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Tissue reactions to bioabsorbable ciprofloxacin-releasing polylactide-polyglycolide 80/20 screws in rabbits' cranial bone

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Abstract The aim of this study was to assess tissue reactions to bioabsorbable self-reinforced ciprofloxacin-releasing polylactide/polyglycolide (SR-PLGA) 80/20 screws in rabbits' cranial bone. Two screws were implanted in each rabbit, one screw on either side of the sagittal suture (n = 28 rabbits). Animals were sacrificed after 2, 4, 8, 16, 24, 54 and 72 weeks, four animals per group. On histological examination the number of macrophages, giant cells, active osteoblasts and fibrous tissue layers were assessed and degradation of the screws was evaluated. At 2 weeks, the highest number of macrophages and giant cells were seen near the heads of the screws. After 4 and 8 weeks, the number of giant cells decreased but that of macrophages decreased from 16 weeks and on. Screws were surrounded by fibrous tissue capsule that progressively was growing in thickness by time. Active osteoblasts were seen around the shaft of the screws with the highest number seen at 4 weeks postoperatively. At 16 weeks, compact fragmentation of the screw heads was seen with macrophages seen inside the screw matrices. After 24 weeks, no polarization of the screws was seen. After one year, PLGA screws had been replaced by adipose tissue, fibrous tissue and "foamy macrophages" which had PLGA particles inside them. After $1\frac{1}{2}$ years, the amount of biomaterial remaining had decreased remarkably. The particles of biomaterial were inside "foamy macrophages." Ciprofloxacin-releasing SR-PLGA 80/20 screws elicited a mild inflammatory reaction but did not interfere with osteoblast activity. No complications were seen when implanted in cranial bone of rabbit.

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Introduction

Despite modern advances in the surgical and antimicrobial armamentarium, the treatment of bone infection remains a formidable challenge for the clinician [1]. Current guidelines for the treatment of acute and chronic osteomyelitis emphasize surgical debridement of all nonviable tissue and prolonged intravenous administration of antibiotics [1–3]. However, achievement and maintenance of adequate therapeutic levels of antibiotic in bone without detrimental systemic effects often is difficult as infected bone has an uncertain blood circulation [2, 4]. Infection leads to circulatory disorder with associated bone necrosis. Necrosis acts

as a good culture medium for bacteria that produce a fibrous glycocalyx material (biofilm) that cannot be overcome by the body's immune systems or with antibiotics [5].

Antibiotic-impregnated polymethylemethacrylate (PMMA) beads have been proved to be effective in delivering concentrated amounts of antibiotic to a local area [2, 6, 7]. But the use of PMMA beads to deliver antibiotics has several disadvantages [2, 7]. The beads have to be removed, which may lead to further damage to the soft tissue [5, 6]. The dead space left within the tissue pose additional risks of infection [2]. Breaking of the beads' chain occurs often during transcutaneous removal [5]. In addition, the fibrous capsule that develops around PMMA beads may hinder elution of the antibiotic [2].

Because of the disadvantages of the antibiotic-containing cement beads, the development of bioabsorbable drug carrier systems is a major research goal [5]. A biodegradable carrier for the antibiotic would obviate the need for this additional surgical procedure and may also potentially reduce the duration and cost of hospitalization [6]. The use of polylactide (PLA), polyglycolide (PGA), and polylactide-co-glycolide (PLGA) matrices for controlled release of therapeutic agents has been explored [1, 3, 8–12].

Although the concept of resorbable bone fixation has considerable appeal, any polymeric device must provide the following tissue characteristics to be of clinical use: biocompatibility, sufficient biomechanical strength to permit bone healing, complete material resorption, and elimination of polymeric residues and metabolic products by a physiologic excretory method [13]. To assess the applicability of osteofixation devices, studying a particular polymer or copolymer's resorption characteristics, as well as its fixation effectiveness, at a specific bone site is of utmost importance prior to clinical use [13]. The aim of the present study was to assess tissue reactions of recently-introduced self-reinforced ciprofloxacin-releasing polylactide-polyglycolide (SR-PLGA) 80/20 screws in cranial bone of the rabbits. Up to our knowledge, they are the first of their kind in the world, and they are expected to make an important addition to surgical tools available today.

Materials and methods

Rabbits and surgical technique

Twenty-eight adult male New Zealand white rabbits, weighing 2.8 to 3.3 kg, were operated on. The animals were anaesthetized with an intramuscular injection of medetomide hydrochloride (Domitor®, Orion, Espoo, Finland), 300 μg/kg, and ketamine hydrochloride (Ketalar®, Parke-Davis, Solna, Sweden), 20 mg/kg. Cefuroxime (Lifurox®, Eli Lilly, Firenze, Italy), 60 mg, was given intravenously as infection prophylaxis at the beginning of the procedure. The operation area was shaved and washed with chlorhexidine, 5 mg/ml (Klorhexol®, Leiras. Turku, Finland). The investigation was approved by the Committee on Animal Experimentation of the University of Oulu.

A longitudinal midline skin incision was made over the sagittal suture. The periosteum was incised in a cross fashion and it was raised off the bone. Holes were drilled in the bone to each side of the sagittal suture (5 mm posterior to the coronal suture and 5 mm lateral to the sagittal suture). The holes were made with an electric drill under continuous flowing saline to minimize heat insult to the bone. Bioabsorbable ciprofloxacin-releasing screws were then applied. The periosteal flaps were turned back over the screw and the skin was closed in layers, subcutis with Vicryl® 5-0 (Ethicon, Norderstedt, Germany) and the skin with Dermalon® 4-0 (Davis & Geck, Sherwood Medical, St. Louis, USA).

The animals were divided into seven follow-up groups, with four rabbits in each group. Follow-up periods were 2, 4, 8, 16, 24, 54 and 72 weeks. The animals were sacrificed with an overdose of pentobarbital (Mebunat®, Orion, Espoo, Finland). Parietal bone blocks of 1×1 cm, with the implant in the center, were taken for examination. One screw was removed from the block and the surrounding bone was grinded and the concentration of ciprofloxacin in the bone was measured. These results will be published later. The other screw with the surrounding bone was taken for histological examination. The specimens were stored in 40% ethanol, mounted in methylmethacrylate, cut into 8 μm thick sections and stained by using a modified Masson-Goldner trichrome staining method. The results were analyzed by light microscopy at magnifications of $\times$16, $\times$40, $\times$100, $\times$250, $\times$400 and $\times$1000, using polarized and ordinary light. The rectangular area around the screw was divided into three smaller rectangular areas: A, B and C. Each of these areas was divided into six smaller areas (Fig. 1). Each of these areas were examined carefully with microscope and the number of macrophages, giant cells, active osteoblasts (seen as cuboidal cells) and

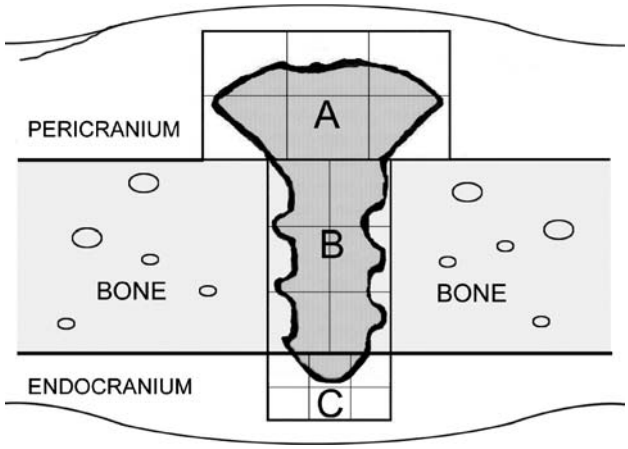

Fig. 1 Examination areas (A, B and C) of histological samples.

fibrous tissue layers around the screw were counted and the degradation rate of the bioabsorbable screw was analyzed. There were three slides from each screw. Each screw was, practically, examined three times in all those 18 areas. Slides were first checked by two observers (pathologist and first author). In the second round the cells and fibroblast layers were counted area by area (18 areas per slide and three slides from each screw). In each follow-up group there were four rabbits, i.e. four screws per follow-up time. Twelve slides from follow-up group were examined. Means of macrophages, giant cells, osteoblasts and numbers of fibrous tissue layers were calculated per follow-up time.

Miniscrews

The investigated prototypes of the antibiotic-releasing self-reinforced miniscrews consist of two components, a bioabsorbable matrix polymer and antibiotic. The bioabsorbable matrix polymer in the antibiotic-releasing miniscrews was commercial PuraSorb® PLG (Purac Biochem bv., Gorinchem, Netherlands), which is a semicrystalline bioabsorbable synthetic PLGA 80/20 polymer. Its inherent viscosity was 6.28 dl/g (0.1%, chloroform, 25°C). The used antibiotic was ciprofloxacin ($C_{17}H_{18}FN_3O_3$), which is a synthetic fluoroquinolone. In *in vitro* tests, all loaded ciprofloxacin was released from studied screws after 23 weeks. During that time, the concentration of released ciprofloxacin per day remained in the range of 0.6–11.6 μg/ml after start-up peak. *In vitro* the maximum concentration value of released ciprofloxacin was recorded in the 8th week [14].

The miniscrews were machined from self-reinforced rods. The geometry of antibiotic-containing miniscrews was the same as that of commercial BioSorbPDX® 1.5 Screws (Bionx Implants Ltd., Tampere, Finland). The length of the screws was 4.0 mm, the thread diameter was 1.5 mm and the core diameter was 1.0 mm. The finished miniscrews were gamma-sterilized before use. Ciprofloxacin has been bacteriologically proved to be bioactive *in vitro* after manufacturing and sterilization, e.g. in studies on S. epidermidis [15].

Results

Postoperative recovery of all rabbits was uneventful. Macroscopically the operative area healed well in all animals. No macroscopically manifest signs of wound infection or other disturbances of wound healing were observed. The screws remained in their original implantation sites.

After two weeks, postoperatively, no changes in the dimensions of the bioabsorbable screws were observed. PLGA material was clearly seen and it had polarizing activity. Many macrophages were seen around the screw heads and few in areas near to screw heads but corresponding to screw shafts. Some fibroblast proliferation comprising 2–3 layers of fibroblasts was seen around screw heads and none seen yet between the screws and bone in areas corresponding to screw shaft and tip areas. There was also the highest number of giant cells (Tables 1 and 3) that was seen near the heads of the screws in area A (Fig. 2). Active osteoblasts (seen as cuboidal cells) were seen at the edges of the bone next to screw threads in area B (Fig. 2, Table 2).

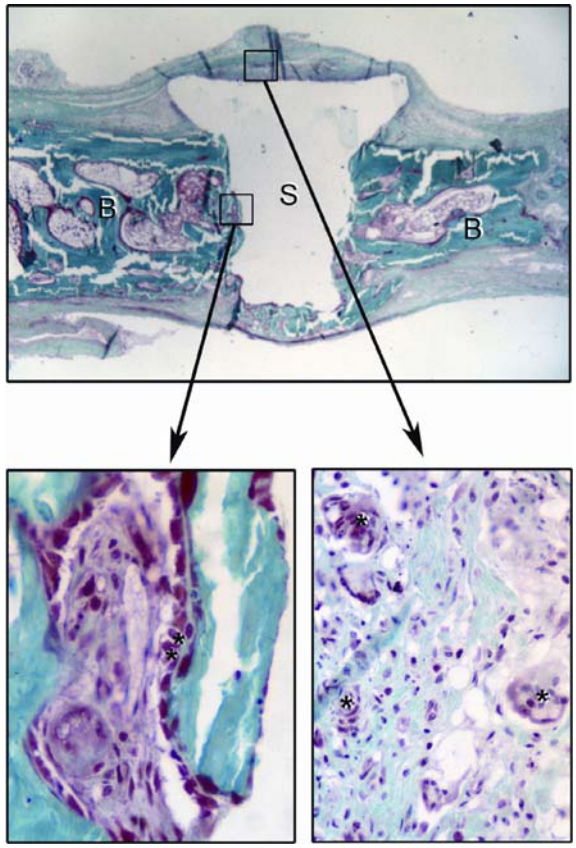

Fig. 2 Macrophages, giant cells and osteoblasts. Macropages and giant cells seen around the head of the biodegradable screws in area A (figure down right). Asterix indicates giant cells. Active osteoblasts seen at the edge of biodegradable screws in area B (figure down left). Asterix indicates active osteoblasts. Follow-up time was two weeks. (S = screw, B = bone). Original magnification 40X and 400X.

After four weeks, the morphology of the bioabsorbable screws had not changed. PLGA material was clearly seen with polarized light. The fibrous tissue surrounding each screw-head was thicker than earlier (more than three layers of fibroblasts) and was started to appear also around screw tips (areas A and C, respectively). Macrophages were seen in area A (around the screw heads) and the number of them started to increase in area B (around the shaft of the screws, Table 1). Giant cells were seen around the heads of screws and they were less in number than earlier (area A). The highest number of active osteoblasts was seen at this time point which was evident around screw shafts (area B, Table 2).

Table 1 Means and standard deviations of numbers of macrophages (MP) and giant cells (GC) seen at different follow-up periods. A, B and C indicate the examination areas in histological sections. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw

Area	Cell type	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	MP	134.7 ± 45.2	64.5 ± 18.5	63.0 ± 11.6	32.0 ± 15.1	13.5 ± 6.4	120.0 ± 42.4	12.0 ± 4.3
	GC	5.5 ± 1.4	4.0 ± 1.7	2.0 ± 0.3	1.0 ± 1.4	0.0	0.0	0.0
B	MP	3.3 ± 2.3	7.5 ± 0.7	12.5 ± 4.5	16.0 ± 3.8	24.0 ± 7.1	59.0 ± 40.5	15 ± 5.9
	GC	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	MP	0.0	0.0	17.0 ± 1.6	9.5 ± 2.5	0.0	0.0	0.0
	GC	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2 Means and standard deviations of numbers of active (cuboidal) osteoblasts seen at different follow-up periods. A, B and C indicate examination areas in histological sections where A is the area around the head of the screw, B is the area around the shaft of the screw, and C is the area below the tip of the screw

Area	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	9.0 ± 7.5	44.5 ± 15.4	23.5 ± 11.6	10.5 ± 3.8	13.0 ± 8.3	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3 Means and standard deviations of numbers of fibrous tissue layers seen at different follow-up periods. A, B and C indicate observation areas in histological sections. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw

Area	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	2.3 ± 0.8	3.1 ± 2.2	3.8 ± 3.2	5.0 ± 5.1	9.0 ± 4.0	6.2 ± 5.7	3.0 ± 2.6
B	0.0	0.0	1.0 ± 2.3	2.2 ± 2.8	2.8 ± 3.3	3.4 ± 3.9	1.3 ± 1.2
C	0.0	2.0 ± 0.7	2.8 ± 2.2	3.1 ± 2.8	2.0 ± 1.6	5.0 ± 3.2	1.5 ± 1.3

After eight weeks, there were no visible changes in the dimensions of the PLGA screws. Polarizing biomaterial was clearly seen. More fibrous tissue surrounded screw-heads and tips (3–4 and 2–3 fibroblast layers respectively) was seen and it was more evident and thicker than before. Fibrous tissue has also appeared in the shaft area between the bone and screw bodies (Table 3). The number of giant cells had decreased further. Macrophages started to form a line around the edges of the screws but their number remained the same as at 4 weeks, (Table 1). The number of active osteoblasts has decreased as compared to 4-week follow-up group (Table 2).

After 16 weeks, fragmentation of the heads of the PLGA screws had started. The fibrous tissue formation was compact in nature, but comprising more fibroblast layers than earlier surrounding the screws all around (all A, B and C areas). Polarization capacity of the screws had decreased remarkably. Although the number of active (cuboidal) osteoblasts has decreased further, they were still around the shaft of the screw (area B). The number of macrophages and few giant cells that were also seen around the edges of the screws has decreased and some macrophages were seen inside the screw matrices (Table 1).

After 24 weeks, a change in the morphology of the PLGA screws had occurred (head of the screw had fragmented). The PLGA material had no remaining polarizing activity. The fibrous tissue capsule was compact in nature and it reached its highest number of fibroblast layers ($n = 9$) around screw heads (Table 3). Active osteoblasts were still seen around the shafts of some screws (Table 1). Although the total number of macrophages has decreased, more macrophages were seen inside the matrix of the head of the screws in area A (Fig. 3). No giant cells were seen.

After one year, there was still some PLGA material left. The position of the PLGA screws was filled with adipose tissue, fibrous tissue and "foamy macrophages," which had intracellular PLGA particles. Capsule fibroblast layers had increased in areas B and C but they had, however, decreased to six layers in area A corresponding to screw-heads. PLGA material was also seen extracellularily (Fig. 4). A fibrous tissue capsule surrounded the remnants of the PLGA screws.

After $1\frac{1}{2}$ years, the amount of PLGA material remaining had decreased remarkably. Nearly all of the remnants of PLGA material which could still be seen, were inside "foamy macrophages." The number of macrophages had decreased (Table 1). No giant cells were seen. The amount of adipose

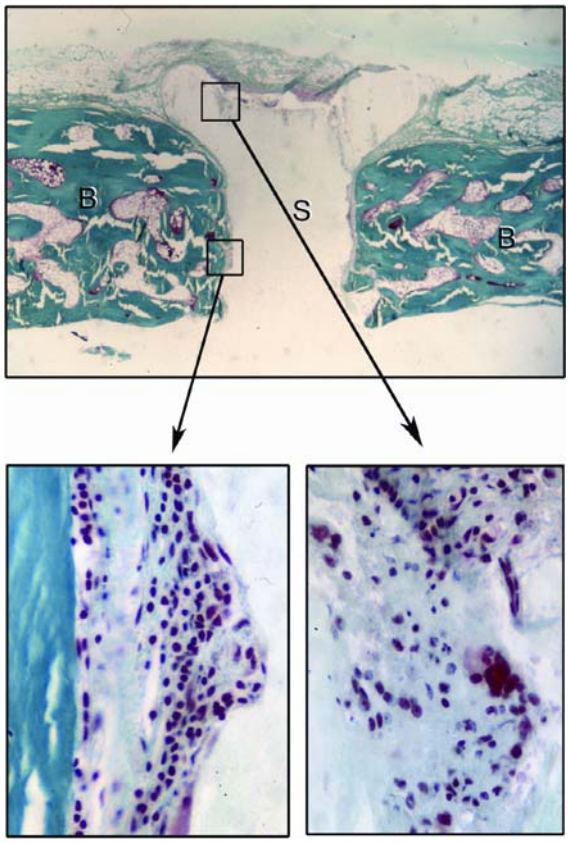

Fig. 3 Macrophages forming a line around the shaft of the screw in area B (figure down left). Macrophages (little red spots) clearly seen inside of the matrix of the head of the screws in area A (figure down right). Follow-up was 24 weeks. Original magnification 40X and 400X.

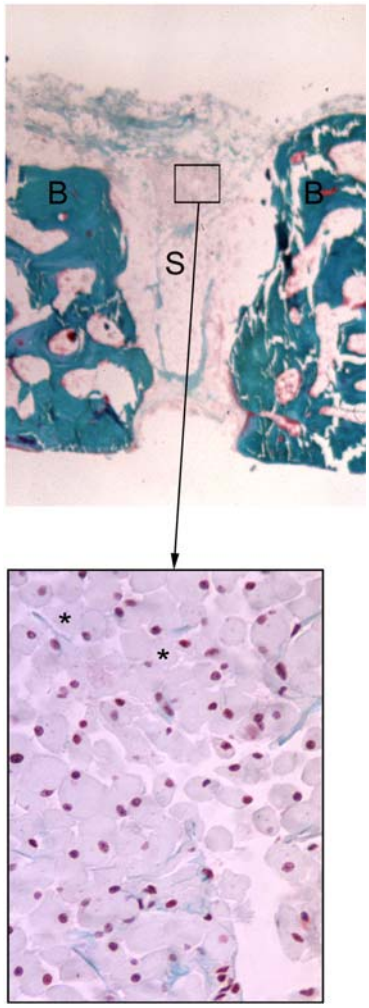

Fig. 4 One year after implantation of PLGA screws, there was still some PLGA material left. "Foamy" macrophages had internalized the PLGA particles, but there was also some extracellular PLGA material. Asterisk indicates biomaterial. (S = screw, B = bone). Original magnification 400X and 40X.

and loose fibrous tissue had increased at the site that was occupied by PLGA screws. However, detected fibroblast layers in the capsule had decreased in all examined areas (A, B and C). No active osteoblasts were seen in any of the examined areas (Table 2).

Discussion

The use of PLA, PGA, and PLGA matrices for controlled release of therapeutic agents has been extensively studied [1, 3, 5, 8, 16]. The delivery of antibiotics locally, directly to the site of their intended action, using a biodegradable carriers, carries the advantages of avoiding systemic side effects and it assures high local tissue levels of the antibiotics. There is no need for repeated injections, no problems with intravenous access or its maintenance and no need for a second surgery to remove the carrier [17].

Biocompatibility refers to the ability of a material to perform with an appropriate host response for a specific application [18]. Extensive animal studies have shown the good biocompatibility of many poly-α-hydroxy acids, like PGAs and PLAs and their copolymers [19]. In this study ciprofloxacin-releasing SR-PLGA (80/20) screws were used. PLGA (82/18) copolymer has been proven to be well tolerated by the body, because it lacks crystallinity and has intermediate absorption characteristics: slow enough to not overwhelm the body's local ability to clear the degradation products, yet fast enough to demonstrate clearance after approximately one year [20].

Ciprofloxacin was selected as the antibiotic of choice for use in this study because it possesses a broad spectrum of activity, including most osteomyelitis-causing pathogens, such as *S. aureus* [5]. Ciprofloxacin has been shown to have greater penetration of cortical bone from polyglycolide carriers than has been reported for gentamicin released from polymethylmethacrylate beads [5]. Released ciprofloxacin from sterilized manufactured PLGA implants was found to be bacteriologically bioactive [15].

Adding antibiotic may however compromise strength of resulting screws. Ciprofloxacin-containing SR-PLGA screws have lower pullout strength (66.8 ± 4.9 N) than corresponding plain conventional SR-PLGA screws (96.3 ± 9.3 N) [10]. Thus it was recommended that screws should be applied in nonload-bearing or slightly load-bearing applications. *In vitro* studies of ciprofloxacin-releasing implants have shown that the screws retain their mechanical properties at the level that ensures their fixation properties at least nine weeks (SR-PLGA rods, Ø 3 mm) [14]. In the most recent studies, new material patches made into tacks have proved that this problem with pullout can be avoided with average strength of ciprofloxacin PLGA is 147 MPa and corresponding control ones 141 MPa [21].

Ciprofloxacin has shown to have inhibitory effects on osteoblastic cells *in vitro* (at concentration 40 μg/ml) [22]. The potentially detrimental level that can adversely affect osteoblast-like cell growth is more than 20 μg/ml [23]. In *in vitro* tests all loaded ciprofloxacin was released from studied screws after 23 weeks. During that time, the concentration of released ciprofloxacin per day remained in the range of 0.6–11.6 μg/ml after start-up peak. *In vitro* the maximum concentration value of released ciprofloxacin was recorded in the 8th week [14]. It has also been shown that experimental fractures exposed to ciprofloxacin have diminished fracture healing when tested in rats' femurs [24]. The authors suggest that administration of quinolones during early fracture repair may compromise fracture healing in humans. Human is a different species and the adverse effect of ciprofloxacin on callus formation may or may not be apparent. One, however, needs to consider this observation when ciprofloxacin screws are taken into clinical trials. Careful clinical and radiological evaluation and follow up of these fractures should be undertaken.

The mechanism of drug release from PLGA compressed matrices is a combination of diffusion and erosion. As drug on the surface of the device diffuses out, exposed polymer hydrolyzes and a greater matrix surface area is exposed [16]. The type of the polymer selected affects the biodegradation rate; therefore, a system can be designed for short or long drug-release periods. The degradation of polylactide and polyglycolide begins with the random hydrolysis of the polymer chains, leading to the reduction of molecular weight and strength properties and fragmentation of the polymeric implant into smaller particles. Enzymes can possibly enhance the degradation [25].

In the body, this also involves a physiological response whereby macrophages phagocytose polymer fragments and metabolize them to naturally-eliminated substances such as water and carbon dioxide [20]. Partly, PGA is also excreted in urine [25]. Macrophages and giant cells are thought to be responsible for the ultimate digestion of the polymeric debris. This is associated with transient mild microscopical foreign body reaction, which is not necessarily clinically-manifested [25]. Macrophages control acute inflammation by their secretion and regulation of elements of the complement cascade, and secretion of cytokines, and metabolites of arachidonic acid. Macrophages exert a regulatory action upon other cells found in granulation tissue such as lymphocytes and fibroblasts. They also possess a phagocytic ability engulfing cellular and molecular debris [26].

In the current study the highest number of macrophages was seen at 2–8 weeks postoperatively. Most of them were seen around the heads of the PLGA screws (area A). This may be due to the fact that small PLGA particles become detached from the surface of the implant, during the implantation process, and elicit a macrophage reaction. As compared to plain (not containing ciprofloxacin) corresponding PLGA screws, similar number of macrophages that was seen at 2 weeks but less at 4 and 8 weeks in the area A (screw head) [27]. The highest number of giant cells occurred at an early stage (2 weeks), after which their number started to decrease, probably taking a lesser role in the reaction. The ultimate digestion and clearing of the decomposing polymeric material later on is probably more dependent on the macrophages that have invaded the implant matrices. At 16 and 24 weeks no difference in the inflammatory reaction profiles could be appreciated as compared to earlier results seen with plain PLGA screws [27]. After one year, the amount of macrophages increased again. The reason for this increase is unclear and needs further research. In comparison, the number of macrophages in corresponding time point with plain SR-PLGA screws was not as prominent as in case of ciprofloxacin-releasing screws in this study [27]. One explanation can be the increased infiltration to clear out the material debris left in the extracellular space, as most of this small debris is formed in the period between 6 and 12 months. This is also supported by the observation with plain PLGA miniscrews (non ciprofloxacin containing) screws studied by authors [27].

It is known that soon after implantation, there is an initial inflammatory response by the body, which is part of the natural healing response. This is followed by encapsulation of the implant in a thin, fibrous membrane, which occurs in response to implants made of any material [12, 20, 28, 29]. In the current study a fibrous tissue capsule was progressed in time having the number of fibroblast layers increasing to reach maximum by 24 weeks, around the heads of screws and by 54 weeks in screw shaft and tip areas. There was also a maturation process as can be seen in organization of the capsule turning to be more compact than loose by time as can be seen for example in 16 weeks specimens. By 54 and 72 weeks, the capsule surrounding the head of screws was thinner comprising less layers of fibroblasts. This can be explained by the fact that the foreign material has degraded and resorbed in this area. In areas corresponding to the shaft of the screws, this decrease in fibrous tissue was observed

after 54 weeks, i.e. 72 weeks Fibrous-tissue capsule seemed to be thicker in area A (screw heads) in a similar manner as seen in a previous study on plain PLGA screws [27]. With both types of PLGA screws (Plain and ciprofloxacin containing) the capsule was thicker and it formed earlier in area A (screw head) than in areas B (screw shaft) and C (screw tip). This may be related to polymer degradation and macrophage reaction and release of fibroblast proliferation.

Unlike permanent (non-absorbable) implants, however, absorbable implants degrade and lose their mass and volume. The body may respond by filling in the resulting space that was occupied by the implant with bone (in case of intraosseous implants) or fibrous tissue, and or a combination of both [28]. In this study after one year, some PLGA biomaterial was still present. PLGA screws had been replaced by adipose tissue, fibrous tissue and "foamy macrophages" which had PLGA particles inside them. After $1\frac{1}{2}$ years, the amount of biomaterial remaining had decreased remarkably. The particles of biomaterial were inside "foamy macrophages." This area was surrounded by fibrous tissue capsule.

Bone infection (osteomyelitis) after surgical procedures and trauma continues to be a serious problem. The combination of surgical intervention and an effective antimicrobial agent remains the mainstay of treatment. Because of the disadvantages of the antibiotic-containing cement beads, the development of bioabsorbable drug carrier systems represents a major advancement [5]. As the diversity of biomaterials increases, new developments are aimed at making these materials becoming interactive rather than passive with respect to the environment they are placed in. The need to accurately and reproducibly assess the response of the body to these materials is apparent [26]. As the majority of biomaterials require surgical implantation, it is convenient and relevant to consider biocompatibility in the light of the mechanisms of the normal wound-healing response, and the influence the presence of an implanted biomaterial has on this process [30].

We developed bioabsorbable screws that can function as local delivery systems, besides their role in osteofixation. This should allow the achievement of high local antibiotic levels at the site of implantation in bone. We have earlier reported that ciprofloxacin-releasing SR-PLGA (80/20) screws have lower pullout strength than corresponding plain SR-PLGA (80/20) screws and they can be used along with conventional SR-PLGA miniscrews for bone fixation and antibiotic delivery [10]. In the present study the biocompatibility of the ciprofloxacin-containing SR-PLGA 80/20 screws was good, as they elicited only a mild inflammatory tissue reaction when implanted in rabbit cranial bone. This mild reaction was also seen in case of plain PLGA screws [27]. No remarkable differences were seen in degradation processes of these two types of screws during the six months follow-up time in this study. According to the results of this study in the current model, we see no contraindications to their clinical use, and thus we have planned our first clinical studies.

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