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A Rapamycin-Releasing Perivascular Polymeric Sheath Produces Highly Effective Inhibition of Intimal Hyperplasia

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Abstract

Intimal hyperplasia produces restenosis (re-narrowing) of the vessel lumen following vascular intervention. Drugs that inhibit intimal hyperplasia have been developed, however there is currently no clinical method of perivascular drug-delivery to prevent restenosis following open surgical procedures. Here we report a poly(ϵ -caprolactone) (PCL) sheath that is highly effective in preventing intimal hyperplasia through perivascular delivery of rapamycin.

We first screened a series of bioresorbable polymers, *i.e.*, poly(lactide-co-glycolide) (PLGA), poly(lactic acid) (PLLA), PCL, and their blends, to identify desired release kinetics and sheath physical properties. Both PLGA and PLLA sheaths produced minimal (<30%) rapamycin release within 50 days in PBS buffer. In contrast, PCL sheaths exhibited more rapid and near-linear release kinetics, as well as durable integrity (>90 days) as evidenced in both scanning electron microscopy and subcutaneous embedding experiments. Moreover, a PCL sheath deployed around balloon-injured rat carotid arteries was associated with a minimum rate of thrombosis compared to PLGA and PLLA. Morphometric analysis and immunohistochemistry revealed that rapamycin-loaded perivascular PCL sheaths produced pronounced (85%) inhibition of intimal hyperplasia (0.15 ± 0.05 vs 1.01 ± 0.16), without impairment of the luminal endothelium, the vessel's anti-thrombotic layer.

Our data collectively show that a rapamycin-loaded PCL delivery system produces substantial mitigation of neointima, likely due to its favorable physical properties leading to a stable yet

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flexible perivascular sheath and steady and prolonged release kinetics. Thus, a PCL sheath may provide useful scaffolding for devising effective perivascular drug delivery particularly suited for preventing restenosis following open vascular surgery.

Keywords

Intimal hyperplasia; poly(ϵ -caprolactone) (PCL) sheath; rapamycin; perivascular drug delivery; open vascular surgery

Introduction

Intimal hyperplasia leads to restenosis, or the pathological re-narrowing of a blood vessel following vascular intervention. Restenosis develops after balloon angioplasty of atherosclerotic lesions, or following open surgical procedures such as bypass or endarterectomy where injury is inflicted to the vessel wall [1]. Neointimal plaque is typically formed by proliferative vascular smooth muscle cells (SMCs) from the media [2] or myofibroblasts that migrate from the perivascular layers into the neointimal space [3]. Despite our in depth understanding of this process as well as the development of inhibitors, treatments for restenotic disease have lagged because of the lack of an optimal clinical means of drug delivery [4].

Over the past decade substantial clinical progress has been made in the treatment of post-angioplasty restenosis using drug-eluting stents. However, these intravascular delivery systems are not applicable to *open* surgical procedures (~300,000 cases per year in the US alone)[5], including bypass, endarterectomy and dialysis access. Even drug eluting stents as a method of drug delivery are imperfect in that residual stenosis remains and there is damage to the endothelium and consequential thrombosis [6, 7]. These limitations as well as the need for options for open surgery have led to attempts to develop perivascular delivery systems. At the time of open surgery, the treated vessel is readily accessible, making application of drug more direct and easily achievable. On the other hand, there remains a conspicuous lack of clinical options to prevent intimal hyperplasia following open vascular surgeries. A major obstacle is the absence of a viable technique for perivascular local drug delivery.

A number of methods have been explored for perivascular delivery of anti-proliferative drugs to reconstructed arteries or veins using a variety of polymers as a vehicle, including drug-releasing polymer gel [8]/depots [9], microspheres [10, 11], cuffs [12], wraps/films [13–16], or meshes [17]. While each method has its own advantages, none has advanced to clinical trials, likely due to various limitations revealed in animal studies, such as moderate efficacy, lack of biodegradation, or mechanical stress to the blood vessel. Thus, there remains an unmet clinical need for a perivascular delivery system that is durable yet biodegradable, non-disruptive to the vessel, can release drug in a controlled and sustained manner, and ultimately, is highly effective in preventing intimal hyperplasia.

The goal of this study was to develop a perivascular deliver system with optimized polymer properties and drug release kinetics to improve the treatment of restenosis. To this end, we

first screened a series of bioresorbable polymers and blends to optimize the *in vitro* release profiles of rapamycin (Sirolimus), an anti-proliferative drug clinically used in drug-eluting stents [18]. We then employed a rat model of intimal hyperplasia to evaluate the efficacy of the prescreened, rapamycin-laden polymer sheaths for inhibition of neointima formation. We found that a poly(ϵ -caprolactone) (PCL) sheath exhibited desirable rapamycin release kinetics *in vitro*, and when applied in the perivascular space, produced a dramatic inhibitory effect on intimal hyperplasia (85% reduction) without the side effect of endothelial damage. Our results suggest that the PCL sheath provides an ideal vehicle for preclinical testing of inhibitors of intimal hyperplasia, as well as a template for designing novel methods of perivascular drug delivery applicable for open vascular surgery.

Materials and Methods

Materials

Rapamycin was purchased from LC Laboratories (Woburn, MA). Poly(D,L-lactic-co-glycolic acid) (PLGA) (85:15, average M_w =50K–70K), poly(L-lactide acid) (PLLA) (M_w =85K–160K), and poly(ϵ -caprolactone) (PCL) (M_w =80K), dimethyl sulfoxide (DMSO), and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO). Chloroform, isopropyl alcohol, phosphate buffered saline (PBS) and sodium azide were from Thermo Fisher Scientific (Hampton, NH). All other reagents were purchased from Thermo Fisher unless otherwise stated.

Preparation of polymeric sheaths with and without rapamycin

Polymeric sheaths were prepared with a solvent casting method (see Figure 1A for schematic). Briefly, 10 mg rapamycin was dissolved in 2.2 ml of chloroform, 220 mg of polymer (PLGA, PLLA, or PCL) was then added into this solution and stirred for 30 min in the dark. The polymer/rapamycin mixture was casted into a 60 mm PTFE dish and kept in a fume hood for 48 h to evaporate the solvent. The casted films were cut into 1cm $\times$ 1cm sheets and subsequently vacuum dried overnight in the dark to eliminate residual solvent. It is estimated that each half-sized polymer sheath used for *in vivo* experiments contains ~100 μ g rapamycin, which is in the range of concentrations proven to be effective for inhibiting restenosis in the rat balloon angioplasty model [19]. Control polymer sheaths were prepared using the same procedures but with no rapamycin added. All types of polymer sheaths were examined by field emission scanning electron microscopy (FE-SEM; LEO 1530, Zeiss, Germany) after sputter coating with gold. Rapamycin-loaded polymeric sheaths were stored at -20°C until use.

In vitro rapamycin release from polymeric sheaths

In order to efficiently screen multiple polymers, we used an *in vitro* system to evaluate their rapamycin release kinetics. In a 0.6 ml microcentrifuge tube, a rapamycin-loaded polymeric sheath (1cm $\times$ 1cm) was incubated in 500 μ l release medium of PBS buffer (pH 7.4) containing 0.02% NaN_3 and 10% isopropyl alcohol (IPA), which was included to inhibit rapamycin degradation. At each indicated time point, 200 μ l of the release medium was replaced with equal volume of fresh medium and the former was transferred into a UV-free 96-well plate (Sigma-Aldrich, St. Louis, MO). The concentration of rapamycin was then

measured by determining the absorbance at 278 nm using a microplate reader (Bio-Rad, Hercules, CA). A calibration standard curve was prepared in the same release medium and used to calculate the amount of released rapamycin (Figure S1). At the end of release experiment, all the polymer sheaths were collected and vacuum dried. FE-SEM was conducted on each type of polymer sample to examine its morphological changes after release of rapamycin.

Rat carotid artery balloon injury model

The experiments involving animal use were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol (Permit Number: M02273) was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Wisconsin-Madison. All surgery was performed under isoflurane anesthesia (through inhaling, flow rate 2 ml/min), and all efforts were made to minimize suffering. Animals were euthanized in a chamber gradually filled with CO₂.

Carotid artery balloon injury was performed in male Sprague-Dawley rats (Charles River, ~350g) as previously described[20]. Briefly, rats were anesthetized, and a 2F Fogarty catheter (Edwards Lifesciences, Irvine, CA) was inserted into the left common carotid artery *via* an arteriotomy in the external carotid artery. To produce arterial injury, the balloon was inflated and withdrew to the carotid bifurcation and this action was repeated three times. The external carotid artery was then permanently ligated, and blood flow was resumed. A half-sized polymeric sheet (1cm × 0.5 cm) loaded with rapamycin was longitudinally placed onto the injured segment (~1.5 cm) of the common carotid artery and wrapped in such a way that a sheath is formed to cover ~ 90% of the outer surface of the vessel (Figure 1, B and C). In control animals, a polymeric sheath without rapamycin was applied to the injured carotid segment equivalent to that in rapamycin-treated animals. The animals used for control and rapamycin treatment were the same litter of rats. The neck incision was then closed using a suture and animals were kept on a 37°C warm pad for recovery.

Morphometric analysis of intimal hyperplasia

Two weeks after balloon injury, animals were anesthetized, and then perfused at a physiological pressure of 100 mmHg, with PBS followed by paraformaldehyde for fixation [20]. Balloon-injured artery segments treated with either a control PCL sheath or a rapamycin-containing PCL sheath were collected from the same part of common carotid arteries in the control animal group or rapamycin treatment group. Paraffin sections (5 μm thick) were excised at equally spaced intervals and stained with hematoxylin-eosin (H&E) for morphometric analysis, as described in our previous reports [20, 21]. Eight sections from each of 4 animals in the control group or 6 animals in the rapamycin treatment group were used. Images were acquired using a Nikon Ti-U Eclipse microscope equipped with the Nikon Elements software packages. The areas enclosed respectively by the external elastic lamina (EEL) and the internal elastic lamina (IEL) and lumen area were measured using the NIH Image J software as previously described [20]. Intimal area (IEL area minus lumen area) and medial area (EEL area minus IEL area) were then calculated. Intimal hyperplasia was assessed with the area ratio of intima versus media. For each of these parameters, data

from all 8 sections were pooled to generate the mean for each animal. The means from all the animals in each treatment group were then averaged, and standard error of the mean (SE) was calculated.

Immunostaining for in vivo assessment of cell proliferation and re-endothelialization

By following our published method [20, 21], immunostaining was performed on paraffin-embedded carotid artery sections collected on day 14 after balloon injury, a time point that represents most rapid neointima accumulation after injury[22, 23]. At least 8 sections from each of 4 (control) or 6 (rapamycin treatment) animals were used. To determine cell proliferation, a rabbit monoclonal antibody against a marker of proliferative cells, Ki67 (Abcam, Cambridge, MA) was used at a 1:500 working dilution. The sections received antigen retrieval using citrate buffer (pH 6) in an 80°C water bath for 2h followed by permeabilization with 0.25% triton. After 1h incubation with the primary antibody at room temperature, the sections were incubated with the ImmPRESS HRP-conjugated goat-anti-rabbit secondary antibody (Vector Laboratories, 1:200 dilution) for 30 min, followed by visualization with 3, 3-diaminobenzidine (DAB) and counterstaining with hematoxylin.

For quantification, on each section images were taken from six different fields (magnification 200×). The Ki67 positive cells in the medial and neointimal layers were manually counted. The number of Ki67-positive cells in each 200× image was defined as Ki67-positive cells per field. The data were pooled to generate the mean and standard deviation for each animal. The means from each of 4 (control) or 6 (rapamycin treatment) animals were averaged, and the standard error (SE) was calculated for each animal group.

To assess re-endothelialization, immunostaining of CD31 (an endothelial cell marker) was performed using a goat anti-CD31 primary antibody (R&D Systems, Minneapolis, MN, 1:150 dilution) and a biotinylated rabbit-anti-goat secondary antibody. Staining of CD31 was visualized by using streptavidin-HRP and DAB. For quantification, the luminal perimeter and the percentage of this perimeter that stained for CD31 on each section were measured using Image J. The percentage of re-endothelialization was scored from 1 to 5 (1: <20%; 2: 20 to 40 %; 3: 40 to 60%; 4: 60 to 80%; 5: 80–100%) and the scores were then averaged [24].

Statistical analysis

All data are presented as a mean $\pm$ standard deviation (SD) or standard error (SE). Statistical analysis was conducted using two-tailed unpaired Student's t-test. A difference is considered statistically significant when a *P* value is < 0.05. Quantification of morphometry and immunostaining was performed by a student blinded to experimental conditions.

Results and Discussion

Characterization of polymeric sheaths

The polymeric sheaths with or without rapamycin loaded were fabricated through a solvent-casting method (Figure 1A). Our pre-tests showed that film thickness between 20–100 μ m could be controlled by the amount of polymer added into the PTFE dish. To produce

sufficient mechanical flexibility necessary for use as a perivascular sheath, the polymer films were prepared with an average thickness of around 50 μm . The incorporation of rapamycin did not significantly change the appearance of these polymer sheaths. Both rapamycin-loaded PLGA and PLLA sheaths appeared to be semi-transparent whereas the PCL sheath appeared opaque due to their intermediate crystallinity (Figure 2, top row). FE-SEM images revealed morphological difference between the 3 types of polymer sheaths at a micro-scale level (Figure 2). The surfaces of PLGA and PLLA sheaths appeared smooth with no evidence of rapamycin precipitate. It is noteworthy that numerous small cracks were observed on PLLA sheath which might have been caused by the vacuum drying process. In comparison, PCL sheaths exhibited a rougher morphology. We can speculate that the rougher morphology on the PCL surface might be due to surface associated rapamycin that has not homogeneously incorporated into the polymer material. The hydrophobicity of the three polymers increases in the following order: PLGA<PLLA<PCL [25]. Probably due to a high hydrophobicity, the solubility of PCL in the chloroform solvent (>100 mg/ml) is much higher than that of rapamycin (~5 mg/ml). As such, PCL might have a good competitive edge over rapamycin for staying dissolved in chloroform by the end of evaporation thus leading to precipitation of rapamycin and surface roughness. With PLGA and PLLA, rapamycin may have remained dissolved until the end of solvent evaporation, resulting in no observed roughness on the surface of these two polymers.

Release of rapamycin from polymeric sheaths in vitro

The choice of polymers had a dominant effect on the release kinetics of rapamycin (Figure 3). The release from PLGA was sustained over the first 30 days and then followed by accelerated release in the last 20 days. While the PLLA sheath provided very slow release of rapamycin throughout, the PCL sheath produced a faster, near-linear release of rapamycin over 50 days (Figure 3A). We found that after 50 days of release, 10% and 46% of rapamycin was released from PLLA and PLGA, respectively, whereas nearly 100% rapamycin was released from the PCL sheath within the same time frame. Analysis of daily release revealed a minor initial burst of rapamycin from all 3 polymers over the first 10 days, although the PCL sheath showed faster release compared to the other two during this period (Figure 3B). The distinct release profiles from different polymers may have been attributable to inherent physicochemical properties of the polymers and the interaction between rapamycin and the polymer. The homogeneous and smooth surface of PLGA and PLLA sheaths suggests that rapamycin was evenly distributed in the polymer matrix, thus the slow release from these materials may have resulted from gradual water penetration into the matrix and associated polymer degradation and resorption. The sudden acceleration of rapamycin release from PLGA after 30 days was likely caused by bulk degradation of the polymer; this is supported by the highly porous structure of the PLGA sheaths after 50 days of rapamycin release (Figure 3C). In contrast, a faster and sustained release of rapamycin from PCL might have resulted from the dissolution of rapamycin from surface-associated or embedded rapamycin precipitates. This speculation is supported by a previous study in which similar release profiles were observed by Khan *et al.* during carrier free rapamycin release from a drug eluting stent [26]. The small pits left on the PCL surface after 50 days could have been caused by dissolution of rapamycin precipitates (Figure 3C).

To further demonstrate our ability to refine the release kinetics of rapamycin from polymeric sheaths, we fabricated a series of PLGA/PCL blends with different ratios (Figure 4A). Pure PLGA and pure PCL showed distinct profiles of drug release that were sustained for at least 45 days (Figure 4A). Interestingly, blending different ratios of PCL into PLGA substantially altered the PLGA release kinetics, resulting in a series of curves demonstrating accelerated release of rapamycin similar to that from pure PCL. These results show that manipulating polymer composition can be an effective approach to modulating drug release kinetics. Taken together, the foregoing *in vitro* release experiments demonstrate that the PCL sheath releases rapamycin gradually over a long period of time (>45 days) and is thus favorable for our goal of producing steady and sustainable drug release.

Rapamycin-laden PCL sheath dramatically reduces intimal hyperplasia without compromising re-endothelialization in the rat carotid artery injury model

We then compared *in vivo* 3 types of polymeric sheaths, PLGA, PLLA, and PCL, each representing distinct physical properties and *in vitro* rapamycin release kinetics. We performed balloon injury in the rat carotid artery, which is a widely accepted animal model of intimal hyperplasia. Immediately following balloon injury, a polymeric sheath loaded with rapamycin, or a control sheath without rapamycin was placed around the artery, and secured in place by surrounding tissues (Figure 1C). Animals were sacrificed on day 14 for morphometric analysis.

While both PLLA and PLGA produced frequent arterial thrombosis, thrombosis was rare with the PCL sheath (found only in 2 animals in the control group). Among 12 rats treated with a PCL sheath, 10 animals (4 for control, 6 for rapamycin) were without apparent pathology (thrombosis, infection, or scarring). We then measured the development of intimal hyperplasia (as an area ratio of intima/media) on H&E-stained carotid sections collected from these animals (Figure 5, A and B). We found that the rapamycin-loaded PCL sheath dramatically reduced intimal hyperplasia by 85% compared to control PCL sheath (0.15 ± 0.05 vs 1.01 ± 0.16) (Figure 5C). As a result, the lumen area was increased by 155% (6.13 ± 0.51 vs 3.98 ± 0.63) (Figure 5D). The efficacy of the rapamycin-loaded PCL sheath is deemed excellent compared to ~50% reduction of intimal hyperplasia generally reported in the literature using various other perivascular delivery systems [8, 13, 17]. In addition, ki67-positive (proliferative) cells were significantly reduced by more than 40% in the medial and neointimal layers in the arteries treated with rapamycin-PCL sheaths, as compared to control arteries (Figure 6). Since an established function of rapamycin is inhibition of SMC proliferation and migration [27], these data manifest that the PCL sheath effectively delivered rapamycin into SMCs in the vessel wall to mitigate the growth of neointimal plaque.

Shrinkage of vessel wall, or constrictive remodeling, is often an important contributor to the loss of lumen size in addition to intimal hyperplasia [28]. There is evidence that rapamycin, when intraluminally applied in drug-eluting stents, is associated with stent-edge constrictive remodeling of the vessel, compromising its efficacy in improvement of lumen patency[29]. With the PCL sheath however, we did not observe constrictive remodeling, as demonstrated by no change in EEL length (Figure 5E), which is a surrogate for overall vessel size.

It has been well documented that intraluminal implantation of rapamycin-eluting stents impair endothelial cell proliferation and hence re-endothelialization, producing the adverse side effect of acute or late vascular thrombosis [30, 31]. To evaluate whether extraluminal delivery of rapamycin *via* a PCL sheath inhibits re-endothelialization, we performed immunostaining for CD31, a marker of the endothelial layer (Figure 7, A and B). We found similar levels of CD31 staining in the arteries treated with a rapamycin-PCL sheath and in those treated with a control sheath (Figure 7C). This indicates that the recovery of endothelium 14 days after denudation caused by balloon injury was not impaired by rapamycin delivered from a perivascular PCL sheath. We postulate that diffusion of rapamycin from a PCL sheath placed outside the artery likely generated a gradient of the drug with higher concentrations in the perivascular layers (adventitia and media) where neointima-forming cells originate, but with diminishing concentrations toward the lumen thereby preserving the luminal endothelium.

Favorable properties of the PCL sheath that facilitate optimal *in vivo* outcomes

There are many limitations of drug eluting stents. Most notable is the inability to use stents in open surgical procedures. Even in situations where stents are appropriate (following angioplasty) they are associated with life-threatening thrombosis. These complexities have prompted efforts to develop stent-free drug delivery strategies, such as perivascular delivery systems. Although a number of perivascular delivery platforms have been developed and tested, there have been various shortcomings. The PCL formulation that we have developed and evaluated in the current study was intended to circumvent some of the major limitations. PCL is a desirable base material for the intended biomedical application, as extensive previous *in vitro* and *in vivo* studies indicate that PCL is a safe, biocompatible polymer. PCL is FDA approved for use in a number of medical devices, including drug delivery devices [12]. PCL also has a relatively long time of biodegradation and is therefore suitable for sustained drug release.

Physical properties of polymers used for perivascular drug delivery are important with regard to the ease of deployment as well as interference with the vessel wall and arterial hemodynamics. At the time of deployment around the artery, both PLGA and PLLA sheaths were very soft whereas the PCL sheath had greater rigidity. Upon collection of carotid arteries 14 days after surgery, both PLGA and PLLA sheaths became so stiff and hardened that it was difficult to extract these sheaths from the artery. In contrast, the rigidity and shape of the PCL sheaths were well maintained without the development of excessive stiffness. These observations regarding polymer physical properties are consistent with the known properties of these materials, as PCL is more ductile than other bioresorbable polymers, and PLLA and PLGA are known to become rigid and brittle after uptake of water. Interestingly, 2 out of 4 animals tested with PLGA and 12 out of 14 animals tested with PLLA developed thrombotic occlusion in the treated carotid arteries (Figure S2). In contrast, in 12 animals treated with a PCL sheath, thrombus was found only in two animals, both in the control group. We regularly encounter thrombosis in a small portion of animals undergoing balloon angioplasty. This drastic difference between PCL and the other two polymers underscores the influence of physical properties of polymer drug carriers on the outcomes of their perivascular application.

Indeed, it is well known that either physical stress imposed onto the vessel wall or disrupted hemodynamics can produce injury to the vessel wall leading to intimal hyperplasia. For example, a PCL cuff has been used to induce intimal hyperplasia in a mouse femoral model [12]; another animal model of intimal hyperplasia is developed by partially ligating the mouse carotid artery to alter the dynamics of blood flow [32]. In the present study, the *in vivo* hardening of PLGA and PLLA sheaths generated a wrinkled surface resulting in constriction of the arterial wall (Figure S2). This likely led to abnormal hemodynamics which is known to damage the inner lining of endothelium producing thrombosis [31]. However, an even and somewhat smooth surface was maintained with the PCL sheath with little impingement on the artery (Figures S2 and S3), which may have helped prevent thrombosis. Moreover, we wrapped the PCL sheath around the artery but the sheath was not fully circumferential so as to leave the polymer sheath open on the top (Figure 1, B and C). This may have provided sufficient flexibility preventing constriction.

Drug-releasing hydrogels (pluronic gel [20], Re-gel [9] and hydrogels with microspheres [10, 11]) have been used to minimize physical stress to the vessel wall and for convenient perivascular application of drug; both approaches have proven effective in mitigating intimal hyperplasia. The major drawback of these materials includes a relatively short life (< 2 weeks) and high mobility that often results in the need for repeated application [9]. The PCL sheath used in the current study remained intact at least for 90 days when subcutaneously embedded (PLGA and PLLA sheaths were partially dissolved at 15 days and 90 days, respectively) (Figure S3). This excellent durability of PCL is a desired feature for sustained drug delivery in humans, where non-regressive intimal plaque develops for up to two years after reconstructive surgery. A rapamycin-eluting collagen wrap was recently tested in humans and deemed safe [33]. However, the lack of appropriate controls mitigated any definitive conclusions regarding efficacy; moreover, low physical strength and durability of this wrap is a major issue. A polyester mesh coated with a copolymer of L-lactic acid and ϵ -caprolactone was developed for perivascular release of rapamycin [17]. While this polymer mesh was flexible and relatively easy to deploy, polyester is not a bioresorbable material, compromising the potential of this mesh for clinical use. Another report revealed that rapamycin released from a PCL stent cuff reduced intimal hyperplasia in mouse femoral arteries [12]. Ironically in this study the PCL cuff was originally devised to interfere with normal hemodynamics in order to induce intimal hyperplasia.

Finally, release kinetics plays an important role in the sustainability and efficacy of any drug delivery system. While most reported drug-eluting polymers demonstrate a typical first order cumulative release curve with a “burst” release in the initial hours/days [10, 12, 16, 34, 35], the PCL sheath revealed a near-linear, zero order release curve, demonstrating steady, sustained release of rapamycin for at least 50 days (Figures 3 and 4). A previous study using a rapamycin-releasing co-polymer of L-lactic acid and ϵ -caprolactone (PLA-CL) produced promising outcomes in reducing intimal hyperplasia [15]. However, it is not clear whether this polymer film produced drug release kinetics that would favor sustainable efficacy. As shown in Figure 4A, only ~20% of rapamycin was released from our preparation of PCL at 2 weeks, which generated a profound inhibitory effect on neointima. Suggesting that our approach will be durable, more than 30% of rapamycin still remained in the PCL sheath

after 45 days. It is thus reasonable to propose that there is a great potential to extend the inhibitory effect of our rapamycin-PCL format on neointimal hyperplasia for periods well beyond 45 days. Taken together, our data show that the PCL delivery system reported herewith confers multiple advantages that help circumvent many of the major shortcomings associated with the previously reported perivascular drug-delivery polymeric vehicles.

Conclusion

We have developed a PCL format for local drug delivery that can be conveniently deployed as a perivascular sheath at the time of open vascular surgery. In a rat model, 2 weeks after application, PCL sheaths loaded with rapamycin dramatically reduced neointima by 85% without showing side effects of either endothelial damage or constrictive remodeling. The excellent efficacy of this perivascular delivery system may be attributable to many factors. The major ones include: 1) Appropriate physical properties keeping the shape as well as flexibility of the PCL sheath suitable for normal vessel wall physiology. 2) Sustained, nearly linear drug release kinetics. 3) Perivascular drug delivery evenly spread along the PCL sheath/artery interface. 4) Excellent durability (at least 3 months *in vivo*). Future studies in a long-term animal model such as rat vein graft [36] are warranted. Moreover, for preclinical studies, using a porcine model [24] that may more closely recapitulate the human conditions should be considered. Thus, our present study suggests that this PCL sheath provides a viable platform for future development of a safe and efficacious perivascular drug delivery method to treat recurrent vascular disease, particularly for patients undergoing open vascular reconstruction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Schematic of polymer sheath fabrication and its perivascular application: (A). Fabrication of polymer sheaths is described in detail in Materials and Methods. (B). Rat carotid artery is intraluminally injured with a balloon catheter, and a polymer sheath is wrapped along the injured segment. Yellow patches represent neointimal plaque. (C). The picture shows a PCL sheath (green arrow, 1 cm × 0.5 cm) wrapped around a balloon-injured rat carotid artery (blue arrow). Note that the sheath does not fully cover the artery, with an open slot generating flexibility.

Figure 2. Gross view of sheaths made of different polymers and their surface morphology: Each polymer sheet is a 1 cm × 1 cm square. SEM images were obtained with a low magnification (scale bar = 100 μm) and high magnification (scale bar = 1 μm).

Figure 3.

Rapamycin release from PLGA, PLLA, and PCL sheaths: (A). Cumulative release measured as percentage of rapamycin. Each data point is a mean $\pm$ SD (n=3). (B). Daily release of rapamycin. Each bar is a mean $\pm$ SD (n=3). (C). SEM images of the polymer sheaths that were recovered from PBS buffer after 50 days of rapamycin release. Low Mag, scale bar = 10 μm ; High Mag, scale bar = 1 μm .

Figure 4. Effect of blending PCL into PLGA on rapamycin release kinetics: (A). Cumulative release measured as percentage of total amount of rapamycin. Each data point is a mean $\pm$ SD (n=3). (B). Daily release of rapamycin. Each bar is a mean $\pm$ SD (n=3).

Figure 5.

Inhibitory effect of rapamycin-releasing PCL sheath on intimal hyperplasia in balloon-injured rat carotid arteries: Morphometric analysis was performed on the H&E-stained sections of rat carotid arteries that were collected on day 14 after balloon angioplasty. A and B show representative sections from arteries treated with control and rapamycin-loaded PCL sheaths, respectively. Arrow indicates external elastic lamina (EEL); arrowheads mark internal elastic lamina (IEL). Neointima is encircled inside IEL. C, D, and E are quantified data of intimal hyperplasia (ratio of intima/media area), lumen area, and EEL length, respectively. Each bar represents a mean ($\pm$ SE) of 4 control or 6 rapamycin-treated animals, * $P < 0.05$.

Figure 6.

Inhibitory effect of rapamycin-releasing PCL sheaths on cell proliferation in balloon-injured rat carotid arteries: Rat carotid cross-sections were obtained from the same experiments as in Figure 5. Sections were immunostained for Ki67 as described in Methods. (A) and (B) show representative microscopic images of Ki67 staining in arteries treated with control and rapamycin-PCL sheaths, respectively. Brown arrow points to Ki67 positive cells (stained brown); arrowhead marks internal elastic lamina (IEL). (C). Quantification of Ki67 positive cell number per field on sections. Each bar represents a mean $\pm$ SE of 4 control or 6 rapamycin-treated animals, * $P < 0.05$.

Figure 7. Perivascular rapamycin-PCL sheaths do not affect re-endothelialization in balloon-injured rat carotid arteries: Rat carotid cross-sections were obtained from the same experiments as in Figure 5. Sections were immunostained for CD31 as described in Methods. (A) and (B) are representative microscopic images of CD31 staining (see brown periluminal lining indicated by a black arrow) of arteries treated with control and rapamycin-PCL sheaths, respectively. Arrowhead marks internal elastic lamina (IEL). (C) Quantification of re-endothelialization was performed by scoring CD31 positive versus total perimeter, as described in Materials and Methods. Each bar represents a mean $\pm$ SE of 4 control or 6 rapamycin-treated animals, * $P < 0.05$.