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Biocompatibility of silver nitrate and ofloxacine coated bioabsorbable SR-PLLA rods

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Abstract The purpose of this study was to evaluate the biocompatibility of silver nitrate and ofloxacine coatings of bioresorbable self-reinforced poly-L-lactic acid (SR-PLLA) rods. SR-PLLA rods coated with pure poly(caprolactone-co-L-lactide) or blended with silver nitrate (10, 5 or 2 weight-%) or ofloxacine (5 or 2 weight-%) were implanted in the dorsal muscles of 25 male rabbits. Tissue reactions caused by implantation trauma were seen 1 week after implantation. The positive control and 10 w-% silver nitrate coating showed the most marked reactions 1 month after implantation. Only sparse reactions were seen 6 months after implantation. Tissue reactions were scored semi-quantitatively. As a result of this study, we concluded that silver nitrate or ofloxacine coatings up to five w-% did not alter the good biocompatibility of SR-PLLA essentially. The method may lead to the possibility of preventing bacterial adhesion to urological stents during insertion.

Key words Bioabsorbable · Stent · Infection

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Abbreviations *Chr* Chronic inflammatory changes · *Ac* Acute · *Eos* Eosinophilic reactions · *Fibr* Fibrosis · *FBR* Foreign-body reaction · *MN* Muscle necrosis around implant · *NF* Necrosis facing implant · *CD* Calcific deposition · *10S* 10 w-% silver nitrate · *5S* 5 w-% silver nitrate · 2S 2 w-% silver nitrate · *5O* 5 w-% ofloxacine · *2O* 2 w-% ofloxacine · *PL* Poly-L-lactic acid · *N* Negative control · *P* Positive control

Introduction

Bioresorbable polymers have been used as surgical suture materials since the 1960s. Kulkarni and coworkers [1] reported the manufacturing of bioresorbable polylactic acid sutures (PLA) in 1966. Since then, various bioabsorbable suture materials, such as polyglycolic acid (PGA, Dexon), copolymerate of polyglycolic and polylactic acid (Vicryl), poly-p-dioxane (PDS), and copolymerate of polyglycolic acid and trimethylene carbonate (Maxon), have been widely used. The clinical use of bioabsorbable implants in traumatology and orthopedics started in Finland by fixation of malleolar fractures by polyglycolide/polylactide rods and sutures [2]. Extensive animal studies have shown good biocompatibility of polyglycolides and polylactides [3]. To give better mechanical strength to bioabsorbable material, several self-reinforcing (SR) techniques can be used [4]. The development of bioabsorbable devices for urologic use started in the late 1980s. In an experimental study of Kemppainen et al. [5], self-reinforced poly-L-lactide spiral (SR-PLLA) stents showed good tissue penetration and biocompatibility properties. Since then, SR-PGA stents have been clinically used after visual laser ablation of prostate (VLAP) [6, 7], transurethral microwave therapy (TUMT) [8], and with free skin urethroplasty for recurrent bulbar urethral strictures [9]. SR-PLLA stents have been used clinically in the treatment of recurrent urethral strictures with optical uretrotomy [10] and combined with finasteride in the treatment of acute urinary retention [11].

The adherence of uropathogens to the uroepithelium or to the surface of prosthetic devices is the key event in the pathogenesis of urinary tract infection [12]. Because during insertion urological stents are temporarily in contact with distal urethra, which is colonized with bacteria, the prevention of bacterial adherence to the stent is very important. Cormio et al. found that immersion in antibiotic solution prevented bacterial adherence [13].

In the studies of our group, both silver nitrate and ofloxacine coatings had the same effect [14, 15]. Before clinical applications, it is essential to investigate the biocompatibility properties of silver nitrate and ofloxacine coated stents. The aim of this study was to test the long-term biocompatibility of silver nitrate and ofloxacine coated SR-PLLA stents by a muscle implantation test.

Material and methods

The bioabsorbable SR-PLLA wire was coated with ε-caprolactone/L-lactide copolymer. ε-Caprolactone/L-lactide copolymer [P (CL/L-LA)] coating consists of a semi-crystalline co-polymer of ε-caprolactone (95 mol-%) and L-lactide (5 mol-%). The molecular weight of the polymer is 107,000 g/mol. The melting temperature is 52° C and the glazing temperature −60° C. The copolymer coating is rigid and hard below −60° C, but becomes elastic at normal body temperature, thus enabling the rapid dilatation of a pre-molded SR-PLLA spiral. Caprolactone/lactide polymers were polymerized according to the method developed in Helsinki University of Technology [16].

ε-caprolactone/L-lactide copolymer coating was blended with three different concentrations (10, 5 or 2 weight-%) of silver nitrate or two different concentrations (5 or 2 weight-%) of ofloxacine. The 1.1-mm-thick SR-PLLA wire was cut into 12.5-mm pieces.

SR-PLLA rods coated with pure ε-caprolactone/L-lactide copolymer were used as controls. Silicone rods were used as negative controls and organotin positive PVC rods as positive controls. SRPLLA test material was manufactured by Bionx Implants Ltd (Tampere, Finland). Ofloxacine was obtained from Hoechst Marion Roussel (Romainville, France) and positive control test material from Portex Limited (Kent, UK).

In all, 25 New Zealand White male rabbits were used as experimental animals. Their weights ranged from 2.8 to 4.0 kg. The animals were anesthetized with Domitor 1 mg/ml (medetomide hydrochloride) 0.3 ml/kg and Ketalar 50 mg/ml (ketamine hydrochloride) 0.3 ml/kg, intramuscularly. A dorsal midline incision was made. The test material pieces were implanted into dorsal muscles via a 2.0 mm i.v., cannula. The implantation sites were marked in the fascia with nonabsorbable sutures, and the skin was closed with resorbable running suture. In accordance with the recommendations of the International Organization of Standardization (ISO) [17], there were 8 pieces of each test material and 8 positive and negative controls per eight rabbits, randomly implanted, with 8–10 implants per animal and each type of material in at least three rabbits. The distance between two implants was at least an inch.

Eight rabbits were killed after 1 week, eight after 1 month, and eight after 6 months, by giving an overdose of Mebunat 60 mg/ml (pentobarbital natrium) intravenously. One rabbit died 2 days after implantation of the test material, for unknown reason. The test material with a 5-mm margin of muscular tissue was excised and fixed in 10% formalin. The specimens were inspected, measured in three dimensions and tissue samples were dissected as perpendicular as possible to the implants for paraffin embedding. Routine 4-μm-thick sections were prepared and stained with hematoxylin and eosin. The histological analysis was performed blindly by an experienced pathologist (KJ). The histological parameters assessed and recorded included acute and chronic

Table 1 Tissue reactions 1 week after implantation: 0 = no reaction, 1 = mild reaction, 2 = moderate reaction, 3 = marked reaction

	Chr	Ac	Eos	Fibr	FBR	MN	NF	CD
10S	000	000	301	222	110	112	000	000
	000	000	100	101	001	012	000	000
	0	0	0	0	1	1	0	0
5S	000	000	020	000	100	120	000	000
	001	000	121	101	011	212	000	000
	0	0	0	0	1	0	0	0
2S	000	000	200	011	110	121	000	000
	001	000	001	000	011	212	000	000
	10	00	10	11	11	20	00	00
5O	000	000	000	010	010	211	000	000
	000	000	010	010	000	121	000	000
	00	00	00	00	10	11	00	00
2O	001	000	000	000	012	211	000	000
	000	000	000	001	110	000	000	000
	0	0	1	1	0	0	0	0
PL	000	000	011	001	020	111	000	000
	100	000	120	111	001	032	000	000
	00	00	10	10	10	21	00	00
N	010	000	031	010	121	020	000	000
	100	000	200	010	222	100	000	000
	10	00	10	10	21	10	00	00
P	101	000	321	111	011	232	000	000
	111	000	111	011	101	212	000	000
	10	00	03	10	11	11	00	00
Significance (Kruskall-Wallis)	0.01	1.00	0.06	0.14	0.01	0.15	1.00	1.00

inflammatory changes, eosinophilic reactions, fibrosis, foreign-body reaction, muscle necrosis around implant, necrosis facing implant, and calcific depositions. The statistical package used was SPSS for Windows 8.0. The Kruskall-Wallis test was used to estimate the differences in tissue reactions, with the statistical significance level at $P < 0.05$.

Results

The semi-quantitative scoring of tissue reactions is shown in Tables 1, 2, and 3. The reactions due to

Table 2 Tissue reactions 1 month after implantation

	Chr	Ac	Eos	Fibr	FBR	MN	NF	CD
10S	111	000	002	101	020	321	030	120
	11	01	22	22	33	23	33	30
5S	100	000	101	211	001	312	100	000
	101	000	110	112	330	222	221	230
	0	0	2	2	0	2	0	0
2S	110	000	002	111	100	221	000	000
	100	100	223	111	112	222	000	111
	1	0	2	1	0	2	0	0
5O	100	100	221	111	130	231	030	120
	010	000	223	121	101	221	000	100
2O	111	000	223	121	011	222	000	011
	011	010	223	111	200	222	000	100
	10	00	33	02	00	11	00	00
PL	111	000	201	001	100	121	000	000
	101	000	200	111	021	122	000	021
	11	00	23	11	11	21	00	00
N	011	001	221	110	001	222	000	001
	111	110	333	211	300	211	000	200
	21	11	33	11	11	22	00	00
P	101	000	221	201	102	322	202	102
	111	011	121	121	133	233	022	122
	11	00	00	11	23	23	32	13
Significance (Kruskall-Wallis)	0.07	0.28	0.00	0.45	0.25	0.05	0.00	0.14

Table 3 Tissue reactions 6 months after implantation

	Chr	Ac	Eos	Fibr	FBR	MN	NF	CD
10S	010	000	000	000	111	000	000	000
	0	0	0	1	2	0	0	0
5S	000	000	000	000	011	000	000	000
	000	000	000	000	111	000	000	000
	000	000	000	000	011	000	000	000
	1	0	0	0	1	0	0	0
2S	001	000	000	000	111	000	000	000
	101	000	000	111	111	000	000	100
	01	00	00	11	12	00	00	00
5O	000	000	000	010	111	000	000	000
	000	000	000	000	001	000	000	000
	0	0	0	0	0	0	0	0
2O	010	000	000	000	110	000	000	000
	010	010	010	000	021	000	000	000
	0	0	0	0	1	0	0	0
PL	000	000	000	000	111	000	000	000
	000	000	000	010	100	000	000	000
N	011	000	000	100	000	000	000	000
	010	000	000	111	111	010	000	000
	0	0	0	1	0	0	0	0
P	000	000	000	110	010	000	000	000
	000	000	001	101	001	000	000	000
	00	00	00	00	00	00	00	00
Significance (Kruskall-Wallis)	0.07	0.41	0.57	0.01	0.02	0.41	1.00	0.53

Table 4 The assessment of tissue reactions[a]

Eosinophils	
0–5 cells	Score 0
6–10 cells	Score 1
11–50 cells	Score 2
> 50 cells	Score 3
Foreign-body giant cells	
0–1 cells	Score 0
1–10 cells	Score 1
11–20 cells	Score 2
r > 21 cells	Score 3
Muscle necrosis	
0–1 fibers	Score 0
2–10 fibers	Score 1
11–50 fibers	Score 2
> 50 fibers	Score 3
Fibrosis	
No	Score 0
> 8 cell layers	Score 1
1/3–1/1 diameter of the implant	Score 2
rExtensive fibrosis	Score 3
Calcific depositions	
No	Score 0
1 deposition	Score 1
1–5 depositions	Score 2
r > 6 depositions	Score 3
Necrosis facing implant	
No	Score 0
A few fragments of necrotic debris	Score 1
Necrotic debris facing the implant	Score 2
Necrotic mass facing the implant	Score 3

[a] Acute and chronic inflammation was assessed according to the guidelines given in the updated Sydney system for classification and grading of gastritis

implantation trauma, such as muscle necrosis and fibrosis, dominated at 1 week after implantation. Acute or chronic inflammatory changes were sparse. Marked eosinophilic reactions were seen with the positive control and 10 w-% silver nitrate, but also with the negative control. Tissue reactions were more severe at 1 month after implantation, even with the negative control. Especially the positive control and 10 w-% silver nitrate showed marked foreign-body and necrosis reactions. At 6 months after implantation, there were generally no tissue reactions in test samples. However, mild-to-moderate foreign-body reactions were seen. Statistical significance was reached at 1 month with eosinophilic reactions and necrosis reactions, and at 6 months with foreign-body reaction. A detailed assessment of the tissue reactions is reported in Table 4.

Discussion

The degradation of bioresorbable polymers proceeds via a random, bulk hydrolysis of the ester bonds in the polymer chains. At the early stage there is a decrease in the molecular weight of the polymer, although there is no change in the appearance of the implant. For example, the SR-PLLA implants with initial molecular weight of 260,000 Da show an average molecular weight of 10,000 Da after 36 weeks in the subcutis of the rabbit. When the molecular weight of the polymer goes below about 5,000 Da, the implant disintegrates into debris, which triggers a nonspecific foreign-body reaction. This includes macrophages, giant cells, and leukocytes. When bioresorption is complete, the inflammatory reaction disappears, leaving only scar tissue [18]. The bioresorption time of PLLA in soft tissue is about 12 months [19]. In hydrolysis, PLA is depolymerized to lactic acid, which in turn is transformed to pyruvate by lactate dehydrogenase. Decarboxylation of pyruvate yields acetyl-CoA, which enters the tricarboxylic acid cycle [20].

In this study, we had some problems with the rods that had been implanted in the fascia, which meant that the pathologist was unable to score the tissue reaction. The International Organization of Standardization recommends the muscle implantation test for studying the local effects of implants. However, it must be kept in mind that tissue reactions at urothelial surfaces may differ from those in muscle. This must be remembered when interpreting our results.

The tissue reactions were most marked at 1 month after implantation. Caprolactone-L-lactide-copolymer coated SR-PLLA showed good biocompatibility; in fact, sometimes it caused less tissue reaction than the negative control. We noted that 10 weight-% silver nitrate coating showed marked tissue toxicity. The other silver nitrate and ofloxacine coatings seemed to possess better biocompatibility properties, but they were usually a little worse than the negative control.

Foreign-body reaction was practically the only tissue reaction at 6 months after implantation, which may reflect the disappearance of the various coatings for two reasons: Firstly, this reaction type is typical in the degradation of pure SR-PLLA in soft tissue at that time.Secondly, the intensity of the foreign-body reaction varied very little between the various coatings. There was often black pigment in the macrophages lying on the rods coated by 10 and 5 w-% silver nitrate. This was presumably caused by the presence of silver compounds, which were thus ingested, then transported from the muscle and later excreted from the body.

There were marked eosinophilic reactions at 1 week and 1 month after implantation. Eosinophilic reactions in rabbit muscle implantation tests have been noticed in both the studies of Talja et al. [21] and Isotalo et al. [22]. Since there were marked eosinophilic reactions with silicone, which is considered very biocompatible, it seems that this type of reaction is of minor importance.

It seems that blending silver nitrate or ofloxacine up to 5 weight-% to caprolactone-L-lactide coating of SR-PLLA does not have any essential effect on the biocompatibility.This is very valuable information, because we know that both silver nitrate and ofloxacine coatings reduce bacterial adherence to SR-PLLA. Further studies are needed to ascertain the biocompatibility in the urethra before clinical applications. We are waiting with

interest to discover whether this new coating method is capable of preventing the bacterial colonization of urethral or ureteral stents during the insertion procedure.

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