Fixation of distal femoral osteotomies with self-reinforced poly(L/DL)lactide 70 : 30/bioactive glass composite rods. An experimental study on rats

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Self-reinforced poly(L/DL)lactide 70 : 30/bioactive glass [SR-P(L/DL)LA/bioactive glass] composite rods, 2 mm in diameter and 36 mm in length, were implanted into the dorsal subcutaneous tissue of 16 rats. Osteotomies of the distal femur were fixed with these rods (2 × 15 mm) in 64 other rats. The follow-up times varied from one week to one year. After sacrifice, three-point bending and shear tests, and molecular weight measurements were performed for subcutaneously placed rods. Radiological, histological, histomorphometrical, microradiographic, and oxytetracycline-fluorescence studies of the osteotomized and intact control femora were performed. At 24 weeks the mechanical properties had decreased significantly. Thirty-nine osteotomies healed uneventfully. One of the 64 evaluated osteotomies showed signs of infection at six weeks, and there were 19 non-unions and six delayed unions. In 20 operations the fixation was loose and out of these 14 non-unions were observed. No gross signs of inflammatory or foreign-body reactions were observed.

The present investigation showed that the mechanical strength and fixation properties of SR-P(L/DL)LA/bioactive glass composite rods are suitable for fixation of cancellous bone osteotomies in rats as long as the operative technique is correct. The present article is the first report on the application of SR-P(L/DL)LA/bioactive glass composite rods for fixation of cancellous bone osteotomies.

1. Introduction

According to previous experimental animal [1] studies, bioabsorbable self-reinforced (SR) polyglycolide (SR-PGA) pins have shown sufficient fixation properties for fixation of cancellous bone fragments. SR-PGA has a degradation time of a few months [2]. Previous experimental [3] investigations have correspondingly demonstrated safe fixation of small fragment osteotomies with self-reinforced poly-L-lactide (SR-PLLA) pins. The ultimate degradation time of polylactide is several years [4, 5]. Despite a slightly weaker initial mechanical strength compared to self-reinforced SR-PGA pins, the mechanical strength of SR-PLLA pins is maintained at a high strength level longer than with SR-PGA pins [6]. Self-reinforced poly (desamino tyrosyl–tyrosine ethyl ester carbonate), Poly(DTE carbonate) rods have also proved suitable in experimental bone fixation [7]. It has also been shown that a synthetic material (called bioactive glass) could bond to bone developing a chemical bonding layer on its surface [8].

The aim of the present study was to examine the use of SR-P(L/DL)LA/bioactive glass composite rods in cancellous bone fixation in the fixation of distal femoral osteotomies on rats.

2. Material and methods

Materials. The bioabsorbable, bioactive implants were made by using the raw materials listed below. Bioabsorbable polymer matrix: Lactide stereocopolymer, poly (L/DL) lactide 70 : 30, Resomer LR 708 (Boehringer Ingelheim, Germany). Inherent viscosity approximately 6.1 dl/g (given by manufacturer).

Bioactive filler. Crushed, bioactive glass 13–93, particles with composition 6 wt % Na_2O, 12 wt % K_2O, 5 wt % MgO, 20 wt % CaO, 4 wt % P_2O_5, 53 wt % SiO_2 [9]. Particle size distribution 50–125 μm.

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TABLE I Initial properties of gammairradiated SR-P(L/DL)LA/bioactive glass composite implants

	Flexural strength, MPa (st dev)	Flexural modulus, GPa (st dev)	Shear strength, MPa (st dev)	i.v., dlg^{-1}	M_w, Da*)	T_g, °C
After gammairradiation	140 (9)	3.2 (0.1)	90 (7)	0.9	42 400	56.9

*PS standards, $\alpha = 0.73$, $K = 5.45 \times 10^{-4}$.

Manufacturing of the implants. SR-P(L/DL)LA/bioactive glass implants were produced by extrusion (Gimac microextruder, Mac.gi, Italy) and self-reinforced by solid state die-drawing (DR approximately 4.5). The weight percentage of bioactive glass was 19 ± 2%. The sizes of the finished SR-P(L/DL)LA/bioactive glass composite rods were: diameter of 2 mm and length of either 15 or 36 mm. The implants were sterilized with gamma irradiation with minimum dose of 25 kGy.

Implant properties. The initial mechanical properties (average of four samples), inherent viscosity (average of two samples), viscosity-average molecular weight, M_w, (average of two injections) and glass transition temperature (average of two samples) of SR-P(L/DL)LA/bioactive glass composite rods are given in Table I.

2.1. Testing of SR-P(L/DL)LA/bioactive glass rods

For the evaluation of the strength retention properties, cylindrical rods (length 36 mm, diameter 2 mm) made of SR-P(L/DL)LA/bioactive glass, were implanted into the dorsal subcutaneous tissue of rats. Sixteen male Wistar rats weighing 410–460 g (mean 432 g) were used. The rats were anesthetized with subcutaneous injections of medetomidine (Domitor, Orion-Yhtymä Oy, Espoo, Finland) 0.5 mg/kg and ketamine (Ketalar, Parke-Davis, Solna, Sweden) 50 mg/kg. During surgery all rats received 11 250 IU of benzathin penicillin and 11 250 IU of benzylpenicillin, procain (Duplocillin LA, Intervet International B.V., Boxmeer, Holland). Small areas on both sides of the back were shaved and scrubbed with antiseptic fluid. The cylindrical rods were implanted into the dorsal subcutaneous tissue, and the two incisions were closed in layers. The anesthesia was brought to an end with atipamezol (Antisedan, Orion-Yhtymä Oy, Espoo, Finland) 0.25 mg/kg.

The follow-up times were 1, 3, 6, 12, 24, 36, 48, and 52 weeks. Each follow-up group consisted of two rats. A total of 32 SR-P(L/DL)LA/bioactive glass rods were implanted subcutaneously, four implants in each follow-up group. After sacrifice the implants were carefully removed from the subcutis and immersed in saline until the strength measurements which were performed within 24 h.

The rods were mechanically tested under bending and shear load with a universal materials tester (Instron 4411, Instron PLC, High Wycombe, England). The three-point bending test was performed with a crosshead speed of 5 mm/min and a span length of 32 mm. The bending strength and modulus were calculated according to standard (SFS-EN ISO 178, 1997), modified for circular cross-section samples. The shear strength test was performed with 10 mm/min according to a modified standard (BS 2782 Method 340B, 1978) by means of a specific tool. The samples were subjected to thermal analysis by a differential scanning calorimeter Perkin-Elmer DSC-7 (Perkin Elmer, Norwalk, USA). The glass transition temperatures of the samples were determined from the second heating run after quenching. The retention of the molecular weight during the follow-up period was examined by gel permeation chromatography using Waters apparatus (Waters, Milford, MA, USA) equipped with one PLgel 5 μm guard column and two PLgel 5 μm mixed-C columns (Polymer Laboratories, Amherst, MA, USA). Narrow polystyrene standards were used for calibration. The bioactive glass was removed from dissolved samples prior to injection.

2.2. Bone fixation properties of SR-P(L/DL)LA/bioactive glass rods

For the evaluation of the bone fixation properties cylindrical rods (length 15 mm, diameter 2 mm) made of SR-P(L/DL)LA/bioactive glass were used in the fixation of the osteotomies of the distal femur of the rats. Sixty-four male Wistar rats weighing 340–550 g (mean, 404 g) were used in the study. The rats were anesthetized and given infection prophylaxis in a similar manner as in the strength retention test. The right hind limb was shaved and scrubbed with antiseptic fluid, and a medial parapatellar incision was made. The patella was dislocated laterally and the articular part of the femur was exposed. A drill channel of 2 mm in diameter was made from the distal part of the femur through the cancellous bone. A horizontal osteotomy was made with an oscillating saw in the cancellous metaphysis of the distal femur. The posterior cortex was broken into a hinge. After reduction, the osteotomy was fixed with a SR-P(L/DL)LA/bioactive glass rod and the incision was closed in layers. The anesthesia was brought to end with atipamezol. The rats were allowed to walk freely in their cages after the operation, and no external splint of the operated limb was used. In 20 operations the fixation was not correct.

The follow-up times were 1, 3, 6, 12, 24, 36, 48 and 52 weeks. There were eight rats in each follow-up group. Two days before death, the rats were given oxytetracycline (OTC) hydrochloride (Terramycin, Pfizer, Cedex, France) 50 mg/kg intramuscularly for OTC labeling studies. The rats were killed with an overdose of sodium pentobarbital by heart puncture (Mebunat, Orion–Farmos, Turku, Finland).

After sacrifice, both femora were taken as specimens with the left femur acting as a control. Roentgenograms were taken in the anteroposterior and lateral positions

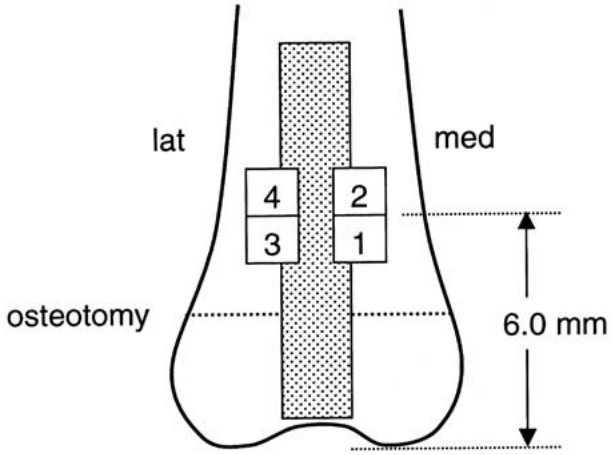


Figure 1 Schematic anterior view of the distal rat femur showing positioning of the osteotomy, the implant, and the four standardized sample fields (1, 2, 3, 4) used in the histomorphometric analysis.

(distance 1.00 m, 35 kV, 3.2 mAs, and 0.03 s). Macroscopic and manual evaluations of the area of the osteotomies were done. The distal portions of both femora were taken as specimens, fixed in 70% alcohol, and embedded in methylmethacrylate. For histologic and histomorphometric analysis, 5 µm longitudinal sections in the frontal plane were cut with a Reichert–Jung micro-tome (Nussloch, Germany) and stained by the Masson–Goldner method [10]. For contact microradiography (Faxitron X-ray system, Model 43855 A, Hewlett–Packard, McMinnville, Oregon; Imtec Pol–Edged H.R.P., ultra flat, type 1A, Imtec Products, Sunnyvale, California, USA) and oxytetracycline (OTC) fluorescence studies, 80 µm thick sections were made with a Leitz Saw Microtome 1600 (Wetzlar, Germany). Fluorescence microscopy was performed using an HBO 220 ultraviolet lamp (Osram, Berlin, Germany) and a BG 812/6 primary filter (Leitz, Wetzlar, Germany).

For semiautomatic quantitative histomorphometrical analysis, an Olympus microscope was linked via a videocamera (Color Cube 12, Soft Imaging System GmbH, Münster, Germany) to a computer (Dell Optilex MMP Pentium, Ireland). Magnifications of × 20.6 and × 125 were used. The image analyzing software was AnalySIS 3.00 (Soft-Imaging System GmbH, Münster, Germany). Four specimens in all follow-up groups were analyzed. Both femora were analyzed in each rat, the left femur acting as a control. Four standardized sample fields were determined in each femur, centralizing 6.0 mm from the distal joint level and 1.5 mm apart in the horizontal direction (Fig. 1). The AnalySIS-program was used in the determination of the corresponding sample field. Within the 0.68 mm^2 sample fields, the histomorphometrical variables were analyzed. The variables were as follows: total tissue area, total area of trabecular bone, total length of the trabecular bone circumference, total length of osteoid and the total length of osteoblast lines. The hypothesis was that the measured variables in the operated femora were higher than those in the controls. The paired *t*-test with one tailed interpretation was used for statistical evaluation due to the hypothesis.

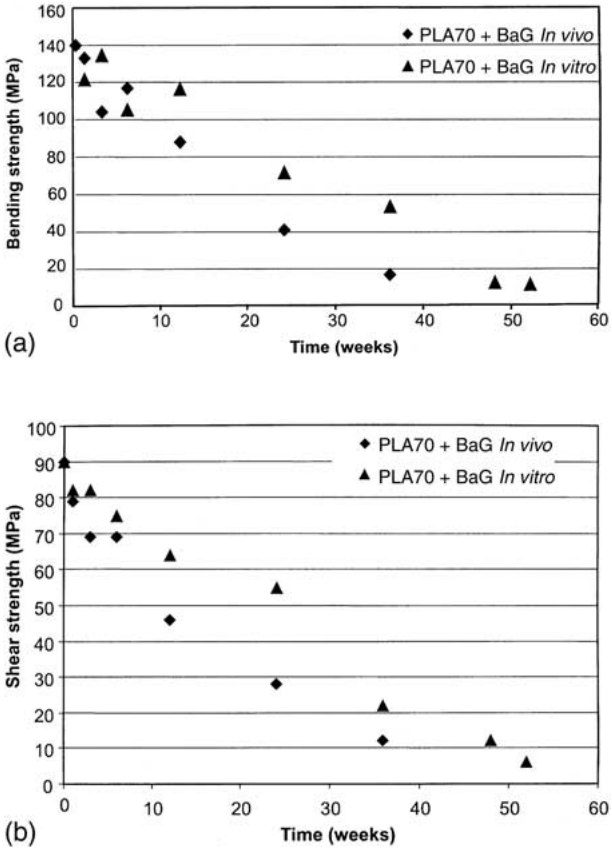


Figure 2 Bending (a) and shear (b) strengths of the SR-P(L/DL)LA/bioactive glass composite rods used for fixation of distal femoral osteotomies in rats. *In vivo* hydrolysis was faster than *in vitro*.

3. Results

3.1. Testing of P(L/DL)LA/bioactive glass composite rods

At the time of sacrifice the mean weight of the rats was 518 g (450–600 g). The mechanical properties had decreased significantly at 24 weeks (Fig. 2). Bending strength had decreased by 70% *in vivo* and by 49% *in vitro*. Shear strength had decreased by 68% *in vivo* and by 37% *in vitro*. After 36 weeks *in vivo* the mechanical properties could not be measured. The studied specimens lost their mechanical properties faster *in vivo* than *in vitro*. Viscosity average molecular weight (M_w) began to decrease immediately after the composite rods were implanted or immersed in buffer solution (Fig. 3). After

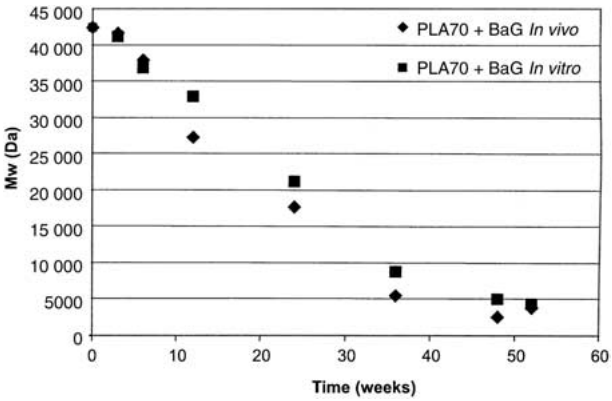


Figure 3 Molecular weight of the SR-P(L/DL)LA/bioactive glass composite rods *in vitro* and *in vivo*.

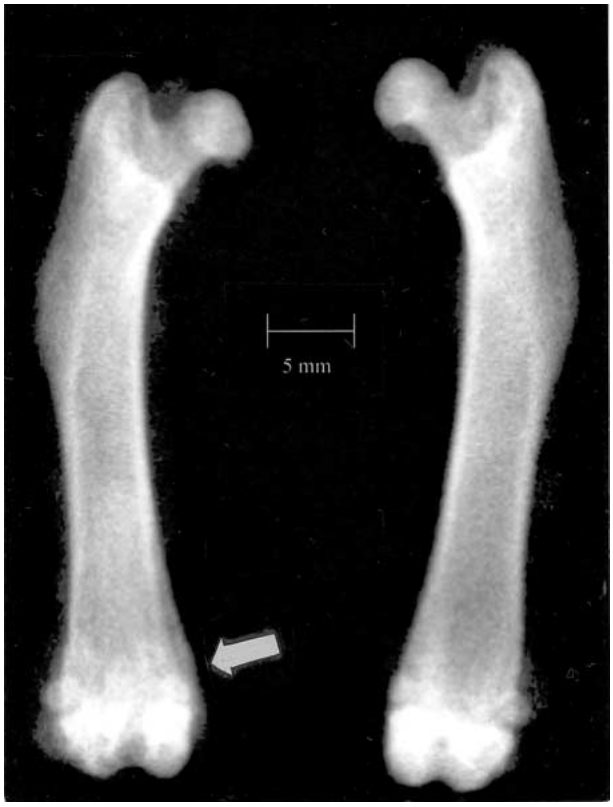
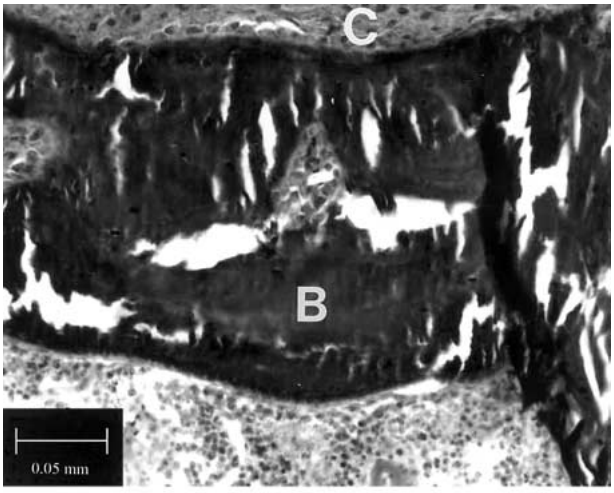


Figure 4 In radiograph (12 weeks) the healing of the osteotomy can be seen. The osteotomy line is not visible. External callus formation can be seen on the medial cortex (white arrow). The operated femur is shorter than the control.

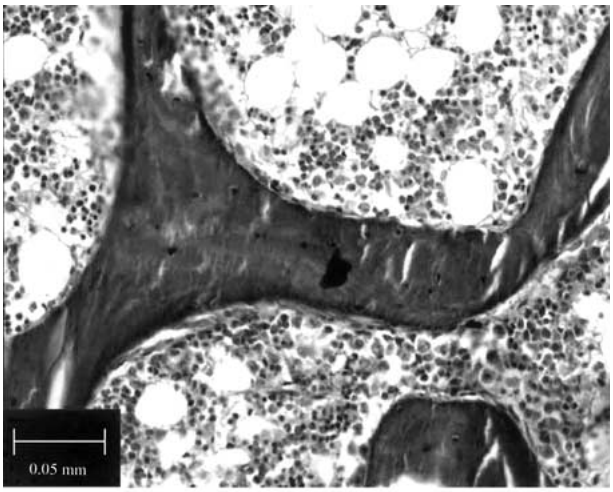
24 weeks, the M_w was reduced to 41% of the initial value *in vivo* and to 50% *in vitro*. The glass transition temperature (T_g) decreased to 51–53 °C during the follow-up time.

3.2. Bone fixation properties of SR-P(L/DL)LA rods

At the time of sacrifice the mean weight of the rats was 497 g (350–770 g). In the macroscopic evaluation, after sacrifice at one week the operated femora seemed quite good and were as long as the controls. At three weeks, four fixations were unstable and the operated femora were on average 0.9 mm (0–1.5 mm) shorter than the controls. At six weeks, five fixations were unstable and one was infected. The operated femora were approximately 1.9 mm (0.5–3.0 mm) shorter. At 12 weeks, all fixations were stable and the operated femora were approximately 2.3 mm (1.0–3.0 mm) shorter. At 24 weeks, one fixation was slightly rotated and one was unstable. The operated femora were 2.7 mm (0–4.0 mm) shorter than the control ones. At 36 weeks, all the operated femora were stable and on average 5.1 mm (4.1–5.5 mm) shorter than the controls. At 48 weeks, one osteotomy was unstable and the operated femora were on average 3.5 mm (1.1–4.6 mm) shorter. At 52 weeks, all the osteotomies were stable but one was slightly displaced and one medial condyle was detached. The operated femora were on average 3.4 mm (2.2–5.3 mm) shorter.



(a)



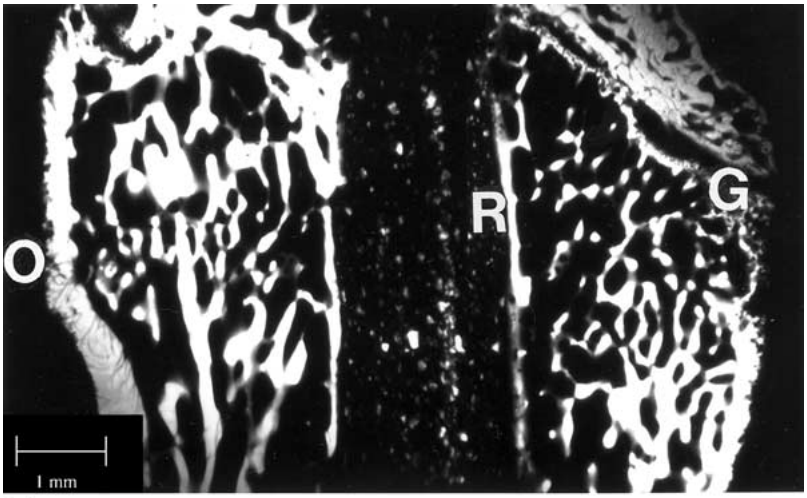
(b)

Figure 5 (a) Photomicrograph of the osteotomized site at 12 weeks showing a mild inflammatory reaction. The osteotomy is consolidated. Connective tissue rim around the implant channel (white letter C). The bony rim around the implant channel is seen clearly (white letter B). The bone structure is much thicker than that in the control (b). (Original magnification × 125.)

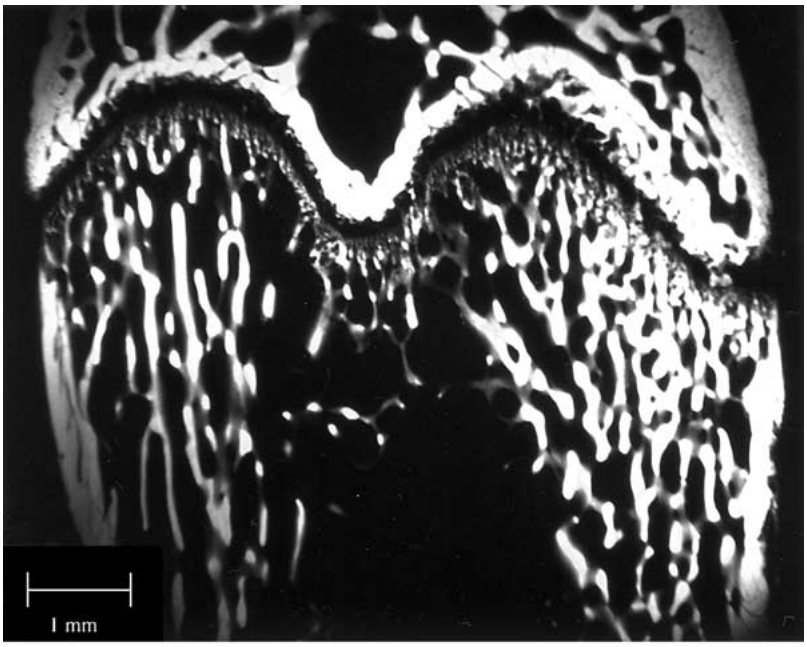
3.3. Radiological, histological, microradiographical and oxytetracycline (OTC) fluorescent results

Three displacements were observed histologically and radiologically. Radiologically the osteotomy line was visible in most operated femora up to 24 weeks. Strong external callus formation was seen up to 12 weeks. After six weeks a bony rim around the implant was seen in most cases. The OTC-uptake was high up to six weeks and at 12 weeks it was only moderate. A total of 19 non-unions and six delayed unions were observed.

At one week, radiologically all osteotomies were properly fixed and the osteotomy line was visible in all cases. Histologically, there was a bony connection in three osteotomies. New bone formation was seen around the implant and in the osteotomy area. The orifice of the implant channel was closed by granulation tissue in six cases. Endosteal and periosteal callus formation was seen microradiographically in the proximal part of the operated femora and only endosteal in the distal part.



(a)



(b)

Figure 6 Microradiograph of the osteotomized site at 12 weeks. The osteotomy (white letter O) is consolidated. Growth cartilage (white letter G). The bony rim around the implant channel is seen clearly (white letter R) (a). The bone structure is thicker compared to the control side (b). (Original magnification × 20.6.)

The OTC-uptake was strong on the proximal side of the osteotomy.

At three weeks, radiologically there were no displacements. The osteotomy line was visible in seven cases. The callus formation was strong in most cases. A bony rim around the implant was seen in four cases. Histologically, there was a bony connection in one case. The orifice of the implant channel was closed in six cases. A thin layer of granulation tissue around the implant was seen in five cases. The formation of new bone around the implant and in the osteotomy area was strong. Microradiographically there was callus formation all around the osteotomy area. There was strong OTC-uptake endosteally and periosteally. There was also OTC-uptake distally from the osteotomy which was not seen at one week. The beginning of the formation of the bony rim around the implant was seen.

After six weeks, there were two displacements and the osteotomy line was seen radiologically in five cases. Strong external callus formation and a bony rim around the implant was seen in all cases. Histologically, one osteotomy had healed properly and one only on the other side. In one case, there was a bony connection and five non-unions were observed. There was also one infection. The orifice of the implant channel was closed by granulation tissue in five cases. Histologically, there was a bony rim around the implant in four cases and rim of granulation tissue in six cases. Mineralized callus was seen microradiographically. The OTC-uptake was as strong as at three weeks.

At 12 weeks, radiologically there were no displacements and the osteotomy line was visible in four specimens. External callus formation was slower. A (Fig. 4) bony rim around the implant was seen in seven cases. Histologically, four osteotomies had healed properly and there were three non-unions. In one case there was a bony connection. The orifice of the implant channel was closed by bone in one case and by

granulation tissue in five cases. A bony rim around the implant was seen in all (Fig. 5) cases and a rim of granulation tissue in five cases. Microradiographically, the new bone was mineralized like normal bone (Fig. 6). The OTC-uptake was only moderate.

At 24 weeks, radiologically all osteotomies had healed well and the osteotomy line was visible in five cases. No callus was observed. A bony rim was well seen in all cases. Histologically, three osteotomies were properly healed and there were five non-unions. The orifice of the implant channel was closed by bone in four and by granulation tissue in four cases. A bony rim around the implant was seen in all cases and a rim of granulation tissue in six cases. The OTC-uptake was low.

At 36 weeks, radiologically there was one displacement and the osteotomy line was visible in three cases. A bony rim was seen in all cases. Histologically, six osteotomies had healed properly and there was a bony connection in one case. One non-union was seen. The orifice of the implant channel was closed by bone in five and by granulation tissue in three cases. A bony rim was seen in all cases a rim of granulation tissue in four cases. Microradiographically, the findings were the same as radiologically. The OTC-uptake was low.

At 48 weeks, radiologically there was one displacement and the osteotomy line was visible in two cases. A bony rim was seen in four cases. Histologically, three osteotomies had healed properly and only one on the other side. There were four non-unions. The orifice of the implant channel was closed by bone in five cases and by granulation tissue in five cases. A bony rim was seen in all cases and a rim of granulation tissue in six cases. The OTC-uptake was low.

At 52 weeks, radiologically, there was one slight displacement and a bony rim was seen in six cases. Histologically, six osteotomies had healed properly and only one on the other side. One non-union was observed. The orifice of the implant channel was closed by bone in four and by granulation tissue in four cases. A bony rim was seen in all cases and a rim of granulation tissue in three. The OTC-uptake was low.

3.4. Histomorphometrical results

The mean total trabecular bone area fraction in the operated femora was much higher than that in the control femora in all follow-up groups ($n = 32$, $t = 6.542$, $p < 0.0001$; Table II) and no dependence on time was seen. The mean trabecular bone circumference fraction in the operated femora was also higher than that in the control femora in all follow-up groups ($n = 32$, $t = 5.935$, $p < 0.0001$). The total osteoid length fraction was higher in the operated femora in all follow-up groups, except for the three and six week groups ($n = 32$, $t = 2.454$, $p < 0.05$). The osteoblast lines practically occurred only in the operated femora and disappeared slowly during the 52 week follow-up time, therefore there was no statistical interest. Especially the trabecular bone circumference fraction and the osteoblast line length fraction in the operated femora had a tendency to start up from high values and to descend to the end stage level at around 24 weeks. In the operated femora, strong osteoblast activity was seen at 1, 3, 6 and 12 weeks.

4. Discussion

The purpose of the present study was to examine the use of SR-P(L/DL)LA/bioactive glass composite rods in the fixation of distal femoral osteotomies in rats. At the time of the operation the rats were still growing and at the time of the sacrifice the operated femora were on average shorter than the controls due to the operation trauma to the growth cartilage. Thirty-nine fixations healed uneventfully and a total of 19 non-unions and six delayed unions were observed. In 20 operations the fixation was loose and out of these were 14 non-unions observed. This indicates that the inadequate operative technique may have affected the results. The callus formation was normal in the cases with proper fixation. The histomorphometrical results showed that the healing happened in normal time and the active osteoid formation was slow after six weeks.

Previous experimental animal studies have shown that the biocompatibility of the two most clinically used implants of bioabsorbable polyesters, polyglycolide [2, 11, 12] and polylactide [4, 13–15], is acceptable for internal fixation. The use of intraosseously implanted SR-PLLA devices does not seem to carry risks for development of foreign-body reactions.

In a previous study, with follow-up times of 20–250

TABLE II Results of histomorphometric analysis of the tissue-implant interface after fixation with SR-P(L/DL)LA/bioactive glass rods of the osteotomized distal femur in rats (mean and SD)

Time (weeks)	Total trabecular bone area fraction over the total tissue area (%)		Trabecular bone circumfrence fraction over the total tissue area (%)		Total osteoid length over the total tissue area (%)		Total osteoblast line length over the total tissue area (%)	
	SR-P(L/DL)LA (SD)	Control (SD)	SR-P(L/DL)LA (SD)	Control (SD)	SR-P(L/DL)LA (SD)	Control (SD)	SR-P(L/DL)LA (SD)	Control (SD)
1	0.29 (0.02)	0.18 (0.06)	8.66 (1.06)	4.61 (0.92)	0.12 (0.23)	0.02 (0.04)	0.12 (0.11)	0.00 (0.00)
3	0.39 (0.03)	0.23 (0.08)	6.70 (2.10)	4.68 (1.04)	0.33 (0.31)	0.39 (0.31)	0.19 (0.20)	0.00 (0.00)
6	0.29 (0.14)	0.24 (0.18)	5.86 (0.80)	3.80 (1.76)	0.20 (0.24)	0.38 (0.46)	0.06 (0.04)	0.01 (0.02)
12	0.28 (0.06)	0.20 (0.12)	5.15 (1.06)	4.11 (2.13)	0.58 (0.37)	0.01 (0.03)	0.17 (0.31)	0.00 (0.00)
24	0.34 (0.03)	0.17 (0.06)	4.60 (1.14)	4.11 (1.46)	0.55 (0.11)	0.12 (0.11)	0.04 (0.07)	0.00 (0.00)
36	0.42 (0.18)	0.08 (0.04)	4.40 (1.09)	2.04 (0.76)	0.22 (0.16)	0.17 (0.17)	0.02 (0.05)	0.00 (0.00)
48	0.28 (0.06)	0.05 (0.06)	3.38 (0.21)	1.03 (1.23)	0.25 (0.13)	0.10 (0.10)	0.04 (0.07)	0.00 (0.00)
52	0.34 (0.15)	0.12 (0.03)	3.84 (0.77)	2.71 (0.59)	0.30 (0.29)	0.09 (0.12)	0.00 (0.00)	0.00 (0.00)

days, the histomorphometrical analysis of the tissue–implant interface showed the osteogenic response to SR-PGA to be vigorous but transitory after internal fixation of a rabbit distal femoral osteotomy with an SR-PGA screw [16]. In another study, no signs of degradation of SR-PLLA pins were observed within the 52-week follow-up times, but the total bioabsorption of SR-PGA pins had occurred between the follow-up periods of 24 and 36 weeks [17]. In the earlier literature only one late foreign-body–tissue reaction to SR-PLLA has been reported in an experimental study in one single rat followed up for a considerably longer time than the other rats [18].

In the present study the biocompatibility of the SR-P(L/DL)LA/bioactive glass composite rods was good, as only a mild inflammatory tissue reaction was seen. The bone, granulation tissue, and connective tissue rim seem to be a bone tissue response to the injury and operation trauma. Using the operated limb causes torque and micro-movement between the implant and bone, which increases the granulation tissue production.

The results of *in vivo* and *in vitro* material studies suggest that the SR-P(L/DL)LA/bioactive glass composite is suitable for fracture fixation as long as the fixation technique is correct. Summarizing the present findings it was observed that SR-P(L/DL)LA/bioactive glass composite rods seem to result in an osteostimulatory response at the tissue–implant interface after implantation into the cancellous bone of the distal rat femur.

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References

1. S. VAINIONPÄÄ, K. VIHTONEN, M. MERO, H. PÄTIÄLÄ, P. ROKKANEN, J. KILPIKARI and P. TÖRMÄLÄ, *Arch. Orthop. Traum. Surg.* **106** (1986) 1.
2. O. BÖSTMAN, U. PÄIVÄRINTA, E. PARTIO, J. VASENIUS, M. MANNINEN and P. ROKKANEN, *J. Bone. Joint. Surg. Am.* **74** (1992) 1021.
3. A. MAJOLA, S. VAINIONPÄÄ, K. VIHTONEN, M. MERO, J. VASENIUS, P. TÖRMÄLÄ and P. ROKKANEN, *Clin. Orthop.* **268** (1991) 260.
4. Y. MATSUSUE, S. HANAFUSA, T. YAMAMURO, Y. SHIKINAMI and Y. IKADA, *ibid.* **317** (1995) 246.
5. M. VERT, P. CHRISTEL, H. GARREAU, M. AUDION, M. CHANAVAZ and F. CHABOT, in ''Polymers in Medicine II'' (Plenum Press, New York, 1986) p. 263.
6. P. TÖRMÄLÄ, *Clin. Mater.* **10** (1992) 29.
7. T. PYHÄLTÖ, M. LAPINSUO, H. PÄTIÄLÄ, M. PELTO, P. TÖRMÄLÄ and P. ROKKANEN, *J. Orthop. Sci.* **7** (2002) 549.
8. L. HENCH, *Biomaterials* **19** (1998) 1419.
9. M. BRINK, Academic dissertation (Åbo Akademi University, Turku, Finland, 1997).
10. J. GOLDNER, *Am. J. Pathol.* **14** (1938) 237.
11. E. J. FRAZZA and E. E. SCHMITT, *J. Biomed. Mater. Res. S.* **1** (1971) 43.
12. J. B. HERRMANN, R. J. KELLY and G. A. HIGGINS, *Arch. Surg.* **100** (1970) 486.
13. D. E. CUTRIGHT, E. E. HUNSUCK and J. D. BEASLEY, *J. Oral. Surg.* **29** (1971) 393.
14. R. K. KULKARNI, K. C. PANI, C. NEUMAN and F. LEONARD, *Arch. Surg.* **93** (1966) 839.
15. R. W. BUCHOLZ, S. HENRY and M. B. HENLEY, *J. Bone. Joint. Surg. Am.* **76** (1994) 319.
16. O. M. BÖSTMAN, U. PÄIVÄRINTA, E. PARTIO, M. MANNINEN, J. VASENIUS, A. MAJOLA and P. ROKKANEN, *Clin. Orthop.* **285** (1992) 263.
17. P. NORDSTRÖM, H. PIHLAJAMÄKI, T. TOIVONEN, P. TÖRMÄLÄ and P. ROKKANEN, *Arch. Orthop. Trauma. Surg.* **117** (1998) 197.
18. R. R. M. BOS, F. R. ROZEMA, G. BOERING, A. J. NIJENHUIS, A. J. PENNINGS, A. B. VERWEY, P. NIEUWENHUIS and H. W. B. JANSEN, *Biomaterials* **12** (1991) 32.

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