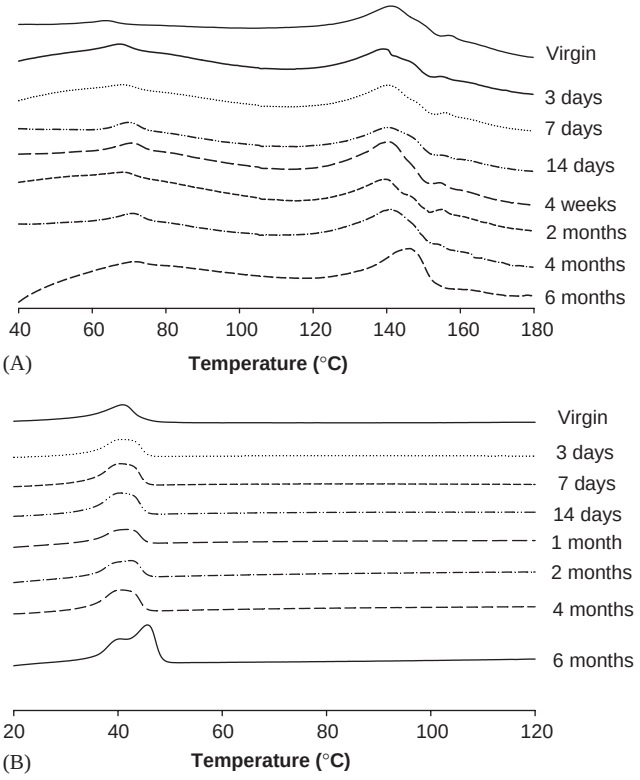

Fig. 8. DSC curves of the PLLA (A) and PCEL (B) impregnated grafts at various implantation times.

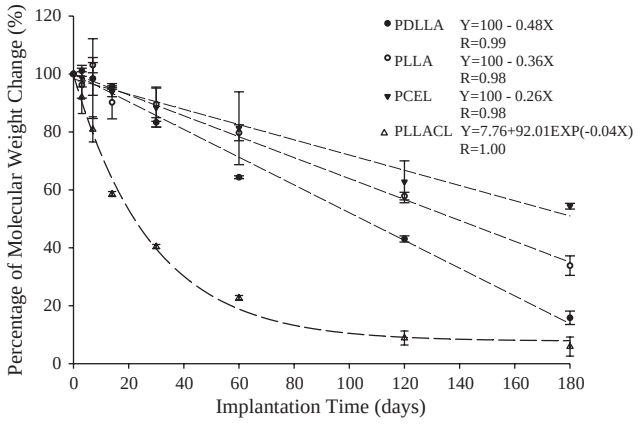

Fig. 9. Percentage of initial molecular weight as function of implantation time and the line regression results.

An important part of the regeneration process is the cellular infiltration into the prosthetic structure and the following deposition of extracellular matrix. The infiltration was much slower into the polymer-impregnated implants than into the gelatin-impregnated implants. In the polymer-impregnated implants, tissue ingrowth was incomplete at 6 months, even when the molecular weight of the polymers had decreased significantly. In the gelatin-impregnated implants, however, the process was complete in 1 month. The slow degradation of the synthetic scaffold apparently delayed tissue infiltration. However, the degradation rate of a synthetic scaffold can be modified by changing the molecular weight, type and ratio of different components in case of copolymers, and the processing conditions as well. The linear and exponential degradation patterns also make it possible to predict the lifetime of a biodegradable scaffold. However, cautions must be excised while performing such a prediction because the degradation pattern of a particular scaffold depends on many factors [57,58] such as the dimension of the polymer, the implantation site, and the intensity of inflammation. Under the present experimental conditions and based on the SEC and SEM observations, among the four types of synthetic polymers, the degradation rate was therefore ranked as follows: PLLACL > PDLLA > PLLA > PCEL. This ranking also reflects the rate of tissue infiltration. We observed better tissue ingrowth into the PLLACL-impregnated grafts than into other three polymer-impregnated grafts. Assuming a constant quality of polymer impregnation in all grafts, the high degradation rate and permeability to small molecules of the PLLACL may have contributed to this phenomenon.

The different surface chemistries of the four types of biodegradable polymers did not record measurable variations in cellular or tissue reaction. Indeed, different surface chemistries may or may not induce different cellular activation, as reported in a comprehensive in vitro study of 12 materials by Sefton's group [59]. The chemistry of the polymers used in this experiment was actually quite similar, with only ester as functional group for PLLA, PDLLA and PLLACL, and ether and ester for PCEL. In vivo cellular reaction, based on our criteria, i.e., total inflammatory cells and the activation of alkaline phosphatase, is more sensitive to leachable components rather than to immobile surface chemical groups.

Early ingrowth of perigraft tissue into the prosthetic wall may have an important role in the early development of well-organized neointima and endothelium [60]. In this regard, the degradation rate of the polymeric sealants used in this study was too slow. On the other hand, rapid sealant degradation, as in the case of gelatin that disappeared completely between 14 and 30 days, induced strong acute inflammation. The authors therefore suggest that future research on synthetic sealants should consider the balance between fast degradation, e.g., early tissue infiltration, and the degree of acute inflammation.

The exponential decay of the molecular weight of the PLLACL, as showed in Fig. 9, suggested that the hydrolytic degradation happened throughout this material, i.e., a bulk phenomenon. This is understandable because polycaprolactone or PCL is well known for its high permeability to small molecules such as drugs [61] or water [62]. The as high as 30% of caprolactone in the PLLACL and the low crystallinity of the CL component

in the PLLACL ensured the uniform and fast degradation of the PLLACL. In comparison, PCEL has much lower CL component of 15%, which was, however, highly crystallized. The slow degradation of the PCEL suggested that instead of enhancing hydrolysis, the 10% hydrophilic PEG and the 15% water permeable CL in the PCEL actually delayed it, likely by increasing water permeability and so diminishing or eliminating the autocatalysis effect. The linear behaviours of other polymers, on the other hand, did not necessarily show a surface erosion process. For example, the hydrolytic degradation of the PCEL is uniform because of the water permeability-enhancing PEG and CL components [63]; but the degradation of the PLLA and PDLLA is heterogeneous, caused by the autocatalysis effect [63].

The crystalline structure of the biodegradable polymers changed little during most of the implantation times. The increase of T_g over time and T_m at 6 months however did demonstrate that hydrolysis caused by implantation effected the aggregation of polymer molecules by making them more compact, i.e., a higher crystallinity [64].

The involvement of biofactors such as enzymes in the degradation of poly(α-hydroxyl acid) and PCL under in vivo condition remains controversy. While in vitro experiment has provided clear evidence that certain enzymes are capable of hydrolyzing PLLA, for example proteinase K can degrade the amorphous region of PLLA [65] and *Pseudomonas lipase* is effective to hydrolyze PCL [65,66], in vivo experiment has provided conflicting results and has not unveiled decisive link between cellular activities and the degradation of those synthetic polymers. For example, in a cage implantation experiment, PLLA was reported not provoking any higher leukocyte or macrophage concentration, or any higher extracellular enzyme activity in comparison with the cages without PLLA [67]. In a most recent report, the molecular weight, crystallinity, mechanical strength and thermal properties of the thermally compressed PLLA pellets and thermally extruded PLLA rods (2 mm diameter) were compared after degradation in both in vitro (PBS, pH7.4 at 37 °C) and in vivo (subcutaneous in rats) models [68]. The authors found no difference between the two models and concluded that there was no enzyme contribution in the in vivo degradation process. In contrary, another recent report examined the in vitro (PBS, pH7.4 at 37 °C) and in vivo (subcutaneous in rats) degradation of PDLLA and bioglass-reinforced PDLLA rods that were 2 mm diameter and were formed by thermal extrusion [64]. This work showed that in vivo environment accelerated the degradation of the specimens and suggested the involvement of biological factors such as enzymes. Nevertheless, direct evidence of cell-mediated degradation of synthetic polymers was demonstrated on polyurethanes. The group of Labow and Santerre showed that elastase-like activity produced by neutrophiles increased the degradation of poly(ester urea-urethane) [69] and that esterase released by monocyte-derived macrophages contributed to the degradation of poly(carbonate urethane) [70]. The groups of Hiltner and Anderson have demonstrated that oxygen radicals released by macrophages and foreign body giant cells attacked the polyether soft segment of polyurethane [71–73]. For the degradable polymers used in this experiment, the authors have found that their degradation was accelerated in vitro by enzymes [63] and in vivo by strong inflammation (unpublished data). By saying so, even though the current work did not try to verify if the observed polymer degradation was at least in part cell-mediated or not, the authors believe that biological factors such as enzymes have played roles and that the observed degradations were biodegradation in nature.

5. Conclusion

This study demonstrated that compared to the gelatin-impregnated prosthetic implants, the polyester vascular graft/synthetic biodegradable scaffold induced a mild acute inflammatory reaction similar to that of the non-impregnated grafts. The degradation of the polymers became intensified at 4 months and was incomplete at 6 months, which reactivated inflammation and delayed the healing process. Considering the strong inflammation caused by gelatin degradation and the genuine risk involved in using animal protein, synthetic biodegradable polymers may represent an option as impregnation materials for vascular prostheses. However, the degradation rate of the synthetic polymers must be modified to achieve faster healing without inducing strong inflammation. Linear and exponential decays were used to describe the in vivo degradation behaviours of the four types of biodegradable polymers.

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