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A histological and immunohistochemical study of tissue reactions to solid poly(ortho ester) in rabbits

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Abstract. In many cases only the temporary presence of a biomaterial is needed in tissue support, augmentation or replacement. In such cases biodegradable materials are better alternatives than biostable ones. At present, biodegradable polymers are widely used in the field of maxillofacial surgery as sutures, fracture fixation devices and as absorbable membranes. The most often used polymers are aliphatic polyesters, such as polyglycolic acid (PGA) and polylactic acid (PLA). Poly(ortho ester) is a surface eroding polymer, which has been under development since 1970, but is used mostly in drug delivery systems in semisolid form.

The aim of this study was to evaluate the tissue reactions of solid poly(ortho ester) (POE), histologically and immunohistochemically. Resorption times and the effect of 2 different sterilization methods (gamma radiation and ethylene oxide) upon resorption were also evaluated. Material was implanted into the tibia and subcutaneously into the mandibular ramus area of 24 rabbits. Follow-up times were 1–10, 14 and 24 weeks.

Histological studies showed that POE induces a moderate inflammation in soft tissue and in bone. At 24 week follow-up, inflammation was mild in soft tissue and moderate in bone. In immunohistochemical studies, no highly fluorescent layer of tenascin or fibronectin was found adjacent to the implant. Resorption of gamma-sterilized rods was faster than ethylene oxide-sterilized rods. The total resorption time was more than 24 weeks in both groups. Clinically the healing was uneventful and the implants the well tolerated by the living tissue. This encourages these authors to continue studies with this interesting new material to search for the ideal material for bone filling and fracture fixation.

Key words: solid poly(ortho ester); bone substitute; foreign body reaction; tenascin; fibronectin.

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Currently, biodegradable materials are widely used in surgery for fixation of fractures and osteotomies[12], as sutures and as bioabsorbable membranes. Bioabsorbable polymer-based bone substitute materials are also commercially available[1,18]. The most often used polymers are aliphatic polyesters like polyglycolic acid (PGA) and polylactic acid (PLA). Bioresorbable fixation is a fascinating alternative for metallic fixation. By using bioresorbable materials it is possible to avoid disadvantages caused by the use of metallic implants, such as pain, infection, possible corrosion and even carcinogenity. If radiotherapy or postoperative imaging is needed, bioresorbable implants do not disturb it. When bioresorbable materials are used, a removal operation is not needed if total resorption is accomplished after tissue has healed. This makes it possible to reduce the hospital costs and morbidity of the patient. The implant material must however fulfil several basic demands. Good compatibility between host tissue and implant is, naturally, essential.

Biocompatibility studies of the most often used absorbable polymers PGA and PLA with laboratory animals have shown that these materials induce only mild nonspecific inflammatory response. Clinical studies in humans have however revealed a relatively high incidence of inflammatory reactions in some studies with pure poly-L-lactide (PLLA) and pure PGA. These reactions have been found some months and even some years after operation[4,6]. The aetiology of this reaction is still unknown, and further studies are ongoing.

Poly(ortho esters) (POE) have been under development since 1970. They are synthetic, bioerodible polymers in which the bioerosion process is limited to the surface of the device. Devices made of POE can be formulated so that it undergoes surface erosion, e.g. the polymeric device degrades only at its surface and becomes thinner with time, rather than crumples[8]. POE hydrolyses in an aqueous environment. Hydrolysis of the polymer is not autocatalytic because the neutral hydrolysis products can diffuse away from the polymer before the carboxylic acid is formed[9]. An erosion process that is confined predominantly to the surface layers has some important consequences. As most of the hydrolysis occurs in the outer layers of the device, acidic hydrolysis products diffuse away from the device and do not accumulate in the bulk material. Thus, the interior of the matrix does not become highly acidic and cause autocatalytic degradation as is the case with bulk hydrolysing poly(lactide-co-glycolide) copolymers or polylactides[8]. Therefore, this study hypothesized that the foreign body reactions might be minor compared to PLA or PGA.

The wax-like POE has been used in experimental studies in the treatment of burns[27] and it has also been studied in many surgical applications[23,22,21,20,16,24,19]. Minor inflammatory tissue reactions have been reported and the polymer has been well tolerated.

In the present study, solid POE implants were implanted into bone and connective tissue. Tissue reactions were studied histologically and immunohistochemically. The influence of sterilization method upon the resorption time was also studied.

Materials and methods

Experimental animals and implant material

The study was approved by the ethics committee of the State Province Office of Southern Finland. Twenty-four adult female rabbits, weighing 3000–4400 g, were used as experimental animals. The solid POE was supplied by Dr Jorge Heller, Advanced Polymer Systems, Redwood City, California, USA. The molecular weight of the polymer was 46,460. The polymer had a composition of 90 mole% trans-cyclohexandimethanol and 10 mole% polyacetal. The synthesis of POE is described by HELLER et al.[9] The polymer powder was ultrasonically moulded to 2-mm-thick plates from which rods were sawn, with a method described earlier by KELLOMÄKI et al.[10] The rods had dimensions approximately 2 mm × 2 mm × 5 mm. The first group was gamma-irradiated with a minimum dose of 25 kGy and the second group was sterilized with ethylene oxide (EO). Implants were made in the Institute of Biomaterials, Tampere University of Technology, Tampere, Finland.

Operative procedure

All animals were fed on standard laboratory food and water *ad libitum*. There was no preoperative fasting. Anaesthesia was induced with a subcutaneous (s.c.) injection of a combination of 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). For infection prophylaxis animals received preoperatively 150,000 IU benzylpenicillin procaine and benzatine penicillin (Duplocillin LA, Gist-Brocades NV, Delt, The Netherlands) s.c. Postoperatively the animals were given buprenorphin 0.05 mg/kg (Temgesic® 0.3 mg/ml, Reckitt & Colman, Hull, U.K.) s.c. The lateral sides of both tibias were shaved and washed with an antiseptic chlorhexidine gluconate solution (Klorhexol® 5 mg/ml, Leiras, Finland). An incision was made laterally on the distal end of the tibia, and the periosteum was reflected down to the bone. A defect (diameter 3 mm) was made with a small cylindrical bur through cortical bone. The implants were placed so that a part of the implant was in the bone marrow and a part was in the cortical bone (Fig. 1). The gamma-sterilized rods were placed into the right tibia (group A), and the ethylene oxide-sterilized into the left tibia (group B). Incision was closed with absorbable sutures (Dexon®). The ramus area of the right mandible was shaved and washed with the same antiseptic solution. An incision was made on skin of the ramus area extraorally and a soft tissue pouch was created subcutaneously. A single gamma-sterilized rod was placed in the pouch. and the incision was closed with the same absorbable sutures.

Postoperative procedure

Postoperatively the animals were given a single dose of buprenorphin 0.05 mg/kg (Temgesic® 0.3 mg/ml, Reckitt & Colman, Hull, U.K.) s.c. Animals were free to move in their cages and they were fed *ad libitum*. No postoperative complications were registered after the operation.

Follow-up times and specimens

The follow-up times were 1–10, 14 and 24 weeks (Table 1). After the follow-up time animals were killed in groups of 2 with overdose of pentobarbital (Mebunat®,

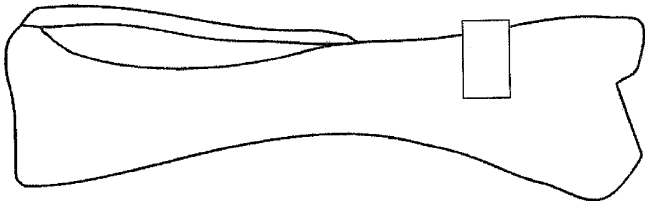

Fig. 1. Schematic drawing of the surgical technique in the tibia of rabbit.