Published in final edited form as:

Macromol Biosci. 2011 May 12; 11(5): 700–709. doi:10.1002/mabi.201000509.

Poly(di-ol-co-citrate)s as Novel Elastomeric Perivascular Wraps for the Reduction of Neointimal Hyperplasia

M. Concepcion Serrano^a,

Biomedical Engineering Department, Northwestern University, Evanston, IL 60208, USA

Ashley K. Vavra^a,

Institute of BioNanotechnology in Medicine, Northwestern University, Division of Vascular Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Michele Jen,

Biomedical Engineering Department, Northwestern University, Evanston, IL 60208, USA

Melissa E. Hogg,

Institute of BioNanotechnology in Medicine, Northwestern University, Division of Vascular Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Jozef Murar,

Institute of BioNanotechnology in Medicine, Northwestern University, Division of Vascular Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Janet Martinez,

Institute of BioNanotechnology in Medicine, Northwestern University, Division of Vascular Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Larry K. Keefer,

Laboratory for Comparative Carcinogenesis/Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702, USA

Guillermo A. Ameer^{*}, and

Biomedical Engineering Department, Northwestern University, Evanston, IL 60208, USA, Fax: +1 847 491 4928, g-ameer@northwestern.edu

Institute of BioNanotechnology in Medicine, Northwestern University, Division of Vascular Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Melina R. Kibbe^{*}

Institute of BioNanotechnology in Medicine, Northwestern University, Division of Vascular Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA, Fax: +1 312 503 1222, mkibbe@nmh.org

Abstract

The synthesis of poly(diols-co-citrate) elastomers that are biocompatible with vascular cells and can modulate the kinetics of the NO release based on the diol of selection is reported. NO-mediated cytostatic or cytotoxic effects can be controlled depending on the NO dose and the exposure time. When implanted *in vivo* in a rat carotid artery injury model, these materials demonstrate a significant reduction of neointimal hyperplasia. This is the first report of a NO-releasing polymer fabricated in the form of an elastomeric perivascular wrap for the treatment of neointimal hyperplasia. These elastomers also show promise for other cardiovascular pathologies where NO-based therapies could be beneficial.

Keywords

biological application of polymers; elastomers; neointimal hyperplasia; nitric oxide; vascular

Introduction

Nitric oxide (NO) was first introduced in the context of biological systems over two decades ago, when several investigators demonstrated its role as a physiological mediator of endothelium-dependent vasodilation.^[1,2] More recently, the pathway involving not only NO but also its stable decomposition products (i.e. nitrite and nitrate) has received significant attention.^[3] NO has been extensively investigated for its key role in the cardiovascular system.^[4] Its protective properties on cardiovascular tissues have prompted extensive research to develop NO-based therapies that can be effectively applied in the treatment of cardiovascular pathologies. Along these lines, NO-releasing vascular grafts may one day be part of the tools available to physicians and surgeons to treat neointimal hyperplasia.^[5-8] Neointimal hyperplasia is the pathologic outcome of the vascular healing response. It involves initial denudation of the vascular endothelium, followed by platelet adhesion and aggregation, leukocyte chemotaxis, proliferation and migration of vascular smooth muscle cells (VSMC), and collagen deposition.^[9,10] More recent research has conferred an important role to adventitial fibroblasts and macrophages, whose phenotypic differentiation into myofibroblasts also contribute to the development of neointimal hyperplasia.^[11,12]

Despite some success to date, many of the NO-based therapies described in the literature for the treatment of neointimal hyperplasia have limited use due to negative systemic side effects, the involvement of viral vectors, and lack of robust or durable efficacy.^[13-16] Local perivascular powder-based treatments have demonstrated significant efficacy relative to other therapies; however, homogeneous application of the NO-donor and its dose are more

difficult to control.^[10,17] Growing interest has been placed recently on the local application of polymer-based or polymer-coated devices that can deliver NO to overcome the limitations of powder-based therapies described above (Table 1).^[10,18-25] We have recently described a novel poly(diols-co-citrate) elastomer based on octanediol that can release NO through the incorporation of diazeniumdiolate groups in the polymer backbone.^[26] Herein, we demonstrate that the choice of diol can determine the NO release kinetics and that these elastomers can be used to fabricate perivascular wraps to inhibit the formation of neointimal hyperplasia following arterial injury. These polymers have mechanical properties adequate for vascular tissues and are biocompatible with vascular cells.

Experimental Part

Chemicals

All chemicals were purchased from Sigma (St. Louis, USA) and used as received unless otherwise indicated.

Synthesis and Preparation of NO-Releasing Poly(diols-co-citrate) Elastomers

The synthesis of NO-releasing elastomers was performed as previously described for octanediol-based elastomers.^[26] Figure 1 summarizes the synthesis of these polymers. Briefly, citric acid (200 mmol), the diol of selection (i.e. 1,8-octanediol for faster NO release or 1,12-dodecanediol for slower NO release) (180 mmol) and *N,N'*-bis(2-hydroxyethyl)ethylenediamine (20 mmol) were polymerized by condensation reaction at 130 °C for 40 min in a N₂ atmosphere to obtain a liquid pre-polymer. The pre-polymer was then dissolved in ethanol (50% solution) and cast into a glass plate by solvent evaporation. The samples were subsequently post-polymerized at 80 °C for 4 d to create the cross-linked POCDA10 (for 1,8-octanediol) or PDDCDA10 (for 1,12-dodecanediol). Prior to diazeniumdiolation, the polymer films were soaked in distilled water for 4 d to eliminate any remaining non-reacted acidic monomers that could affect the stability of the diazeniumdiolate groups and subsequently lyophilized for 2 d. Then, the samples were treated for 4 d in a custom-built chamber pressurized with NO gas (70 psi) in the presence of anhydrous acetonitrile. After treatment, all samples were removed from the chamber, rinsed with fresh acetonitrile three times and dried under vacuum overnight. The resulting NO-releasing materials are referred to as POCDA10-NO and PDDCDA10-NO, respectively.

Chemical and Mechanical Characterization

Fourier-transform infrared (FT-IR) spectra were obtained at room temperature using a FTS40 FT-IR spectrometer (BioRad Hercules, CA, USA). Both pre-polymer samples (50% solution in ethanol) and post-polymerized samples (solid polymer discs post-polymerized as previously described) were analyzed. Elemental analysis was performed to verify the incorporation of the diaminediol and to quantify the nitrogen content in the polymers by X-ray photoelectron spectroscopy (XPS). XPS spectra were collected using Oµm ESCA-LAB (Oµm, Taunusstein, Germany) with a monochromated AlK_α (1486.6 eV) 300 W X-ray source. Analysis consisted of broad survey scan as well as high-resolution N(1s), C(1s) and O(1s) scans. Tensile mechanical tests were conducted according to ASTM D412a on an Instron 5544 mechanical tester equipped with 500 N load cell (Instron Canton, MA, USA).

Briefly, dog-bone-shaped samples (26 mm in length, 4 mm in width, 1 mm in thickness) were pulled at a rate of $500 \text{ mm} \cdot \text{min}^{-1}$. Values were converted to stress/strain and Young's modulus was calculated from the initial slope. Four to six samples were measured and averaged. To evaluate the hydrophobicity of the samples, water-in-air contact angles were measured at room temperature using the dynamic sessile drop method with a Rame' Hart goniometer and an imaging system (Rame' Hart, Inc., USA). At least four independent samples were averaged.

Quantification of NO Release

NO release was monitored by using the Griess reaction, as nitrite is a major degradation product of NO.^[27] Briefly, the specimens were incubated in phosphate-buffered saline (PBS) at 37 °C for up to 4 weeks and supernatant samples were collected at different time points. Fresh PBS was added after each collection and the Griess reaction performed on the supernatants. Samples were prepared as discs of 0.6 cm in diameter, ca. 1 mm in thickness and $35 \pm 4 \text{ mg}$ in weight. At least three independent experiments were performed and samples were run in triplicate.

In vitro Biocompatibility Studies with Non-Treated Elastomers

Prior to cell culture, polymer discs were sterilized under UV-C radiation (Hg lamp, 30 W) for 15 min per side and incubated in culture media at 37 °C for 4 d in order to eliminate any non-reacted acidic residues. Human umbilical vein endothelial cells (HUVEC) and human aortic smooth muscle cells (HASMC) were cultured on the materials at a density of $5 \times 10^4 \text{ cells} \cdot \text{mL}^{-1}$ in the appropriate culture media (i.e. endothelial growth medium-2 and smooth muscle growth medium-2, respectively, Lonza, Walkersville, USA). Cultures were then maintained under CO₂ (5%) atmosphere at 37 °C for different time points. The morphology of the cultures was monitored over time and recorded with an inverted light microscope (Nikon Eclipse, TE2000-U) equipped with a Photo-metrics CoolSNAP HQ camera (Silver Spring, MD, USA). Cell proliferation was quantified via DNA concentration measurements with the Quant-I T PicoGreen dsDNA assay kit (Invitrogen, Eugene, OR, USA) and cell viability tested by using the Live/Dead viability/cytotoxicity kit (Invitrogen, Eugene, OR, USA). For quantifying cell viability in the cultures, the number of live (green fluorescence) and dead (red fluorescence) cells were counted for each culture condition ($n = 5$) and the percentage of live cells calculated relative to total number of cells. Tissue-culture polystyrene (TCP) was used as a control surface.

In vitro Biocompatibility Studies with NO-Releasing Elastomers

Subconfluent cultures of HUVEC and HASMC were exposed to either NO-releasing POCDA10 (POCDA10-NO) or NO-releasing PDDCDA10 (PDDCDA10-NO) polymer discs through the use of a transwell permeable support (polycarbonate membranes, 8.0 μm pore size, 6.5 mm diameter, Corning, USA). Prior to the experiment, polymer discs ($\approx 6 \text{ mm}$ diameter and $\approx 1 \text{ mm}$ thickness) were sterilized under UV-C radiation (Hg lamp, 30 W) for 15 min per side. Different NO fluxes were achieved in the culture media by using either POCDA10 or PDDCDA10 polymers and varying the exposure time. The total nitrite released from these samples (measured by Griess reaction) was: $0.4\text{--}0.7 \mu\text{mol} \cdot \text{cm}^{-2}$ (low

dose), $3 \mu\text{mol} \cdot \text{cm}^{-2}$ (intermediate dose) and $7\text{--}11 \mu\text{mol} \cdot \text{cm}^{-2}$ (high dose). The effects on HUVEC and HASMC were investigated by evaluation of cell morphology in culture, DNA concentration as an indicator of cell number and cell viability, as described above. For these experiments, cell proliferation was referred to the initial DNA concentration of each culture condition.

In vivo Implantation in a Rat Carotid Artery Model of Neointimal Hyperplasia

All animal procedures were performed in accordance with the principles outlined in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication National Academy press, 1996) and approved by the Northwestern University Animal Care and Use Committee. Ten-week-old male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 300–400 g were anesthetized with inhaled isoflurane (0.5–2%). Prior to the procedure, atropine ($0.1 \text{ mg} \cdot \text{kg}^{-1}$) and carprofen (Rimadyl TM, $0.15 \text{ mg} \cdot \text{kg}^{-1}$) were administered subcutaneously to decrease airway secretions and for pain control, respectively. Equaline sterile lubricant (Boise, ID) was also applied to the animal's eyes. A midline incision was made in the neck and, using a combination of blunt and sharp dissection, the left common, internal, and external carotid arteries were identified. After distal ligation of the external carotid artery, the internal and common carotid arteries were occluded with atraumatic clamps and a 2 French arterial embolectomy catheter (Edwards Life-sciences, Irvine, CA, USA) was inserted into the external carotid artery proximal to the point of ligation and advanced into the common carotid artery. Uniform injury was created by inflating the balloon to 5 atm for 5 min. After removal of the balloon, the external carotid artery was ligated proximal to the arteriotomy and blood flow restored. Treatment groups included injury alone ($n = 7$), injury + untreated PDDCDA10 ($n = 6$) and injury + NO-treated PDDCDA10 ($n = 7$). Following restoration of blood flow, untreated PDDCDA10 or PDDCDA10-NO films (50 mm long, 5 mm wide and ≈ 0.2 mm thick; previously sterilized by UV-C radiation for 15 min per side) were wrapped around the common carotid artery at the site of injury a minimum of three times and secured with a 7.0 prolene tie. Finally, the incision was closed in two layers with 4.0 vicryl and 3.0 nylon suture respectively. For injury alone groups, the neck incision was closed following restoration of blood flow. Rats were sacrificed at a 2-week time point to assess for neointimal hyperplasia.

Tissue Processing

Carotid arteries were harvested at 2 weeks following in situ perfusion-fixation with PBS and 2% paraformaldehyde. Vessels were placed in paraformaldehyde at 4°C for 1 h, then 30% sucrose in PBS at 4°C for cryo-protection. The tissue was quick-frozen in Optimal Cutting Temperature compound (Tissue Tek, Hatfield, PA) and 5- μm sections were cut throughout the entire injured segment of the common carotid artery using a Microm HM550 cryostat.

Morphometric Analysis

Carotid arteries harvested at two weeks were examined histologically for evidence of neointimal hyperplasia using routine hematoxylin/eosin (H&E) staining. Digital images were collected with light microscopy using an Olympus BHT microscope (Melville, NY)

with 4 ×, 10 × and 40 × objectives. Six evenly-spaced sections through each injured carotid artery were analyzed. Intima area was measured as the area between the lumen and the internal elastic lamina. Media area was measured as the area between the internal and external elastic lamina. Neointimal hyperplasia was also quantified as the ratio of intima to media area. Luminal area and arterial circumference (external elastic lamina circumference) were also measured. These values were obtained (arbitrary units) using ImageJ software and all analysis was done by a single individual.

Statistics

Statistical analysis was performed by using the Statistical Package for the Social Sciences software (SPSS, version 17.0). Comparisons were done by analysis of variance (ANOVA) and post-hoc Scheffé or Student-Newman-Keuls test to discriminate differences among groups. In all in vitro experiments, at least three independent replicates were measured and averaged. For all statistical evaluations, $p < 0.05$ was considered statistically significant.

Results and Discussion

Polymer-based therapies capable of delivering NO locally for the prevention of neointimal hyperplasia have shown limited success to date. Herein, we describe the synthesis of poly(diols-*co*-citrate)s that show controlled release of NO and feasibility to be prepared as elastomeric perivascular wraps for the treatment of neointimal hyperplasia. FT-IR spectra of the polymeric samples confirmed the esterification reaction (Figure 2). The broad peaks at 3 200–3 400 cm^{-1} were attributed to non-reacted carboxyl and hydroxyl groups from citric acid, whereas peaks centered at 1 730 cm^{-1} referred to non-reacted carboxyl groups from citric acid and ester bonds formed after condensation reaction of the monomers. Sharp peaks centered at 1 050–1 150 cm^{-1} were also attributed to non-reacted hydroxyl groups from citric acid. The low percentage of amine groups incorporated into the polymer network made it difficult to detect their presence via FT-IR. For that reason, elemental analysis using XPS was performed to demonstrate the incorporation of the diaminediol and quantify the nitrogen content in the polymers. Broad survey scans indicated the presence of nitrogen in both POCDA10 and PDDCDA10, while nitrogen was not detected in the polymers without diaminediol content. Further, high-resolution XPS N(1s), C(1s) and O(1s) scans suggested a surface nitrogen content of $0.93 \pm 0.13\%$ for POCDA10 and $0.98 \pm 0.04\%$ for PDDCDA10. These experimental values are close to the theoretical values of 0.87 mol% for POCDA10 and 0.75 mol% for PDDCDA10. The mechanical properties of these polymers are summarized in Table 2. Both POCDA10 and PDDCDA10 materials showed elastomeric properties in the range of those described for vascular tissues.^[28–30] Tensile strength values ranged from 2.7 to 3.5 MPa, Young's modulus of 2.8 to 8.0 MPa and elongations at break of 195 to 290% depending on the diol of selection. Based on these results, the incorporation of the diaminediol monomer into the network tends to confer more stiffness to the polymer network (i.e. higher tensile strength and Young's modulus) when compared to original poly(1,8-octanediol-*co*-citrate) (POC) elastomers. As expected, this significant increase in stiffness is accompanied by a decrease in the elongation at break. Contact angle measurements demonstrated a significantly higher hydrophobicity for PDDCDA10 materials relative to their POCDA10 counterparts in terms of advancing contact angles (Table 2). No

receding contact angles could be reported because of the complete wetting of the polymer surface without distinction among materials.

Figure 3 shows the NO release kinetics from these elastomers as determined by Griess reaction.^[31] A maximum flux of $35.4 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ was obtained for POCDA10-NO and $6.6 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ for PDDCDA10-NO elastomers. The total release was 10.45 and $4.20 \text{ } \mu\text{mol} \cdot \text{cm}^{-2}$, respectively. When evaluating the duration of the release, a more extended release was obtained from PDDCDA10-NO elastomers (up to 21 d), whereas a faster burst release was obtained for POCDA10-NO materials (completed after 2 d). Since the decomposition of the diazeniumdiolated groups and subsequent NO production depends on hydrolysis,^[32] the higher hydrophobicity of PDDCDA10 elastomers demonstrated by contact angle studies was expected to extend the NO release, as previously reported for other polymers.^[33-38] Furthermore, NO generation from diazeniumdiolates of the type described here is acid-catalyzed.^[32] Despite the presence of carboxylic acid groups in the elastomers, the shelf-life was such that substantial amounts of NO were retained in the dry material until freed by immersion in neutral phosphate buffer. Nevertheless, the differential acidity of these elastomers may play a role in the kinetics of the NO release.

Since these materials are envisioned to be used as NO-based therapies in the cardiovascular system, the biocompatibility of the amine-containing poly(diols-co-citrate)s was investigated by culturing HUVEC and HASMC on the polymers. Both cell types attached, spread and proliferated on the surface of these materials while maintaining their characteristic cobblestone and spindle-like morphology, respectively (Figure 4). HUVEC proliferation on POCDA10 and PDDCDA10 was not different from proliferation on TCP as cells reached confluence by day 4 on all surfaces. However, decreased cell proliferation, quantified via DNA concentration, was observed when HASMC were seeded on both POCDA10 and PDDCDA10 materials relative to TCP (Figure 5). Cell viability of HUVEC and HASMC cultured on POCDA10 and PDDCDA10 elastomers was above 95% after 4 d in culture. Therefore, the significant inhibition of HASMC growth in the amine-containing poly(diols-co-citrate)s is not attributed to an increase in cell death. However, both cell types seeded on PDDCDA10 elastomers showed a light but significant decrease in cell viability at 24 h that was reversed after 4 d in culture (Table 3). Further studies concerning cell/polymer interactions are necessary to better understand cell type-dependent responses to the polymer surface.

NO is an upstream signaling molecule involved in the activation of either protective or destructive cellular pathways depending on its dose, derivatives and target cell.^[39] We have found distinctive cellular effects based on the dose of NO released from the materials, the time of exposure and the target cell type. When subconfluent cultures of HUVEC and HASMC were exposed to POCDA10-NO and PDDCDA10-NO polymers with different doses of NO, cell proliferation showed a dose-dependent response. In the particular case of HUVEC cultures (Figure 6A), low doses ($0.4\text{--}0.7 \text{ } \mu\text{mol} \cdot \text{cm}^{-2}$) supported cell proliferation, intermediate doses ($3 \text{ } \mu\text{mol} \cdot \text{cm}^{-2}$) did so only during the first 24 h, and high doses ($>7 \text{ } \mu\text{mol} \cdot \text{cm}^{-2}$) induced cell death. It is important to note that even the low doses used in this study are orders of magnitude higher than the physiological NO concentrations, estimated to be in the range of 10^{-10} to $5 \times 10^{-9} \text{ M}$.^[40] As expected,^[41] HASMC proliferation was

significantly inhibited in a dose-dependent response even at low NO concentrations (Figure 6B). When analyzing the viability of the cultures by live/dead staining (Figure 7), the percentage of cell death significantly increased in a similar dose-dependent response in both HUVEC and HASMC cultures exposed to the NO-releasing materials. The variation within dose is attributed to the standard variability between samples. A biphasic effect of exogenous NO was also observed in keratinocyte proliferation and differentiation.^[42] Low concentrations of NO donors ($0.01\text{--}0.25 \times 10^{-3} \text{ M}$) promoted keratinocyte proliferation, while a cytostatic effect was observed at high concentrations ($0.5 \times 10^{-3} \text{ M}$). Converse effects were found for keratinocyte differentiation. Therefore, these *in vitro* results confirm that the biological effects of NO and its degradation metabolites are not only dose-dependent but also time-dependent. Based on the NO dose generated, NO-releasing poly(diols-*co*-citrate)s can be used as either NO-mediated cytostatic or cytotoxic materials, as previously described for other NO-releasing materials.^[43,44]

Because of the NO release pattern we observed and the favorable mechanical properties of PDDCDA10-NO elastomers, these materials were prepared as thin films (thickness < 0.2 mm) and applied as a perivascular wrap to balloon-injured rat carotid arteries. As seen in Figure 8, the wraps were placed around the periadventitial surface of the common carotid artery and secured with a tie. We were able to achieve close proximity of the wrap to the artery. Representative $400 \times$ histological cross-sections of injured carotid arteries not treated or treated with either PDDCDA10 or PDDCDA10-NO periadventitial wraps for 2 weeks are shown in Figure 9. As it can be appreciated, a significant and consistent inhibition of neointimal hyperplasia was observed after 14 d in the NO-treated wrap group when compared to the injury alone and injury plus untreated wrap. Compared to injury alone, PDDCDA10-NO wraps reduced intimal area and the intima-to-media (I/M) area ratio by 45 and 38%, respectively ($p < 0.001$). Although the untreated wraps also reduced intimal area by 24% ($p = 0.009$), neither wrap reduced medial area and the I/M area ratio was not reduced following exposure to the untreated wraps. These results confirm that the NO wraps resulted in a beneficial biological outcome by inhibiting the development of neointimal hyperplasia. Based on the NO release kinetics, this inhibitory effect may consist of two different phases. During the first few hours following injury, the PDDCDA10-NO wraps may provide a significant NO release to the vascular wall that is sufficient to impact in the inhibition of neointimal hyperplasia. The perivascular administration of the short half-life donor PROLI/NO also suggests a critical role for early NO-delivery in the development of neointimal hyperplasia.^[10] Interestingly, similar results have been reported for burn wound healing applications.^[45] Since inorganic nitrite has been demonstrated to inhibit vascular smooth muscle proliferation,^[46] the extended release of NO-derived nitrites from these PDDCDA10-NO elastomers (up to 21 d *in vitro*) could also play a significant effect on the long-term inhibition observed. In our *in vivo* model, NO degradation to nitrites/nitrates is likely diminished because of the reduced exposure to NO-oxidizing molecules (i.e. oxygenated-hemoglobin and ceruloplasmin). We also know that, in low oxygen conditions, non-enzymatic conversion of nitrite to NO is favoured over the oxygen dependent nitric oxide synthase production of NO from L-arginine.^[3] Therefore, the potential low oxygen tension conditions created by the presence of a perivascular wrap, along with the acidic nature of the polymer due to the non-reacted carboxyl groups of citric acid, could also favor

the reconversion of nitrites to NO,^[3] thus extending the exposure of the vascular wall to NO. More extensive studies should be done to fully assess the clinical impact of this approach. In this sense, further studies are currently in progress to quantify the extent of this neointimal inhibition and to investigate the tissue response to the perivascular wrap, as well as the feasibility of using this strategy in a large animal model.

Conclusion

In summary, we report the synthesis of poly(diols-co-citrate) elastomers capable of providing different NO release profiles. When compared to previous NO-based therapies for inhibiting neointimal hyperplasia, these materials: (1) avoid the side effects of systemically administered NO-donors and the safety concerns of viral-based NO-therapies, (2) have elastomeric mechanical properties that are compatible with vascular compliance and facilitate their clinical use as polymer-based NO therapies, (3) allow for a better control of the NO dose administered due to the incorporation of the NO-donor in the polymer backbone (versus powder-based NO-therapies), and (4) have controllable NO release. Furthermore, NO-mediated cytostatic or cytotoxic effects can be controlled depending on the choice of diol and the release kinetics. These elastomers show also promise for the treatment of other cardiovascular pathologies where NO-based therapies can be beneficial.

Acknowledgments

FT-IR and XPS work was performed in the Keck-II facility of *NUANCE Center* at Northwestern University. *NUANCE Center* is supported by NSF-NSEC, NSF-MRSEC, Keck Foundation, the State of Illinois, and Northwestern University. In addition, this project has been funded in part with Federal funds from the *National Institutes of Health* (NIH, under contracts 1 K08HL084203 – MRK-, N01-CO-2008-00001 with SAIC-Frederick, Inc., and HHSN261200800001E – LKK), the *American Vascular Association* (MRK), the *Alpha Omega Alpha Honor Medical Society* (JM), the *National Cancer Institute* (NCI), and by the *Intramural Research Program of the NIH*, NCI, Center for Cancer Research (LKK). It was also supported in part by the generosity of Mrs. *Hilda Rosenbloom*. The authors would like to thank Dr. *Messersmith's laboratory* for the assistance with the contact angle measurements. M. C. S. is greatly indebted to the *Ministerio de Ciencia e Innovación of Spain* for a postdoctoral fellowship.

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Figure 1.
Reaction scheme of the synthesis of NO-releasing poly(diols-co-citrate) elastomers.

Figure 2. FT-IR spectra of POCDA₁₀ (A,B) and PDDCDA₁₀ (C,D) elastomers. Pre-polymerized (A,C) and post-polymerized (B,D) materials are shown.

Figure 3. Representative release profile from POCDA₁₀-NO and PDDCDA₁₀-NO elastomers as assessed by Griess reaction.

Figure 4. Morphology of HUVEC and HASMC cultured on amine-containing poly(diols-co-citrate) elastomers. Scale bar: 25 μ m.

Figure 5. Proliferation kinetics of HASMC cultured on amine-containing poly(diols-*co*-citrate) elastomers. Statistical significance: $p < 0.05$. Differences relative to control samples (*) and between polymers (#) at a similar time point.

Figure 6.

Cumulative exposure to NO-releasing poly(diols-co-citrate) elastomers: Effects on HUVEC (A) and HASMC (B) proliferation. Statistical significance among doses: $p < 0.05$.

Differences relative to control samples (*), to low doses (#), and to intermediate doses (\$) at a similar time point.

Figure 7.

Cumulative exposure to NO-releasing poly(diols-co-citrate) elastomers: Effects on HUVEC and HASMC viability after 48 h exposure to different NO fluxes. The total nitrite released was measured by Griess reaction as: low ($0.4\text{--}0.7\text{ }\mu\text{mol} \cdot \text{cm}^{-2}$), intermediate ($3\text{ }\mu\text{mol} \cdot \text{cm}^{-2}$) and high dose ($7\text{--}11\text{ }\mu\text{mol} \cdot \text{cm}^{-2}$). Scale bar: $25\text{ }\mu\text{m}$.

Figure 8.

Drawing of the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA), with blood flowing from the CCA to the ICA and ECA (first panel). The drawing depicts how the wraps were placed around the artery and secured with a tie. Also shown are photographs of the actual wraps that were placed and secured around the CCA at the time of surgery (second and third panels). Note the close proximity of the wrap to the adventitial surface of the artery. The last panel shows an intact wrap at harvest, 2 weeks following arterial injury and placement of the wrap.

Figure 9.

(A) Representative histological haematoxylin and eosin stained sections of the injured carotid arteries with the different treatment groups 14 d after arterial injury (400 ×). Also shown is the morphometric analysis of the (B) intimal area, (C) intima-to-media area ratio, and (D) medial area. * $p < 0.05$ vs. injury (I) alone.

Table 1

Polymer-based NO therapies for inhibiting neointimal hyperplasia.

Authors	NO source ^{a)}	Method of administration	Animal model	Delivery system ^{d)}	Inhibition		Time point	Ref.
					%	d		
Fulton et al.	SNAP	local	rabbit	Pluronic gel	41 ^{b)}	28	[18]	
Chaux et al.	SPER/NO	perivascular	rabbit	PLA/PGA gel	40 ^{b)} (wall thickness)	28	[19]	
Kaul et al.	SPER/NO	perivascular	rat	PLA/PGA matrix	73 ^{b)}	14	[20]	
Yoon et al.	sodium nitroprusside	intravascular	pig	Polyurethane-coated stent	0	28	[21]	
Do et al.	NHEA	intravascular	rabbit	silane/methacrylate-coated stent	46 32	7 28	[22]	
Bohl Masters et al.	S-nitrosocysteine	perivascular	rat	PEG diacrylate-based hydrogels	75	14	[23]	
Lipke and West	S-nitrosocysteine	perivascular	rat	PEG/cysteine hydrogel	76	14	[25]	
Kapadia et al.	PROL/NO	perivascular	rat	nanofiber gels	77	14	[24]	
	DPTA/NO				45			
Pearce et al.	PROL/NO gel	perivascular	rat	powder-based	65	14	[10]	
	PROL/NO powder			gel-based	86			
	PAN/NO gel			(poloxamer 407)	40			
	PAN/NO powder				67			
	PROL/NO + PAN/NO				80			
Serrano et al.	PDDCDA10-NO	perivascular	rat	polydiolcitrate elastomer	38	14		

^{a)}DPTA/NO: [N-(3-aminopropyl)-N-(3-aminopropyl)]diazene-1-ium-1,2-diolate, NHEA: N-ethyl-2-(1-ethyl-2-hydroxy-2-nitrosodihydroxy-2-ethanamine, PAN/NO: poly(acrylonitrile)/NO, PEG: poly(ethylene glycol), PGA: poly(glycolic acid), PLA: poly(lactic acid), PROL/NO: disodium 1-[(2-carboxylato)pyrrolidin-1-yl]diazene-1-ium-1,2-diolate, SNAP: S-nitroso-N-acetylpenicillamine, SPER/NO: spermine/NO;

^{b)}Percentages estimated based on reported values.

Table 2

Mechanical properties and water-in air contact angle of amine-containing poly(diols-*co*-citrate) elastomers.

Elastomer	<u>Tensile strength</u>	<u>Young's modulus</u>	<u>Elongation at break</u>	<u>Advancing contact angle</u>
	MPa	MPa	%	°
POC	2.9 ± 0.09	1.9 ± 0.09	367 ± 15.0	82.1 ± 4.20
POCDA10	3.5 ± 0.27	8.0 ± 0.42 ^{a)}	290 ± 39.0 ^{a)}	71.8 ± 6.14 ^{a)}
PDDCDA10	2.7 ± 0.32	2.8 ± 0.20	195 ± 20.8 ^{a)}	84.0 ± 5.70
cardiovascular tissues ^[28-30]	0.069–8.3	0.17–44.7	49–300	–

^{a)} Statistical significance: $p < 0.05$.

Table 3

Cell viability studies of HUVEC and HASMC cultured on amine-containing poly(di-ol-co-citrate) elastomers.

Cells	Time h	TCP		POCDA10		PDDCDA10		Statistics
			%		%		%	
HUVEC	24		100 ± 1		95 ± 4		80 ± 8	$p = 0.008$
	96		100 ± 1		100 ± 1		97 ± 3	$p = 0.195$
HASMC	24		99 ± 1		97 ± 4		91 ± 1	$p = 0.000$
	96		99 ± 1		97 ± 2		99 ± 1	$p = 0.171$