J Mater Sci: Mater Med (2007) 18:1543–1547
DOI 10.1007/s10856-007-3060-3

Tissue biocompatibility of new biodegradable drug-eluting stent materials

Ilkka Uurto · Andres Kotsar · Taina Isotalo · Joonas Mikkonen · Paula M. Martikainen · Minna Kellomäki · Pertti Törmälä · Teuvo L. J. Tammela · Martti Talja · Juha-Pekka Salenius

Received: 15 May 2005 / Accepted: 5 June 2006 / Published online: 17 April 2007

Abstract Drug-eluting stents are a recent innovation for endovascular and endourethral purposes. The aim of this study was to assess the biocompatibility of new biodegradable drug-eluting stent materials in vivo. Rods made of SR-PLDLA (self-reinforced poly-96L,4D-lactic acid) covered with P(50L/50D)LA and rods made of 96L/4D SR-PLA and covered with P(50L/50D)LA including indomethacin 3.3 μg/mm^2 or dexamethasone 1.5 μg/mm^2, were inserted into the dorsal muscles of 20 rabbits serving as test animals. Rods made of silicone and organotin-positive polyvinylchloride were used as negative and positive controls. The animals were sacrificed after 1 week, 1 month, 2 months or 4 months. Histological changes attributable to the operative trauma were seen in all specimens at 1 week and 1 month. At 2 months both dexamethasone and indomethacin induced less fibrosis than the plain SR-PLDLA covered with P(50L/50D)LA without drug. At 4 months dexamethasone induced both chronic inflammatory changes and foreign body reaction, whereas the reactions in the indomethacin and drug-free plain SR-PLDLA groups were insignificant. The new biodegradable drug-eluting stent materials are highly biocompatible. Drug-eluting biodegradable stents may offer a promising new treatment modality for vascular and urethral diseases. However, further studies are needed to demonstrate their feasibility and efficacy.

I. Uurto
Department of Surgery, Satakunta Central Hospital, Pori, Finland

A. Kotsar · T. L. J. Tammela
Department of Urology, Tampere University Hospital and Medical School, University of Tampere, Tampere, Finland

I. Uurto · J.-P. Salenius
Department of Vascular Surgery, Tampere University Hospital and Medical School, University of Tampere, Tampere, Finland

T. Isotalo · M. Talja
Department of Surgery, Päijät-Häme Central Hospital, Lahti, Finland

J. Mikkonen · M. Kellomäki · P. Törmälä
Institute of Biomaterials, Tampere University of Technology, Tampere, Finland

P. M. Martikainen
Department of Pathology, Centre for Laboratory Medicine, Tampere University Hospital, Tampere, Finland

I. Uurto (✉)
Research unit, Tampere University Hospital, P.O. Box 2000, Tampere, Finland
e-mail: ilkka.uurto@uta.fi

Introduction

Neointimal hyperplasia is the key phenomenon leading to restenosis after endovascular procedures such as stenting [1]. Drug-eluting stents have been introduced to reduce this negative development and results in coronary artery disease have been excellent [2]. The restenosis rate has fallen below 10% after successful revascularization of the target lesion with drug-eluting stents [3, 4].

A variety of endourethral stents have been used to treat patients with recurrent urethral strictures, but no ideal device has yet been introduced. To solve this problem the development of spiral biodegradable urethral stents commenced in Finland in the late 1980s. The biodegradable materials used in urology were high-molecular-weight polymers of polylactide (PLA) or polyglycolide (PGA) [5]. Today stents materials are the same as previously but these

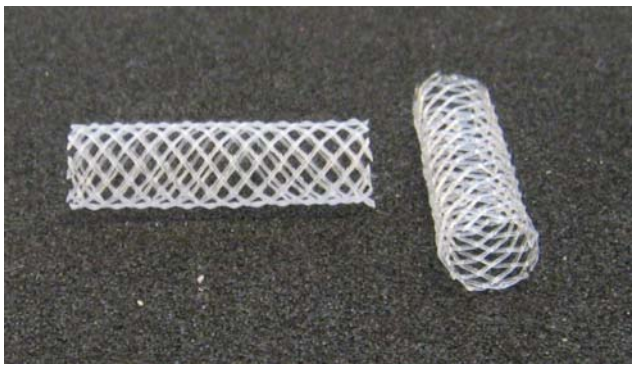

Fig. 1 Braided biodegradable self-reinforced PLA stent

are made by braiding monofilaments over a mandrel to form a mesh design (Fig. 1).

Lactic acid exists as two enantiomers L (+) -lactic acid and D (–) -lactic acid and the degradation rate of the copolymer can be adjusted by selecting the ratio of the eneatiomers [6]. Copolymers with molecular chains consisting of repeating units of both of these monomers can be polymerized from the two isomers.

To achieve good mechanical properties stents have been developed from self-reinforced (SR) PGA and (SR) PLA wires by the extrusion and solid-state drawing techniques, this significantly enhancing the elasticity and toughness of biodegradable polymers [7]. The viscoelastic property of self-reinforced biodegradable polymers confers expansion capacity on biodegradable stents. The expansion rate depends on the material, the internal arrangement of molecular chains, the diameter of the stent wire, the initial outer diameter of the stent and the processing conditions [8]. The biodegradation time of stents may vary from 3 (PLGA) to 12 (PLA) months depending on the materials and the processing methods used [9].

Biodegradable vascular stents have been used clinically in the treatment of coronary artery occlusive disease [10], while in urology urethral stents have been used in the treatment of recurrent urethral strictures [11, 12] and benign prostatic hyperplasia [13–15].

New materials have been investigated with an eye to local drug delivery. In this context biodegradable materials offer interesting prospects [10, 16]. The coating and the matrix can deliver different types of drugs which may have different targets and pharmacokinetics, leaving no foreign material at the implantation site. This offers a good opportunity to affect different tissue mediators on different time scales.

The present aim was to evaluate the tissue biocompatibility of new biodegradable self-reinforced poly-96L,4D-lactide acid (SR-PLDLA) drug-eluting stent materials for endoluminal use in a rabbit dorsal muscle implantation test.

Materials and methods

The self-expandable biodegradable drug-eluting stent materials investigated here were designed and made at the Institute of Biomaterials, Tampere University of Technology, Finland. The rods of stent material implanted in the dorsal muscle were manufactured of 96L/4D SR-PLA with 96 parts L-lactide and four parts D-lactide (Purac Biochem, Netherlands). During self-reinforcing the rods were processed into ellipsoidal shape of size 1.0/0.8 mm and cut to a length of 50 mm. They were then coated using different solutions. The first solution contained 100 mg of dexamethasone (Orion Pharma, Finland), 500 mg of P(50D/50L)LA with a L-lactide portion of 50% and a D-lactide portion of 50% (Boehring Ingelheim, Germany), with 10 ml of acetone as solvent. The second solution contained 200 mg of indomethacin (Orion Pharma, Finland), 500 mg of P(50D/50L)LA and 10 ml of acetone, and the third contained 500 mg of P(50D/50L)LA and 10 ml of acetone, functioning as reference material for the drug-containing coatings. The rods were coated by dipping them into the coating solution and air dried between dipping procedures to achieve a sufficient thick coating (on average 7 μm) on the rods. The acetone was carefully evaporated from the coatings by drying in vacuum, and finally the rods were cut into 11-mm-long pieces. Thereafter the rods were sterilized using gamma irradiation at 25 kGy, temperature remaining below 42°C. The drug amount in the rods was in the case of dexamethasone 1.5 μg/mm^2, and in indomethacin 3.3 μg/mm^2.

All animal protocols were reviewed and approved by the institutional committee for animal research and by the Western-Finland Provincial Government. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

A total of 20 male rabbits (New Zealand White) were used as test animals. The animals were anesthetized with medetomidine hydrochloride 0.3 ml/kg i.m. (Domitor® 1 mg/ml, Orion Pharma, Finland) and ketamine hydrochloride 0.3 ml/kg i.m. (Ketalar® 50 mg/ml, Pfizer, USA). All animals received a single dose of enrofloxacin 5 mg/kg s.c. (Baytril vet.® 100 mg/ml, Bayer, Germany) as antibacterial prophylactic. A dorsal midline incision was made and the implants were placed under visual control longitudinally on both sides of the dorsal muscle, using a hollow needle (2.0 mm) and pushing with a trocar. Eight rods were implanted per animal. Each animal had one positive (organotin polyvinylchloride), one negative (silicon) and six study materials randomly selected according to the ISO standard [17]. The implantation sites were marked on the fascia with 4–0 non-absorbable polypropylene (Prolene®, Ethicon Inc, USA) sutures.

The animals were harvested at 1 week, 1 month, 2 months and 4 months after implantation by administering an overdose of pentobarbital sodium i.v. (Mebunat® 60 mg/ml, Orion Pharma, Finland). The test specimens with surrounding muscular tissue were excised. After fixation in 10% phosphate-buffered formalin the samples were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Histological specimens were blindly analyzed by a pathologist. The biological response parameters assessed and recorded included acute inflammatory changes, necrosis, chronic inflammatory changes, foreign body reaction, fibrosis and eosinophilia. Analysis was performed according to ISO standards which doesn't include statistical analysis. Tissue reactions were scored semi-quantitatively according to the following criteria: 0 = no reaction, 1 = mild reaction, 2 = moderate reaction and 3 = severe reaction.

All results are presented as mean ± standard deviation (SD) unless otherwise stated.

Results

All samples were perceptiple after the follow-up. Acute inflammatory reactions and chronic inflammatory changes due to operative trauma were seen in all specimens at 1 week (Fig. 2). The acute inflammatory reactions had disappeared at 1 month, whereas the disappearance of chronic inflammatory changes took place at 2 months. At 4 months dexamethasone was seen to have induced more chronic inflammatory changes than the other materials (Fig. 2).

The most rapid decrease in fibrosis formation was seen at 1 month in the indomethacin group (Fig. 2) and at 2 months both indomethacin and dexamethasone induced clearly less fibrosis than the drug-free plain SR-PLDLA material.

Decreasing slight eosinophilic reactions were seen with all specimens during the follow up (Table 1). Dexamethasone induced mild necrosis at 1 week and 4 months as well as mild to moderate foreign body reactions throughout the study (Figs. 2, 3, Table 1).

Discussion

Drug-eluting stents have revolutionized the endovascular treatment of coronary arteries. A variety of bioactive agents have been studied, but it seems that antiproliferative agents act most effectively [2]. The local delivery of these agents has inspired researchers to seek innovative solutions. Biodegradable stents and coatings offer one possible means for local delivery.

Pre-clinical evaluation of new stent materials is fundamental in demonstrating their safety and efficacy. Tests protocols should be standardized and reporting methods reliable [18]. Cytotoxicity and biocompatibility testing should be undertaken with every new biomaterial. Cell culture models are the most widely used methods to demonstrate toxicity [19] and standardized animal implantation tests are essential in biocompatibility testing prior to clinical studies [20].

We have previously shown that poly-L-lactide (PLLA) causes no toxicity in cell cultures [21]. This present study confirms our previous results on the good biocompatibility of polylactide [22]. PLLA material can thus be used safely as a stent platform.

Indomethacin and dexamethasone are well-known agents which have been used clinically for many years. Recent work suggests that besides anti-inflammatory properties non-steroidal, anti-inflammatory drugs

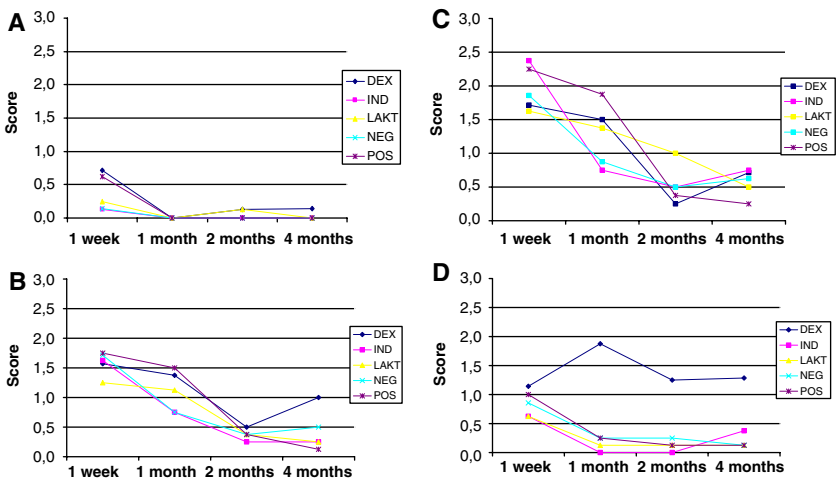

Fig. 2 Semiquantitative analysis of histological changes. (**A**) Acute inflammatory changes. (**B**) Chronic inflammatory changes. (**C**) Fibrosis. (**D**) Foreign body reaction

Table 1 Semiquantitative histological screening at 1 week, 1 month, 2 months and 4 months

		Fibrosis (±SD)	Eosinophilia (±SD)	Chronic inflammation (±SD)	Acute inflammation (±SD)	Necrosis (±SD)	Foreign body (±SD)
1 week	DEX	1.71 (0.95)	0.71 (0.95)	1.57 (0.53)	0.71 (0.95)	0.29 (0.49)	1.14 (1.07)
1 week	IND	2.38 (0.74)	0.38 (0.74)	1.63 (0.52)	0.13 (0.35)	0.00 (0.00)	0.63 (0.52)
1 week	PLA	1.63 (1.19)	0.88 (0.83)	1.25 (0.89)	0.25 (0.46)	0.00 (0.00)	0.63 (0.74)
1 week	NEG	1.86 (0.69)	0.71 (0.76)	1.71 (0.49)	0.14 (0.38)	0.00 (0.00)	0.86 (0.69)
1 week	POS	2.25 (0.71)	0.25 (0.46)	1.75 (0.46)	0.63 (0.74)	0.00 (0.00)	1.00 (0.53)
1 month	DEX	1.50 (1.07)	0.13 (0.35)	1.38 (0.74)	0.00 (0.00)	0.00 (0.00)	1.88 (1.25)
1 month	IND	0.75 (0.89)	0.75 (1.04)	0.75 (0.89)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
1 month	PLA	1.38 (1.06)	0.38 (0.74)	1.13 (0.83)	0.00 (0.00)	0.00 (0.00)	0.13 (0.35)
1 month	NEG	0.88 (0.83)	0.50 (0.76)	0.75 (1.04)	0.00 (0.00)	0.00 (0.00)	0.25 (0.46)
1 month	POS	1.88 (0.83)	0.38 (1.06)	1.50 (0.93)	0.00 (0.00)	0.00 (0.00)	0.25 (0.46)
2 months	DEX	0.25 (0.71)	0.13 (0.35)	0.50 (0.53)	0.13 (0.35)	0.63 (0.52)	1.25 (1.04)
2 months	IND	0.50 (0.93)	0.25 (0.71)	0.25 (0.46)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
2 months	PLA	1.00 (1.07)	0.00 (0.00)	0.38 (0.52)	0.13 (0.35)	0.00 (0.00)	0.13 (0.35)
2 months	NEG	0.50 (0.76)	0.00 (0.00)	0.38 (0.52)	0.00 (0.00)	0.00 (0.00)	0.25 (0.46)
2 months	POS	0.38 (0.74)	0.00 (0.00)	0.38 (0.74)	0.00 (0.00)	0.00 (0.00)	0.13 (0.35)
4 months	DEX	0.71 (0.76)	0.00 (0.00)	1.00 (0.58)	0.14 (0.38)	0.57 (0.53)	1.29 (0.76)
4 months	IND	0.75 (0.89)	0.00 (0.00)	0.25 (0.46)	0.00 (0.00)	0.00 (0.00)	0.38 (0.74)
4 months	PLA	0.50 (0.76)	0.00 (0.00)	0.25 (0.46)	0.00 (0.00)	0.00 (0.00)	0.13 (0.35)
4 months	NEG	0.63 (0.74)	0.25 (0.71)	0.50 (0.76)	0.00 (0.00)	0.00 (0.00)	0.13 (0.35)
4 months	POS	0.25 (0.46)	0.00 (0.00)	0.13 (0.35)	0.00 (0.00)	0.00 (0.00)	0.13 (0.35)

Dexamethasone (DEX), Indomethacin (IND), Polylactic acid (PLA), Negative control (NEG) and Positive control (POS)

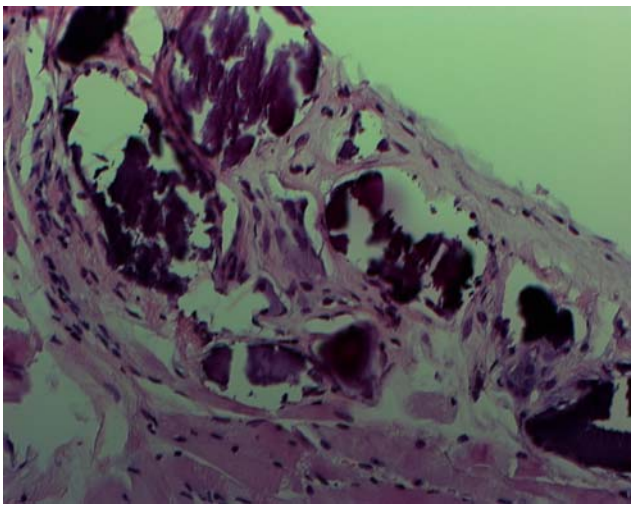

Fig. 3 Histological picture of SR-PLDLA dexamethasone rod inducing giant cell reaction, calcification and minimal fibrosis at 4th month (hematoxyllin eosin, original magnification ×40)

(NSAIDs) such as indomethacin have an antiproliferative effect on vascular smooth muscle cell growth [23]. To our knowledge there have been no studies using indomethacin eluting stents. Dexamethasone is a potent corticosteroid agent which exerts anti-inflammatory, immunosuppressive and antiproliferative effects. Strecker et al. implanted dexamethasone-eluting stents into canine femoral arteries and found that they significantly reduced intimal hyperplasia [24]. Also controversial results have been published [25]. Liu et al. has published the first human pilot trial (STRIDE) with dexamethasone-eluting coronary stents [26]. These stents had a concentration of 0.5 μg/mm^2 dexamethasone and the 6-month binary restenosis rate was 13.3%. Their study demonstrated the feasibility and safety of dexamethasone eluting stents.

Our study showed increased chronic and foreign body reactions during the follow-up with the dexamethasone-eluting rods compared to the plain lactide rods. This may be a question of drug doses. The amount of dexamethasone used in this study was 1.5 μg/mm^2, that is, a three times higher concentration than that used in the STRIDE trial [26]. Our finding indicates that dexamethasone can cause undesirable tissue changes in high doses, but that the safe tolerance for dosing is obviously wide and further studies are needed to establish the optimal level. The indomethacin-eluting rods were highly biocompatible, showing no more inflammatory reactions than the controls.

Fibroblast proliferation is one of the key phenomena in the formation of restenosis. Both tested drugs had a strong inhibitory effect on fibrosis formation after 2 months follow-up, suggesting beneficial effects in the prevention of restenosis.

Organotin polyvinylchloride (PVC) is a standard positive control material that recommended [27]. The PVC material showed no changes which could distinguish it from the negative control silicon, which weakens this study reliability. However, in our previous studies PVC caused marked chronic inflammatory changes and foreign body reactions increasingly during follow-up [28].

The biodegradable platform offers many opportunities compared to biostabile platforms such as the feasibility of local drug delivery. There remain many questions regarding this new treatment modality, for example the release kinetics and tissue concentrations of various drugs. In the current study we evaluated tissue reactions in a healthy muscle, whereas the real benefits of the new biodegradable drug-eluting stents must be evaluated in sclerotic vessels and strictured urethras. Standardized animal implantation tests must be performed before clinical studies.

We conclude that the new biodegradable drug-eluting stent materials showed good biocompatibility and can be used as a stent platform for local drug delivery. The dose of dexamethasone and indomethacin must be balanced against potential side-effects. Further studies with different drug concentrations needs to be evaluated and further studies are needed to demonstrate the feasibility and efficacy of this new self-expandable biodegradable drug-eluting stent.

Acknowledgements This study was financial supported by The National Technology Agency of Finland (TEKES). The drugs used (indomethacin and dexamethasone) were donated by Orion Pharma, Finland.

References

1. M. R. BENNET, *Coron. Dis.* **89** (2003) 218
2. S. T. LAROIA and A. T. LAROIA, *Cardiol. Rev.* **12**(1) (2004) 37
3. M. C. MORICE et al., *N. Engl. J. Med.* **346**(23) (2002) 1773
4. A. COLOMBO et al., *Circulation* **108** (2003) 788
5. M. TALJA et al., *J. Endourol.* **11** (1997) 1
6. D. E. CUTRIGHT et al., *Oral. Surg.* **37** (1974) 142
7. P. TÖRMÄLÄ, *Clin. Mater.* **10** (1992) 29
8. T. Välimaa and P. Törmälä, 1998, Stenting the urinary system (Oxford: Isis Medical Media Ltd), p. 53
9. T. VÄLIMAA and S. LAAKSOVIRTA, *Biomaterials* **25** (2004) 1225
10. H. TAMAI et al., *Circulation* **102**(4) (2000) 399
11. T. ISOTALO et al., *J. Urol.* **160** (1998) 2033
12. T. ISOTALO et al., *J. Endourol.* **16** (2002) 759
13. M. TALJA et al., *J. Urol.* **154** (1995) 2089
14. A. PETAS et al., *J. Urol.* **157** (1997) 173
15. A. Petas et al., *Br. J. Urol.* **80** (1997) 439
16. K. TSUJI et al., *Int. J. Cardiovasc. Interv.* **5**(1) (2003) 13
17. Biological evaluation of medical devices. Part 6. Tests for local effects after implantation (1994) ISO 10993-6
18. Biological evaluation of medical devices. Part 5. Tests for in vitro cytotoxicity, 1999, ISO 10993-5
19. I. UURTO et al., *J Endovasc Therapy.* **10** (2003) 1110
20. Biological evaluation of medical devices. Part 1. Evaluation and testing, 2003, ISO 10993-1
21. T. ISOTALO et al., *Urol. Res.* **27** (1999) 149
22. T. ISOTALO et al., *J. Endourol.*. **13**(7) 1999 525
23. G. BROOKS et al., J. *Pharm. Pharmacol.* **55** (2003) 519
24. E. P. STRECKER et al., *Cardiovasc. Interv. Radiol.* **21**(6) (1998) 487
25. A. M. LINCOFF et al., *J. Am. Coll. Cardiol.* **29**(4) (1997) 808
26. X. LIU et al., *Catheter. Cardiovasc. Interv.* **60**(2) (2003) 172
27. Biological evaluation of medical devices. Part 12. Sample preparation and reference material, 2002, ISO 10993-12
28. S. LAAKSOVIRTA et al., *J. Urol.* **167** (2002) 1527