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Intramedullary bone formation after polylactic acid wire implantation

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Semi-crystalline poly-L-lactic acid (PLLA) wire was implanted intramedullary in rat tibiae to evaluate the fracture healing processes and the tissue reaction on the PLLA in a fractured bone. The fracture healing after PLLA implantation was compared to a sham-operated group of animals. In all animals with intramedullary PLLA wire implantation newly formed bone was seen immediately against the implant 2 and 6 months after implantation. None of the sham-operated animals showed newly formed intramedullary bone formation other than fracture healing callus and normal trabecular bone.

1. Introduction

Because metallic fracture fixation materials can cause stress shielding [1], corrosion problems [2] and even sensitivity [3], bioresorbable materials without these disadvantages and without the need of a removal operation could be very useful in fracture healing treatment. Bioresorbable suture materials developed in the 1960s, can theoretically completely be metabolized in a biological system [4, 5]. Then there is no need for an operative removal procedure, which will give a medical, financial and psychological advantage to the patient. Excellent reviews on these bioresorbable materials have been previously published [6–8]. PLLA is well suited for fracture fixation because of its slow degradation rate which causes little tissue reaction and long-standing mechanical characteristics and was described for this application for the first time in 1971 [9, 10]. The purpose of this study was to evaluate the tissue response on intramedullary implanted PLLA wire under fracture healing circumstances.

2. Materials and methods

2.1. Implantation materials

Poly-L-lactic acid wire with a draw ratio of 5:1 and a diameter of 0.3–0.8 mm was supplied by PURAC Biochem b.v., The Netherlands. The initial molecular weight was 160 kD (determined by viscosity measurement), intrinsic viscosity (in chloroform, $T = 25\,^{\circ}C$) 3.43, specific rotation (in chloroform, $T = 20\,^{\circ}C$) -159.6°, melting range 169.7–179.6 °C, and heat of fusion 52 J g^{-1}. Crystallinity was 55% as calculated from the heat of fusion, compared to that from fully crystalline PLLA, 93.7 J g^{-1} as estimated by Fisher *et al.* [11]. After sterilization by gamma radiation with a dose of 25 kGy the characteristics of the PLLA were changed considerably (Table I). The average implanted mass of PLLA wire per animal was 13.7 ± 2.8 (SD) mg.

2.2 Animals

Twenty-four adult female Wistar rats with an average mass of 250 ± 31 (SD) g were used. Throughout the first week postoperative the animals were caged individually, after one week the animals were caged in groups of eight. The rats were fed standard rat pellets and normal tap water ad libitum.

2.3. Operative procedure

After ether induction and anesthesia with ketamine (Aescoket^{R}) 75 mg kg^{-1} i.m. and xylazine (Rompun^{R}) 8 mg kg^{-1} i.m., under sterile conditions the tibial plateau was exposed and the tibial medullary cavity reamed by means of a 1.1 × 40 mm (19G × 1½) disposable needle (Monoject^{R}) that was introduced manually. After removal of the needle PLLA wires were implanted below the level of the tibial plateau. The knee capsule and the skin were closed with silk sutures (Permahand Seide^{R}). The sham operation procedure was the same except for omitting the PLLA wire implantation. Immediately after the implantation or sham operation procedure the tibia fracture was produced with specially built three-point bending pliers.

2.4. Evaluation of the fracture healing

Fracture healing was evaluated by means of weekly clinical and radiologic examination under anaesthesia during the first 4 weeks postoperative. At the sixth and eighth week these examinations were repeated. A final examination was made at the time of sacrifice of the animal. At clinical examination the mobility of the fracture was determined carefully. The radiological consolidation was determined independently by two trauma surgeons and the investigator by evaluating the appearance of the fracture line and the amount of callus on the X-ray picture.

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TABLE I Specifications of the PLLA wire used

Specification	Before gamma radiation	After gamma radiation
Molecular mass	160.000	35.200
Intrinsic viscosity	3.43	1.13
Melting range (°C)	169.7–179.6	170.4–174.8

TABLE II Consolidation of tibial fractures

Type of consolidation	PLLA group	Sham group	Non-reaming group
	(period post-fracture to reach consolidation in 100% of animals)		
Clinical	10 days	21 days	15 days
Radiological	8 weeks	8 weeks	8 weeks

2.5 Histological examination

After sacrificing the animals with an overdose of pentobarbital (Nembutal^{R}), the fractured tibiae were immediately taken out and fixed in 70% ethanol, dehydrated in a series of ethanol concentrations up to 100% and embedded in methylmetacrylate. Undecalcified sections, with a thickness of 10–15 μm each, were sawn with an innerlock diamond saw [12]. The sections were stained with methylene blue and basic fuchsine or with Masson's trichrome for light microscopy.

2.6. Experimental design and statistics

Three groups of eight rats were used in this experiment. In the first group only a closed tibial fracture was produced. The second group also underwent reaming of the medullar cavity, while the third group received PLLA wire as intramedullary implant. After 2 months two rats in each group were sacrificed while the others were sacrificed after 6 months. The average weights, the time periods of the radiological and clinical consolidations and the average shortening of the tibia of the different treatment groups were compared by using an analysis of variance and a *t*-test.

3. Results

In the PLLA group and in the non-reaming group one rat died because of anaesthiological complications. From the first day postoperative the rats walked around on three legs, after three weeks all the rats presented full weight bearing on the fractured limb again. Between the second and the third week all fractures showed clinical consolidation, while radiological consolidation was complete in all animals at the eighth week (Table II). None of the animals showed delayed union or infection.

Shortening of the fractured tibia caused by angulation at the fracture site occurred in all rats because of the early loading of the fracture without fixation. The average shortening was 4.6 ± 1.8 mm (SD) as compared to the right tibia of the same animal at the moment of termination. The average consolidation times for the different groups are given in Table II. At histological examination, in all implanted animals lamellar bone formation around the PLLA wire was observed (Fig. 1a) after 2 months as well as after 6 months. None of the sham-operated or non-reamed animals showed circular intramedullary new bone formation other than in the fracture area (Fig. 1b). The newly formed bone around the implants was calcified mature bone which had connections to the cortical bone in some areas. No differences in the amount of newly formed bone between the distal and proximal part of the tibia was observed. At the region of the fracture the medullar cavity was filled with newly formed bone with active remodelling. In some animals cartilage was visible in the remnants of the fracture line. At sagital section the bony sheath around the PLLA wire was shown to be nearly continuous (Fig. 1c). Using light microscopy the newly formed bone around the implants seemed to be apposed directly against the PLLA; no connective tissue or cartilage was detected at the interface by light microscopy after a follow up period up to 6 months (Fig. 1d). At the discontinuous sections of the bony sheath some osteocytes and fibroblasts were observed near the implant (Fig. 1e and 1f). Only mild cellular infiltrations of the tissue around the PLLA were observed.

4. Discussion

The sterilization procedure changed the chemical characteristics of the PLLA dramatically. It is clear that PLLA after gamma radiation does not possess the same chemical and mechanical properties as before. Gamma radiation induces chain scission as well as crosslinking in PLLA and decreases the crystallinity [13]. These findings are in accordance with earlier described effects of gamma radiation on polylactic acid [14, 15]. Therefore we suggest that determination of the physico-chemical properties of biodegradable implants should be done after the sterilization procedure. For comparison between *in vivo* and *in vitro* research the same sterilization procedures should be used. Tissue reactions on PLLA implants are described as mild foreign body type reactions initially [5, 16]. The formation of a well-defined fibrous layer around massive intramedullary PLLA implants in pigs was described recently [17]. Also a fibrous layer between transcortical implanted PLLA and cortical bone was described [18]. Matsusue [19] reported the formation of a bony shell around intramedullary implanted PLLA rods in intact rabbit femora. Bony shell formation was also present around different intramedullary implanted metals in intact rat femora [20]. New bone formation directly against PLLA coated self-reinforced polyglycolic acid rods implanted in intact rabbit femora was already reported [21]. According to Brown and Mayor [22] intramedullary implanted metallic and polymeric rods in fractured rabbit tibia showed bone formation only around the metallic rods; it was suggested by these authors that the bone formation occurred as a reaction to transfer

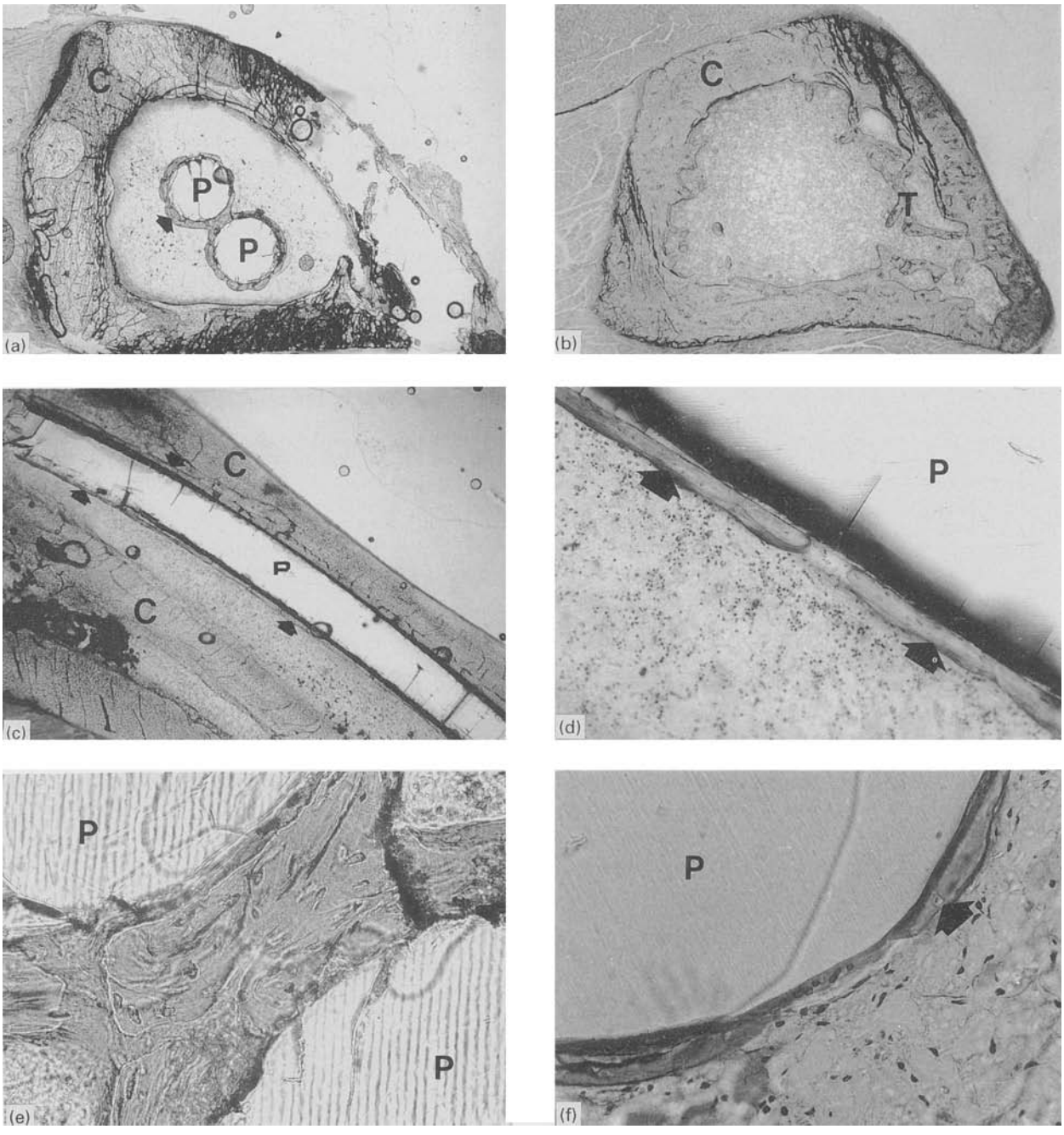

Figure 1 Histological sections of tibia 6 months after implantation. Newly formed bone is indicated by arrows, PLLA by P, cortical bone by C, trabecular bone by T. (a) Overview of newly formed bony sheath (arrow) around PLLA wires (P) proximal from fracture (M = ×25). (b) Overview of proximal tibial medullary cavity of sham-operated animals shows absence of newly formed circular bone, normal trabecular bone (T) can be seen along the cortex (M = ×25). (c) Bony sheath around PLLA wire just distal from fracture, arrows indicate bony sheath (M = ×25). (d) Enlarged part of (1c), newly formed bony sheath along PLLA wire, osteocytes are visible in the bony sheath (M = ×200). (e) Higher magnification of interface of (1a), osteocytes are visible in the newly formed lamellar bony shell that has direct contact with the PLLA (M = ×300). (f) Enlarged part of the interface between PLLA and newly formed bony sheath around PLLA wire. The newly formed bone seems to have direct contact with the PLLA wire. An osteocyte in its lacuna (arrow) can be seen in the bony sheath, some fibroblasts can be seen in the gap between the two parts of the bony sheath (M = ×250).

of load from the metaphysial bone to the rods. The PLLA wire implanted in this study was not as stiff as a solid rod but some load could have been transferred to the PLLA wire during at least the first weeks of the fracture healing period. Load transfer might be a cause of the bone formation around the PLLA wire as observed in this study, but because intramedullary implanted PLLA lost its initial bending strength almost completely within 20 weeks according to Matsusue *et al.* [19], load transfer alone is not a good explanation for the bony sheath formation. New bone formation around intramedullary implanted PLLA in rat tibia has never been described before. Cracks have been visible in the PLLA wire at histological examination but the volume of the wire did not appear to have changed significantly. The cracks may indicate the beginning of degradation but can also be artifacts caused during histological processing. Our observations confirm earlier observations made on the biocompatibility of PLLA. In histological examinations no evidence of a toxic effect or severe foreign body reaction was observed. Our data even suggest osteoconductive properties of intramedullary implanted PLLA wire.

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