In vivo compatibility and degradation of crosslinked gelatin gels incorporated in knitted Dacron

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Abstract: Gelatin gels were applied to porous Dacron meshes with the aim of using these gels for local drug delivery. In this article, the biocompatibility and degradation of gelatin gels with different crosslink densities applied in Dacron were studied *in vivo* by subcutaneous implantation in rats. Dacron discs were treated with carbon dioxide gas plasma to improve hydrophilicity, and subsequently impregnated with gelatin type B. The gelatin samples were crosslinked to different extents using various amounts of water-soluble carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). After 6 h, 2, 5, and 10 days, and 3, 6, and 10 weeks of postimplantation, the tissue reactions and biodegradation were studied by light microscopy. The early reaction of macrophages and polymorphonuclear cells to crosslinked gelatin was similar to or milder than Dacron. Giant cell formation was predominantly aimed at Dacron fibers and was markedly reduced in the presence of a crosslinked gelatin coating. At week 10 of implantation, the crosslinked gelatin gels were still present in the Dacron matrix. The gelatin degradation was less for samples with the highest crosslink density. The gelatin gel with the lowest crosslink density showed clear cellular ingrowth, starting after 6 weeks of implantation. The intermediate and high crosslinked gelatin gels showed little or no ingrowth. In these gels, giant cells were involved in the phagocytosis of gelatin parts at week 10. Application of carbodiimide crosslinked gelatin gels in Dacron is suitable for medical applications because of the good biocompatibility of the gels and the possibility of adapting the degradation rate of gelatin to a specific application. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 51, 136–145, 2000.

Key words: gelatin; crosslinking; carbodiimide; biodegradation; tissue reaction

INTRODUCTION

Prosthetic valve endocarditis is an infrequent but serious complication of cardiac valve replacement. Adherence of bacteria to the valve is considered to be the first step in the development of infection. Infections that give rise to clinical signs within 60 days after surgery are designated as early-onset infections, as opposed to late infections, which become apparent after more than 60 days. Early-onset infections are generally caused by bacteria that enter the body during surgery.[1–3]

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Recently, it was discovered that thrombin-stimulated platelets secrete lysozyme as well as other bactericidal proteins, which are able to clear bacteria from vegetations and kill pathogens.[4] Application of these antibacterial proteins in a controlled-release system—for example, in the Dacron sewing ring of prosthetic heart valves—offers a highly specific system to fight early endocarditis. A minimal release time of 24–48 h after surgery (which is the normal prophylaxis time) is needed to kill the bacteria that enter the body during surgery. Furthermore, the release system has to be nontoxic and degrade within several months after implantation to enable adequate tissue ingrowth into the sewing ring.

A delivery system was developed using crosslinked gelatin as release matrix. Gelatin is a natural polymer extracted from collagen[5] that has been used for a variety of biomedical applications (e.g., as sealant for

vascular prostheses[6–11]) and in drug delivery (e.g., as hydrogel[12,13]) or microspheres.[14–18] Gelatin was crosslinked using *N,N*-(3-dimethylaminopropyl)-*N'*-ethyl carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) to improve the stability of the gels, and the controlled-release properties of the gels were studied as a function of the crosslink density.[19]

The patency of gelatin-coated Dacron vascular grafts has been extensively studied. In these studies, the clinical performance of gelatin-sealed vascular prostheses versus unsealed[6,9] or albumin-,[7] collagen-,[11] or fibrin glue[8]–sealed vascular prostheses was described. The gelatin coatings were generally crosslinked using aldehydes such as formaldehyde[8] and glutaraldehyde.[6,7,9] The coatings all showed fast degradation (within 3 weeks), thus enabling rapid tissue ingrowth into the graft, and all studies reported no adverse tissue reactions against crosslinked gelatin. The crosslink density of sealants is adapted to achieve a fast degradation after implantation. For diffusion-controlled release systems, however, the crosslink density is important not only for the degradation characteristics, but also for the release properties.

In this study, the biocompatiblity and degradation of carbodiimide crosslinked gelatin gels applied in Dacron for controlled-release purposes are related to the crosslink density of the gels. Although we aimed to achieve a relatively fast degradation, this degradation should not interfere with the controlled release during the first days of implantation. The consideration that the Dacron ring of a prosthetic heart valve is sewn into tissue and not subjected to flow conditions[20] led to the choice of a subcutaneous implantation model.

Plain Dacron was treated with a gas plasma of carbon dioxide to improve its hydrophilicity,[21] enabling homogeneous impregnation with gelatin. Plasma-treated Dacron was impregnated with gelatin, which was then crosslinked with EDC and NHS to various crosslink densities. EDC is a zero-length crosslinking agent, which means that crosslinks are introduced into the matrix without incorporating foreign compounds.[22,23] The biocompatibility and degradation of these crosslinked gelatin gels applied in a Dacron mesh were studied *in vivo* by subcutaneous implantation in rats for up to 10 weeks. Plasma-treated Dacron and plasma-treated Dacron impregnated with non-crosslinked gelatin were used as control materials.

MATERIALS AND METHODS

Materials

Pharmaceutical-grade gelatin type B (Batch no. 39238; Sanofi, France) was kindly supplied by Vascutek (Scotland). Single knitted Dacron was a gift from Sorin Biomedica (Italy). 2,4,6-Trinitrobenzene sulfonic acid solution (TNBS) (1*M*) and NHS were purchased from Fluka (Buchs, Switzerland). Phosphate buffered saline (PBS) (pH 7.4, [NaCl] = 0.140*M*) was purchased from NPBI (Emmer Compascuum, The Netherlands). Phosphate buffer used in swelling and loading of the gels was a solution containing 66 m*M* phosphate with a pH of 7.0. Deionized water was obtained from a Milli-Q plus apparatus (Millipore, Molsheim, France). All other reagents were obtained from Merck (Darmstadt, Germany).

METHODS

CO_2-plasma treatment of Dacron

The plasma treatment was performed in a tubular reactor (length 80 cm, internal diameter 6.5 cm) using three capacitor-coupled, externally placed electrodes. The hot electrode was positioned at the center of the reactor and the cold electrodes were placed at 18.5 cm to the sides of the hot electrode. The electrodes were connected to an rf (13.56-MHz) generator through a matching network. The Dacron sheets (20 × 10 cm) were placed on glass holders put in the center of the reactor between the hot and cold electrodes. The reactor was evacuated to a pressure of 1×10^{-5} mbar and flushed with carbon dioxide, after which a carbon dioxide flow of 25 sccm/min was established through the reactor. After 10 min equilibration, the sheets were treated with a pulsed carbon dioxide plasma, i.e. 40 pulses (t_{on} = 1 s, t_{off} = 1 s, 50 W, 0.32 mbar). Following plasma treatment, the carbon dioxide flow was maintained for 2 min, after which the reactor was brought to atmospheric pressure. The samples were removed from the reactor and washed for 3 × 2 h with deionized water to remove unstable groups. The sheets were dried overnight in a dessicator and immediately used for impregnation. The wettability of treated and non-treated Dacron was qualitatively tested in a hydration experiment.

Plasma-treated Dacron as such, or impregnated with gelatin, was used in all the following procedures and experiments.

Impregnation of Dacron with gelatin

CO_2–plasma-treated Dacron was punched into samples with a diameter of 47 mm and weighed. The samples were impregnated by pressing a solution of gelatin in deionized water (10 w/w%, 50°C) through the Dacron mesh using a syringe coupled to a filter holder (diameter 47 mm), in which the Dacron was placed. This procedure was carried out four times with a pause of 40 min between each impregnation. The impregnation was carried out in a laminar flow box. After drying under laminar flow, and subsequently in a dessicator, the weight of impregnated gelatin was determined.

Crosslinking of gelatin with EDC and NHS

Crosslinking of gelatin-impregnated Dacron samples with EDC and NHS was carried out in 2-morpholinoethane sulfonic acid (MES) buffer (pH 5.3, 0.05*M*). Sample wetting in the crosslinking solution was enhanced by positioning the solutions in a dessicator under reduced pressure, and when all samples were thoroughly wetted they were placed at 4°C under atmospheric pressure. The gelatin concentration was constant in all crosslinking experiments at 1 g in 50 mL of solution. Molar ratios of EDC to carboxylic acid groups of gelatin ($COOH_{gelatin}$) of 0.8, 1.6, and 3.0 were used; the molar ratio of NHS to EDC was kept at 0.2. The crosslinking reaction was performed for 16 h at 4°C. The reaction mixture was then replaced with a solution containing 0.1*M* disodium hydrogenphosphate and 1*M* sodium chloride for 2 h (pH 8.5, 50 mL) to quench the crosslinking mixture, and the impregnated mesh was washed four times for 1 h with deionized water (50 mL). All washes were performed at 4°C. The films were dried and punched into samples with diameters of 8 mm (implantation) and 3 mm (characterization).

γ-Sterilization

The Dacron samples were dried for at least 1 day in a dessicator before γ-sterilization was carried out. The samples were weighed, sealed in plastic bags, and exposed to 60 kGy (6.0 Mrad) γ-irradiation from a ^{60}Co source (Gammaster, Ede, The Netherlands).

Degree of swelling

Crosslinked samples (diameter 3 mm) were dried at reduced pressure for 1 day and weighed (W_0). The films were swollen in phosphate buffer for 2 h at 22°C (after which equilibrium was reached), tissue-dried, and weighed again (W). The degree of swelling (S) of the crosslinked gelatin was calculated by the following formula:

$$S = \frac{(W - W_0)}{(W_0 - W_D)} \quad (1)$$

In this calculation, the Dacron weight (W_D = 0.97 ± 0.05 mg for a sample with a diameter of 3 mm) was subtracted from the dry weight of the sample. Experiments were carried out in triplicate.

Number of free amine groups using TNBS[24]

Dried gelatin samples (diameter 3 mm) were weighed and reacted with a sodium hydrogencarbonate solution (pH 8.2, 4 w/v %, 2 mL) containing TNBS (0.01*M*) for 2 h at 40°C before hydrochloric acid (6*M*, 3 mL) was added to hydrolyze the gelatin films for 1.5 h at 60°C. After cooling to room temperature, deionized water (5 mL) was added to the solution. The absorbance was measured against a TNBS solution without gelatin which had been treated exactly the same as the crosslinked gelatin samples, at λ 345 nm with an Uvikon 930 spectrometer. With the absorption coefficient for 2,4,6-trinitrophenyl–derivatized (hydroxy)lysine residues (ε = 14,600 L/mol · cm), the amount of free amine groups per 1000 amino acids was calculated, assuming a molecular weight of 10^5 g/mol for a gelatin molecular chain of 1000 amino acids. The Dacron weight was subtracted from the total sample weight. The experiments were performed in triplicate.

Subcutaneous implantations

All animal studies were performed according to the National Institute of Health guidelines for the care and use of laboratory animals (NIH 85-23 Rev. 1985). γ-Sterilized Dacron disks (diameter 8 mm), as such (Dacron) or impregnated with gelatin B and crosslinked with a concentration of either 0.8, 1.6, or 3.0 EDC/COOH (designated Dacron-gel-0.8, Dacron-gel-1.6, and Dacron-gel-3.0) were incubated in PBS for 1 h at room temperature. The Dacron disk impregnated with noncrosslinked gelatin B (Dacron-gel-0.0) was incubated in PBS for 1 min. Four-month-old male Wistar rats (250–300 g) were ether-anesthetized, and a total of five subcutaneous pockets were made to the right and left of three midline incisions on the back. The series of five Dacron disks was implanted per rat into the pockets (always in the same position) at a distance of approximately 1 cm from the incisions.

After implantation intervals of 6 h, 2, 5, or 10 days, or 3, 6, and 10 weeks, the animal was ether-anesthetized and implants with surrounding tissue were carefully dissected from the subcutaneous site. The implants were immediately immersion-fixed in 2% glutaraldehyde (v/v in PBS) and kept at 4°C for at least 24 h.

Microscopy

The implants were cut into halves, dehydrated in graded alcohols, and embedded in glycol methacrylate, enabling the midregion of the implant to be examined. Semithin sections (2–3 μm) for light microscopy were routinely stained with Toluidine blue.

With regard to the tissue reactions, representative areas are always described, i.e., not the punched sides of the specimens. Cell type and cell number evaluation was performed by three researchers and processed into several graphs. Comparisons between cell numbers in different samples were made for each cell type, and expressed as arbitrary units (au) ranging from 0 to 3 (0.0 = cells not observed, 0.5 = low numbers, 1.0 = some cells observed, 2.0 = cells present all over, 3.0 = relatively high numbers).

RESULTS

Initial properties

Carbon dioxide plasma treatment of Dacron led to the introduction of oxygen-containing functional

groups (alcohol, aldehyde, carboxylic acid, and epoxide)[21] which improved the wettability of the Dacron surface. Plasma-treated Dacron was impregnated with gelatin which was subsequently crosslinked with different concentrations of reagents. After crosslinking and sterilization, the films were characterized with respect to swelling and free amine group content. Table I shows that an increasing concentration of EDC and NHS resulted in a decreased number of free amine groups and reduced swelling. The ratio between gelatin and Dacron weights was calculated to account for effects of weight variations on the biocompatibility and degradation of the gels.

Subcutaneous implantations: Macroscopic evaluation

Especially at 6 h, but also at day 2, disks were loosely present in the subcutaneous pocket. At day 5, capsule formation had started resulting in firm encapsulations at day 10 and week 6. Capsules were always thin and appeared similar

Subcutaneous implantations: Microscopic evaluation (Fig. 1)

At 6 h, all materials consisted of tighter and looser bundles of Dacron fibers in mostly the cross-section, but also in longitudinal section. Both Dacron and Dacron-gel-0.0 [Fig. 2(a)] contained only Dacron material (with Dacron-gel-0.0, noncrosslinked gelatin was not retrieved). Crosslinked gelatin gels were recognized as clear solid films in which the Dacron fibers and bundles were neatly embedded. The film surface varied from slightly wavy (Dacron-gel-0.8) [Fig. 2(b)] to increasingly wavy (Dacron-gel-1.6) [Fig. 2(c)], to a more irregular indented surface (Dacron-gel-3.0) [Fig. 2(d)].

In Dacron, low extravasation of polymorphonuclear cells (PMNs) and macrophages occurred from blood vessels in the subcutaneous tissue (Fig. 1) (0.5 au). Wound fluid was also present in the subcutaneous tissue further from the material. Only a few PMNs and macrophages were present between the fibers (Fig. 1). Dacron-gel-0.0 contained similar numbers of PMNs but clearly more macrophages in the tissue and between the fibers and bundles (Fig. 1).

Cellular infiltration did not occur inside the crosslinked gelatin samples [Fig. 2(b–d)] except in the filling of cracks with wound fluid and a few PMNs and macrophages, which was especially observed with Dacron-gel-3.0 [Fig. 2(d)]. Further tissue interactions were similar, consisting of the same low number of PMNs but twice as many macrophages as observed in Dacron (Fig. 1).

After 2 days' implantation, tissue reactions had clearly proceeded in each sample. The subcutaneous tissue showed a tighter fit, with many small blood vessels and the onset of fibroblast activation. PMN numbers had increased slightly in Dacron and markedly in Dacron-gel-0.0, whereas the samples containing crosslinked gelatin had attracted similar or even lower numbers than at 6 h. The number of macrophages remained at the same level (Dacron-gel-0.0, Dacron-gel-1.6, and Dacron-gel-3.0) or slightly increased (Dacron and Dacron-gel-0.8). The number of lymphocytes was low in Dacron, Dacron-gel-0.0, and Dacron-gel-0.8, in Dacron-gel-1.6 somewhat increased, and was hardly present in Dacron-gel-3.0 (Fig. 1). In Dacron and Dacron-gel-0.0 samples, cells were concentrated around the tighter Dacron bundles. This was most obvious in Dacron-gel-0.0, which showed more cellular infiltration than Dacron. For the crosslinked gelatin gels, sometimes part of the gel split off.

At day 5, the tissue reactions were dominated by encapsulation and foreign-body processes, i.e., marked by fibroblasts, and foreign-body giant cells formed by fusion from macrophages. PMNs were rare; only a low number were observed in Dacron-gel-0.0 (Fig. 1).

Fibroblasts (not shown in Fig. 1) showed similar activation and ingrowth into the Dacron and Dacron-gel-0.0. Plain Dacron contained more space filled with

TABLE I
Characteristics of Implanted Dacron Disks (average ± mean; $n = 8$)

Sample	Weight (mg)	Gelatin/Dacron (w/w)*	$EDC/COOH_{gelatin}$ (mol/mol)	NH_2/1000 Amino Acids	S
Dacron	5.2 ± 0.2	0			
Dacron-gel-0.0	13.7 ± 0.4	1.63		31.5 ± 2.5	⩾†
Dacron-gel-0.8	10.7 ± 0.7	1.06	0.82	10.1 ± 2.1	3.4 ± 0.1
Dacron-gel-1.6	12.0 ± 0.4	1.31	1.60	4.9 ± 0.9	2.4 ± 0.1
Dacron-gel-3.0	11.1 ± 0.4	1.13	3.00	3.4 ± 0.7	2.2 ± 0.0

*The ratio between gelatin and Dacron content was calculated from the increase in weight of the different samples compared to plain Dacron.

†The swelling of noncrosslinked gelatin films could not be measured because these films slowly dissolve in aqueous solution.

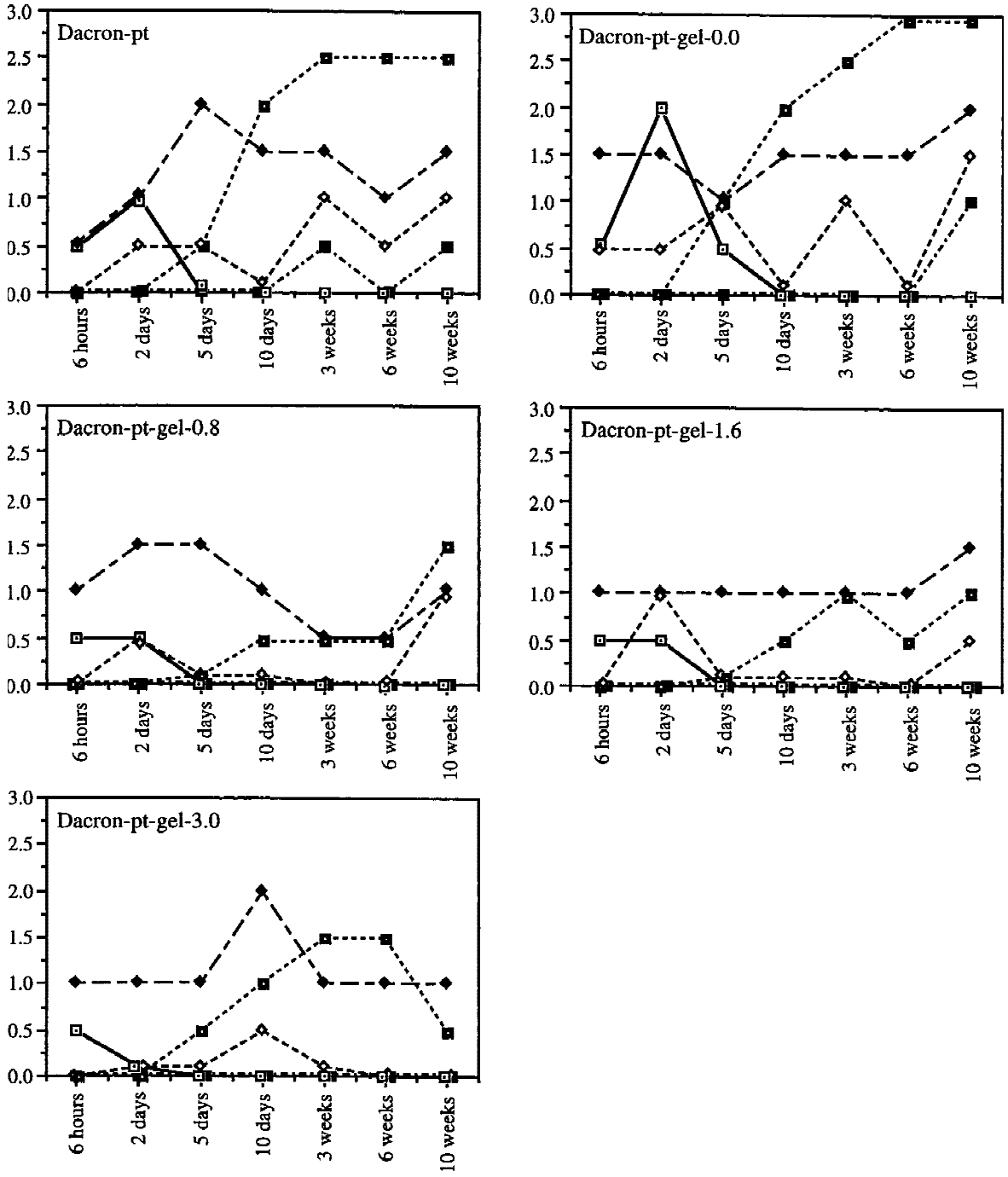

Figure 1. Trends of cellular reactions to the Dacron and Dacron-gelatin samples during the implantation period. Total cell numbers (from surrounding tissue, interface, and inside of samples) are expressed as arbitrary units: PMN (□), macrophages (♦), giant cells (◘), lymphocytes (◈), and plasma cells (■).

fibrin, as the Dacron fibers and bundles were further apart, probably owing to the absence of a gel. Macrophage numbers increased in Dacron and decreased in Dacron-gel-0.0. In both samples, a giant cell reaction was obvious, especially at the outside where fibers were completely surrounded by giant cells, but with Dacron-gel-0.0 it was also on the inside surrounded complete fiber bundles [Fig. 3(a)]. This material also induced some attraction of lymphocytes (Fig. 1).

In contrast, crosslinked gelatin induced less giant cell formation. Dacron-gel-0.8 showed macrophage adherence to the surface and indications of fusion; however, clear giant cells were not observed. Dacron-gel-1.6 showed more indications of macrophage fusion; only Dacron-gel-3.0 sometimes showed clear giant cells [Fig. 3(b)]. As on day 2, the number of macrophages was higher with Dacron-gel-0.8 than with the two other crosslinked gelatins.

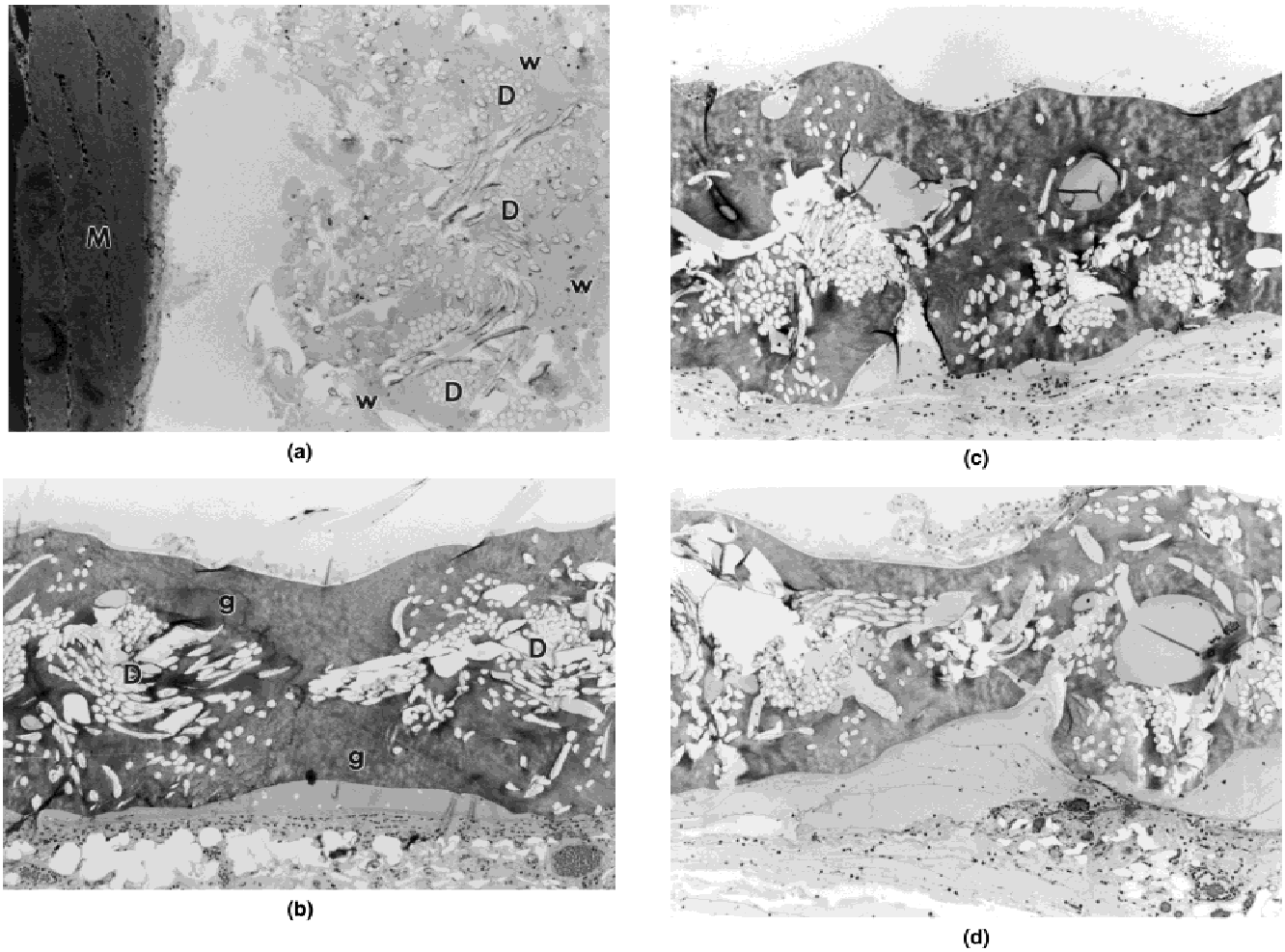

Figure 2. Dacron (a), Dacron-gel-0.8 (b), Dacron-gel-1.6 (c), and Dacron-gel-3.0 (d) at 6 h of implantation (original magnification ×10). D = Dacron fiber (bundle)s; g = crosslinked gelatin; W = wound fluid; M = back muscle.

At day 10 after implantation, Dacron still contained more fibrin-filled areas than Dacron-gel-0.0 (related to the larger space), although macrophage numbers were similar. Giant cell numbers were also similar for both samples, but had clearly increased compared to day 5. Thus, Dacron-gel-0.0 showed almost complete ingrowth with giant cells, whereas the fibrous capsule around the material, consisting of fibroblasts alternating with newly formed collagen bundles, was tighter and more mature than observed in Dacron.

The fibrous capsule around Dacron-gel-0.8 was tight in some areas and contained several macrophages. Macrophages were dominant at the interface, whereas only a few giant cells were present. The capsule around Dacron-gel-1.6 was the tightest of the three crosslinked gelatin samples, closely following the wavy structure of the film. Macrophages dominated at the interface, but giant cells were also seen. Within the series of crosslinked gelatin gels, the highest numbers of macrophages, giant cells, and lymphocytes were observed with Dacron-gel-3.0 (Fig. 1).

At week 3 implantation, Dacron showed giant cell occupation throughout the material. Well-vascularized fibrous tissue and some unoccupied areas were present between cells. In Dacron-gel-0.0, less fibrous tissue and more (mature) giant cells were observed. Both samples contained some lymphocytes. Moreover, plasma cells, i.e., immunoglobulin-producing B cells, were found in low numbers with Dacron.

Dacron-gel-0.8 was surrounded by a thick fibrous capsule that had detached from the material. A thin layer with some macrophages and giant cells was present at the interface. The gelatin gel size was reduced and voids around the Dacron fibers and bundles were observed. Dacron-gel-1.6 did not show obvious size reduction. The fibrous capsule was thinner than for Dacron-gel-0.8; it showed increased numbers of macrophages and giant cells. More giant cells were observed with Dacron-gel-3.0, also inside the cracks in the crosslinked gelatin. Dacron-gel-3.0 was surrounded by a thin capsule equal to Dacron-gel-1.6, containing similar numbers of macrophages. A reduction in gelatin size was not observed.

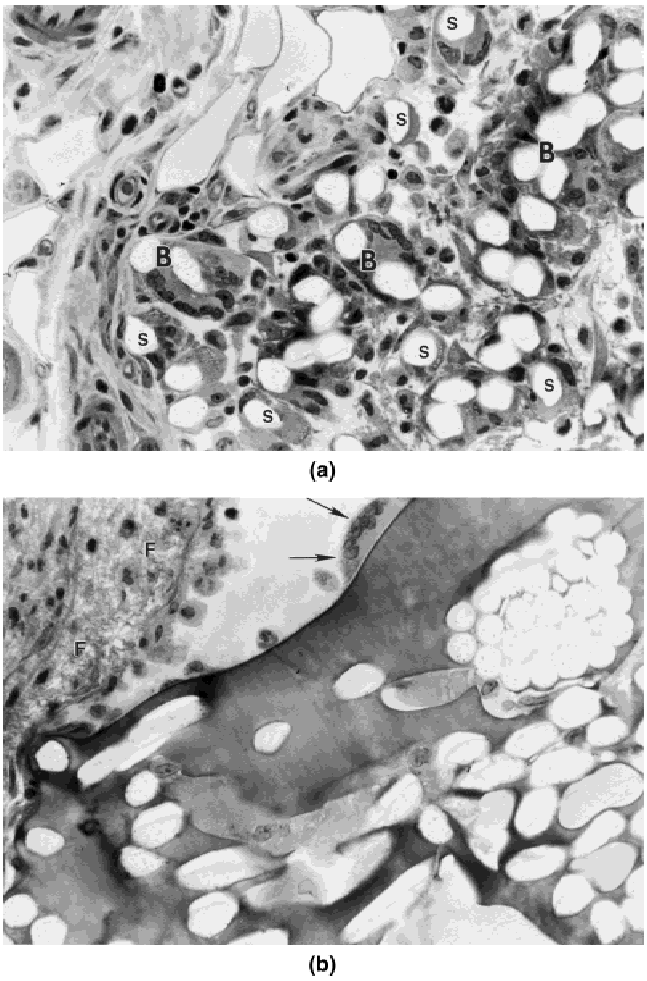

Figure 3. Dacron-gel-0.0 (a) and Dacron-gel-3.0 (b) at day 5 of implantation (original magnification ×40). s = single Dacron fibers; B = Dacron fiber bundles; → = giant cell with at least seven nuclei.

At 6 weeks' implantation, fibrous tissue was abundantly present as a capsule around the complete Dacron disk and around the giant cells between the Dacron fiber bundles [Fig. 4(a)]. However, in small central areas, fibrin was observed. The capsule was less vascularized than at week 3. Dacron fibers were sometimes localized outside the capsule. Dacron-gel-0.0 clearly showed less fibrous tissue but more and larger giant cells [Fig. 4(b)]. The fibrous tissue sometimes consisted of thick collagen layers. No lymphocytes were observed (Fig. 1).

The crosslinked gelatin samples clearly decreased in size. Dacron-gel-0.8 showed the largest decrease, as judged from voids at the interface and around Dacron fibers and fiber bundles, from the lightest staining, and from the flipping-over of film edges [Fig. 4(c)]. At some sites, macrophages and giant cells were found inside the gelatin gel. Cell presence inside the gelatin was not observed with Dacron-gel-1.6 and Dacron-gel-3.0. Dacron-gel-3.0 showed the most giant cells. The darkest staining and lowest number of voids were observed with Dacron-gel-3.0 [Fig. 4(d)].

At week 10, both Dacron and Dacron-gel-0.0 showed complete ingrowth, with giant cells surrounding Dacron fibers and fiber bundles, which were themselves surrounded by fibrous tissue. The fibrous tissue, which was more prominent in Dacron than Dacron-gel-0.0, showed higher vascularization with more macrophages than at week 6. Few lymphocytes and twice as many plasma cells were also present. As in week 3 and 6, more and larger giant cells were observed with Dacron-gel-0.0 (Fig. 1). One area with giant cells seemed to show some degradation of Dacron fibers.

All crosslinked gelatin samples contained gelatin. Dacron-gel-0.8 showed the least gelatin coating (size equal to week 6), with staining varying from light to dark. Cellular activity clearly increased, showing tissue penetration by capillaries, macrophages and giant cells, fibroblasts, and some lymphocytes at many light stained sites of the material [Fig. 5(a)]. The macrophages and giant cells adhered to Dacron fibers. The capsule showed higher cellular activity where tissue had penetrated the material.

Dacron-gel-1.6 showed more dark-stained areas than Dacron-gel-0.8, although light-stained areas were also present. The amount of gelatin had decreased. In contrast to week 6, some ingrowing tissue was present (in the light-stained areas). Capillaries were not observed inside the gelatin, and giant cells were less obvious at Dacron fibers, but sometimes seemed to surround the phagocytositic parts of gelatin [Fig. 5(b)]. The Dacron-gel-1.6 capsule showed more macrophage activity than that of Dacron-gel-0.8 and Dacron-gel-3.0.

Dacron-gel-3.0 showed the most dark-stained gelatin. Cracks were slightly more pronounced than at week 6. Ingrowth in the gelatin film was not observed; one larger part of gelatin gel seemed to be separated by fibrous tissue.

DISCUSSION

Biocompatibility

The biocompatibility of materials is determined by cellular activity at the materials. The early tissue reaction (up to 2 days) to crosslinked gelatin impregnated in Dacron was similar to or milder than Dacron and Dacron-gel-0.0, as observed from PMN numbers (Fig. 1). For Dacron and Dacron-gel-0.0, the number of giant cells increased markedly after 5 days of implantation, owing to a foreign-body reaction to exposed Dacron. It appears that this foreign-body reaction to Da-

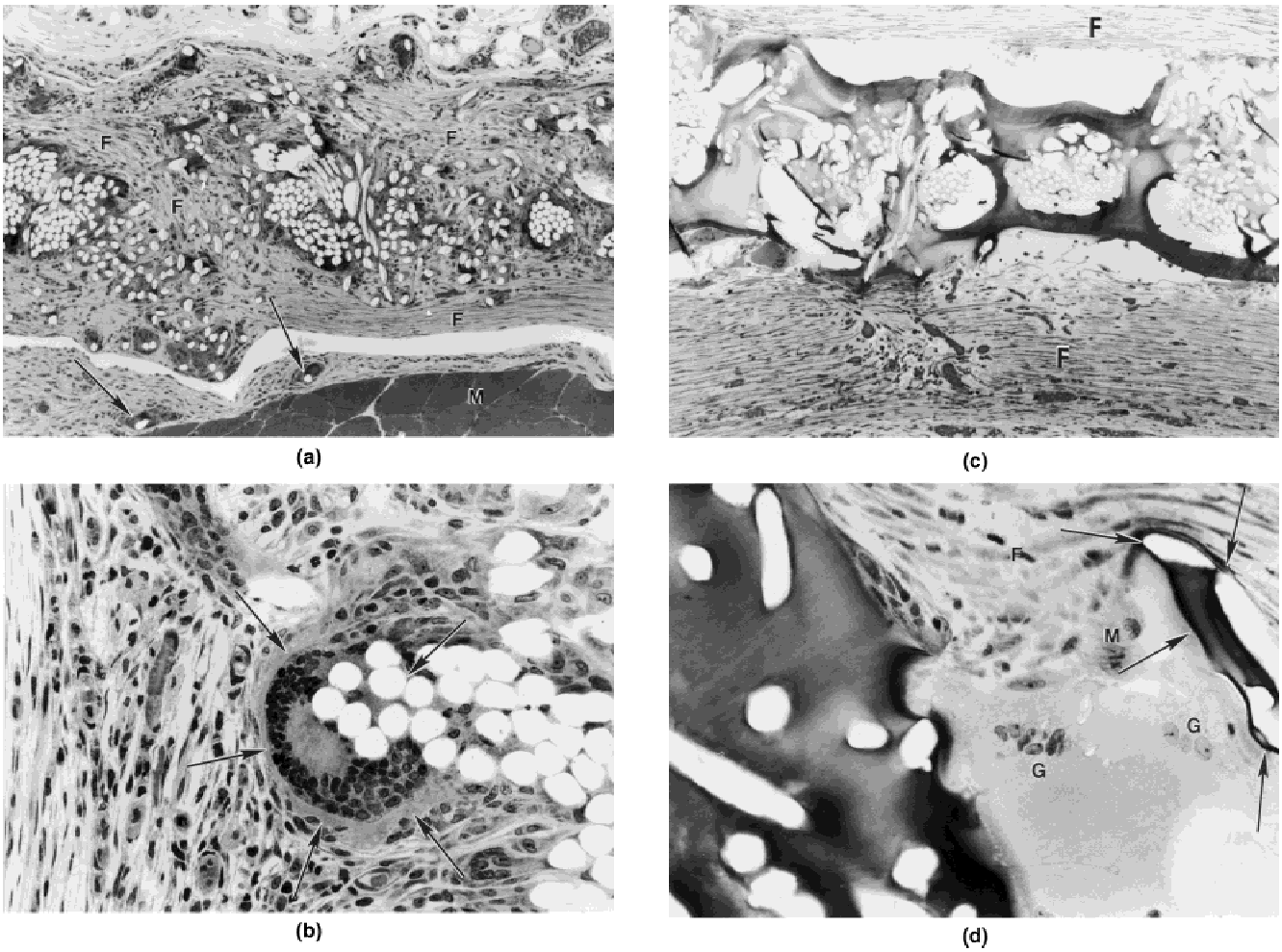

Figure 4. Dacron [(a), ×10], Dacron-gel-0.0 [(b), ×40], Dacron-gel-0.8 [(c), ×10], and Dacron-gel-3.0 [(d), ×40] at week 6 of implantation. (a–c) F = fibrous tissue; → = giant cells; M = back muscle. (d) F = fibroblasts; M = macrophages; G = giant cells; → = separation of two Dacron fibers within part of the gelatin.

cron is delayed when the Dacron fibres are coated by crosslinked gelatin, until the gelatin becomes degraded and Dacron is exposed to the surrounding tissue. During at least 6 weeks after implantation, the cellular activity to crosslinked gelatin is low compared to Dacron, with a low number of giant cells, lymphocytes, and plasma cells. It is not clear whether this tissue reaction to gelatin-coated Dacron will become similar to Dacron after complete degradation of gelatin.

Dacron-0.8 attracted most macrophages early on, which seems related to its low crosslink density. Gelatin contains binding sites for fibronectin and probably for other serum and cellular proteins and macromolecules, which may act as intermediates for cellular adhesion. With increasing crosslink density, fibronectin-binding domains are shielded from the surface of the gelatin film, resulting in a reduction in cellular adhesion.[25,26] The higher number of macrophages in Dacron-gel-0.8, however, did not result in a concomitant formation of giant cells.

Giant cell formation was hardly observed with the samples containing crosslinked gelatin, in contrast to the control samples. Giant cells were formed by fusion from macrophages, which were sufficiently available at the surfaces. However, the differentiation of macrophages is dependent on the form, composition, and topography of the implanted surface: The surface response to fabric and relatively rough surfaces generally results in the formation of giant cells, whereas relatively smooth and flat surfaces do not induce giant cell formation.[27] In this study, giant cell formation predominantly occurred on Dacron fibers. Although some giant cells were observed with all crosslinked gelatin samples, an increase in crosslink density, which resulted in a rougher surface, caused an increase in giant cell formation.

Biodegradation

Biodegradation is related to cellular infiltration and ingrowth. After 10 weeks of implantation, the control

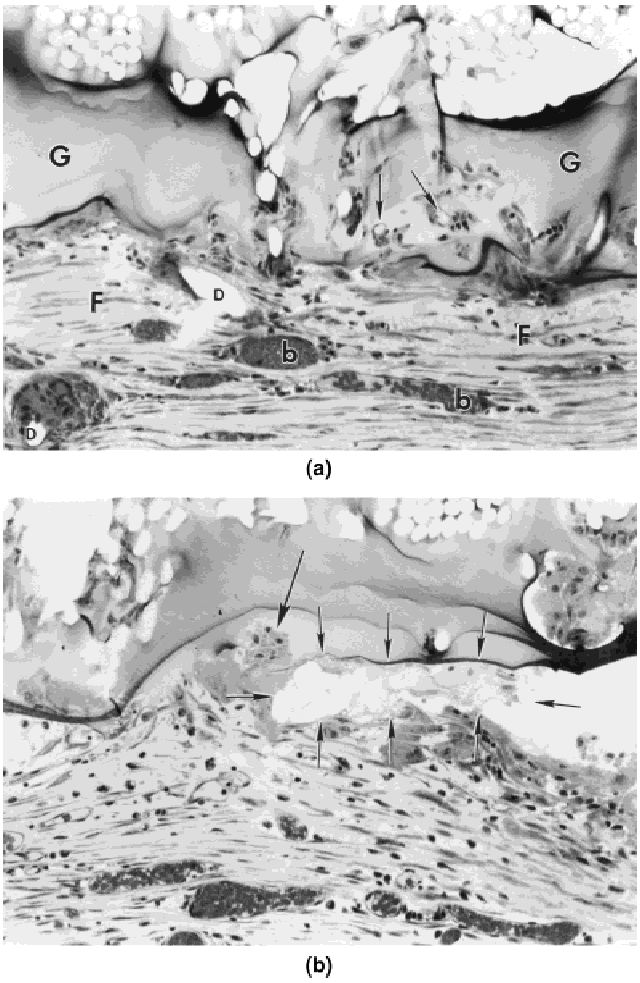

Figure 5. Dacron-gel-0.8 (a) and Dacron-gel-1.6 (b) at week 10 of implantation (original magnification ×20). (a) G = gelatin; → = capillaries; D = Dacron fibers; b = blood vessels; F = fibrous capsule. (b) Large arrows = ingrowth of tissue; small arrows = surrounding of larger gelatin fragments by giant cell pseudopods.

samples showed complete ingrowth of Dacron, with giant cells and fibrous tissue. Although the degradation rate of the crosslinked gelatin coating was not fast, a decrease in degradation rate and cellular ingrowth with increasing crosslink density were observed. Compared to the Dacron control sample, however, tissue ingrowth in the crosslinked gelatin gels was minimal after 10 weeks of implantation. The observed voids were probably caused by shrinkage of partly hydrolyzed gelatin gel during processing of the samples before light-microscopic evaluation.[7]

Gelatin gels need a minimal crosslink density to prevent dissolution. Taking this minimal crosslink density into consideration, the *in vivo* degradation rate of gelatin can be varied from 1 to several months, depending on the desired application. For short-term controlled release of pharmacological compounds from a prosthetic heart valve, a relatively high degradation rate and fast tissue ingrowth are preferred and, thus, a low crosslink density. The degradation of gelatin may be faster *in situ* in a prosthetic heart valve than in a subcutaneous pocket because of the higher strain and the occurrence of some blood flow.

CONCLUSIONS

The *in vivo* compatibility and biodegradation of carbodiimide crosslinked gelatin-impregnated Dacron for application as a biodegradable controlled-release system in a prosthetic heart valve were studied by subcutaneous implantation in rats.

The tissue reactions with Dacron and gelatin-coated Dacron were relatively mild during implantation. Although Dacron is a well-accepted biomaterial, the presence of a crosslinked gelatin coating in the Dacron mesh had a definite positive effect on the biocompatibility of the samples. After 3 weeks, the number of giant cells, macrophages, and lymphocytes observed in the crosslinked Dacron-gel samples was lower than for the control samples. It is not clear, however, whether this improved biocompatibility was transient and would disappear upon degradation of the crosslinked gelatin gel. No correlation was observed between the biocompatibility and crosslink density of the gelatin gels, except that increased gelatin crosslink density resulted in rougher surfaces, thereby slightly promoting giant cell formation. The crosslink density, however, determines the degradation rate of the crosslinked gelatin gels and cellular ingrowth into the gels. Dacron-gel-0.8, which had the lowest crosslink density, degraded faster than Dacron-gel-1.6 and Dacron-gel-3.0, and showed most cellular ingrowth. after 10 weeks of implantation. Although the weight of the implanted gelatin gels varied to some extent (Table I), these variations had no effect on the biocompatibility or degradation of the gels.

For a diffusion controlled–release system, the release characteristics (such as drug loading and release time) also depend on the crosslink density of the gel. Therefore, the optimal crosslink density of these gelatin gels is determined by a combination of the required release characteristics and degradation rate, depending on the therapeutic drug and application. EDC/NHS crosslinking is a suitable method to prepare biocompatible crosslinked gelatin gels for controlled-release applications because the crosslink density can easily be varied. The controlled release of antibacterial proteins to prevent prosthetic valve endocarditis is one example of the possible applications of this system.

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