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Fixation of experimental osteotomies with bioabsorbable SR-polylactide–polyglycolide (80/20) polymeric rods

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Abstract Self-reinforced polylactide–polyglycolide (80/20) composite rods, 2 mm in diameter and 36 mm in length, were implanted into the dorsal subcutaneous tissue of 20 rabbits. Osteotomies of the distal femur were fixed with these rods (2 × 15 mm) in the rabbits. The follow-up times varied from 3 to 104 weeks. After sacrifice, three-point bending and shear tests and molecular weight measurements were performed for subcutaneously placed rods. Radiological, histological, microradiographic, oxytetracycline-fluorescence, and histomorphometrical studies of the osteotomized and intact control femora were performed. After 6 weeks the mechanical properties had decreased significantly, but osteotomies had healed uneventfully. The present investigation showed that the mechanical strength and fixation properties of SR-Polylactide–glycolide (80/20) rods are suitable for fixation of cancellous bone osteotomies in rabbits provided that the operative technique is correct. The present article is the first report on the application of these rods for fixation of cancellous bone osteotomies.

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Introduction

The use of bioabsorbable polymers as a suture material started nearly 50 years ago [1]. Polyglycolic acid (PGA) has been used worldwide as a biodegradable suture material since 1970. Bioabsorbable devices are mostly made of the polymers polylactide, polyglycolide, and their copolymers (polyglycolide–co-polylactide and P(L/DL)LA).

Previous experimental animal [2–5] and clinical studies [6, 7] have shown that bioabsorbable self-reinforced (SR) polyglycolide (SR-PGA) pins have sufficient fixation properties for fixation of cancellous bone fragments. SR-PGA has a degradation time of a few months [8]. Similar experimental [9] investigations have demonstrated good fixation of small fragment osteotomies with self-reinforced poly-L-lactide (SR-PLLA) pins. The degradation time of polylactide is several years [10, 11]. The mechanical strength of SR-PLLA pins is maintained at a high strength level longer than with SR-PGA pins [12].

The aim of the present study was to examine the use of SR-Polylactide/polyglycolide (80/20) polymeric rods in cancellous bone fixation in the fixation of distal femoral osteotomies on rabbits.

Materials and methods

Self-reinforced poly-L-lactide-polyglycolide (SR-PLGA) rods (with lactide–glycolide ratio 80:20) were supplied by Linvatec Biomaterials (Tampere, Finland). The rods were 2 mm in diameter and 50 in length. Before use, the rods were cut into two pieces of the length of 36 or 15 mm with an electric loop specially designed for this purpose.

Testing of SR-PLGA rods

For in vivo testing the rods were sterilized by gamma irradiation at a minimum dose of 25 kGy (NN, MM). The rods were mechanically tested under flexural and shear load with a universal materials tester (Instron 4411, Instron PLD, High Wycombe, Great Britain). The three-point bending test was performed with a cross-head speed of 10 mm/min and a span length 16 times the diameter of the sample. The flexural strength and modulus were calculated according to ASTM D790M-93, modified for circular cross-section samples. The shear strength test was performed with 10 mm/min according to BS2782 method B.

For evaluation of the strength retention properties cylindrical rods (length 36 mm, diameter 2 mm) made of SR-PLGA were implanted into the dorsal subcutaneous tissue of rabbits. Twenty mature New Zealand rabbits of both sexes weighing 2,040–5,040 g (mean 4,238 g) were used. The rabbits were anaesthetized with subcutaneous injections of medetomidine (Domitor®, Orion-yhtymä Oy, Espoo, Finland) 0.375 mg/kg and ketamine (Ketalar®, Parke-Davis, Solna, Sweden) 25 mg/kg. During the anaesthesia all rabbits received an intramuscular injection 0.15 mg/kg of benzyl penicillin procaine as an infection prophylaxis. A small area on the back was shaved and wiped with antiseptic fluid. Two cylindrical rods were implanted into the dorsal subcutaneous tissue via two incisions which were then closed in layers with either 4-0 Vicryl® (NN, MM) or 4-0 Vicryl® rapid.

The follow-up times where 3, 6, 12, 24, 48, and 104 weeks. Each follow-up group consisted of three rabbits. A total of 40 SR-PLGA rods were implanted subcutaneously, two implants in each rabbit and, thus, six 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. Three rabbits died during the experiment due to reasons not connected with the experiment.

Bone fixation properties of SR-PLGA rods

For evaluation of the bone fixation properties, cylindrical rods (length 15 mm, diameter 2 mm) were used in the fixation of the osteotomies of the distal femur of the rabbits. The previously mentioned 20 rabbits were used, and the surgery was performed during the same anaesthesia as the implantation of the rods into the dorsal subcutaneous tissue. The knee region of the right hind leg was shaved and wiped with antiseptic fluid. A medial prapatellar incision was made, the patella dislocated laterally, and the articular surface of the distal femur exposed. A drill channel of 2 mm in diameter was made through the medial and lateral condyles. A horizontal osteotomy was made with an oscillating saw in the cancellous metaphysis of the distal femur (Fig. 1). The posterior cortex was left intact. The osteotomy was fixed with two SR-PLGA rods, and the excess parts of the rods were removed with the oscillating saw. The patella was reduced and the incision closed with the above-mentioned, absorbable sutures. The rabbits were allowed to walk freely in their cages after the operation, and no external support of the operated limb was used.

The follow-up times were 3, 6, 12, 24, 48, and 104 weeks. There were three rabbits in each follow-up group. Two days before the sacrifice the rabbits received an intramuscular injection of oxytetracycline (OTC) hydrochloride 50 mg/kg (Terramycin®, Pfizer, Amboise, France) for OTC-labelling studies. For the sacrifice the rabbits were sedated with ketamine (Ketalar®, Parke-Davis, Solna, Sweden) and medetomidine (Domitor®, Orion-yhtymä Oy, Espoo, Finland). The rabbits were then killed with an overdose of sodium pentobarbital (Mebunat®, Orion-Farmos, Turku, Finland) administered intravenously.

After sacrifice, both femora were taken as specimens. Radiographs were taken in the anteroposterior and lateral positions (distance 1.00 m, 40 kV, 3.6 mAs, and 0.04 s). Macroscopic evaluation of the area of the osteotomies was done. The distal parts of the femora were taken as

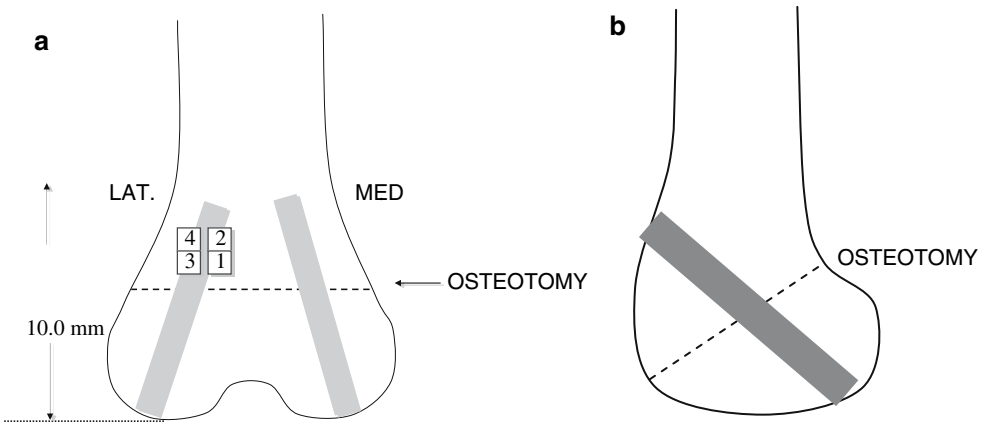

Fig. 1 Schematic anterior (**a**) and lateral view of the distal rat femur showing positioning of the osteotomy, the implant, and the four standardized sample fields (1, 2, 3, 4) used in the histomorphometric analysis

specimens, fixed in 70% alcohol, and embedded in methyl methacrylate. For histological and histomorphometric analysis, longitudinal sections measuring 5 μm in thickness were cut in a frontal plane with a Reichert-Jung microtome (Nussloch, Germany) and stained according to the Masson-Goldner method [13]. For contact microradiography (Faxitron X-ray system, Model 43855 A, Hewlett-Packard, McMinnville, Oregon; USA; Imtec Pol-Edged H.R.P., ultra flat, type 1A, Imtec Products, Sunnyvale, California, USA), and OTC fluorescence studies, 80-μm-thick sections were made with a Leitz Saw Microtome 1600 (Wetzlar, Germany). Fluorescence microscopy was performed with an HBO 220 ultraviolet lamp (Osram, Berlin, Germany) and a BG 812/6 primary filter (Leitz, Wetzlar, Germany).

For semiautomatic quantitative histomorphometric analysis, a Leiz 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 analysing software was AnalySIS 3.00 (Soft-Imaging System GmbH, Münster, Germany). Six specimens in all follow-up groups were analysed. Both femora of each rabbit were analysed, the left femur serving as control. Four standardized sample fields were determined in each femur (Fig. 1). The AnalySIS-program was used for the determination of the corresponding sample field. Within the 1.06 mm × 0.84 mm (0.89 m^2) sample fields, the histomorphometrical variables were analysed. 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 total length of osteoblast lines. The paired *t*-test was used for statistic evaluation.

Results

Testing of self-reinforced Polylactide–polyglycolide (80/20) polymeric rods. PLGA pins expanded during the first 6 weeks due to hydrolysis. Peak load started to decreased meaningfully after 3 weeks. Stress load and bending modulus decreased gradually during the 6 weeks. All these changes were statistically meaningful. The total change of diameter (expansion), peak and stress load and bending modulus (decrease) was statistically meaningful during the first 6 weeks. While the pins expanded at the same time their strength properties gradually became worse (Table 1). PLGA pins expanded during the first 6 weeks. After that the change in diameter was statistically not meaningful. Stress load decreased meaningfully in 12 weeks except between 3 and 6 weeks. The same phenomena was noticed in shear stress measurements. The over all changes in diameter (expansion), stress load and shear stress (decrease) were meaningful in 12 weeks (Table 2)

Bone fixation properties of self-reinforced Polylactide–polyglycolide (80/20) polymeric rods

At the time of sacrifice the mean weight of the rabbits was 3,984 g (2,740–5,180 g). In the macroscopic evaluation after sacrifice all fixations were stable.

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

Three rabbits died during the experiment. No displacements were observed radiologically or histologically. Radiologically the osteotomy line was visible in the operated femora up to 26 weeks. Strong external callus formation was seen up to 12 weeks. After 6 weeks a bony rim around the implant was seen in most cases. The OTC-uptake was high up to 6 weeks but at 12 weeks it was only moderate.

Three weeks. Radiologically there were no displacements. The osteotomy line was visible in all cases. The callus formation was strong in most cases. A bony rim around the implant was seen. Histologically there was a

Table 1 Statistical analysis: in vivo 3-point bending test of PLGA pin 2.0 mm in diameter

		Change of diameter (mm)		Change of peak load (*N*)		Change of stress load (MPa)		Change of modulus (GPa)	
Unpaired *t*-test	0 week versus 3 week	0.019	*	0.0577	ns	0.0075	**	0.0002	***
	0 week versus 6 week	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
	3 week versus 6 week	0.41	Ns	<0.0001	***	0.0001	***	0.0003	***
Tukey-Kramer multiple comparison test	0 week versus 3 week	$p < 0.05$	*	$p > 0.05$	ns	$p < 0.05$	*	$p < 0.01$	**
	0 week versus 6 week	$p < 0.01$	**	$p < 0.001$	***	$p < 0.001$	***	$p < 0.001$	***
	3 week versus 6 week	$p > 0.05$	ns	$p < 0.001$	***	$p < 0.001$	***	$p < 0.001$	***
		ANOVA $p = 0.0040$	*	ANOVA $p < 0.0001$	***	ANOVA $p = 0.0001$	***	ANOVA $p < 0.0001$	***

Table 2 Statistical analysis: in vivo shear test of PLGA pin 2.0 mm in diameter

		Change of diameter (mm)		Change of stress load (MPa)		Change of shear stress (MPa)	
Unpaired *t*-test	0 week versus 3 week	0.1763	ns	0.0198	*	0.0156	*
	0 week versus 6 week	0.0003	***	0.003	***	0.0001	***
	0 week versus 12 week	0.0831	ns	<0.0001	***	<0.0001	***
	3 week versus 6 week	0.042	*	0.9801	ns	0.7874	ns
	3 week versus 12 week	0.7067	ns	0.0018	**	0.0022	**
	6 week versus 12 week	0.0772	ns	<0.0001	***	<0.0001	***
Tukey-Kramer multiple comparison test	0 week versus 3 week	$p > 0.05$	ns	$p < 0.05$	*	$p < 0.01$	**
	0 week versus 6 week	$p < 0.05$	*	$p < 0.05$	*	$p < 0.01$	**
	0 week versus 12 week	$p > 0.05$	ns	$p < 0.001$	***	$p < 0.001$	***
	3 week versus 6 week	$p > 0.05$	ns	$p > 0.05$	ns	$p > 0.05$	ns
	3 week versus 12 week	$p > 0.05$	ns	$p < 0.001$	***	$p < 0.001$	***
	6 week versus 12 week	$p > 0.05$	ns	$p < 0.001$	***	$p < 0.001$	***
		ANOVA $p = 0.0181$	*	ANOVA $p = 0.0001$	***	ANOVA $p < 0.0001$	***

bony connection in two cases. A thin layer of granulation tissue around the implant was seen. The formation of new bone around the implant and in the osteotomy area was strong. Microradiographically there was callus formation around the osteotomy area. The OTC-uptake was strong endosteally and periosteally.

Six weeks. There were no displacements, and the osteotomy line was seen radiologically in one case. Strong external callus formation and a bony rim around the implant could be seen. Histologically all osteotomies had healed properly. The orifice of the implant channel was closed by granulation tissue. Histologically there was a bony rim around the implant. Mineralized callus was seen microradiographically. The OTC-uptake was as strong as at 3 weeks.

Twelve weeks. Radiologically there were no displacements, and the osteotomy line was not visible (Fig. 2). A bony rim around the implant was seen. Histologically all osteotomies had healed properly. The orifice of the implant channel was closed by bone in one case and by granulation tissue in two cases. A bony rim around the implant was seen in all cases. Microradiographically the new bone was mineralized as normal bone (Fig. 3). The OTC-uptake was only moderate.

Twenty six weeks. Radiologically all osteotomies healed well, and the osteotomy line was not visible. No callus was observed. A bony rim was well seen in all cases. Histologically the osteotomies were properly healed. The orifice of the implant channel was closed by bone. A bony rim around the implant was seen in all cases. The OTC-uptake was low.

Fifty two weeks. Radiologically there were no displacements, and a bony rim was seen. Histologically the osteotomies had healed properly. The orifice of the implant channel was closed by bone. A bony rim was seen in all cases. The OTC-uptake was low.

Hundred and four weeks. Radiologically, histologically, and microradiographically the osteotomies were healed. The OTC-uptake was low.

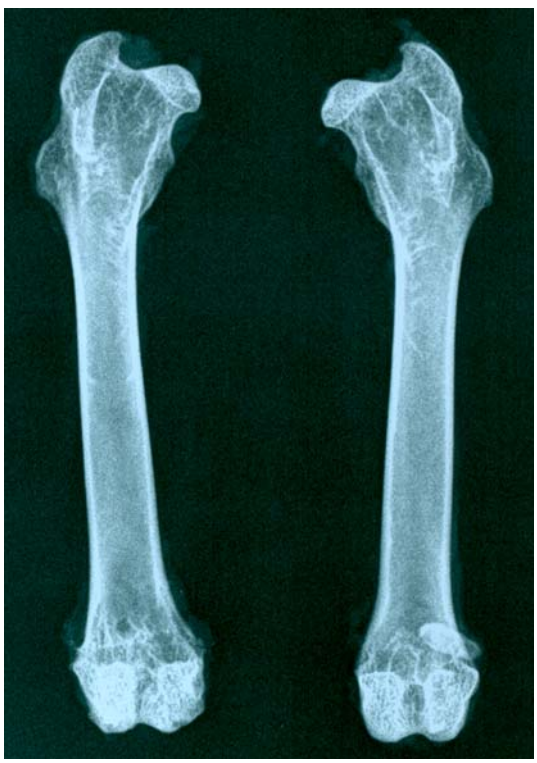

Fig. 2 In the radiograph (12 weeks) the healing of the osteotomy can be seen. The osteotomy line is not visible. External callus formation can be seen on the medial cortex (white arrow). The operated femur is shorter than the control

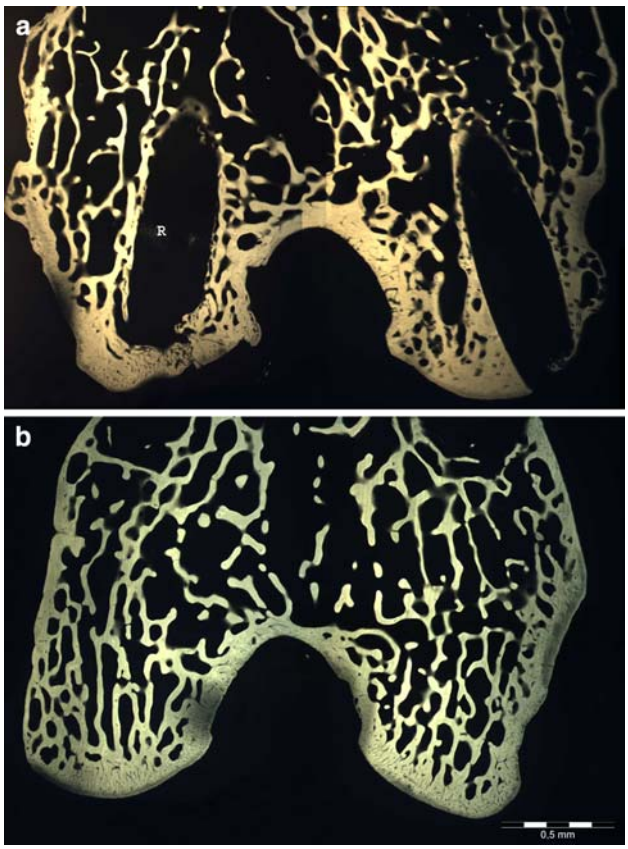

Fig. 3 Microradiograph of the osteotomized site at 12 weeks. The osteotomy (O) is consolidated. Growth cartilage (G). The bony rim around the implant channel is seen clearly (white letter R) (**a**). The bone structure is thicker compared to the control side (**b**). (Original magnification 20.6×)

Histomorphometrical results

The difference of the trabecular area divided by the total area between the operated side and the control side was calculated (B/A differ). Statistically meaningful changes were noticed when comparing the results of week 3 to those of weeks 12, 26, 52 and 104. Also the values of week 6 compared to those of weeks 12, 26, 52, and 104 were statistically significant, which indicates that the trabecular area is mainly decreased between the weeks 3 and 12. The same phenomenon was noticed when the C/A differ was analysed. The D/A differ was not statistically significant in any of the measurements (Tables 3–5).

Discussion

The purpose of the present study was to examine the use of self-reinforced polylactide–polyglycolide (80/20) composite rods in the fixation of distal femoral osteotomies in rabbits. Sixteen of the seventeen fixations healed uneventfully; there was only one non-union. The histomorphometrical results showed that the osteotomies were healed within normal time and the active osteoid formation was slow after 6 weeks.

Previous experimental animal studies have shown that the biocompatibility of the two clinically used implants of bioabsorbable polyesters, polyglycolide [9, 14, 15] and polylactide [1, 10, 16, 17], is acceptable for internal fixation. The use of intraosseously implanted SR-PLLA

Table 3 Results of the histomorphometric analysis of tissue implant interface after fixation of distal femoral osteotomy with PLGA 80/20 rods in rabbits

Week	B/A differ mean	SD	C/A differ mean	SD	D/A differ mean	SD
3	0.31	0.13	4.17	0.84	2.75	0.94
3	0.32	0.08	2.75	3.34	2.83	1.11
3	0.23	0.20	1.55	1.70	0.94	0.97
6	0.10	0.20	1.74	2.87	0.29	0.79
6	0.34	0.05	5.44	1.48	1.59	1.64
6	0.19	0.21	4.76	1.23	1.64	0.75
12	0.20	0.06	0.09	0.93	0.96	0.59
12	0.01	0.08	−0.59	1.03	–	–
12	−0.01	0.11	0.41	0.91	−0.29	0.47
26	0.03	0.19	−0.41	1.90	–	–
26	−0.02	0.10	1.02	0.97	0.28	0.20
26	0.19	0.09	0.06	1.18	–	–
52	−0.03	0.20	0.79	0.66	0.59	0.00
52	−0.01	0.17	0.53	2.07	0.07	0.48
104	−0.05	0.13	0.69	0.67	–	–
104	−0.84	1.45	0.27	2.22	–	–

A = Total area, B = Trabecular area, C = Length of bone edge, D = Length of osteoids line

Table 4 Results of the histomorphometric analysis of tissue implant interface after fixation of distal femoral osteotomy with PLGA 80/20 rods in rabbits: the mean values of each week measured

Week		B/A differ mean	SD	C/A differ mean	SD	D/A differ mean	SD
3		0.28	0.14	2.82	2.29	2.00	1.28
	Number	12		12		7	
6		0.21	0.19	3.98	2.46	1.13	1.16
	Number	12		12		11	
12		0.07	0.12	−0.03	0.97	0.54	0.83
	Number	12		12		3	
26		0.06	0.15	0.24	1.44	0.28	0.20
	Number	11		11		2	
52		−0.02	0.17	0.66	1.43	0.24	0.45
	Number	8		8		3	
104		−0.45	1.02	0.48	1.48	–	–
	Number	6		6		–	–

A = Total area, B = Trabecular area, C = Length of bone edge, D = Length of osteoids line

Table 5 Statistical results of the histomorphometric analysis of tissue implant interface after fixation of distal femoral osteotomy with PLGA 80/20a rods in rabbits

B/A differ *t*-test			C/A differ *t*-test			D/A differ *t*-test		
3 versus 6	0.2835	ns	3 versus 6	0.2480	ns	3 versus 6	0.1584	ns
3 versus 12	0.0005	***	3 versus 12	0.0007	***	3 versus 12	0.1125	ns
3 versus 26	0.0012	**	3 versus 26	0.0043	**	3 versus 26	0.1142	ns
3 versus 52	0.0004	***	3 versus 52	0.0294	*	3 versus 52	0.0544	ns
3 versus 104	0.0226	*	3 versus 104	0.0386	*	3 versus 104	–	–
6 versus 12	0.0358	*	6 versus 12	$p < 0.0001$	***	6 versus 12	0.4297	ns
6 versus 26	0.0429	*	6 versus 26	0.0003	***	6 versus 26	0.3386	ns
6 versus 52	0.0119	*	6 versus 52	0.0030	**	6 versus 52	0.2266	ns
6 versus 104	0.0400	*	6 versus 104	0.0060	**	6 versus 104	–	–
12 versus 26	0.8528	ns	12 versus 26	0.6044	ns	12 versus 26	0.7080	ns
12 versus 52	0.2017	ns	12 versus 52	0.2128	ns	12 versus 52	0.6129	ns
12 versus 104	0.0934	ns	12 versus 104	0.3886	ns	12 versus 104	–	–
26 versus 52	0.3267	ns	26 versus 52	0.5341	ns	26 versus 52	0.9159	ns
26 versus 104	0.1189	ns	26 versus 104	0.7438	ns	26 versus 104	–	–
52 versus 104	0.2591	ns	52 versus 104	0.8252	ns	52 versus 104	–	–

devices does not carry risks for development of severe foreign-body reactions [18]. In a previous study, with a follow-up time up to 250 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 [19]. No signs of degradation of SR-PLLA pins were observed within the 52-week follow-up time, but the total bioabsorption of SR-PGA pins had occurred between the follow-up periods of 24 and 36 weeks [20].

Self-reinforced poly (desamino tyrosyl—tyrosine ethyl ester carbonate), Poly(DTE carbonate) rods have also proved suitable in experimental bone fixation [21]. It has been shown that a synthetic material (called bioactive glass) can bond to bone developing a chemical bonding layer on its surface [22].

In the present study the biocompatibility of the bioabsorbable self-reinforced polylactide–polyglycolide (80/20) 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.

Conclusions

Summarizing the present findings it can be stated that SR-polylactide–polyglycolide (80/20) composite rods seem to result in an osteostimulatory response at the tissue-implant interface after implantation into the cancellous bone of the distal rabbit femur.

The results of in vivo and in vitro material studies suggest that the bioabsorbable self-reinforced polylactide/polyglycolide (80/20) rods are suitable for osteotomy fixation provided that the fixation technique is correct.

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