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A Tailorable In-Situ Light-Activated Biodegradable Vascular Scaffold

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Abstract

Biodegradable vascular scaffolds (BVS) are novel treatments for obstructive atherosclerotic cardiovascular disease that have been developed to overcome the limitations of traditional metallic drug-eluting stents (DES). The mechanical properties of bioabsorbable polymers used for the production of novel BVS are a key consideration for the clinical translation of this emerging technology. Herein, we describe the engineering of an *in situ* light-activated vascular scaffold (ILVS) comprised of a biodegradable citric acid-based elastomeric polymer, referred to as methacrylated poly-diol citrate (mPDC), and a diazeniumdiolate chitosan nitric oxide donor (chitoNO). *In vitro* studies demonstrate that the mechanical properties of the ILVS can be tailored to meet or exceed those of commercially available self-expanding bare metal stents (BMS). The radial compression strength of the ILVS is higher than that of a BMS despite undergoing degradation at physiologic conditions for 7 months. ILVS containing chitoNO provides sustained supraphysiologic levels of NO release. Lastly, ILVS were successfully cast in porcine arteries *ex vivo* using a custom designed triple balloon catheter, demonstrating translational potential. In

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Conflict of Interest:

MA, JY, GA and MK are listed as inventors on patent applications filed by Northwestern University for this technology.

Author Contributions

MSA, JY, JHB, NAM, GAA, and MRK contributed to designing the research studies, conducting experiments, acquiring data, and analyzing data.

MRK and GAA obtained funding and provided overall supervision for the project.

MSA, JHB, GAA, and MRK participated in writing the manuscript and figures.

MSA, JY, JHB, GAA and MRK all participated in critical revision of the manuscript and figures.

conclusion, these data demonstrate the ability of an ILVS to provide tunable mechanical properties and drug-delivery capabilities for the vasculature, and thereby support mPDC as a promising material for the development of novel BVS platforms.

Introduction

Current generation metallic drug-eluting stents (DES) have led to dramatic reductions in the need for repeat revascularization procedures to restore arterial blood flow in patients with both obstructive coronary artery disease (CAD) and peripheral artery disease (PAD) (1, 2). Despite the therapeutic benefits of currently available DES, concerns remain regarding the risk of restenosis, stent thrombosis, and delayed arterial healing associated with incomplete endothelialization and abnormal vasomotion (3–5). Biodegradable vascular scaffolds (BVS) have been developed to address the limitations of existing DES by providing short-term vessel scaffolding followed by complete dissolution to support arterial healing, favorable arterial remodeling, and to restore normal arterial physiology (6–9). However, emerging data from clinical trials of various BVS platforms suggest that the performance of these novel devices is inferior with respect to device delivery success, mechanical properties, material degradation profile, and clinical outcomes compared to state-of-the-art metal DES (10, 11). Thus, development of novel BVS materials and delivery systems is warranted to overcome these limitations. The optimal BVS platform should be based on a bioabsorbable material capable of providing dynamic vascular mechanical scaffolding in order to resist the radial and bending forces of the treated artery under various anatomic circumstances (12). Additionally, the biocompatibility properties and drug-delivery capabilities of the BVS material should support normal arterial healing during and after BVS dissolution.

We have previously developed a novel ultraviolet (UV) light-activated citric acid-based elastomeric polymer for use as a vascular biomaterial (13). Methacrylated poly-diol citrate (mPDC) is a highly viscous pre-polymer that when mixed with a photoinitiator and exposed to light is cross-linked (or photopolymerized) to form a biocompatible and biodegradable elastomeric polymer with modifiable mechanical properties and drug delivery capabilities. We have further engineered mPDC to optimize its properties as the material for a novel BVS platform referred to here as an *in situ* light-activated vascular scaffold (ILVS) that is capable of being photopolymerized using visible blue light rather than UV light, which may potentially cause endothelial injury (14). The tailorable mechanical properties and the nitric oxide (NO)-releasing capability of the ILVS described herein represent significant advances in the design and fabrication of BVS. NO has been shown to inhibit a variety of processes that contribute to deleterious arterial healing, and therefore the NO-releasing properties of the ILVS may improve arterial patency following endovascular intervention (15–18). The ILVS consists of a liquid pre-polymer mPDC and a NO donor, and is designed for percutaneous delivery into the endovascular space using a custom-engineered catheter where it is cross-linked to the luminal surface of an artery following balloon angioplasty to form a BVS.

Here we describe the development and characterization of the components of the ILVS including the visible blue light-activated formulation of mPDC, a novel chitosan-based NO

donor (chitoNO) capable of providing sustained NO release, and a specialty designed triple balloon catheter capable of delivering the ILVS to the intravascular space. We hypothesize that a NO-releasing biodegradable ILVS will possess highly tailorable mechanical properties and achieve strength similar to current metallic arterial stents. Lastly, we demonstrate the translational potential of our technology by casting an ILVS into a porcine artery *ex vivo*.

Results

Synthesis and in vitro characterization of a photopolymerizable elastomeric polymer, mPDC

mPDC was synthesized by reacting citric acid with 1,12 dodecanediol, followed by methacrylation with glycidyl methacrylate, and purification in deionized water (19). Synthesis of mPDC (Figure 1A) produced a highly viscous pre-polymer that upon blending with a blue light photoinitiator allowed for crosslinking of mPDC into a tubular elastomeric polymer (Figure 1B) using a blue light wavelength of 465 nm. Infrared spectroscopy (IR) and proton nuclear magnetic resonance (¹H-NMR) were used to characterize the purified mPDC polymer. Successful incorporation of the methacrylate group and functionalization of the poly (1,12-dodecamethylene citrate (PDDC) pre-polymer were confirmed by the presence of the characteristic peaks on FTIR and NMR (Supplemental Figure 1A and B). The methacrylation efficiency of the PDDC pre-polymer approximated 80%.

mPDC-based ILVS have tunable mechanical and flexibility properties

In vitro testing of ILVS demonstrated the ability to modulate radial compression and flexibility performance compared to a commercial self-expanding bare metal stent (BMS) with the same diameter and length (Figure 2). The radial compression strength of the ILVS was modulated by changing the photopolymerization time and energy (Figure 2A and 2B), with the radial strength increasing with greater photopolymerization time and energy. Additionally, alteration of the thickness and diameter of the ILVS affected the radial compression strength. In the context of this manuscript, thickness refers to the difference between the inner and outer radii of the ILVS, and diameter refers to the outer diameter of the ILVS. As the ILVS thickness increased (Figure 2C and 2D) or the ILVS diameter decreased (Figure 2E and 2F), the radial strength was increased. Three-point bending tests demonstrated that the ILVS has more flexibility when compared to BMS (Figure 3A). Comparison of the slope of compressive force versus deflection reduces the potential for erroneous flexibility comparisons due to non-linear force versus deflection data. Furthermore, the flexibility of the ILVS can be modulated by photopolymerization intensity (Figure 3B). All of the data from these experiments show that it is possible to tune the mechanical properties of mPDC.

ILVS is degradable yet retains mechanical properties over time when exposed to physiological conditions

In vitro degradation studies showed that mPDC has a mass loss of 27% after 28 weeks (Figure 4A). Despite that mass loss, mechanical testing performed during the degradation studies demonstrated the stability of the radial strength of the ILVS compared to BMS (Figure 4B). Furthermore, we demonstrated the ability to modulate degradation time. ILVS

photopolymerized for 30 seconds had an approximately 5% greater mass loss at 2 weeks compared to scaffolds photopolymerized for 45 seconds (Figure 4C).

Rheological properties of mPDC are dependent on the concentration of mPDC

Rheological analysis of mPDC was performed at room temperature and physiologic temperature to replicate the conditions prior to and during catheter delivery of mPDC into the endovascular space. The viscosity of various mPDC concentrations was decreased with increasing temperatures (Figure 5A), and was stable across a range of shear rates (Figure 5B). The decrease in mPDC viscosity with increasing temperature provides a means to facilitate catheter injection of concentrated polymer blends.

mPDC-chitoNO ILVS provided sustained NO-release

Reaction of unmodified chitosan with NO in acetonitrile yielded an N-diazeniumdiolate NO donor form of chitosan (chitoNO). Low-molecular weight chitosan was utilized to minimize the impact of chitosan on the viscous properties of the mixture of chitoNO with mPDC. Loading of mPDC with 100 mg of chitosan per milliliter of liquid pre-polymer mPDC generated a NO-releasing form of mPDC (mPDC-chitoNO), which functions as the drug delivery form of the ILVS. The NO release profile of mPDC and mPDC-chitoNO ILVS was evaluated under physiologic conditions using the NO analyzer. mPDC-chitoNO ILVS exhibited an initial peak of NO release followed by a sustained flux of NO for greater than 3 hours (Figure 6A). Average NO flux from mPDC-chitoNO (100 mg/ml chitoNO) ILVS was sustained at $21.8 \pm 1.8 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ (Figure 6A). Furthermore, mechanical tests demonstrated that the radial strength of mPDC-chitoNO is greater than mPDC alone, and can be increased with increasing concentrations of chitoNO (Figure 6B). The viscosity of mPDC is affected similarly, as the viscosity of mPDC-chitoNO 70 wt% was higher than mPDC 70 wt% (Figure 6C).

ILVS can be engineered for intravascular delivery using a custom-designed illuminating triple balloon catheter

In anticipation of large animal studies to demonstrate pre-clinical feasibility, a significant amount of time and resources were committed to overcoming the engineering challenges faced in developing a specialty catheter and optimizing mPDC for catheter-based endovascular delivery of the ILVS (Figure 7). The design of the specialty catheter includes 6 lumens: two polymer injection/aspiration ports, two balloon ports, one port for the guide wire, and one port for the laser diffuser (Figure 7B). The steps involved in ILVS delivery include: 1) proximal and distal occlusion balloon inflation to isolate the arterial segment targeted for ILVS delivery; 2) evacuation of blood through the polymer infusion/aspiration lumens by irrigation with saline; 3) injection of the mPDC into the endovascular space through the polymer infusion lumen of the specialty catheter; 4) inflation of the inner angioplasty/casting balloon to create the inner lumen of the ILVS; 5) passage of the laser-coupled fiber through the light lumen of the catheter into the space between occlusion balloons and activation of the laser light source (Figure 7E); 6) photopolymerization of the ILVS *in situ* (Figure 7F); 7) deflation of the middle balloon followed by irrigation of the space with saline to remove any unreacted polymer; and 8) deflation of the occlusion balloons to restore flow.

mPDC can be easily injected into the balloon catheter

As noted above, the diameter of intravascular catheters is limited by the diameter of the artery targeted for endovascular intervention. As mPDC is highly viscous in the pre-polymer state, we identified the conditions necessary for intravascular delivery within the confines of the 1.3 mm diameter polymer infusion lumen in our 9 French specialty catheter. After test injecting various concentrations of mPDC (diluted with ethyl acetate) into commercial angiographic catheters of various inner diameters, we selected a 1.3 mm polymer injection lumen for our specialty catheter and a 80 wt% mPDC formulation of mPDC for the ILVS which was easily hand-injected using a 3 ml syringe down the length of the 42 cm catheter.

ILVS can be formed within an artery

Ex vivo casting of an ILVS in a porcine artery (Figure 8A) using a modified balloon angioplasty catheter set-up provided mechanical scaffolding that exceeded the radial strength provided by BMS scaffolding (Figure 8B). Histological processing using a paraffin embedding system confirmed the presence of the mPDC as a thin polymer coating along the periphery of the artery's inner wall (Figure 8C). Of note, tissue processing conditions resulted in some detachment of the polymer from the wall of the artery. Taken together these data show the potential of the ILVS as a novel device for endovascular therapy.

Discussion

We have described the material properties and engineering of a highly novel endovascular tool known as the ILVS. The biomaterial foundation of the ILVS is the photocurable mPDC that can be functionalized to deliver NO by doping with a diazeniumdiolate chitosan-based NO donor. We have demonstrated that mPDC has tailorable mechanical and degradation properties depending on the photopolymerization conditions and physical dimensions of the ILVS.

Biodegradable vascular scaffolds are an emerging technology developed to overcome the limitations of current DES, including delayed arterial healing, impaired endothelialization, stent malapposition and fracture, and vessel caging causing abnormal vasomotion, that contribute to persistent neointimal formation, neoatherosclerosis, and stent thrombosis (11). The properties of bioresorbable vascular scaffolds may be advantageous in dealing with common mechanisms of endovascular treatment failure in both CAD and PAD. Coronary stent thrombosis related to chronic inflammation and possibly stent malapposition are theoretically mitigated by BVS resorption (5, 11). Although understudied as a mechanism of peripheral stent failure, stent thrombosis may result from the pro-thrombotic environment associated with metal stents and stent fractures (20). Distinct mechanisms of stent failure in PAD may also be minimized through resorption of the BVS thereby reducing the presence of chronic outward radial force and fractured stent struts that serve as chronic stimuli for neointimal formation and eventual stent failure (21, 22).

In both patients with CAD and PAD the superiority of existing BVS constructed of various materials has not been demonstrated (23–25). Stent thrombosis, inadequate radial strength, and a combination of neointimal hyperplasia and thrombus appear to underlie the various

mechanisms of BVS failure in patients with obstructive atherosclerotic disease (23, 24, 26, 27). Together these findings suggest mechanical durability and arterial healing responses are important factors in the long-term patency of BVS for the treatment of obstructive atherosclerotic cardiovascular disease.

No existing peripheral revascularization technology possesses the capability to customize BVS properties at the time of delivery. The material properties of mPDC allow for on-demand modification of the radial strength, flexibility, and degradation properties during ILVS delivery. The ability of mPDC to resist elastic recoil can be modulated to address the conditions of the artery being mechanically revascularized. Photopolymerization time and intensity of cross-linking of the ILVS may be increased to enhance scaffold radial strength and oppose the high degree of compression associated with calcified arterial plaques. Conversely, photopolymerization time and intensity may be reduced for lesser degrees of radial support in an effort to minimize chronic outward radial forces while preserving mechanical support. Additionally, matching ILVS radial strength and flexibility to the mechanical properties of the artery being treated may have important implications for long-term arterial patency (6, 28–31). Because additional photopolymerization further cross-links mPDC, bolsters scaffold radial strength, and prolongs degradation time, re-exposure of ILVS can be performed immediately after device delivery in cases where post-implantation minimal lumen diameter is suboptimal. Thus, it is conceivable that early scaffold degradation could be treated by re-exposure of a previously implanted ILVS with additional photopolymerization ‘treatments’. Of course, cross-linking of ILVS that are in the process of biodegradation *in vivo* will need to be examined in future studies.

ILVS are also designed to coat the entire luminal surface of the target vessel. The advantages to this approach are the greater volume and surface area available for drug delivery compared to the strut design of BMS and pre-formed BVS. Coating the entire luminal surface reduces the likelihood of scaffold malapposition and under-expansion that has been observed to increase the risk of coronary poly(lactic acid) scaffold thrombosis in clinical studies of patients with acute myocardial infarction (32). Importantly, inhomogeneous drug release resulting from scaffold malapposition may affect arterial healing, and the risk of restenosis and thrombosis of implanted bioresorbable vascular scaffolds (33, 34). Furthermore, the endovascular coating provided by the ILVS may reduce the risk of restenosis by providing a physical barrier to neointimal growth, and may also reduce the risk of distal embolization in the treatment of ulcerated or thrombotic lesions. (35).

No clinically viable therapies have been developed that permit sustained, non-toxic, and targeted NO release to tissues of interest. The localized arterial delivery of NO has a well-recognized potential for improving the efficacy of endovascular interventions by reducing neointimal hyperplasia and improving arterial endothelialization, thereby reducing the risk of restenosis and thrombosis, respectively (36, 37). In addition, NO is a potent vasodilator and will thereby combat the elastic recoil often observed following arterial intervention. Despite the description of diazeniumdiolates over fifty years ago, clinical translation of diazeniumdiolate NO donors has not been realized for various reasons, including concerns of carcinogenicity related to their N-nitroso derivatives, instability of NO release, lack of potency, and complexity of preparation (38, 39). We have developed a NO donor derived

from chitosan, a biocompatible polysaccharide biopolymer that does not require chemical modification in order to achieve diazeniumdiolation during NO gas exposure. We observed that chitoNO provides prolonged release of NO from mPDC at levels that exceeds the reported physiological NO flux levels from endothelial cells ($0.5\text{--}4 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$) (40). When generated from NO donors, HNO reduced NO release efficiency, produced endothelial toxic effects, and increased mortality in an animal model of intimal hyperplasia (41, 42). As opposed to other types of chitosan-based NO donors reported in the literature (43–45) derived from complex reactions to introduce secondary and tertiary amines with unknown *in vivo* toxicity profiles, chitoNO is derived from a naturally occurring substance with minimal synthetic modifications (43–45). The release of NO from mPDC will promote endothelial recovery, minimize neointimal hyperplasia, and promote positive arterial remodeling following scaffold implantation (46).

The objective of this work was to demonstrate the feasibility of this novel strategy to stent arteries using the ILVS. Although the results are promising, there are some limitations to this study. The control of the mechanical strength and elasticity is due to the extent of radical polymerization of the methacrylates grafted onto the PDC, which depends on the degree of methacrylation, the initiator type and content, and the presence of other components in the system such as NO, which may inhibit the initiation or chain propagation steps. Therefore, direct comparisons between the ILVS with and without NO loading should not be made. For the vascular stenting application discussed herein, although the ILVS radial strength can be tailored to achieve the radial strength of commercial stents, we hypothesize that the ILVS may not require the radial strength of conventional metallic (or even other biodegradable) stents since the release of NO may promote vasodilation reducing the need for outward scaffolding forces. To enable comparison among various ILVS and assess the impact of individual components or composition, a relationship between the polymer crosslink density and scaffold mechanical properties could potentially be devised. The crosslink density (n) can be calculated according to the theory of rubber elasticity using the equation $n = E_0 / 3RT$, where n represents the number of active network chain segments per unit volume (mol/m^3), R is the universal gas constant (8.3144 J/mol K) and T is the absolute temperature (K). Future studies should focus on characterizing how the mechanical properties of the scaffold vary with the degree of polymer crosslinking.

In summary, the intravascular delivery of ILVS represents a potential paradigm shift in the field of endovascular therapy. No existing therapy has demonstrated the ability to address the unique mechanical and arterial healing challenges of endovascular interventions in the peripheral arteries. Future long-term large animal models of atherosclerosis examining the efficacy and safety of the ILVS platform will further clarify the translational potential of this technology.

Methods

Synthesis of methacrylated poly (1,12-dodecamethylene citrate) (mPDC)

Poly (1,12-dodecamethylene citrate) (PDDC) was synthesized by polycondensation of 154 g (0.8 mol) of citric acid (Sigma-Aldrich) and 81 g (0.4 mol) of 1,12-dodecanediol (Sigma-Aldrich). Citric acid and 1,12-dodecanediol were heated to 165°C for 15 minutes under a

nitrogen atmosphere. After melting, the reaction was continued for an additional 30 minutes at 140°C. The viscous PDDC pre-polymer was allowed to cool and dissolved with 150 mL of ethanol. PDDC was purified by precipitation in 1500 mL of deionized water, followed by lyophilization. Next, PDDC was methacrylated by dissolving 66 g of PDDC in 540 mL of tetrahydrofuran (Sigma-Aldrich). After dissolution, 2448 mg (36 mmol) of imidazole (Sigma-Aldrich) was added, followed by drop-wise addition of 53 mL (0.4 mol) of glycidyl methacrylate (Sigma-Aldrich). The mixture was stirred for 6 hours at 60°C and was then placed on a rotary evaporator for 30 minutes at 60°C. The resulting mPDC was purified using deionized water and was lyophilized overnight. Methacrylation efficiency of the PDC was determined using NMR.

Characterization of mPDC

The proton nuclear magnetic resonance (¹H-NMR) spectra of PDDC and mPDC were recorded with an Au400 NMR spectrometer (Agilent, Santa Clara, CA) at ambient temperature, using dimethylsulfoxide-*d*₆ as a solvent and a reference. The Fourier transform infrared (FTIR) transmission spectra of PDDC and mPDC were recorded on a Nexus 870 spectrometer (Thermo Nicolet, Madison, WI, USA) by accumulation of 64 scans, with a resolution of 8 cm⁻¹.

Preparation of blue light photoinitiator

300 mg of photoinitiator camphorquinone (Sigma-Aldrich) and 300 mg of co-initiator 4-ethyl N, N-dimethylaminobenzoate (Sigma-Aldrich) were dissolved in 3 mL of ethyl acetate (anhydrous, 99.8%; Sigma-Aldrich) and blended with mPDC immediately prior to photopolymerization.

In vitro mPDC scaffold synthesis

mPDC was dissolved in ethyl acetate (10–20 wt%) and blended with the blue light photoinitiator (1–2 wt%). Scaffold diameter (outer diameter: 4, 5, and 6 mm) and thickness (0.2, 0.5, and 0.75 mm) were controlled using polycarbonate scaffold molds and PTFE rods of varying diameter to create the ILVS lumen. Photopolymerization was achieved by external illumination using a continuous wave blue laser diode (455–470 nm; Necsel, Milpitas, CA) positioned at a distance of 1 cm from the polymer scaffold mold with varying light intensities (200–900 mW/cm²), measured with a power meter (FieldMaster® coherent Model, Coherent Inc., Santa Clara, CA, USA). Photopolymerization energy and time were varied to modulate mechanical properties of the cured ILVS. For mPDC ILVS containing chitoNO, the photoinitiator was increased to 3 wt% to ensure complete polymerization. All ILVS were cut to 2.1 cm to match the length of the nitinol BMS (Cordis Precise, 5 mm diameter, 0.22 mm thickness).

Mechanical testing of mPDC and mPDC-chitoNO scaffolds

Mechanical compressive tests of *in vitro* and *ex vivo* mPDC scaffolds were performed using an Instron 5544 mechanical tester equipped with a 500 N load cell at a rate of 5 mm/min. Radial compression testing was performed by compressing mPDC stents a total of 2 mm corresponding to 33% to 50% displacement depending on the outer diameter of the stent. A

three-point bend test apparatus (a cylindrical actuator in the middle of two cylindrical end-supports at a distance of 20 mm) was used for flexibility (inverse of stiffness) testing, which was performed on a MTS Sintech 20/G Universal Testing Machine with a 210 N load cell at a crosshead rate of 10 mm/min. The maximum bending angle was set at 48°. All testing and measurements were performed in accordance with American Society of Testing and Materials (ASTM) guidelines for testing vascular stents (F2079, F2477, and F2606).

Degradation study of mPDC scaffold and serial radial compression tests

In vitro mass loss was performed by incubation of mPDC scaffolds in 10 mL of PBS at 37°C and pH 7.4 for various time points. Phosphate buffered saline was refreshed daily. Three mPDC scaffolds were used per time point. The effect of polymeric degradation on mechanical properties was determined using the Instron Mechanical Tester for radial compression (as described above). Scaffolds were weighed before and after lyophilization, and the dry weight was used to calculate mass loss.

Rheological characterization of mPDC and mPDC-chitoNO

The rheological properties of mPDC with and without chitoNO were characterized using an Advanced Rheometer AR 550 (TA Instruments). A 20 mm diameter plate geometry was used for all experiments with a total sample volume of 150 μ L, and a solvent trap cover to minimize sample evaporation. A temperature ramp experiment was performed from 15 to 45°C with a heating rate of 5°C/min while monitoring the viscoelastic moduli under small amplitude oscillatory shear at an applied frequency of $\omega=50$ rad/s and strain amplitude of $\gamma_0=1.5\%$. Subsequently, a flow ramp experiment was performed for 0.1 to 142.665 rad/s at 37°C to determine the dynamic viscosities of mPDC in different dilutions.

Synthesis of nitric oxide-releasing chitosan (chitoNO)

1 g of chitosan (low molecular weight; Sigma-Aldrich) suspended in 6 mL of acetonitrile in a Parr hydrogenation bottle was flushed with argon and then let to stir for 4 days under pressurized NO gas at 70 PSI. The suspension was then filtered with 0.2 micron filter paper (Millipore), and the recovered chitoNO was let to dry overnight at room temperature.

Preparation of nitric oxide-releasing mPDC (mPDC-chitoNO)

mPDC-chitoNO discs were made for NO release experiments by incorporating chitoNO at various concentrations (0 and 150 mg/ml) into liquid mPDC containing 10 wt% ethyl acetate. The blue light photoinitiator was incorporated into the mPDC-chitoNO at 1 and 3 wt % depending upon the absence or presence of chitoNO, respectively. The liquid mPDC-chitoNO was crosslinked in PTFE dishes to obtain a disc diameter of 1.5 cm and thickness of 0.5 cm.

Nitric oxide release measurements

NO release from the mPDC-chitoNO discs was measured using a Sievers chemiluminescence Nitric Oxide Analyzer (NOA), model 280 (GE Instruments; Boulder, CO). A mPDC-chitoNO disc was placed in the NOA purge vessel containing 2 mL of PBS buffer at 37 °C. NO liberated from the disc was continuously swept from the headspace of

the sample cell and purged from the buffer with a nitrogen sweep gas and bubbler into the chemiluminescence detection chamber. The flow rate was set to 200 mL/min with a chamber pressure of 6 Torr and an oxygen pressure of 6 psi.

Engineering the ILVS for Catheter-Based Endovascular Delivery

A critical engineering challenge of the ILVS was to evaluate the feasibility of injecting the viscous mPDC pre-polymer into a small diameter lumen (typically <2 mm) utilized in endovascular catheter systems. The rheometric properties of pure and diluted (ethyl acetate) mPDC pre-polymer at various temperatures and shear rates were evaluated as described below. Then the most concentrated dilution of mPDC that was easily hand-injected at 37°C down the length of various commercial angiography catheters with differing inner diameters (range: 0.9-1.3 mm ID; length 90-130 cm) was identified. This information was used to approximate the ID of the polymer infusion/aspiration lumens of our specialty catheter as described below.

Specialty Catheter Design and Specifications

The specialty catheter (Vention Medical®) was a 9F catheter fabricated from flexible polyether block amide (PEBAX) and was developed specifically for the endovascular delivery of the ILVS. The catheter contains multiple lumens and three balloons (proximal/distal occlusion balloons and inner angioplasty/casting balloon) (Figure 7). The zone of catheter between the occlusion balloons is transparent, allowing light to pass through the catheter to polymerize the polymer. The guidewire lumen is 0.71 mm in diameter, allowing the use of a 0.018" guidewire. The light fiber lumen is 0.71 mm in diameter to permit passage of our fiber optic light diffuser (1cm active tip, 200 µm glass core, Pioneer Optics, Co; Bloomfield, CT). The proximal and distal occlusion balloon lumens are 0.41 mm in diameter, and the inner angioplasty/casting balloon lumen is 0.41 mm. The polymer infusion/aspiration lumens are 1.3 mm in diameter. The inter-occlusion balloon distance is 2.5 cm, and the inner angioplasty/casting balloon is 1 cm in length. The occlusion balloons are made from Yulex, a very compliant, latex substitute material. The occlusion balloons can be inflated to above 5 mm in diameter. The angioplasty/casting balloon is made from Nylon, a non-compliant material. Two types of catheters were fabricated with 5 or 6 mm diameter inner angioplasty/casting balloon diameter to treat various arterial diameters. Functionality of the specialty catheter was assessed using a mock blood vessel made from 6 mm ID silicon tubing (Figure 7F).

Ex vivo mPDC scaffold fabrication

mPDC scaffolds were photopolymerized in porcine iliac arteries (approximately 6 mm diameter) using a commercial peripheral angioplasty balloon (4 mm × 4 cm). A suture was used to tie the distal artery to prevent leakage of polymer. mPDC was poured into the artery around the balloon which was then inflated to nominal pressure. A laser-coupled fiber optic diffuser (Pioneer Optics, Bloomfield, CT) was then inserted into the catheter wire lumen to the level of the balloon. The mPDC scaffold was photopolymerized for 45 seconds using 287 mW/cm² of light energy. The ILVS-treated artery was cut to 2.1 cm for radial compression testing.

Tissue processing and histology

Porcine iliac arteries with photopolymerized mPDC were fixed with 10% formalin overnight. A series of acetone solutions (75%, 90%, and 100% for two times; 15 min each) was used for the dehydration steps. The samples were then embedded in paraffin using routine procedures, cut into 10-12 μ m sections, and stained with hematoxylin and eosin (H&E). Images of the samples were collected with light microscopy using a Zeiss Imager.A2 microscope (Hallbergmoos, Germany).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Synthesis and chemical characterization of mPDC

(A) Reaction scheme of mPDC synthesis. (B) Cured mPDC tubes formed from liquid mPDC.

Figure 2. Mechanical characterization of mPDC scaffolds

(A) Radial strength of mPDC increases with increasing photopolymerization times. * $P < 0.01$ vs 30 sec and Nitinol BMS; $P < 0.004$ vs 30 sec, Student's t test. (B) Radial strength of mPDC increases with increasing photopolymerization intensity. * $P < 0.004$ vs 0.65 mW/cm^2 for Nitinol BMS and 0.9 mW/cm^2 ; ** $P < 0.05$ vs 0.65 mW/cm^2 and Nitinol BMS, Student's t test. (C) Radial strength of mPDC increases with increasing scaffold thickness. * $P < 0.0005$ vs all samples, 1-way ANOVA with post-hoc Bonferroni. (D) Image showing increasing thickness of mPDC scaffold. (E) Radial strength of mPDC decreases with

increasing scaffold diameter. * $P < 0.005$ vs all samples; ** $P < 0.01$ vs 6 mm and Nitinol BMS, 1-way ANOVA with post-hoc Bonferroni. (F) Image showing increasing diameter of mPDC scaffold. Data represent mean $\pm$ SEM of 3 independent experiments. BMS=bare metal stent; sec=seconds

Figure 3. Flexibility characterization of the ILVS compared to commercial nitinol bare-metal stents (BMS)

(A) Representative bending flexibility plots for the ILVS (0.5 mm thickness, 5 mm outer diameter, and cured for 45 s). (B) Flexibility of mPDC increases with increasing photopolymerization times. Data are representative of 3 independent experiments. s=seconds

Figure 4. Degradation profile of mPDC

(A) Mass loss of mPDC scaffold over time. Note that mPDC scaffolds have a mass loss of 27% after incubation in pH 7.4 PBS at 37 °C for 28 wks. (B) Radial strength of mPDC scaffolds is retained after incubation in pH 7.4 PBS at 37 °C for 28 wks. (C) Mass loss of mPDC scaffolds is decreased after incubation in pH 7.4 PBS at 37 °C for 28 wks when photopolymerization time is increased from 30 to 45 s. Data represent mean $\pm$ SEM of 3 independent experiments. wks=weeks; s=seconds

Figure 5. Rheology of varying concentrations of mPDC

(A) Viscosity of mPDC solution decreases with lower mPDC concentrations and increasing temperatures. (B) Viscosity of mPDC remains the same with varying concentrations of mPDC over a range of shear rates. Data are representative of 3 independent experiments.

Figure 6. Characterization of mPDC-chitoNO

(A) NO surface flux from mPDC with 100 mg/mL of chitoNO shows sustained release for 180 min. Data represent average $\pm$ SEM of 3 independent experiments. (B) Radial strength of mPDC scaffolds is increased with increasing amounts of chitoNO in the scaffold. Data represent mean $\pm$ SEM of 3 independent experiments. (C) Viscosity of mPDC 70 wt% is increased with addition of chitoNO (50 mg/mL). Data are representative of 3 separate experiments. min=minutes

Figure 7. Design of the illuminating triple balloon specialty catheter

(A) Prototype drawing of the triple balloon specialty catheter. (B) The specialty catheter has 6 lumens, where P = polymer injection/aspiration port, W = guide wire port, B = proximal/distal occlusion balloon port and middle casting balloon port, and L = laser fiber port. (C) Representative image of occlusion balloons (yellow) and middle casting balloon (pink). (D) Ten specialty catheters shown with the 6 ports on the left and the balloons on the right. (E) Laser fiber diffuser turned on. (F) Functionality of the specialty catheter was assessed using a mock blood vessel (6mm ID silicon tubing).

Figure 8. *Ex vivo* mPDC scaffold experiments

(A) mPDC photocured inside a porcine artery. (B) Radial strength of the mPDC coated artery is greater than that of nitinol BMS and the artery alone. Data represent mean $\pm$ SEM of 3 independent experiments. (C) Hematoxylin and eosin stain of the porcine artery coated along the luminal surface with mPDC. Scale bar: 100 μ m. BMS=bare metal stent