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A biodegradable perivascular wrap for controlled, local and directed drug delivery

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Abstract

Perivascular delivery of anti-proliferative agents is an attractive approach to inhibit hyperplasia that causes stenosis of synthetic hemodialysis grafts and other vascular grafts. Perivascular drug delivery systems typically release drugs to both the vascular wall and non-target extravascular tissue. The objective of this study was to develop a biodegradable, perivascular delivery system for localized, sustained and *unidirectional* drug release in the context of synthetic arteriovenous (AV) grafts used for chronic hemodialysis. To this end, a dense non-porous polymer barrier layer was laminated to either i) a drug-loaded non-porous polymer layer, or ii) a porous polymer layer. To provide tuneability, the porous layer could be loaded with drug during casting or later infused with a drug-loaded hydrogel. The polymer bilayer wraps were prepared by a solvent casting, thermal-phase inversion technique using either polylactide-co-glycolide (PLGA) or polycaprolactone (PCL). Sunitinib, a multi-target receptor tyrosine kinase inhibitor, was used as a model drug. In a modified transwell chamber system, the barrier function of the non-porous PLGA backing was superior to the non-porous PCL backing although both markedly inhibited drug diffusion. As assessed by *in vitro* release assays, drug release duration from the drug-loaded non-porous PCL construct was almost 4-fold greater than release from the porous PCL construct infused with drug-laden hydrogel (22 days vs. 5 days); release duration from the drug-loaded non-porous PLGA construct was prolonged approximately 3-fold over release from the porous PLGA construct infused with drug-laden hydrogel (9 days vs. 3 days). Complete *in vitro* degradation of the PLGA porous and non-porous constructs occurred by approximately 35 days whereas the PCL constructs remained intact even after most drug was released (49 days). The PLGA non-porous bilayer wrap containing 143±5.5 mg sunitinib in the inner layer was chosen for further pharmacokinetic assessment *in vivo* where the construct was placed around the external jugular vein in a porcine model. At one week, no drug was detected by HPLC/MS/MS in any examined extravascular tissue whereas high levels of drug were detected in the wrapped vein segment (1048

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ng/g tissue). At four weeks, drug was detected in adjacent muscle (52 ng/g tissue) but 13-fold greater amounts were detected in the wrapped vein segment (1742 ng/g tissue). These results indicate that the barrier layer effectively impedes extravascular drug loss. Tensile testing showed that the initially flexible PLGA construct stiffened with hydration, a phenomenon also observed after *in vivo* placement. This characteristic may be useful to resist undue circumferential venous tensile stress produced in AV grafting. The PLGA wrap bilayer formulation is a promising perivascular drug delivery design for local treatment of hemodialysis AV graft hyperplasia and possibly other hyperplastic vascular disorders.

Keywords

hyperplasia; drug delivery; perivascular wrap; inflammation; sunitinib; arteriovenous graft

Introduction

Neointimal hyperplasia (NH), a overgrowth of cells into the intimal layer of blood vessels, remains a common pathology affecting interpositional vein grafts, catheterized veins, and arteries treated with balloon angioplasty, often resulting in stenosis and occlusion. NH is a particularly pervasive problem afflicting synthetic arteriovenous (AV) grafts used for chronic hemodialysis, resulting in thrombotic occlusion of up to 50% of grafts within the first year of placement [1]. Yet no effective prevention strategies exist in clinical practice. Stenosis typically occurs at the graft-venous anastomosis, caused by proliferation and migration of adventitial fibroblasts and medial smooth muscle cells (SMCs) to the intima [2]. Many factors likely trigger NH development, including (i) the presence of the synthetic graft material that initiates a foreign body response, (ii) introduction of arterial blood into the vein causing flow disturbances and elevated tensile stress, (iii) frequent needle punctures into the graft for chronic dialysis, and (iv) persistent uremia. That these factors are chronic and not acute or transient is significant to the clinical problem and sustained prevention of NH in the AV graft may require repeat treatments.

The first-line treatment for AV graft stenosis is percutaneous angioplasty; yet this treatment does not improve graft survival [3, 4]. Improved restenosis rates with stent placement after angioplasty compared to angioplasty alone have been reported, but similar thrombotic occlusion rates were observed between the two groups [5]. Systemic drug strategies to prevent AV graft stenosis have produced largely disappointing results [6]. As the outer adventitial and medial layers of the vascular wall are considered to be primary contributors of cells that participate in NH development in AV grafts [7], a *perivascular delivery approach* for anti-hyperplastic agents is logical. We have reported using ultrasound-guided perivascular injection of a drug-loaded polymer gel to graft-vein anastomoses in animal models of AV graft stenosis [8, 9]. High concentrations of drug were achieved in the vessel wall but also in non-target tissue, and the polymer gel was found to migrate from the site of application. A localized perivascular wrap would better target and retain drug at the AV site. A *biodegradable* wrap is also desirable so that the venous tissue would be accessible to later perivascular delivery of drugs by injection since repeat applications may be needed to counteract chronic pro-hyperplastic factors that afflict AV grafts. Lastly a means to inhibit release of drug from the wrap to the extravascular space would prevent off-target drug effects.

Edelman and others have reported use of ethylene-vinyl acetate (EVA) polymer wraps for perivascular drug delivery [10, 11]. Of note, an EVA wrap was used to deliver paclitaxel to the graft-vein anastomosis to significantly decrease luminal stenosis in a porcine AV graft model [12]. As the hemodialysis AV graft is subjected to repeated and chronic insults,

repeat exposure to drug may be necessary to maintain patency over the graft lifetime. However, EVA films are non-degradable and would inhibit repeat perivascular application of drug to the vein wall. Biomedical polymers, such as polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA), are biodegradable and are readily fabricated into thin flexible sheets suitable for an implantable perivascular wrap. Inhibition of NH was reported with perivascular delivery of paclitaxel from a PCL wrap to a rat carotid artery after balloon injury [13], or to a sheep AV graft model using a PLGA wrap [14]. However, a large multi-center clinical trial was initiated to test the efficacy of the PLGA-based paclitaxel-eluting wrap for preventing stenosis in AV grafts, but was prematurely terminated due to increased infection incidence. Most recently, in a small clinical trial, a collagen-based wrap was used to deliver sirolimus to AV grafts [15]. Although the trial was too small to determine efficacy, no adverse events were reported.

Platelet-derived growth factor (PDGF) is a potent mitogen that plays an essential role in initiating SMC migration and proliferation [16, 17]. PDGF and PDGF receptor expression is up-regulated in the vasculature after injury [18, 19] and a polyclonal antibody specific for PDGF inhibited NH in a rat arterial injury model [20]. Systemically delivered pharmacological inhibitors of PDGF receptor tyrosine kinase activity (e.g., imatinib) also inhibit arterial NH [21, 22]. Of particular relevance to this study, one group has reported perivascular delivery of a tyrosine kinase inhibitor, tyrphostin AG-51, via a Pluronic gel to attenuate hyperplasia formation in a rabbit interpositional bypass vein graft model [23]. Sunitinib, a multi-target receptor tyrosine kinase inhibitor of both PDGF receptor subtypes and all three VEGF receptor subtypes (KIT, FLT3, and CSR-1R [24]). VEGF has also been shown to contribute to NH by increasing smooth muscle cell activation at the anastomotic region [25]. Thus, the capabilities of sunitinib to inhibit *both* PDGF and VEGF activities could prove beneficial to treating AV stenosis. However, the therapeutic efficacy of sunitinib in preventing NH has not yet been evaluated. Sunitinib with its plurality of actions may be a better drug than imatinib for inhibiting NH in AV grafts and was utilized in the present proof-of-concept studies.

This manuscript describes development of a biodegradable perivascular wrap as a drug delivery device intended to limit loss of drug to the non-target extravascular space while delivering drug to the target vessel. The wrap is biodegradable so not to obstruct later repeat perivascular drug application that could counteract chronic pro-proliferative forces present in the AV graft setting. We characterized resorbable and tunable polymer vascular wraps comprising dense and porous bilayers of either PCL or PLGA for directed perivascular delivery of drug. Unidirectional drug release from a drug-loaded polymer layer is focused on the vascular wall by a dense monolithic polymer barrier backing that blocks drug release to non-vascular tissue. The second drug-loaded layer overlaid onto the first polymer barrier layer during wrap fabrication can be tailored to release drug with varying kinetics and loads. *In vitro* drug release profiles for sunitinib were assessed for each wrap formulation. Additionally, wrap mechanical properties and *in vivo* tissue distribution of sunitinib from a PLGA non-porous bilayer wrap formulation over a one-month time course in a porcine model are reported.

Methods

Materials

Sunitinib malate (>99% purity) and sunitinib free-base (FB) (>99% purity) (LC Laboratories, Woburn, USA), drug standard sunitinib-d10 (Toronto Research Chemicals, North York, Canada), Glycosil™ (thiolated hyaluronic acid (HA)) and Extralink™ (polyethylene glycol diacrylate (PEGDA)) (Glycosan Biosystems, Salt Lake City, USA), bovine testicular hyaluronidase (HAse), acetonitrile (ACN), methyl-tert-butyl ether

(MTBE), ethanol, and acetone (HPLC grade) and polycaprolactone (PCL) of 14 kDa, 45 kDa, and 80 kDa mol wt was from Sigma-Aldrich (St. Louis, USA). Poly(lactic-co-glycolic acid) (PLGA) (inherent viscosity 0.65 dL/g, 106 kDa) of LA/GA copolymer ratio 50:50 was from Surmodics Pharmaceuticals (Birmingham, USA).

Preparation of PCL and PLGA drug-loaded and drug-free polymer wrap constructs

Four distinct types of resorbable polymer vascular wraps were prepared (see Fig. 1): i) a non-porous sunitinib-loaded PCL monolayer (*Construct 1*), ii) a laminated PCL bilayer comprising a porous monolayer with a non-porous drug-free barrier layer (*Construct 2*), iii) a laminated PLGA bilayer comprising a non-porous sunitinib-loaded monolayer and a non-porous barrier layer (*Construct 3*), and iv) a laminated PLGA bilayer comprising a porous sunitinib-loaded monolayer and non-porous barrier layer (*Construct 4*). Non-porous formulations (*Constructs 1 and 3*) were loaded with sunitinib during construct fabrication, while porous composites could be loaded with drug after fabrication by infusion with the drug-loaded hyaluronic acid hydrogel (HG) carrier (*Constructs 2HG and 4HG*). Solvent casting and thermally induced phase inversion techniques were used to create all polymer wrap variations. Construct 1: PCL was dissolved at 20% w/v in acetone with stirring at 45°C. Three PCL molecular weights were used: 45 kDa, 80 kDa, and a blend of 14 kDa:80 kDa (2:1). Upon complete dissolution of the polymer in acetone over a period of ~1 hour, an 8% v/v water non-solvent was added with continued stirring (final composition v/v 7.4% water/92.6% acetone). After this solution reached optical homogeneity, sunitinib was added at 0.2% w/w and dissolved with vigorous stirring. The polymer/drug solution was cast into a Pyrex dish and degassed by ambient bubble dissipation. The dish was then placed in a -20°C freezer overnight to instigate and complete phase inversion of the polymer solution. Solvent was evaporated from the resulting monolith for 48 hours at r.t. in a chemical fume hood to produce the final spongy monolithic slab. Formation of non-porous layers used in *Construct 2 and 2HG* were identical to 1 except that no drug was added to 2 and 2HG.

Construct 2: A layer of non-porous PCL was cast in a Pyrex dish as described above, but without loaded drug or solvent evaporation. Identical polymer solution in acetone mixed with NaCl solid crystal poragen (>425 µm particle sieve size) as a slurry was directly layered over the cast PCL solution and allowed to settle under gravity. This construct was then phase-inverted at -20°C and placed in a series of DI water baths for 24 hours to extract the salt poragen and the solvent, then air-dried, and cut to size as a porous film. For construct 2HG, a sunitinib-laden hydrogel was then infused into the porous polymer layer (described below: 2HG).

Construct 3: Construct 3 was processed similarly to the non-porous PCL preparations except: a PLGA solution of 50% w/v in acetone and ethanol was employed as the non-solvent species at 30% v/v (final composition v/v 77% acetone/23% ethanol). Drug was added to the PLGA used for the upper layer prior to solvent evaporation. The bilayer composites were fabricated by solvent-welded lamination of the non-porous barrier layer to the drug-loaded layer using solvent acetone applied by spray bottle. The resulting bilayer was placed in vacuum at r.t. overnight.

Construct 4: For porous upper layer fabrication, the PLGA polymer solution was processed slightly differently than the PCL analogue (vida supra): PLGA solution was mixed with NaCl poragen (>425 µm particle sieve size) to form a 35 mL polymer-salt slurry in ethanol/acetone. This mixture was centrifuged at 500 rcf for 5 min, after which 15 mL of DI water non-solvent was added and then re-centrifuged at the same settings for 30 min to complete the system phase inversion. The poragen-polymer network was extracted in multiple excess DI water baths over 24 hrs. The resultant porous network was air-dried and welded to a PLGA non-porous barrier film using acetone applied by spray bottle. This provided a dense

polymer backing layer and upper porous polymer layer for the bilayer composite wrap. Samples were solvent evacuated in a chemical fume hood to remove residual solvent and punched to sample size. For construct 4HG, a sunitinib-laden hydrogel was then infused into the porous layer as described below.

Hyaluronic acid hydrogel-loaded drug preparation

HA-based commercial biomedical grade hydrogels were prepared per manufacturer's instructions (Glycosan Biosystems). Briefly, a 1% w/v HA solution in DI H₂O was mixed with dry sunitinib or sunitinib FB powder prior to hydrogel crosslinking (5 mg drug/mL hydrogel for the PCL constructs; 3 mg drug/mL hydrogel for PLGA constructs). The solution was then spontaneously crosslinked using 2% w/v PEG-diacrylate (Extralink, Glycosan Biosystems) in DI H₂O at a volume ratio of 4:1 by Michael addition chemistry. Directly after addition of the crosslinking agent and prior to gelling, the HA hydrogel/drug solutions were infused into the exposed porous polymer layers (for only *Constructs 2HG and 4HG*) under vacuum and then allowed to gel overnight. As a control to examine drug release from porous PCL without hydrogel, sunitinib FB suspended in the same volume of DI water was vacuum pulled into the porous PCL (Construct 2) and allowed to dry.

Scanning Electron Microscopy (SEM) Imaging

SEM was performed on cross-sections and surfaces of each fabricated polymer matrix. After conductive gold layer deposition, samples were imaged (PCL matrices at 3 kV and PLGA at 10 kV, Hitachi S3000-N).

In vitro drug release

For drug release experiments from *Construct 1* monolithic drug-laden PCL samples, 250 μ L of each PCL molecular weight or PCL blend was cast into 1.5 mL microfuge tubes. Drug release was also tested from polymer *Constructs 2, 2HG, 3, or 4HG*. Ten-mm biopsy punches of resulting PCL and PLGA constructs were used for release assays. Constructs were incubated at 37°C with gentle shaking in borosilicate vials with 5 mL release medium (1 $\times$ PBS with 2% bovine serum albumin (BSA)). Sample medium (5 mL) was removed and replaced daily. The release profile of sunitinib FB from HA hydrogel alone (crosslinked without underlying PCL or PLGA support polymer construct) was performed in the presence (5 mU/mL) or absence of HAse [26]. HAse-containing medium (1 $\times$ PBS with 2% BSA) was made fresh daily. Release media samples (1 mL) were spiked with an internal drug standard (sunitinib-d10) and final spiked drug concentrations of all HA samples were 5 mg of sunitinib per mL of hydrogel. Drug was extracted from the media by vortexing with MTBE (4 mL) for 2 min and centrifugation at 25°C at 2000 $\times$ g for 15 min. The organic layer was collected and evaporated at 42°C then reconstituted in 200 μ L (65:35) ACN:H₂O containing 0.1% formic acid. Sample recovery of >90% was attained. Cumulative release from *in vitro* experiments done in triplicate was expressed as the mean $\pm$ standard deviation.

PCL or PLGA barrier-mediated restriction of drug diffusion

To test the efficacy of the barrier layers to restrict drug diffusion, Costar Transwell® cell culture inserts (Corning) were modified by replacing the polycarbonate insert membranes with either PCL or PLGA polymer monolith discs (see Fig. 1 in the Online Data Supplement). Drug or a lipophilic dye (Oil Red O) was placed in release media in the upper chamber and release media alone was in the lower chamber. The lower chamber was assayed for solute (dye or drug) content over time using HPLC MS/MS or UV spectrometry (NanoDrop 2000 spectrophotometer, Thermo Scientific).

Polymer mechanical testing

PLGA Construct 3 (2cm × 3.5mm in the middle at the breakpoint; 0.5mm thickness) cast in the absence or presence of drug, were placed in PBS at 37°C for 0, 1 or 7 days, then subjected to tensile testing (Instron 4465, Instron Corp., Norwood, USA) at a speed of 1 cm min⁻¹ for all samples except for the 7-day drug-free PLGA constructs that were assessed at 1 mm min⁻¹. Force elongation was measured until sample breakage (five replicates). Young's modulus of elasticity is calculated by the Instron algorithm.

In vivo pharmacokinetic studies

PLGA Construct 3 was used in *in vivo* studies. The drug-free barrier layer of each construct was cast as 3cm × 3cm and the upper layer was 3 cm × 2.5 cm. The drug-free barrier layer was solvent-welded to either a i) non-porous drug-loaded (sunitinib malate (0.1% w/w, 143±5.5 µg drug)) PLGA layer, or ii) a non-porous drug-free PLGA layer (control). Drug loading in final bilayer polymer constructs was analyzed by completely hydrolyzing samples in NaOH for 48 h and extracting sunitinib for assay by HPLC MS/MS using spiked standards as a reference curve. These polymer bilayer “wraps” were sterilized using a STERRAD® system (ASP, Irvine, USA) under low temperature hydrogen peroxide plasma.

All *in vivo* studies were performed as approved by the Institutional Animal Care and Use Committee of the University of Utah and Veterans Affairs Salt Lake City Healthcare System and followed guidelines specified by the Guidelines for the Care and Use of Laboratory Animals. A porcine model was utilized for perivascular polymer wrap placement as it is the same animal species used for hemodialysis arteriovenous (AV) graft stenosis studies [9, 27, 28]. Briefly, three-month old Yorkshire cross-domestic swine (~30kg) were anesthetized using a mixture of ketamine (4mg/kg; Hospira Inc., Lake Forest, USA), xylazine (4 mg/kg; Lloyd Laboratories, Shenandoah, USA), and tiletamine/zolazepam (4mg/kg; Fort Dodge Animal Health, Fort Dodge, USA). Isoflurane (1–3%) was administered via tracheal intubation. Under sterile conditions, external jugular veins (EJV) were dissected, bilaterally and the adventitial layer was removed to elicit an injury response. A sunitinib-loaded Construct 3 bilayer wrap was placed around one EJV with the drug-loaded layer directly contacting the vascular tissue and monolithic barrier facing outward. A drug-free Construct 3 bilayer wrap was placed on the contralateral EJV site in each pig as an internal control. To seal the bilayer wrap edge, medical grade adhesive was applied along the length of the wrap edge. Wounds were assessed daily for adverse effects. In the first animal, in order to increase the amount of tissue pharmacokinetic data obtained, drug-loaded and contralateral drug-free wraps were also placed around the femoral veins in the hind limbs in addition to the wraps placed in the neck. However, edematous swelling occurred in the hind limbs after surgery that required draining. The swelling did not impact the animal's mobility, but it was decided in other pigs that wraps would only be placed in the neck. Blood was drawn weekly for systemic concentrations of sunitinib.

In vivo drug distribution in tissues

At one, two, and four weeks after wrap placement, animals were euthanized by the intravenous injection of pentobarbital sodium (80–100 mg/kg) and the EJVs and surrounding tissues were explanted and processed. Tissue from one animal was used for each time point. The wrap, underlying venous tissue and any surrounding fibrous tissue were isolated together and retained for drug extraction. Sections of vein both proximal and distal to the wrapped EJV segment were also isolated for separate drug extraction. The ipsilateral sternocleidomastoid muscle adjacent to the EJV was removed from the drug-treated side and separated into six segments. Lateral tissue adjacent to the veins was also isolated.

Tissue was homogenized in 1X PBS for 1 min. To monitor recovery, 200 μ L sunitinib-d10 (internal standard (IS) solution, 500 ng/mL) in running buffer was added to 0.5 mL of tissue homogenate. The extraction procedure was the same as for extracting sunitinib from release media, but was repeated once more by adding 100 μ L of 0.05 N NH_4OH and 4 mL TBME to the aqueous layer. The two organic fractions were combined and evaporated to dryness at 42 $^{\circ}\text{C}$ overnight. Samples were reconstituted in 200 μ L of 65:35 ACN:H₂O running buffer containing 0.1% formic acid and 10 μ L was injected into the HPLC MS/MS system.

Drug sample analysis by HPLC MS/MS spectrometry

The drug sample analysis protocol was modified and adapted from previously published protocols [29–31]. The analytical separation of sunitinib and sunitinib-d10 (IS) was performed using an Atlantis dC₁₈ column (2.1 $\times$ 30 mm, 3.0 μ m) (Waters Corp., Milford, USA), protected by a 4mm $\times$ 2mm C₁₈ packed guard column (Phenomenex) at 25 $^{\circ}\text{C}$ on an Acquity 2695 HPLC system (Waters Corp.) or on an Acquity UPLC H-Class system (Waters Corp.) both equipped with a refrigerated autosampler. The mobile phase consisted of acetonitrile–deionized H₂O (65:35, v/v) containing 0.1% formic acid and was run isocratically at a flow rate of 0.3 mL/min. Sample run times were 6 min on the Acquity 2695 HPLC and 4 min on the Acquity UPLC H-Class and were followed by a 2-min needle wash to prevent possible carryover. The IS and sunitinib eluted at 1.2 min on the Acquity 2695 HPLC. For the Acquity UPLC H-Class, the IS and sunitinib eluted at 0.42 min. Two independent triple quadrupole tandem mass spectrometers (Micromass® Quattro II or Acquity TQD, Waters Corp.) equipped with an electrospray ionization (ESI) interface were used as described in further detail in the Online Data Supplement.

Data analysis

Calibration curve linear regression, graphing, and statistical significance calculation for *in vitro* drug release comparing sunitinib release from various molecular weight PCL construct 1 were performed using Graphpad Prism® software (La Jolla, USA). A two-way ANOVA was used to determine statistical significance (p value <0.05) between various MW PCL samples at each time point for drug release.

Cumulative release data from each formulation tested were fitted by non-linear curve fitting using Graphpad Prism® software. For all formulations tested, except sunitinib FB from hydrogel or PLGA Construct 3, cumulative release was fitted using one-phase exponential association. A linear fit (indicating zero order release) was used to fit release of sunitinib FB from the hydrogel. Drug release from the PLGA Construct 3 demonstrated a two-phase release mechanism, consisting initially of drug diffusion out of the polymer matrix, followed by more rapid release of drug due to bulk degradation of the PLGA matrix (dominates release in this phase) and diffusion. A two-phase model to describe the release of ganciclovir from PLGA microspheres was used to fit the drug release data [32]. The following equation was applied:

$$F=A[1-\exp(-K_1 * T)] + \frac{B}{\{1+\exp[-K_2 * (T - T_{50})]\}} \quad (1)$$

where F is the fraction of total drug released, A is the percentage of overall drug released during phase I, K₁ is the rate constant of drug release in phase I, B is the percentage of drug released during phase II, K₂ is the drug release rate constant during phase II, and T₅₀ is the time taken to release 50% of the loaded drug.

Results

Three primary goals were set for perivascular polymer wrap performance: 1) drug release would occur primarily unidirectionally toward the vessel wall, 2) drug delivery would be sustained for three weeks or longer, similar to what is provided by drug-eluting stents, and 3) that the bilayer wrap would undergo degradation soon after drug depletion so that the re-injection of drug to the vessel site would be unimpeded by the presence of wrap material. To these ends, bilayer wraps were created by laminating a polymer *drug reservoir* layer, for sustained drug release to the blood vessel wall, onto a *drug-free* barrier layer serving to block drug diffusion to the extravascular space. Two types of polymer layers were investigated for use as the drug reservoir layer: i) A non-porous layer fabricated with drug prior to lamination onto the barrier layer; or ii) a porous layer fabricated without drug for later infusion with different drug-laden vehicles. The porous upper layer in Constructs 2 and 4 permits tuning of drug delivery strategies. Images of the different polymer constructs are shown in Fig. 2. Constructs 2HG and 4HG have been infused with sunitinib-laden HA hydrogel (HG). Sunitinib is yellow thus the drug-laden constructs are yellow. The PLGA non-porous monolayer appears amber even in the absence of drug. The lower barrier layer of the PCL construct 2HG, *initially white (upper row, middle image)*, absorbed sunitinib from the upper drug-laden porous layer over time. SEM analysis revealed small pores in the PCL dense barrier layer even in the absence of a poragen during casting (Fig. 3A), thus explaining the drug's ability to enter the PCL barrier layer. In contrast, the PLGA monolayer exhibited a solid uniform surface (Fig. 3B) with no notable porosity. The porous PCL construct contains both large pores formed by the NaCl poragen and smaller pores within the intervening material (Fig. 3C), whereas the porous PLGA construct contains only large pores formed by the poragen (Fig. 3D). In light of the undesired drug partitioning into the “non-porous” PCL barrier layer, a PCL wrap consisting of a drug-laden non-porous layer laminated to a drug-free barrier layer was not constructed. Hereon, the term “porous” describes polymer constructs created with the poragen and “non-porous” describes polymer constructs created in the absence of poragen, despite the observation that PCL constructs formed in the absence of poragen have micropores.

Restriction of compound diffusion by PCL or PLGA barrier layers

Modified Transwell chambers were used to investigate the ability of the non-porous PCL or PLGA barrier monolayers to curtail penetration and diffusion of drug. Drug was added to media in an upper chamber separated from a lower chamber by either PCL or PLGA non-porous polymer films. As shown in Table 1, a very small amount of sunitinib was detected in media collected at 72 h from the lower chamber below the PCL insert (~0.01% of the amount of drug detected in the upper chamber). However, no drug was detected in the chamber beneath the PLGA insert indicating that the non-porous PLGA layer was a more effective barrier to drug penetration and diffusion.

In vitro drug release profile from the HA hydrogel

The porous layers of PCL Construct 2 or PLGA Construct 3 can be loaded after fabrication with different drugs and carriers. In the current studies, the porous polymer layers were infused with drug-loaded Glycosil, a biomedical commercial HA-based crosslinked hydrogel with an extensive published history of medical use [33, 34]. Release kinetics for the salt form of sunitinib (sunitinib malate) and the free-base (FB) form were examined. Both forms inhibited PDGF-induced smooth muscle cell proliferation (IC₅₀ of 5 nM and 17 nM, respectively, data not shown). Sunitinib malate readily dissolved into the HA hydrogel whereas sunitinib FB remained in particulate form suspended within the gel. The cumulative *in vitro* release profiles of either form of sunitinib are shown in Fig. 4. Nearly complete release of sunitinib malate was observed from the hydrogel by five days. Thus hydrogel

alone was not an effective vehicle for sustained release of sunitinib malate. In contrast, release of sunitinib FB was much slower, with only 50% release occurring by day 15 and 100% release occurring 30 days, at which point the hydrogel had hydrolyzed completely. Drug release was enhanced in the initial 5-day time period with addition of HASE, an enzyme present in wound tissue that breaks down the major hydrogel component, hyaluronate [35]. However, complete release of drug in the presence of HASE occurred by 24 days, similar to that seen in the absence of HASE.

In vitro drug release profiles from PCL polymer formulations

Kinetics of drug release from the PCL-based constructs are shown in Fig. 5. Nearly complete release of sunitinib from the non-porous PCL Construct 1 occurred between 22–30 days (triangles). In contrast, release from PCL Construct 2HG, where drug-loaded hydrogel was infused into a porous PCL layer, resulted in 80% release by 49 days, slower than that from hydrogel alone (Fig. 5, circles vs. diamonds). Both constructs provided release profiles satisfying the desired goal set for drug release duration for the polymer constructs.

Release data shown in Fig. 5 were from constructs made from 80 kDa PCL which resists *in vivo* degradation and remains intact within the body for years [36, 37]. And in fact, no degradation of the PCL material was observed at 50 days in our *in vitro* studies. However, as AV grafts are subjected to continued pro-hyperplasia insults, repeat application of drug may be required at time points beyond the period where drug depletion from a perivascular wrap occurs. The presence of intact wrap material could complicate reapplication of drug via injection to the perivascular region. Non-porous constructs made from lower mol wt PCL or blends of PCL would likely have faster degradation kinetics. PCL constructs were formed with 45kDa, 80kDa, or a blend of 67% 80kDa/33% 14kDa mol wt PCL. Sunitinib release from these constructs demonstrated rapid drug release within the first seven days followed by slower continuous release (Supplemental Fig. II). All drug was released from the lower mol wt construct and the blend construct near Day 10. Attempts to form polymer layer constructs from 14 kDa PCL failed as the polymer had little mechanical integrity and readily crumbled after thermal phase inversion and drying. Thus, the constructs formed using lower mol wt PCL either released drug too quickly or had insufficient mechanical integrity than the constructs made from 80 kDa PCL.

In vitro drug release from PLGA constructs

As the 80 kDa mol wt PCL remains intact long after drug was depleted, and mechanical integrity was lost when lower mol wt PCL was used for construct formation, wraps were then made from PLGA; the degradation rates of PLGA can be readily altered by adjusting the polylactide to glycolide ratios [38]. Release of sunitinib was tested from PLGA Construct 3 (non-porous) and Construct 4HG (porous infused with drug-laden hydrogel) (Fig. 6). Drug release from Construct 4HG was complete by 15–20 days before polymer degradation was grossly observable. In contrast, complete drug release did not occur until 35–37 days from PLGA Construct 3. This construct initially provided a steady release of drug, similar to commercially available drug-eluting stents [39] but thereafter bulk degradation occurred, as determined by visual assessment, accompanied by rapid drug release.

Drug release rate constants (k) and half-lives ($t_{1/2}$) for drug release from polymer

The release rate constant (k) and the half-life ($t_{1/2}$) of drug release from the different polymer constructs are presented in Table 2. These data illustrate a wide variety of release profiles accomplished by not only the use of different polymers for construct formation but also by combining drug-loaded hydrogel within the porous layers. Sunitinib release from PLGA Construct 3 exhibited a pronounced biphasic release profile (Fig. 6) and release rate

constants for each phase are shown. The following release parameters were obtained from the curve fit of equation 1 (shown in Methods): A was 30%, k_1 was 0.081 day^{-1} , B was 76.4%, k_2 was 0.65 day^{-1} , and T_{50} was 33.1 days. Drug release from the PCL construct 2HG had the longest $t_{1/2}$. However, PCL construct 2HG is also predicted to have a very long *in vivo* persistence based on other reports [36, 37]. As PLGA Construct 3 i) showed controlled, sustained *in vitro* drug release (~30 days), ii) yet underwent bulk degradation by 30 days that would allow access to the perivascular space for subsequent re-application of drug by ultrasound-guided injection, and iii) contained an effective barrier-layer that promoted unidirectional drug release, this construct was chosen for further proof-of-concept *in vivo* assessments in the porcine EJV implant study.

In vivo sunitinib distribution from PLGA Construct 3

Sterile PLGA bilayer wraps (Construct 3) ($3 \text{ cm} \times 3 \text{ cm} \times 1 \text{ mm}$) (inner layer loaded with $143 \pm 5.5 \text{ mg}$ sunitinib) were placed around the EJV in swine (Fig. 7). Drug-free wraps were placed on the contralateral EJV as controls (not shown). No infections or remarkable swelling or delays in wound healing in the neck area were noted in any animals at any time after wrap placement. Moreover, at explant, no signs of infection in the surgical area near the wraps were observed. After one week, the PLGA wrap was intact as determined by visual assessment after explant but by four weeks, the wrap was only visually detectable as small flakes of material that had become incorporated with tissue. Distribution of sunitinib into the EJV wall, surrounding fibrous tissue, the sternocleidomastoid muscle, and tissue adjacent to the EJV, was assessed as illustrated in Fig. 8. Drug in the wrapped EJV segment was detectable as early as one week and remained elevated at four weeks. Sunitinib concentrations at all three time points in the wrapped portion of the EJV (the target tissue) were much greater (Fig. 8) than the experimentally determined *in vitro* IC_{50} (~2 ng drug/g tissue (~5 nM)) needed to inhibit proliferation of PDGF-stimulated SMCs.

At two and four weeks after placement, sunitinib was detected in the sternocleidomastoid muscle, but at concentrations much less than in the wrapped vein (Fig. 8). Sunitinib was undetectable at any time points in the distal portion of wrapped vein segments, tissue adjacent to the vein, and in all tissue sections on the control side. These results illustrate that drug was preferentially released toward the vessel wall and away from the extravascular space, fulfilling an initial goal set for wrap performance. Systemic drug concentrations were undetectable except at four weeks after wrap placement (3.6 ng/mL detected at this time point). This animal was the same (and only) animal receiving two drug-laden wraps: one in the hind-limb and one in the neck. The contribution of the wrap in the hind-limb to systemic concentrations was inseparable from the contribution of the wrap in the neck. As there was significant fluid accumulation in the hind-limb area, this wrap may have contributed more drug to systemic circulation. Other animals with wraps placed only in the neck showed no systemic drug levels.

Polymer mechanical properties

While the wraps were flexible prior to implantation, every wrap was stiff at explant. Thus, the effect of water exposure on the PLGA wrap elasticity was measured. Young's modulus of elasticity is a measure of material stiffness. Table 3 lists the Young's modulus for drug-free or drug-loaded non-porous monolayer PLGA constructs at various time points before and after incubation in $1 \times \text{PBS}$. Prior to incubation in PBS, the drug-free PLGA was elastic and flexible; after seven days in PBS the Young's modulus increased indicating the PLGA became brittle with exposure to PBS. Addition of sunitinib into the PLGA matrix further decreased the elasticity of the PLGA. Also, increased hydration time of the drug-loaded constructs (7 days) resulted in samples becoming so brittle that testing of elasticity failed since the material fragmented when attempting to place it in the Instron clamps.

Discussion

The objective of this work was to develop a resorbable drug-loaded perivascular wrap that provides directed, local drug release towards the graft and vascular wall to inhibit AV graft hyperplasia. A polymer bilayer wrap design employing a drug-free non-porous outer barrier layer laminated to either a porous or non-porous drug-loaded inner tissue-contacting layer, was anticipated to provide unidirectional drug delivery towards the vessel while minimizing drug loss to surrounding extravascular tissue. Limiting drug encroachment into surrounding non-target tissues should decrease unwanted toxicities and preserve loaded dose for extended targeted release directly to the vascular tissue site of desired drug action. Recently, the development of a multilayer drug delivery system was reported that contained a barrier polymer layer to restrict drug loss. The system was created for use as a drug-eluting stent *within* tubular structures such as blood vessels [40, 41]. The current polymer bilayer constructs reported herein differ from the previously reported work in that they i) were developed to be applied *perivascularly*, ii) have greater tunability since the system could be made with porous or non-porous layers, and iii) can provide a faster degradation profile. Importantly, the current system was shown to be well-tolerated *in vivo* whereas the previous work did not report *in vivo* results.

The polymer solvent casting and thermal phase inversion techniques used to fabricate the wraps in this study are versatile since both porous and non-porous constructs could be made using two relevant degradable polymers. For this application, polymer formulations were assigned to provide a strong but flexible construction for both layers that could be easily wrapped around a surgically accessed vessel. An additional benefit of these methods is the use of toxicologically benign solvent systems. Typically, harsh chlorinated solvents (e.g., chloroform or dichloromethane) are used to create various PCL and PLGA matrices [42–44]. High levels of residual solvent can still remain in these matrices even after extensive removal efforts [45], leading to potential toxicity risks. Therefore, employing solvent systems that are i) facile to eliminate from the polymer wrap, and ii) innocuous if left behind in trace amounts, is desirable.

The versatility of the various wrap iterations permits incorporation of drug i) directly into the porous or nonporous polymer during casting or ii) into hydrogels that then can be incorporated into the porous polymer network. The HA hydrogel matrix used to load and suspend sunitinib in the porous layer is a naturally occurring polysaccharide and a major component of the extracellular matrix (ECM) present in mammalian tissues. This nonsulfonated, polyanionic GAG regulates cell motility and adhesion [46], and maintains water homeostasis within the ECM [47]. HA hydrogels have been developed that are biocompatible, biodegradable, and injectable by making thiolated HA derivatives crosslinked with PEGDA [48]. Attempts were made to create a polymer wrap from HA gel material directly but its mechanical properties proved unsuitable for this application. However, the HA hydrogels proved easy to infuse into the porous layer of the polymer bilayer wraps and provided another means to influence the drug-release profiles.

The dissimilar release profiles of the salt and FB sunitinib forms from hydrogel are likely due to distinct interactions occurring between the salt and non-salt forms of sunitinib and the hydrogel. Derivatized HA in the Glycosil hydrogel is thiol-functionalized via the available carboxylic acid groups, but only 42% of these moieties become modified [35]. At pH 7, the unreacted carboxylic acid groups are likely deprotonated while FB sunitinib (pKa~ 8.95) should possess one protonated amine group, leading to an electrostatic interaction between the two. Thus, release of FB sunitinib from this matrix is governed by ionic interactions, simple diffusion, and polymer degradation. In the case of the malate salt form, the presence

of malate anion reduces the potential for ionic interaction via the amines thus promoting faster diffusion.

Breakdown of the HA hydrogel occurs via i) hydrolysis of the PEG acrylate esters present after HA Michael addition crosslinking [49, 50], and ii) enzymatic cleavage of the glycosidic linkage by HAse. Since HAse is endemic to wound sites, an earlier release of sunitinib from the hydrogel-infused construct would be expected *in vivo* over that observed *in vitro*.

At 2 weeks, 34% of drug was released from the wrap *in vivo*, compared to ~20% of drug in the *in vitro* experiments. Unlike the *in vitro* assay sink conditions, acidic polymer degradation products (lactic and glycolic acids) can accumulate around the PLGA wrap *in vivo*, reducing the local pH and resulting in an autocatalytic effect, accelerating polymer degradation and subsequently drug release [43, 51]. Throughout the four weeks, only ~38% of the total 145µg drug loaded in the wraps was accounted for. The unaccounted drug was likely eliminated from the vascular tissue by metabolism, diffusion through the vein into systemic circulation, or dispersed into non-analyzed surrounding tissue.

Design improvements to inhibit further drug loss to the extravascular space could include: i) increasing the thickness and/or density of the barrier layer, ii) increasing the copolymer lactide to glycolide ratio to decrease the wrap degradation rate, and iii) increasing the PLGA molecular weight. Also, lower drug concentrations could be loaded so that drug release to the extravascular space would be well below IC₅₀ levels.

The PLGA polymer is initially stiff, but after casting, the PLGA film was ductile. The increased ductility is likely due to a change in the polymer chain conformation after casting, allowing for greater interchain mobility. However, it was observed after both *in vitro* and *in vivo* testing that the PLGA constructs were much less flexible after exposure to an aqueous environment, revealing an anti-plasticizing effect of water [52]. The incorporation of sunitinib into the PLGA further decreased material extensibility, probably due to drug-polymer interactions that reduce polymer chain mobility in the matrix similar to previous reports [44, 53]. Explanations for the loss in film extensibility upon hydration include i) thermodynamically-driven polymer chain rearrangement to minimize contact with the infiltrating water molecules [52], and/or ii) the loss of drug and replacement of residual solvent molecules that enable high degrees of chain network mobility relative to water. Others have reported the phenomena of residual solvents altering mechanical properties in various degradable polymers [54, 55]. Regardless of the cause, the increased film stiffness may be advantageous in decreasing hyperplasia at the vein-graft anastomosis. It has been postulated that enhanced circumferential tensile stress that occurs in a vein after introduction of arterial flow may significantly contribute to subsequent NH formation [56]. Others have shown that restricting venous distension by application of perivascular supportive stents inhibits NH in vein interposition grafts exposed to arterial flow [56]. Hence, stiffening of the bilayer PLGA wrap after application around the vein-graft anastomosis could be mechanically beneficial in addition to pharmacological benefits for decreasing hyperplasia by inhibiting subsequent circumferential expansion.

Conclusions

A resorbable polymer-based bilayer perivascular drug delivery wrap was created that degrades over 30 days, provides uni-directional local drug delivery due to the incorporation of an impermeable polymer backing, is well-tolerated *in vivo*, and tunable to provide different drug release rates by either direct incorporation of drug, or by infusion of a drug-loaded hydrogel into a porous layer. The PLGA construct was found to undergo stiffening

after *in vivo* placement that may be useful as a perivascular support against circumferential tensile stress in the vein. This study also revealed a novel interaction of the positively charged free base form of sunitinib with a selectively modified natural HA-hydrogel, indicating that the hydrogel could be used for prolonged delivery of cationic or basic small molecule drugs. The PLGA construct presented herein is a promising perivascular drug delivery system for the local treatment of AV graft NH and possibly other hyperplastic vascular disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of polymer wrap constructs. **PCL wraps:** *Construct 1*- a non-porous monolayer loaded with drug during fabrication. *Construct 2*- a bilayer wrap containing a top polymer porous layer welded to a bottom non-porous drug-free layer. The porous layer of construct 2 can be loaded with different drug carriers. *Construct 2HG*- construct 2 infused with a drug-laden hydrogel. The hydrogel is loaded as a sorbed droplet. **PLGA wraps:** *Construct 3*- a bilayer wrap containing a top non-porous layer loaded with drug during fabrication then welded to a bottom non-porous drug-free barrier layer. *Construct 4*- a bilayer wrap containing a top porous layer solvent-welded to a bottom non-porous drug-free barrier layer. *Construct 4HG*- construct 4 sorbed with a drug-laden hydrogel in the overlying porous layer.

Figure 2.

PCL and PLGA constructs. *PCL constructs (first row).* *Construct 1*-(left) non-porous monolayer with drug added during fabrication. *Construct 2*-(center) porous/non-porous bilayer fabricated without drug; (right) *Construct*-same as construct 2 except drug-laden HA hydrogel(HG) was infused into the porous top layer. Sunitinib is bright yellow. *PLGA constructs (second row).* *Construct 3*-(left) top layer, a non-porous polymer fabricated with drug, laminated to a non-porous barrier layer with no drug. *Construct 4*-(center) porous/non-porous bilayer fabricated without drug. *Construct 4HG*-same as Construct 4 except drug-laden hydrogel was infused into the top porous layer.

Figure 3. Morphology of PCL (A,C) and PLGA (B,D) polymer constructs formed in the absence (A, B) or presence (C,D) of porogen as determined by SEM.

Figure 4.

In vitro release of sunitinib malate or sunitinib free-base (FB) from HA-based hydrogel (droplet). Sunitinib malate (circles), sunitinib FB (diamonds), and sunitinib FB in the presence of hyaluronidase (HAse) (5mU/mL) (triangles).

Figure 5.
In vitro release profiles of sunitinib from PCL construct 1 (triangles), 2HG (circles) or hydrogel alone (diamond). Sunitinib = sunitinib malate; FB = free base.

Figure 6.
In vitro release of sunitinib from both PLGA Construct 3 (triangles) and 4HG (circles). FB= free base.

Figure 7. Sunitinib-loaded Construct 3 applied to EJV in aporcine model. Panel A) The dissected EJV prior to wrapplacement (upper). Construct 3 wrapped around the EJV (lower). Panel B) Construct 3 *in situ* after dissection at 1, 2, or 4 weeks after placement. Lower row shows cross-sectional images taken after explant of the same constructs shown in upper row.

Figure 8.

Sunitinib concentrations in segments of porcine tissue. Upper panel: a graphical representation of sunitinib concentrations (represented in yellow) in the PLGA wrap, the EJV that was surrounded by the wrap, the sections of vein that were proximal and distal to the wrapped section of EJV, the muscle, and adjacent tissue at 1, 2 or 4 weeks. Color scale bar indicates drug concentrations. Sunitinib concentrations in each tissue segment are listed in the table.

Table 1

Small molecule diffusion through non-porous layer.

Polymer material	Transwell chamber	Sunitinib (mg/mL) LC/MS/MS*	Oil Red O Optical density (521 nm)
PCL	Upper	20.5 ± 7.5	0.232 ± 0.13
	Lower	0.018 ± 0.003	b.d.
PLGA	Upper	129 ± 17.6	0.401 ± 0.028
	Lower	b.d.	b.d.

b.d. = below detection

*
calibrated against spiked known standards

Table 2Drug release rate constants (k) and half-lives ($t_{1/2}$)

Delivery system	k or k_1	$t_{1/2}$ (days)	k_2	$t_{1/2}$ (days)	Goodness of fit (r^2)
sunitinib FB from PLGA construct 4HG	0.21	3.27	-	-	0.937
sunitinib FB from PCL construct 1	0.15	4.61	-	-	0.878
sunitinib FB from PCL construct 2	0.12	5.77	-	-	0.916
sunitinib from HA-hydrogel plus Hase	0.086	8.05	-	-	0.976
sunitinib from PLGA construct 3	0.081	8.56	0.65	1.06	0.982
sunitinib FB from PCL construct 2HG	0.031	22.26	-	-	0.993
* sunitinib FB from HA-hydrogel	3.68% release/day	13.6	-	-	0.989

“sunitinib” indicates sunitinib malate;

“sunitinib FB” indicates sunitinib free-base

* indicates a linear fit, with zero-order release, whereas other fits are exponential and first order

Table 3

Effects of hydration and drug loading on monolithic PLGA film mechanical strength.

Samples	Days hydrated	Young's modulus (MPa)
Drug-free PLGA	0 days	1.12±0.14
Drug-free PLGA	1 day	2.53±0.69
Drug-free PLGA	7 days	30.13±2.88
Drug-loaded PLGA	0 days	40.15±0.35
Drug-loaded PLGA	1 day	51.9±11.3
Drug-loaded PLGA	7 days	Too brittle to test