Biocompatibility of solid poly (ortho ester)

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In previous studies poly (ortho ester) (POE) has shown promise as a resorbable device, a hemostatic sealant and as a carrier for drugs in bone surgery. The aim of this study was to evaluate the tissue reactions of solid poly (ortho ester) implanted into both tibiae of 17 rabbits. One half of the rods were sterilized by gamma radiation and the other half by ethylene oxide. The follow-up times were from 1 week to 21 weeks, after which the animals were killed and the bony specimens examined histologically. The connective tissue samples were examined immunohistochemically in order to study the occurrences of two extracellular matrix glycoproteins, tenascin and fibronectin. The results showed that solid poly (ortho ester)s induce a moderate inflammatory reaction for 9 weeks. Tenascin and fibronectin were present in samples from 1 week up to 4 weeks. It was also found that gamma sterilized POE was resorbed at week 7 and ethylene oxide sterilized POE at week 13.

1. Introduction

Today some fractures and osteotomies can be fixed with biodegradable devices instead of metallic ones. The polymers most often used are poly (lactic acid) (PLA) and poly (glycolic acid) (PGA). As the devices do not need to be removed, patient suffering and the costs to the hospital can be reduced. However, abacterial inflammatory reactions have been noticed after the clinical introduction of these devices [1–5]. Both swelling and pain at the site of implantation have also been reported. The etiology of this inflammatory reaction is still unknown despite numerous studies [3, 5, 6]. An ongoing search for new biocompatible materials is thus necessary.

Poly (ortho ester)s (POE) are a group of synthetic bioerodible polymers. Devices made of poly (ortho ester)s can be formulated so that the device undergoes surface erosion, e.g., the polymeric device degrades only at its surface and becomes thinner with time rather than crumpling. This may be the reason for minor foreign body reaction compared with PLA or PGA. In studies where wax-like poly (ortho ester)s have been implanted into rats, tissue reactions have been reported to be moderate [7–9]. In this study, we have assessed tissue reactions of solid POE both histologically and immunohistochemically. The devices were implanted into bone. Also resorption time of the material from the tissue and the influence of sterilization method are reported.

2. Materials and methods

2.1. Experimental animals and implant material

Seventeen adult rabbits (New Zealand White) of both sexes, weighing from 3000 g to 4000 g were used as experimental animals. Solid poly (ortho ester) was used as an implant material (SRI International, Menlo Park, CA, USA). The molecular weight of the polymer was 140 000 and its chemical structure is shown in Fig. 1. It has a structure comprised of a 20/80 ratio of linear and cyclic blocks. Polymer synthesis has been described by Heller *et al.* [10].

Implant samples were prepared in the Biomaterials Laboratory, Tampere University of Technology, Finland. The polymer power was ultrasonically melted to 2 mm thick plates from which rods were sawn. The rods had dimensions approximately 2 mm × 2 mm × 5 mm. The first group of rods was sterilized by gamma radiation (Kolmiset Oy, Ilomantsi, Finland) with a minimum dose of 25 kGy. The second group of devices was sterilized with ethylene oxide (Bioscience Oy, Tampere, Finland).

2.2 Operative procedure

There was no preoperative fasting. The rabbits were anesthetized with subcutaneous (s.c.) medetomidine 0.3 mg/kg (Domitor® 1 mg/ml, Lääkefarmos, Turku, Finland) and ketamine hydrochloride 50 mg/kg (Ketalar® 50 mg/ml, Parke-Davis, Barcelona, Spain). They also received 150 000 IU bentzylpenicillin procaine and 150 000 IU bentzatine penicillin (Duplocillin la®, Gist-Brocades NV Delt-Holland) s.c. preoperatively for infection prophylaxis. The lateral sides of both tibias were shaved and scrubbed with an antiseptic solution, chlorhexidine gluconate (Klorhexol® 5 mg/ml, Leiras, Finland). An incision was made laterally on the distal end of the tibia and the

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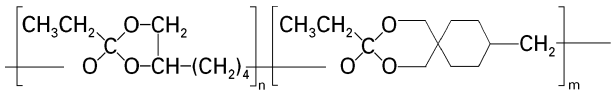

Figure 1 The chemical structure of studied poly (ortho ester).

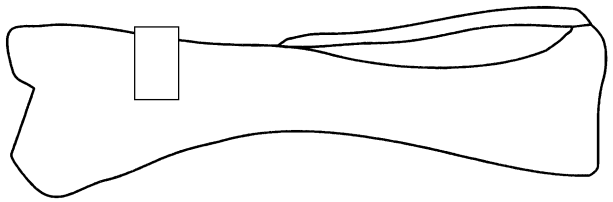

Figure 2 Schematic drawing of the surgical technique.

periosteum was reflected down to the bone. A small defect was drilled with a cylindrical bur through cortical bone to the bone marrow. Implants were inserted into the bone so that part of the implant was placed into the bone marrow and another part into the cortical bone (Fig. 2). The gammasterilized rods were inserted into the right tibia (group A) and the ethyleneoxide sterilized (group B) into the left tibia. Incisions were closed by absorbable sutures (Dexon®).

2.3. Postoperative procedure

All animals were fed *ad libitum* after the surgical procedure and they were free to move in their cages.

2.4. Follow-up times and specimens

Follow-up times were 1, 2, 3, 4, 7, 8, 9, 13, 17 and 21 weeks (Table I). After the follow-up time the animals were killed by administering an overdose of pentobarbital (Mebunat®, Orion, Turku, Finland) and both tibias were exarticulated. For histologic studies the bony specimens were fixed in 70% alcohol and embedded in methylmetacrylate [11]. They were cut into 5 μm sections, stained by the Masson–Goldner method [12] and evaluated by light microscopy. The formation of osteoid, growth of granulation tissue, amount of inflammatory and giant cells and foreign body reaction were evaluated from the specimens and graded from 0 to 3 (0 = none, 1 = slight, 2 = moderate, 3 = abundant). The average grade for each specimen was then calculated.

After careful dissection a sample for immunohistochemistry was taken from the connective tissue facing the implant. The samples were snap frozen in precooled isopentane contained in vials in a slurry of ice and stored at $-70\,^{\circ}C$. Cryosections 5 μm thick were cut, air dried and briefly fixed with cold acetone. Indirect immunofluorescence microscopy was performed by using monoclonal antibodies (Mabs) 100EB2 [13] against human tenascin and 52DH1 [14] against human cellular fibronectin. The specimens were incubated with the Mabs for 30 min. Fluorescein isothiocyanate (FITC)-coupled sheep anti-mouse IgG antiserum (Jackson Laboratories, West Grove, PA) was then applied. The specimens were evaluated by

TABLE I Follow-up schedule

Follow-up time (weeks)	Number of rabbits
1	1
2	2
3	2
4	2
7	2
8	3
9	2
13	1
17	1
21	1

Leitz Aristoplan© fluorescence microscope equipped with appropriate filters for immunohistochemistry.

3. Results

3.1. Histologic study

The site of implantation was clearly visible in all samples during the first two weeks (Fig. 3a). After that the region became more difficult to recognize until week 17, when it was no longer visible. Bone formation was strongest at weeks 1, 2 and 3 (Figs. 3b, 3c and 4) in both groups. Some osteoid formation was also seen at weeks 4, 7, 8 and 9. Slight formation of granulation tissue around implants was seen until week 8 and week 13 in groups A and B, respectively (Figs. 3d and 5). A few inflammatory cells were present until week 13 and week 9 in group A and group B, respectively (Fig. 6). The number of giant cells was highest during the first four weeks, and after week 13 they were no longer found (Fig. 7). The foreign body reaction was stronger in group B (ethylene oxide sterilized POE) than in group A (gamma sterilized POE) and it lasted longer (group A week 8 and group B week 17, Fig. 8).

3.2. Immunohistochemical study

Some tenascin and fibronectin immunoreactivity was detected in connective tissue bordering POE-implant from first to fourth postoperative weeks (Fig. 9a). However, no highly fluorecent tenascin or fibronectin layer facing the implant was seen. After fourth postoperative week no tenascin or fibronectin immunoreactivity was detected in connective tissue (Fig. 9b).

4. Discussion

The use of metallic devices for fracture fixation is considered to be a reliable method for achieving undisturbed fracture healing. However, there are several disadvantages in the use of these metallic implants. One is bone athropy from stress shielding by the rigid bone plates and screws. Other disadvantages are pain, infection, the possibility of corrosion and even carcinogenic potential. Also they need to be removed after the fracture has healed. Bioresorbable fracture fixation devices are an alternative to metallic devices. In the beginning, poor strength of biodegradable

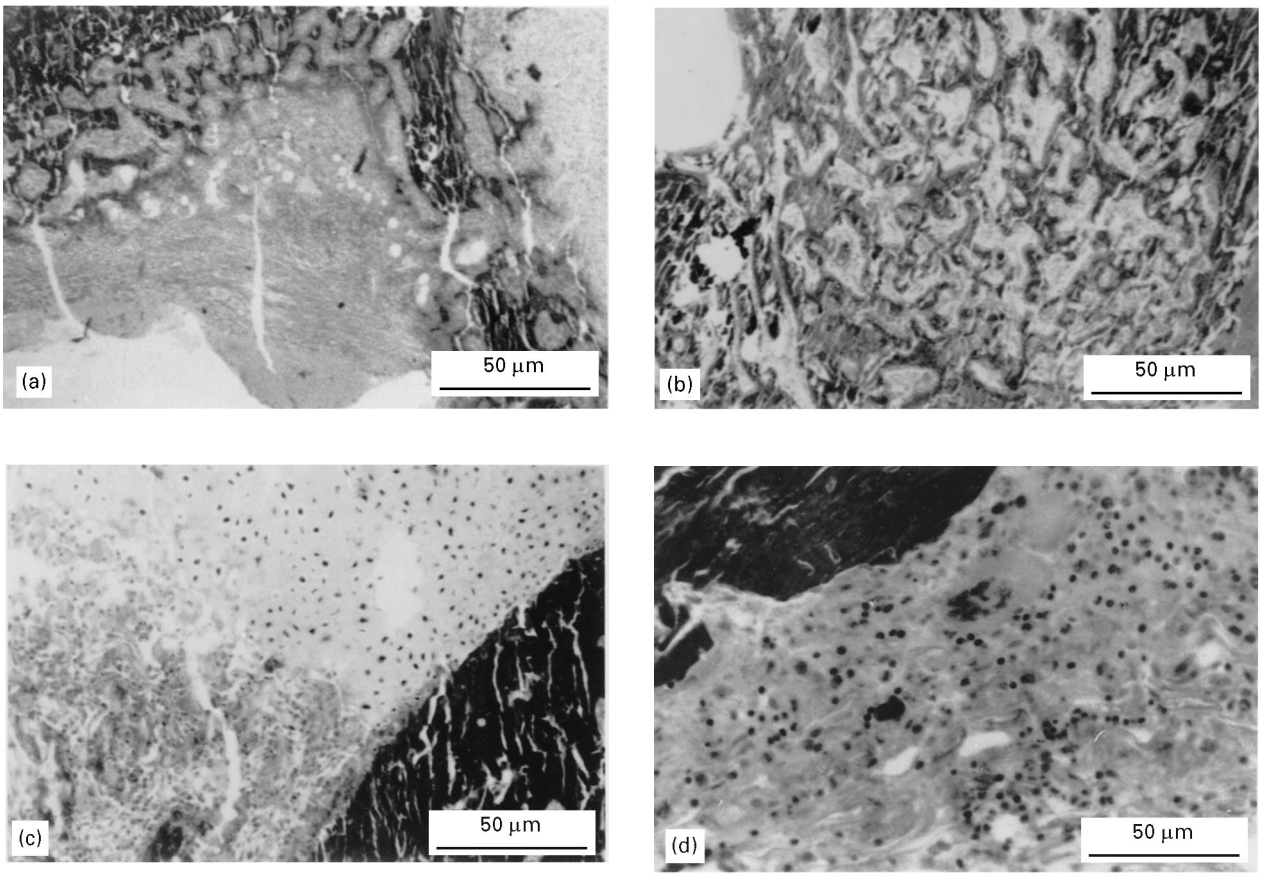

Figure 3 (a) Implant still visible surrounded by fibrotic granulation tissue and osteoid. (b) Abundant osteoid formation in a specimen taken one week after implantation. (c) Strong bone formation in a 3-week specimen. (d) A thick layer of granulation tissue around the implant area. Several giant cells are found in inflammatory reaction.

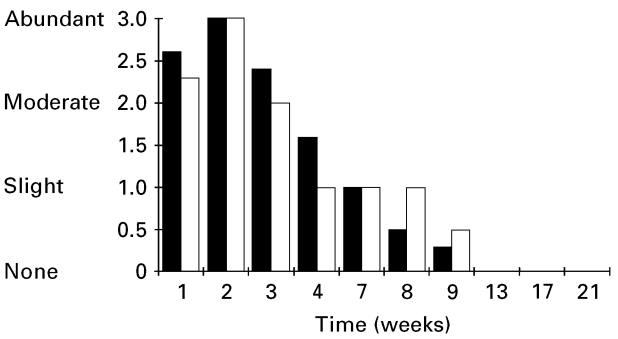

Figure 4 Formation of osteoid: ■ gamma sterilized; □ ethylene oxide sterilized.

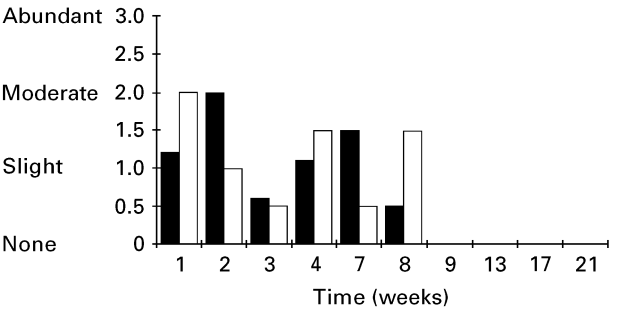

Figure 6 Inflammatory cell infiltration: ■ gamma sterilized; □ ethylene oxide sterilized.

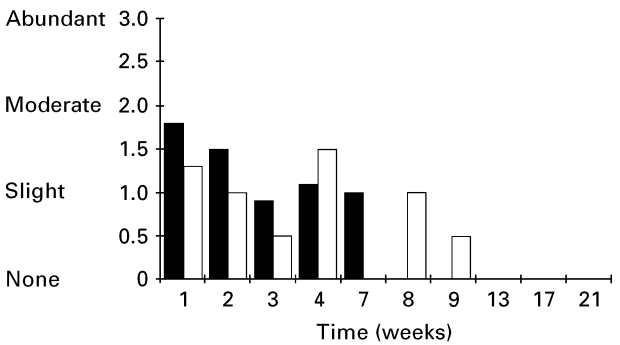

Figure 5 Presence of granulation tissue: ■ gamma sterilized; □ ethylene oxide sterilized.

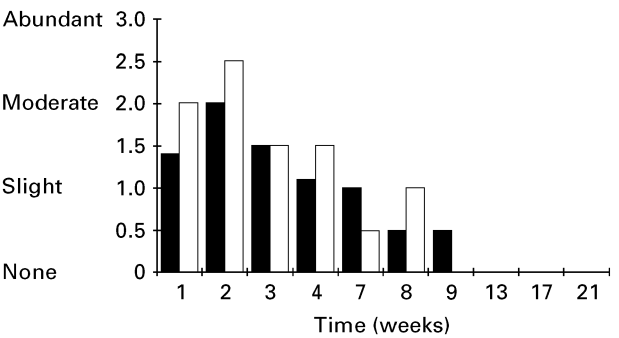

Figure 7 Presence of giant cells: ■ gamma sterilized; □ ethylene oxide sterilized.

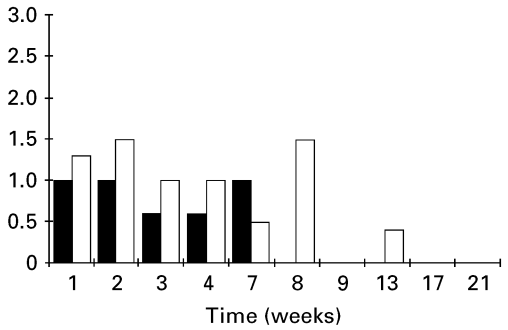

Figure 8 Foreign body reaction: ■ gamma sterilized; □ ethylene oxide sterilized.

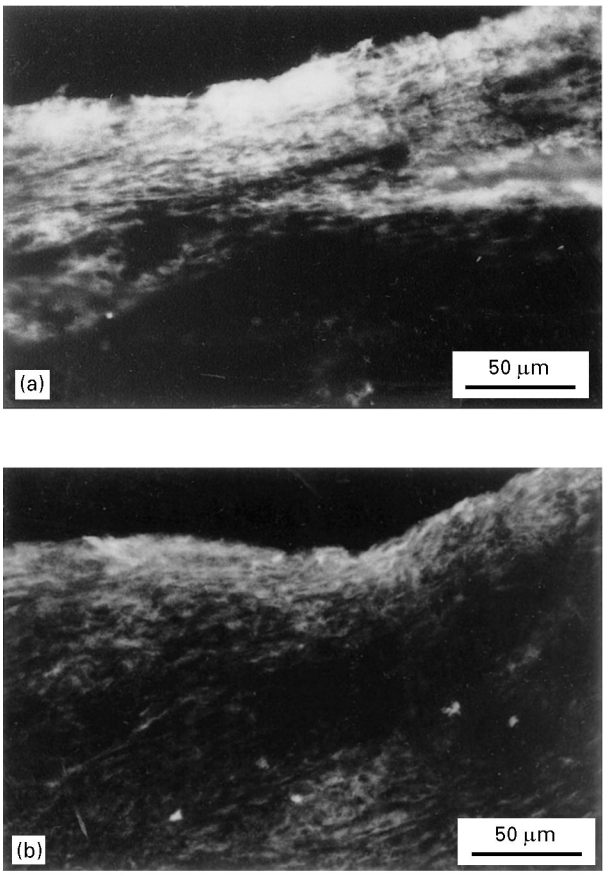

Figure 9 Immunofluorescence micrographs: (a) demonstrating fibronectin immunoreactivity close to the implant in a 2-week specimen; (b) showing no clear fibronectin immunoreactivity detectable in a 9-week specimen.

devices was a problem, but by better material management that has now been resolved [15]. Unfortunately after clinical introduction of these devices, unaccountable abacterial inflammatory reactions have been noticed and about 6% of patients operated on with polyglycolide devices have developed a tissue reaction at the site of implantation [3]. Microscopic examination showed an intense inflammatory response composed of neutrophilic polymorphonuclear leukocytes and small lymphocytes. Another common finding was the occurrence of monocyte-macrophages and foreign-body-type giant cells. These reactions have been found some months [3] or even some years after operation [1, 5] with PGA and PLA, respectively. Yet, no such reactions have so far been detected with self-reinforced poly (L-lactide) fracture fixation devices (Törmälä, personal communication). The reason for this tissue reaction is not yet known, but it has been suggested that local tissue tolerance and transport potential are important [2]. Also, the incidence of inflammatory reaction around the implants has been shown to vary with different anatomic regions [2].

In this study, we have found that POE causes minimal tissue reaction, which is in agreement with previous studies [7–9]. Only a mild inflammatory reaction with some giant cells was noted. Resorption of materials sterilized with ethylene oxide was slower than that of gamma sterilized materials. Gamma irradiation reduces molecular weight so that resorption times are shorter. Ethylene oxide sterilization does not reduce molecular weight. This finding is in agreement with the studies of Lähde *et al.* [16]. The resorption times for solid ethylene oxide sterilized POE and for solid gamma sterilized POE were 13–17 weeks and 8 weeks, respectively.

We also analysed the expression of two extracellular matrix glycoproteins, tenascin and fibronectin. Tenascin is absent from most normal adult tissues but is strongly expressed during inflammatory processes, wound healing and in neoplastic tumors including sarcomas, melanomas and carcinomas [17, 18]. It is also expressed during acute and chronic tissue rejections [19]. During wound healing, tenascin is regularly detectable in wounds older than 5 days and is generally dispersed within 21–45 days. There after it is regarded as pathological [20, 18]. Fibronectin is not found in normal adult tissue, but is present in fetal tissue. It is expressed during wound healing, rejection and in tumor stroma [19, 21]. In this study neither tenascin nor fibronectin were found in the specimens after follow-up at 4 weeks. Kontio *et al.* [22] studied connective tissue capsules around PLA-implants, and they found tenascin and fibronectin during the whole 48-week follow-up period. They suggested that PLA induces a prolonged tissue response different from conventional wound healing. The high expression of tenascin and fibronectin close to the implants might be an early sign of subclinical rejection. If this is shown to be true, the biocompatibility of POE might be better than that of PLA.

We conclude that POE causes only a moderate inflammation reaction for 9 weeks. Giant cells are also found during the first 4 weeks. More studies with different experimental animals are needed to confirm the biocompatibility of POE and to estimate mechanical properties and the possible use of POE as fracture fixation devices.

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