Abstract

The introduction of drug-eluting stents (DES) to prevent in-stent restenosis is one of the major advances in interventional cardiology. Currently many types of DES are under evaluation for effectiveness and safety, a time-consuming and difficult procedure in humans. An animal model that allows rapid evaluation of the present and upcoming therapeutic approaches to prevent in-stent restenosis is most valuable and still lacking.

Here, a perivascular cuff to induce restenosis was constructed of a poly(ε-caprolactone) (PCL) formulation suitable for the controlled delivery of drugs. Placing the PCL cuff around the femoral artery, in vivo, resulted in reproducible restenosis-like lesions containing predominantly smooth muscle-actin positive cells. Loading the cuff with the anti-restenotic compounds paclitaxel and rapamycin resulted, in vitro, in a sustained and a dose-dependent release for at least 3 weeks. Paclitaxel- and rapamycin-eluting PCL cuffs placed around the femoral artery of mice in vivo significantly reduced intimal thickening by 76±2% and 75±6%, respectively, at 21 days. Perivascular sustained release of both anti-restenotic agents is restricted to the cuffed vessel segment with no systemic adverse effects or effect on cuffed contralateral femoral arteries. Drug-eluting poly(ε-caprolactone) cuffs provide an easy and rapid tool to evaluate anti-restenotic agents to be used in combination with the DES strategies.
Local perivascular delivery of anti-restenotic agents from a drug-eluting poly (ε-caprolactone) stent cuff

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Introduction

Percutaneous transluminal coronary angioplasty (PTCA) was introduced in the late 1970’s as a method to restore coronary blood flow in atherosclerotic coronary arteries in patients with (symptomatic) stenoses. Since then it has become widely accepted as an effective and safe treatment modality for single and multivessel coronary atherosclerotic disease. However, a major drawback to PTCA has been the occurrence of restenosis of the treated vessels, resulting in renewed symptoms and the need for repeated intervention in up to 50% of patients [1]. The introduction of intracoronary bare-metal stents reduced the restenosis rate within 6 months, however a smaller portion of the patients (20-30%) still suffered of so called in-stent restenosis [2,3]. Recently, drug-eluting stents (DES) loaded with the anti-proliferative compounds paclitaxel and rapamycin were introduced very successfully in interventional cardiology. The restenosis rate dropped from 20-30 to 1-3% at one year [4,5]. Many new anti-proliferative, anti-inflammatory, anti-migratory or pro-healing compounds to be loaded onto stents are currently under evaluation. These DES are supposed to inhibit inflammation and neo-intimal growth and subsequently in-stent restenosis. However, little is known concerning the potential adverse effects of these anti-restenotic agents on vessel wall integrity and (re-)healing, atherosclerotic lesions formation, progression, and plaque stability [see 6 for detailed review].

An animal model that allows rapid evaluation of the present and upcoming therapeutic approaches to prevent in-stent restenosis is most valuable and still lacking. One well-defined mouse model of restenosis consists of the placement of a non-constrictive perivascular polyethylene cuff around the mouse femoral artery, which results in a reproducible and concentric intimal thickening within 2 to 3 weeks, mainly consisting of rapid induction of smooth muscle cell proliferation [7-9].

Drug loaded polymer formulations, as the ones present in the majority of the DES coatings, are a rational technique to deliver compounds locally for a prolonged period of time to the vessel wall to inhibit intimal hyperplasia. Local application of drugs for anti-restenotic compounds evaluation is also possible using gelatin or pluronic (F-127) gels. A substantial disadvantage of these methods is that they are water-based, which restricts the half-life of the delivery system. Using a cuff made of a polymer suitable for eluting anti-restenotic compounds instead of the polyethylene cuff would be an important step towards a useful animal model for preclinical evaluating new DES strategies in mice. Poly(ε-caprolactone) (PCL) is a biocompatible and biodegradable polymer belonging to the aliphatic polyester family [10,11]. Extensive in vitro and in vivo biocompatibility and efficacy studies have been performed, resulting in U.S. Food and Drug Administration (FDA) approval of number of medical and drug delivery devices composed of PCL [12-15]. PCL has a relatively long biodegradation time and is therefore suitable for drug-eluting purposes [16,17]. In addition, PCL formulations have also been investigated as a stent eluting coating for paclitaxel in a rabbit model of restenosis [18] and in the Boston
Scientific DES program (TAXUS™) [19]. Polymeric formulations consisting of PCL blended with poly(ethylene glycol) (PEG) have been developed in the past for local delivery of anti-oncogenic drugs [20-22]. The relatively hydrophilic PEG dissolves into the aqueous medium and open channels within the PCL matrix through which water can penetrate and drugs can be sustainably diffused out.

In the present study the non-constrictive perivascular cuff to induce restenosis was constructed of a blended polymeric formulation of PCL and PEG suitable for controlled drug delivery. The novel drug-eluting PCL cuff described here simultaneously induces reproducible intimal hyperplasia and allows local delivery of anti-proliferative compounds to the vessel wall. This new approach gives the possibility to evaluate the effects of the tested compounds on neointima formation, vessel wall integrity, and potential side effects. We show that, in vitro, paclitaxel and rapamycin-eluting PCL cuffs give a sustained release of the drug for at least 3 weeks. Consequently, this sustained release resulted in a substantially reduced neointima formation for both anti-restenotic agents tested, in vivo, with no systemic adverse effects or effect on cuffed contralateral femoral arteries.

Materials and methods

Materials

Poly(ethylene glycol) 300 (PEG; H(OCH2CH2)nOH; MW 285–315) was obtained from J.T. Baker (Philipsburg, USA). Poly(ε-caprolactone) (PCL; [-O(CH2)5CO-]n; MW 10,000–20,000) was purchased from Polysciences Inc. (Warrenton, USA). Paclitaxel was kindly provided by Bristol-Myers Squibb Company (New Jersey, USA) and rapamycin was obtained from LC Laboratories (Woburn, USA). Phosphate-buffered saline (PBS) pH 7.4 was obtained from B. Braun (Melsungen, Germany) and n-octanol (C8H17OH; >99.0%) was supplied by Merck (Darmstadt, Germany).

Preparation of drug-eluting PCL cuffs

The PCL-based drug delivery cuffs were manufactured as previously described [20,23]. In brief, paclitaxel or rapamycin were first blended with PEG before this blend was mixed with molten PCL at 70°C. The PCL:PEG ratio was 4:1 (w/w). Drug-loaded polymer cuffs were made from the different blended molten drug-polymer mixtures and designed to fit around the femoral artery of mice. Drug-eluting PCL cuffs had the shape of a longitudinal cut cylinder with an internal diameter of 0.5mm, an external diameter of 1mm, a length of 2mm, and a weight of approximately 5mg.
In vitro release profiles of paclitaxel and rapamycin

PCL cuffs were loaded with 0.5%, 1%, 2.5%, and 5% (w/w) paclitaxel (n=5) or rapamycin (n=5) and in vitro release profiles for both drugs were performed as previously described [22]. Cuffs of each composition were placed in 20ml glass scintillation vials and cooled to 4°C. Five milliliters of iced-cold PBS pH 7.4 containing 0.2% bovine serum albumin (fraction V, Roche Diagnostics, Mannheim, Germany) were placed on top of the cuffs followed by 5ml of n-octanol. The n-octanol formed an upper immiscible phase on top of the PBS so that any drug released into the PBS would partition into the octanol phase. The vials were capped and incubated at 37°C. The concentration of either paclitaxel or rapamycin in the octanol phase was analyzed by UV-VIS absorbance methods (Pharmacia LKB Ultrospec III, Peak Tek Inc., Glenside, USA). This octanol phase was replaced back into the vial. UV-VIS analyses were performed by determining the absorbance at the specified wavelength for both paclitaxel (229nm) and rapamycin (277nm) using a double beam UV/VIS spectrophotometer (UVIKON 933, Kontron Instruments Ltd, Milan, Italy). Calibration graphs of both drugs in n-octanol were established by measuring the absorbance of a set of standards of each drug in octanol in the 0-50mg/ml concentration range. Both drugs are far more soluble in n-octanol than in PBS. This substantial difference in solubility ensured rapid release of the drug into the octanol phase.

In vivo release of paclitaxel

Paclitaxel of 5% (w/w) loaded PCL cuffs (n=4) was quantitatively extracted before and 21 days after placement in the animals by incubating the cuffs in 25ml of n-octanol overnight at 37°C. The paclitaxel concentration was determined by UV-VIS absorbance methods as described above and the percentage of paclitaxel released was calculated.

Femoral artery cuff mouse model

Male C57BL/6 mice, aged 10-12 weeks, were used in this study. At the time of surgery, mice were anaesthetized with an intraperitoneal injection of 5mg/kg Dormicum (Roche, Basel, Switzerland), 0.5mg/kg Dormitor (Orion, Helsinki, Finland) and 0.05mg/kg Fentanyl (Janssen, Geel, Belgium). The femoral artery was dissected from its surroundings. A non-constrictive polyethylene cuff (Portex, Kent, UK, 0.40mm inner diameter, 0.80mm outer diameter, 2.0mm length), an empty PCL cuff, a paclitaxel-, or a rapamycin-loaded PCL cuff (0.5%, 1%, 2.5%, and 5% (w/w)) was placed loosely around the femoral artery (n=6/group) [8]. The committee on animal welfare of TNO approved all animal experiments.

Blood parameters

Blood samples were collected in EDTA-coated vials (Sarstedt, Nümbrecht, Germany) by tail bleeding at the time of sacrifice (n=6/group). Plasma cholesterol and triglyceride
levels were measured enzymatically using commercially available kits (Roche Diagnostics, Mannheim, Germany) following standard protocols. Plasma alanine aminotransferase (ALAT) concentration was measured enzymatically (Reflotrons, Roche Diagnostics, Mannheim, Germany) as described by the manufacturer. Total blood leukocyte (CD45+), T-cell (CD3+), B-cell (CD19+) and monocyte/granulocyte (CD11b+) numbers were determined by fluorescence-activated cell sorting (FACS) analysis (FACSCalibur, BD Biosciences, California, USA) of whole blood using a PerCP-CY5.5-conjugated rat anti-mouse CD45 monoclonal antibody, a fluorescein isothiocyanate (FITC)-conjugated hamster anti-mouse CD3 monoclonal antibody, a R-Phycoerythrin (R-PE) conjugated rat anti-mouse CD19 monoclonal antibody, and an allophycocyanin (APC)-conjugated rat anti-mouse CD11b monoclonal antibody, respectively following standard protocol (TruCOUNT, BD Biosciences, California, USA). Haematocrit (HT) tubes (Hawksley, West Sussex, UK) were used to collect blood and HT percentage was calculated as the ratio between erythrocytes and total blood.

Histological assessment of intimal lesions

Animals were sacrificed after 21 days. The thorax was opened and a mild pressure-perfusion (100mmHg) with 4% formaldehyde in 0.9% NaCl (v/v) for 5 minutes was performed by cardiac puncture. After perfusion, femoral artery was harvested, fixed overnight in 4% formaldehyde, dehydrated and paraffin embedded. Equally spaced cross-sections (200mm; 5mm thick) were used throughout the entire length of the cuffed femoral artery for histological analysis. All samples were routinely stained with haematoxylin-phloxine-saffron (HPS). Weigert’s elastin staining was used to visualize elastic laminae. Smooth muscle cells were visualized with a smooth muscle cell actin staining (1:1600, Roche, Mannheim, Germany). Anti-PECAM-1 antibodies (1:200, Sigma, St. Louis, USA) were used as endothelial cell marker and AIA 31240 macrophage staining (1:3000, Accurate Chemical, Wesbury, USA) was used to detect monocytes/macrophages.

Quantification of intimal lesions in sections of cuffed femoral artery

Six equally spaced cross-sections (200mm; 5mm thick) were used in all mice to quantify intimal lesions. Using image analysis software (Leica Qwin, Wetzlar, Germany), total cross sectional medial area was measured between the external and internal elastic lamina; total cross sectional intimal area was measured between the endothelial cell monolayer and the internal elastic lamina. All data are presented as mean±SEM. Data were analyzed using the Mann-Whitney U-test (SPSS 11.5 for Windows). P-values less than 0.05 were regarded as statistically significant.
Results

Characterization and quantification of intimal lesions in polyethylene and PCL cuffed femoral arteries

To evaluate if a PCL cuff was able to induce reproducible neointima formation equally to the established polyethylene cuff, mice received either a polyethylene or a PCL cuff around the femoral artery. Twenty-one days after the placement of a polyethylene or a PCL cuff, light microscopy of transverse sections through the cuffed femoral artery revealed a comparable thickening of the intimal region, while proximal and distal sections of the cuffed femoral artery possessed normal histology (data not shown). This intimal thickening was two to four cell layers thick and consisted predominantly of α-smooth muscle cell actin-positive cells. Both polyethylene and PCL cuff-induced neointima showed an intact endothelial cell layer and inner elastic lamina. Furthermore, macrophage infiltrates were not detected in the neointimal area, but were identically present in the granulation tissue within the cuff, indicating an equal inflammatory response induced by both cuff materials (Figure 1). Quantification of intimal lesions induced either by a polyethylene or PCL cuff revealed no significant differences in neointima formation between both cuffs (4.7±0.9 vs. 4.7±0.5x10^3 μm^2, p=0.6). Moreover, no differences in media (12.2±1.2 vs. 11.0±0.6x10^3 μm^2, p=0.1) or intima/media ratio (0.37±0.07 vs. 0.43±0.04, p=0.4) were observed in intimal lesions induced by both polyethylene and PCL cuffs.

In vitro and in vivo release of anti-restenotic agents from drug-eluting PCL cuffs

Paclitaxel

PCL cuffs were loaded with 0.5%, 1%, 2.5%, and 5% (w/w) paclitaxel and their in vitro release profile was determined. As shown in Figure 2a, paclitaxel showed a sustained and dose-dependent release from the PCL cuffs for a 21-day period. Paclitaxel percentage release was almost complete for the lower loading dosages (0.5%: 92.1±10.1%; 1%: 96.9±55.1%) whereas the cuffs with the higher loadings still contained paclitaxel (2.5%: 67.2±0.9%; 5%: 57.3±3.4%) after 21 days of in vitro release. Similarly, it was possible to extract paclitaxel from a PCL cuff placed in vivo. A 5% (w/w) paclitaxel-eluting PCL cuff that was placed around the femoral artery for 21 days and subsequently extracted with n-octanol showed that in this time period a comparable amount of paclitaxel was released in vivo (22.9±2.4 μg released/mg cuff, corresponding with 47.7±1.1% still present in the cuff) as in vitro. These data demonstrate that after 3 weeks similar amounts of paclitaxel were released both in vitro as well as in vivo from the PCL cuffs.
Figure 1. Cross-section of cuffed murine femoral artery 21 days after placement of either a polyethylene or a PCL cuff.

Weigert’s elastin staining. Cuffs constructed with both materials (polyethylene or PCL) show comparable intimal hyperplasia and an intact inner elastic lamina. Haemotoxilin-phloxine-saffron (HPS) staining. A multiple cell layer thick intimal hyperplasia is observed in both types of polymer cuffs. Alpha smooth muscle cell actin staining for smooth muscle cells. Intimal hyperplasia predominantly consists of alpha smooth muscle cell positive cells in both cuffs. PECAM-1 staining for endothelial cells. In both mice receiving a poly-ethylene or PCL cuff endothelial cells are present at the luminal side of the intimal lesion. AIA 31240 staining for macrophages. Macrophages are present in granulation tissue within both cuffs. Magnification 200x.
Rapamycin

To investigate if rapamycin could also be loaded into and released from our drug-eluting PCL cuffs they were loaded with 0.5%, 1%, 2.5%, and 5% (w/w) rapamycin and their in vitro release profile was assessed. Rapamycin, as paclitaxel, showed a sustained and dose dependent release from the PCL cuffs for a 21-day period (Figure 2b). Release followed zero-order kinetics (amount released proportional with time) with minimal burst and little inter-cuff variability. Total release after 21 days was: 0.5%: 75.2±5.3%; 1%: 82.9±4.8%; 2.5%: 66.1±0.6%; 5%: 39.4±2.5%.

Effect of anti-restenotic agents perivascular delivery on neointima formation in vivo

Paclitaxel

To assess the effect of paclitaxel perivascular delivery using a drug-eluting PCL cuff on neointima formation, PCL cuffs were loaded with 0.5%, 1%, 2.5%, and 5% (w/w) paclitaxel and placed around the femoral artery of C57BL/6 mice for 21 days. Morphometric analysis of the cuffed femoral artery revealed that, after 3 weeks, a neointima had formed in mice receiving an empty PCL cuff. Mice receiving a 0.5% (w/w) paclitaxel-eluting PCL cuff developed a neointima with comparable size (PCL cuff: 4.7±0.5; 0.5%: 4.2±0.5x10³ μm², p=0.6; Figure 3). Most importantly, animals receiving a 1%, 2.5%, and 5% (w/w) paclitaxel-eluting PCL cuffs showed a strongly reduced development of neointimal tissue or almost complete absence of intimal hyperplasia (1%: 1.3±0.4, p=0.002; 2.5%: 0.9±0.2, p=0.001; 5%: 0.6±0.2, p=0.001).

Figure 2. In vitro release profiles of PCL cuffs loaded with increasing percentages of (A.) paclitaxel and (B.) rapamycin for a 21-day period.

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Values are shown as mean±SEM of five individual cuffs.
No significant effects were observed in media size of the murine cuffed femoral arteries. Intima/media ratios from the empty PCL cuff and 0.5% (w/w) paclitaxel-eluting PCL cuff were similar (0.43±0.04 vs. 0.32±0.04, p=0.1), whereas higher paclitaxel loadings resulted in a significant reduction in intima/media ratio (1%: 0.11±0.03, p=0.001; 2.5%: 0.09±0.01, p=0.001; 5%: 0.08±0.02, p=0.002) as compared to empty PCL cuff.

Rapamycin

To evaluate if rapamycin perivascular delivery with our drug-eluting cuff could also inhibit neointima formation, PCL cuffs were loaded with 0.5%, 1%, 2.5%, and 5% (w/w) rapamycin and placed around the femoral artery of mice for 3 weeks. In animals receiving an empty PCL cuff the neointima was two to four cell layers thick, whereas in animals receiving a rapamycin-eluting PCL cuff the neointima is maximally one or two cell layers thick (Figure 4). Quantification revealed a significant difference in neointima formation between empty PCL cuff and all rapamycin loading dosages tested (PCL cuff: 4.4±0.5; 0.5%: 1.4±0.2, p=0.001; 1%: 1.4±0.4, p=0.003; 2.5%: 0.9±0.2, p=0.001; 5%: 0.9±0.2x10^3 μm^2, p=0.001; Figure 4). No decrease in media size was observed. Rapamycin perivascular treatment resulted in a significant decrease in intima/media ratios for all loading dosages (PCL cuff: 0.36±0.06; 0.5%: 0.16±0.02, p=0.007; 1%: 0.17±0.04, p=0.027; 2.5%: 0.13±0.02, p=0.004; 5%: 0.14±0.02, p=0.004) as compared to empty PCL cuff.

Systemic effects of perivascular delivery of anti-restenotic drugs

Both anti-restenotic compounds tested in our drug-eluting PCL cuff are known to have systemic adverse effects. Systemic delivery of paclitaxel (Taxols, Bristol-Myers Squibb Company, New Jersey, USA) is known, according to the manufacturer, to induce bone marrow suppression (primarily neutropenia) and, to a minor extent, to elevate hepatic enzymes levels. To test whether locally delivered paclitaxel in our new model had any adverse systemic effect, specific blood and liver parameters were analysed in animals receiving either an empty PCL or a 5% (w/w) paclitaxel-eluting PCL cuff. No differences in total blood leukocyte (CD45+) (PCL cuff: 11.0±1.9, 5%: 10.2±1.0x10^6 cells/ml, p=0.5), T-cell (CD3+) (PCL cuff: 1.9±0.1, 5%: 1.9±0.3x10^6 cells/ml, p=1.0), B-cell (CD19+) (PCL cuff: 6.5±0.5, 5%: 5.8±1.3x10^6 cells/ml, p=0.3) and monocyte/granulocyte (CD11b+) (PCL cuff: 2.6±0.3, 5%: 2.6±1.0x10^6 cells/ml, p=0.5) or in plasma alanine aminotransferase (ALAT) levels (PCL cuff: 23.5±1.3, 5%: 23.7±2.7U/l, p=0.9) were observed between groups.

As for paclitaxel, oral rapamycin (Rapamunes, Wyeth-Ayerst Laboratories, New Jersey, USA) treatment is identified to cause hypercholesterolemia, hypertriglyceridemia and anaemia, as described in manufacturer product information sheet. For that reason, also the effects of locally rapamycin delivery on these systemic parameters were evaluated. No differences in plasma cholesterol (PCL cuff: 1.9±0.1; 5%: 2.1±0.1mM, p=0.2),
triglycerides levels (PCL cuff: 0.7±0.1; 5%: 0.7±0.2mM, p=0.8) or haematocrit percentage (PCL cuff: 48.6±1.2; 5%: 49.2±2.4%, p=0.6) were observed between animals receiving an empty PCL or a 5% (w/w) rapamycin-eluting PCL cuff. In addition, to investigate if anti-restenotic drugs perivascular delivery with our drug-eluting PCL cuff had some systemic effects on neointima formation, four mice received a 5% (w/w) paclitaxel-eluting PCL cuff in the right femoral artery and an empty PCL cuff in the contralateral left femoral artery. As shown in Figure 5, perivascular delivery of paclitaxel using a PCL cuff had no effect on neointima formation on the left contralateral femoral artery (PCL cuff: 5.3±1.1; 5%: 0.9±0.1×10³μm², p=0.02) indicating a high perivascular localized delivery of paclitaxel to the cuffed vessel segment.
Discussion

In the present study we demonstrate that the use of drug-eluting stents (DES) can be mimicked in a mouse model of restenosis by using a drug releasing perivascular cuff constructed of a blend of poly(ε-caprolactone) (PCL) with poly(ethylene glycol) (PEG) [20]. This PCL-based cuff can induce reproducible restenosis-like lesions in the femoral artery similarly to the established polyethylene cuff (Figure 1) and can easily be loaded with anti-restenotic compounds, i.e. paclitaxel and rapamycin, to give an in vitro sustained and dose-dependent release for at least 3 weeks (Figure 2). Cuffs containing higher loadings of paclitaxel (1-5% (w/w)) reduced intimal thickness by 76±2% at 21 days (Figure 3). Likewise, locally released rapamycin resulted in an inhibition of neointima formation by
75±6%, for all tested concentrations (Figure 4). Moreover, our experiments demonstrate that perivascular sustained release of both anti-restenotic drugs studied is restricted to the cuffed vessel segment with no systemic adverse effects or effect on cuffed contralateral femoral arteries (Figure 5).

Paclitaxel-eluting stents used in human studies [6] are coated with polymeric formulations containing paclitaxel at a concentration of 1μg/mm². Although the physical structure of our drug-eluting PCL cuff is different from that of a DES, e.g. the thickness of the polymer layer is quite different, we tried to estimate the paclitaxel eluted from the inner layer of the PCL cuff. From these calculations it appeared that the 0.5% (w/w) paclitaxel-eluting PCL cuff delivers approximately 2.5μg/mm² paclitaxel to the cuffed vessel segment in a 3 weeks period. In our model this concentration failed to suppress neointima formation probably due to the difference in physical structure or due to the fact that the delivery is periadventitial instead of intraluminal as in the DES. However, paclitaxel delivered in this way at higher concentrations inhibited neointima formation. Studies by Winternitz et al. [20] showed that lower paclitaxel loadings in PCL:PEG formulation are completely dissolved but at higher loadings paclitaxel is present in the polymer matrix as monolithic dispersions. As a result, the mechanism of paclitaxel release from cuffs loaded with low concentrations is different from that of higher loadings (Figure 2a). This difference could also contribute to the failure of 0.5% (w/w) paclitaxel-eluting PCL cuff to inhibit intimal

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**Figure 5.**

Total intimal area of contralateral empty PCL and 5% (w/w) paclitaxel-eluting PCL cuffed murine femoral arteries 21 days after cuff placement. Total intimal area was quantified by image analysis using six serial sections in each cuffed artery and expressed in mm² (mean±SEM, n=4). * p<0.05.
hyperplasia. Remarkably, the 0.5% (w/w) rapamycin-eluting PCL cuff showed to be effective in inhibiting restenosis. This might be explained by a higher effectiveness of rapamycin as an anti-restenotic agent when compared to paclitaxel.

Local delivery of drugs is preferred to systemic therapy for the treatment of restenosis mainly because of the systemic side effects associated with the antirestenotic compounds used. Local application of drugs for antirestenotic compounds evaluation is also possible using gelatin or pluronic (F-127) gels. The latter delivery system has been demonstrated to be an effective carrier for drugs to be applied locally to the adventitia [24-27]. Nevertheless, a substantial disadvantage of this method is that it is a water-based gel which restricts the half-life of this delivery system. As an example, Fulton et al. [24] found that after 5 days, 80% of the antisense oligonucleotides to proliferating cell nuclear antigen (PCNA) were released from the pluronic gel. In the same way, Ishizaka et al. [25] found that 7 days after cilostazol local delivery the drug concentration was 96% decreased when compared to the first day. To our opinion when working with pluronic gels it is difficult to achieve control release in time especially around small murine blood vessels. Moreover, high concentrations of hydrophobic compounds, such as rapamycin and paclitaxel, are difficult to attain given that there is limited physical space where the gel can be applied perivascularly to the vessel wall. Recently, Schachner et al. [26] locally applied a pluronic gel with a low and high dose of rapamycin for 1, 2, 4 and 6 weeks in a mouse model of vein graft disease. Interestingly, only the high dose was statistically significant in reducing neointimal hyperplasia in experimental vein grafts at 1 and 2 weeks. Oppositely, our new drug-eluting PCL cuff is enhanced when compared to the pluronic gel delivery system given that it is not water-based, and shows a sustained and controlled release of both paclitaxel and rapamycin for at least a 3 week period.

Presently, several drugs are under investigation in animals and humans to be used in combination with DES to prevent restenosis. Some of these drugs, such as paclitaxel and rapamycin, have proven to be clinically effective in preventing neointima formation and are currently used worldwide. However, not all drugs screened so far have been successful in preventing restenosis in humans. These results urge that preclinical studies are necessary to further evaluate the efficacy of DES. To our opinion, a model that allows an easy and rapid assessment of the present and forthcoming anti-restenotic agents is most valuable to efficiently evaluate the efficacy of these drugs before the start of expensive and time-consuming human clinical trials. Another current concern of the DES is clinical safety. Only limited pathological data on human coronary arteries with DES is available. We do think that our novel drug-eluting PCL cuff is helpful to evaluate the effects of anti-restenotic agents on the vessel wall pathology and on progression of the restenotic process, which in humans is not possible.
Conclusions
In the present study we report the development of a polymeric drug-eluting cuff that simultaneously induces reproducible intimal hyperplasia and allows local perivascular delivery of compounds to the vessel wall with no systemic adverse effects. This new approach provides the possibility to evaluate the effectiveness and safety of new anti-restenotic agents to be used in combination with the DES strategy in a rapid and easy animal model of restenosis, as we demonstrated for paclitaxel and rapamycin.

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