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Development of drug-coated balloon for the treatment of multiple peripheral artery segments

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ABSTRACT

Objective: Peripheral artery disease is the second most common cardiovascular disease. It can often occur in complex form when there is a presence of long, diffuse, and multiple lesions. Current treatments use either single long drug-coated balloons (DCBs) or multiple DCBs; however, treatment success is limited. The purpose of this study was to investigate the preclinical feasibility of our multiple-release Tailored Medical Devices DCB (MR-TMD-DCB) to treat multiple arterial segments using a single DCB.

Methods: The MR-TMD-DCBs were developed using a two-layer coating approach. The DCBs were developed in a certified Current Good Manufacturing Practices facility using presterilized materials and reagent and then characterized for coating morphology, thermal and chemical changes, and in vitro particulate shedding. The drug loss, tissue uptake, and undelivered drug amounts were analyzed using an in vitro peripheral artery flow model and explanted pig arteries. Then, an in vivo survival study was performed using a healthy porcine model to measure the short-term drug uptake (seven swine; 14 treatments at day 1) and retention (seven swine; 14 treatments at day 7) in two different arterial segments after treatment with a single MR-TMD-DCB.

Results: The coating on the MR-TMD-DCB was smooth and homogeneous with paclitaxel molecularly dispersed in its amorphous state. A negligible number of particulates were shed from the MR-TMD-DCB coating. A similar amount of drug was accurately delivered into two separate explanted arteries using a single MR-TMD-DCB during the in vitro flow model testing (707 ± 109 ng/mg in the first explanted artery and 783 ± 306 ng/mg in the second explanted artery). The MR-TMD-DCB treatment resulted in equivalent drug amounts in the two arterial segments at day 1 (63 ± 19 ng/mg in the first treatment site and 59 ±19 ng/mg in the second treatment site) and at day 7 (9 ± 6 ng/mg in the first treatment site and 10 ± 6 ng/mg in the second treatment site). In addition, the drug levels at each time point were in the clinically relevant range to prevent neointimal hyperplasia.

Conclusions: The MR-TMD-DCBs provided equivalent and clinically relevant drug retention levels into two different arterial segments. Thus, MR-TMD-DCBs can be used to accurately deliver drug into multiple arterial segments with the use of a single DCB. The clinical outcomes of these findings need further investigation. Future long-term pharmacokinetics and safety studies will be performed to evaluate the safety and efficacy of the MR-TMD-DCB. (J Vasc Surg 2020;71:1750-7.)

Clinical Relevance: Peripheral artery disease is frequently associated with complex lesions including long, diffuse, and multiple blockages that currently require the use of two or more drug-coated balloons (DCBs) for treatment. The use of multiple DCBs increases the procedural cost, time, and complications. The multiple-release DCB developed in this study demonstrated the feasibility to safely and effectively treat two arterial sites using a single device. The multiple-release DCB will be of great benefit, both procedurally and financially, for treatment of complex peripheral lesions. The multiple-release DCB should be further investigated for clinical importance in long-term porcine models against commercially available DCBs.

Keywords: Drug-coated balloon; Peripheral artery disease; Multiple release; Atherosclerosis; Angioplasty

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Author conflict of interest: The institutions of J.A.A., S.L., T.R., and P.K. have a patent issued for the drug-coated balloon discussed in the article. In the future, there is a possibility that the institutions could license the drug-coated balloon to a medical device company. The invention is associated with Sanford Research and University of South Dakota. There has been no financial compensation to date from any company for use of the drug-coated balloon discussed.

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Drug-coated balloons (DCBs) are currently used to treat atherosclerotic vessels, mainly lesions in the peripheral arteries.[1-4] Mostly, the balloons are coated with a drug formulation containing a mixture of the drug paclitaxel (PAT) and an excipient. There has been great interest in novel technologies in DCB development that can precisely control the delivery of drug to provide better tissue uptake and longer tissue retention.[5,6] We recently developed a DCB that contains a polymeric coating (polyethylene oxide [PEO]) to precisely control the delivery of drug (PAT) from the balloon, which has shown inhibition of smooth muscle growth for prevention of neointimal hyperplasia.[7] The PEO-based DCB has been shown to be safe and effective through minimal particulates shed and therapeutic amounts of drug retained in the tissues at day 30, respectively.[8] This demonstrates its potential as an alternative to the currently available DCBs to treat a single lesion.

Peripheral artery disease can often occur in complex forms. In patients with diabetes and heart diseases, there is a greater chance that the lesions are long and diffuse and that they occur at multiple locations within an artery.[9-13] The treatment of these complex lesions is difficult and associated with limited long-term success. Current endovascular treatments of complex lesions often include plain balloon angioplasty, stenting, atherectomy, DCBs, or any combination thereof. In the case of DCBs, either single long DCBs or multiple DCBs are needed to treat complex lesions (Supplementary Fig 1, online only). Vascular surgeons are often unable to treat longer lesions because of the limited sizes of DCBs available to them. Also, vascular surgeons try to avoid the use of DCBs longer than 12 cm in most cases as the blood vessels vary in diameter and the balloons are typically noncompliant. On the other hand, the use of multiple DCBs may lead to increased cost, time, and procedural complications. Hence, there is a need in the marketplace for an innovative DCB able to provide multiple episodes of drug delivery, which may be a benefit, both procedurally and financially, to the patients and surgeons.

We previously developed a single-release Tailored Medical Devices DCB (SR-TMD-DCB) and compared the drug retention in the tissue with a commercially available DCB until day 30.[8] In this study, we have developed multiple-release Tailored Medical Devices DCBs (MR-TMD-DCBs) for the treatment of two arterial segments. The DCB coating was carefully formulated, and a suitable multilayer dip coating approach was used to obtain the MR-TMD-DCB with the desirable controlled drug release properties. The particulate safety and drug loss/tissue uptake were investigated through an in vitro peripheral artery flow model using explanted porcine arteries. Short-term survival studies were then conducted in a porcine model to determine drug retention at 1 day and 7 days in two different arterial locations treated using an MR-TMD-DCB.

ARTICLE HIGHLIGHTS

- **Type of Research:** In vitro and in vivo assessment of a new chemical platform for next-generation drug-coated balloons
- **Key Findings:** The newly developed multiple-release drug-coated balloon delivered and retained equivalent and clinically relevant drug levels in two arterial sites using a single device while also limiting the number of particulates shed from the coating.
- **Take Home Message:** A next-generation multiple-release drug-coated balloon has demonstrated the feasibility to safely and effectively deliver multiple episodes of drug with the use of a single device.

METHODS

Materials

PEO (average Mv, 100,000), ethanol (200 proof), methanol, phosphate-buffered saline, high-performance liquid chromatography (HPLC)-grade water, endotoxin-free water, and acetonitrile were purchased from Fisher Scientific (Waltham, Mass). PAT was purchased from ChemieTek (Indianapolis, Ind). The balloon catheters were purchased from Medtronic (Santa Rosa, Calif). The balloon inflation device was received from Boston Scientific (Marlborough, Mass). All chemicals purchased were used as received.

Preparation of MR-TMD-DCB

The coating formulation and balloons were prepared in a certified Current Good Manufacturing Practices (cGMP) facility (University of South Dakota cGMP cleanroom, Sioux Falls, SD) to produce sterile devices for the animal studies. All the components needed to make the MR-TMD-DCBs were sterilized before use. The PEO powder was sterilized using ethylene oxide (performed by Nelson Laboratories, Inc, Salt Lake City, Utah), and the PAT-ethanol solution was sterile filtered through a 0.2-μm syringe filter in the cGMP laboratory. The instruments and items needed for the MR-TMD-DCB preparation were depyrogenated and autoclaved before use. All manufactured lots were tested for sterility and endotoxin. The sterile testing methods are described in the Supplementary Methods (online only); the sterility and endotoxin results of the MR-TMD-DCBs are provided in Supplementary Fig 2 (online only) and the Supplementary Table (online only), respectively.

The formulation and dip coating method were optimized by analyzing a range of drug-polymer ratios, dipping time, and drying time to achieve desirable drug release properties required for multiple inflations. The optimized coating strategy used the two-layer MR-TMD-DCB approach. The angioplasty balloon catheters (EverCross percutaneous transluminal angioplasty balloon catheters, 5-8 mm × 20-40 mm; Medtronic)

were coated with a drug-polymer layer (first layer) and a polymer-only layer (second layer). The solution used to coat the balloons was prepared as described in the Supplementary Methods (online only). The dip coating method was used to prepare the MR-TMD-DCB as described in the Supplementary Methods (online only). A schematic representation of the MR-TMD-DCB is provided in Supplementary Fig 3 (online only). Thus prepared MR-TMD-DCBs were used in this study.

Characterization of MR-TMD-DCBs

The MR-TMD-DCBs were characterized for their surface morphology, thermal properties, and chemical composition using scanning electron microscopy, differential scanning calorimetry, and Fourier transform infrared spectroscopy, respectively. The details of each of the characterizations are provided in the Supplementary Methods (online only).

In vitro particulate analysis of MR-TMD-DCB

An in vitro bench-top model was used to analyze the particulates shed from three of the MR-TMD-DCBs. The methods of the model are provided in our previous study.[8] The particulates were imaged using an Olympus SZX10 stereo microscope equipped with Olympus DP22 camera (Olympus America, Center Valley, Pa), and the number of particulates and average size of the particulates were quantitatively analyzed (>100 μm) using ImageJ software (National Institutes of Health, Bethesda, Md). For proper visualization, the white particulates were artificially colored green.

Testing of MR-TMD-DCB in an in vitro model using explanted arteries

An in vitro peripheral artery flow model (Supplementary Fig 4, online only) was used along with explanted porcine carotid arteries (received from John Morrell & Co, Sioux Falls, SDak) to determine drug uptake into two separate arteries using an MR-TMD-DCB (5 mm × 40 mm) at the initial time point. The methodologic details of the in vitro model are described in the Supplementary Methods (online only). At least four MR-TMD-DCBs were used to measure the drug release and drug uptake in the model.

The coating distribution on the MR-TMD-DCB before each inflation in the explanted artery was also analyzed. The methodologic details of the imaging are provided in the Supplementary Methods (online only). At least three MR-TMD-DCBs were used, and the representative images were chosen.

Pharmacokinetic study in porcine model

The in vivo protocol was approved by the Institutional Animal Care and Use Committee and the Institutional Review Board, and the animal care complied with the *Guide for the Care and Use of Laboratory Animals* (Protocol No. 121-10-19D). The swine (healthy Yucatan miniature female; average body weight, 30-45 kg) used in this study were purchased from LoneStar Laboratory Swine (Sioux Center, Iowa) and housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. The MR-TMD-DCBs were developed in the University of South Dakota cGMP cleanroom as described before. The in vivo animal operations were carried out as previously described (Supplementary Methods, online only).[8]

Treatment of porcine arteries using MR-TMD-DCB. The pretreatment of the arteries was performed as previously described.[8] Because of the delicate nature of the vessels in the miniature swine and not having access to ultrasound, cutdowns were used to perform the angioplasty instead of performing the procedure percutaneously. To access the left and right iliac arteries, bilateral flank incisions of 1.5 to 2.0 inches were made in the groin area. Open exposures were made with dissection carried down to the left and right iliacs bilaterally. A 5F Micro-Stick Introducer set (MedComp, Braunfels, Germany) followed by a wire and 7F sheath (Terumo, Tokyo, Japan) was used for access. A 5- to 6-mm × 20-mm MR-TMD-DCB was inserted into the upper portion of the left external iliac artery (first treatment site) and inflated for 1 minute. After the first inflation period, the balloon was deflated and transferred down to the second treatment site, keeping a 0.5-cm nontreatment gap between the first and second treatment sites (internal iliac artery used as a separation landmark). The balloon was inflated at the second treatment site for 2 minutes. This was repeated on the right external iliac artery using a different MR-TMD-DCB. The treatment sites in the arteries are provided in Fig 1, *A*. After the treatments, the MR-TMD-DCB was deflated and removed. The incisions were closed in layers with 5-0 Prolene and standard vascular closing procedures. The survival swine were then treated with 1 day of postoperative ketorolac tromethamine (1 mg/kg; Hospira, Lake Forest, Ill) for pain. The swine were housed and monitored throughout the study.

Per the protocol, the swine were euthanized at the day-1 and day-7 time points using Fatal-Plus solution (intravenous injection of 50-100 mg/kg) in accordance with the Institutional Animal Care and Use Committee-approved guidelines established by the American Veterinary Medical Association. After the swine were euthanized, the arteries were collected, frozen, and evaluated for PAT retention using HPLC analysis as described previously.[8] The median sacral artery was retrieved and used as a nontreated control artery. A sample of 5 mL of blood was collected from the day-1 animals to measure the plasma drug levels using HPLC. A total of 14 swine were used, of which 7 swine were used at each day-1 and day-7 time point (Fig 1, *B*). Because two

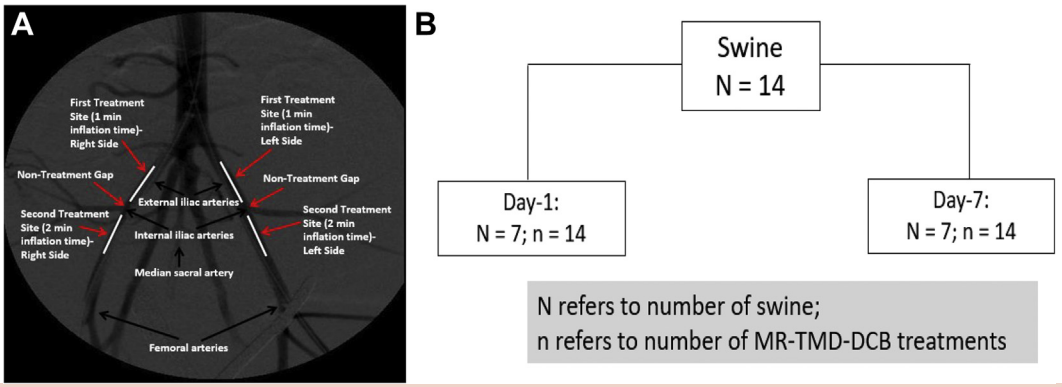

Fig 1. In vivo efficacy study design. **A,** The lower anatomy of the swine used during treatment with the multiple-release Tailored Medical Devices drug-coated balloon (*MR-TMD-DCB*). The inflation sites, arteries, and nontreatment zones are shown. **B,** The study design of the in vivo procedure using the porcine models. A total of 14 swine were used, of which 7 swine were used at each day-1 and day-7 time point. Because two treatments can be performed in each animal, a total of 14 MR-TMD-DCB treatments were performed at each time point.

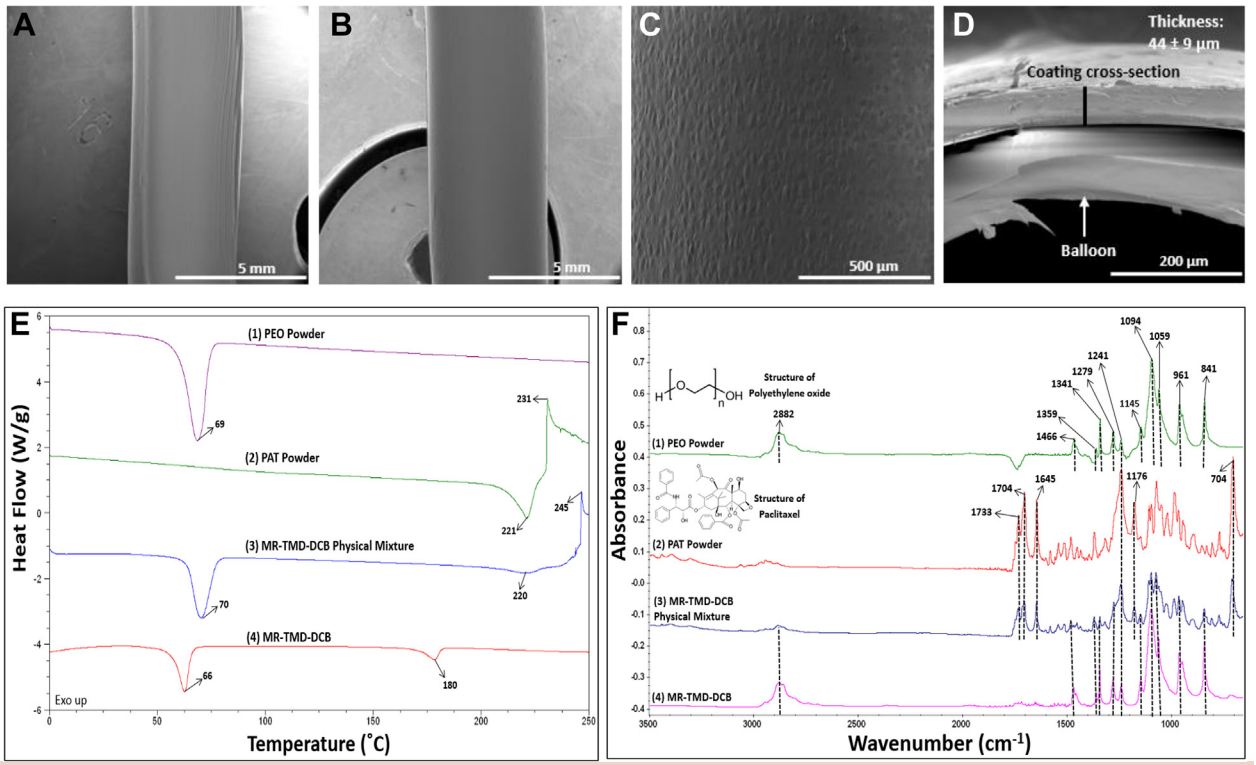

Fig 2. Characterizations of multiple-release Tailored Medical Devices drug-coated balloons (*MR-TMD-DCBs*). Scanning electron microscopy images of the surface morphology of the MR-TMD-DCB deflated **(A)**, inflated **(B)**, magnified **(C)**, and in cross section **(D)**. The thermal properties in the differential scanning calorimetry spectra **(E)** and chemical composition in the Fourier transform infrared spectra **(F)** are provided for the different forms of polyethylene oxide (*PEO*) and paclitaxel (*PAT*) powders and MR-TMD-DCB.

treatments were performed in each animal, a total of 14 MR-TMD-DCB treatments were performed at both day 1 and day 7, giving a total of 28 treatment balloons.

Statistical analysis

The experiments were carried out using at least three balloons in this study unless otherwise specified. Per a power analysis, a total of 14 swine (7 at each day-1 and day-7 time point) were recommended on the basis of a correlation of 0.9 and a power of 0.9. For the in vitro studies, a one-way analysis of variance was used to determine significance between groups at $P < .05$. For the in vivo studies, a paired two one-sided test method was used to determine equivalence at ±50% of the first

treatment artery using a 90% confidence interval.[14] The experimental data are represented as mean ± standard deviation in this study.

RESULTS

MR-TMD-DCB characterizations

The morphology, thermal properties, and chemical composition of the coatings on the MR-TMD-DCB are provided in Fig 2. In the scanning electron microscopy images, the coatings are smooth and homogeneous without any visible cracks present on the surface (Fig 2, *A-C*). The cross section of the DCB shows a coating thickness of 44 ± 9 μm (Fig 2, *D*).

The differential scanning calorimetry spectra of PEO powder, PAT powder, physical mixture, and balloon coating of the MR-TMD-DCB are provided in Fig 2, *E*. The melting peak of PEO was found at ~69°C for all the PEO-containing samples. For PAT powder, an endothermic melting peak at 221°C was observed in PAT powder and the physical mixture powder used in the same concentration as that used to make the coating on the MR-TMD-DCB. However, the melting peak for PAT disappeared when the coating was incorporated onto the balloon. This is consistent with our previous study and further confirmed the amorphous state of PAT in the PEO-PAT formulation prepared using our previously designed protocol.[8]

Fig 2, *F* shows the Fourier transform infrared spectra of the control powders and coating obtained from the MR-TMD-DCB. The characteristic peaks of PEO were observed for all the PEO-containing samples.[7] The characteristic peaks of PAT were present for the control PAT powder and the physical mixture powder used to make the MR-TMD-DCB.[7] However, no characteristic PAT peak was observed for the MR-TMD-DCB coating. This could indicate that the PAT in the MR-TMD-DCB coating is molecularly dispersed within the PEO matrix as observed in our previous studies.[7,8]

MR-TMD-DCB testing in flow models

Particulate formation. The images and quantitative analysis of the particulates shed by the MR-TMD-DCB are provided in Fig 3. The particulate analysis results from our previous study[8] of a control DCB (IN.PACT Admiral; Medtronic) are also provided in Fig 3 for a direct comparison to the MR-TMD-DCB developed in this study. Based on the images, the control DCB had more particulates and larger particulates shed from its coating compared with the MR-TMD-DCB coating, which had smaller particulates collected on the filter paper (Fig 3, *A*). The quantitative analysis confirmed the results, showing that the MR-TMD-DCB had significantly fewer particulates and that the average size of particulate was significantly smaller than that of the control DCB (Fig 3, *B*). After 1 hour, the particulates from the MR-TMD-DCB disappeared on the filter paper, indicating that the observed particulates were formed from the hydrophilic PEO and not the hydrophobic PAT. However, the high number of large particulates observed from the control DCB is believed to be due to the formation of PAT crystals as it was still evident on the filter paper after 1 hour. Because PEO is a biocompatible, hydrophilic polymer, the particulates will quickly degrade in the body and provide a reduced potential for downstream embolism. These results suggest that the MR-TMD-DCB developed in this study shed negligible drug particulates and thus can safely deliver the drug during treatment.

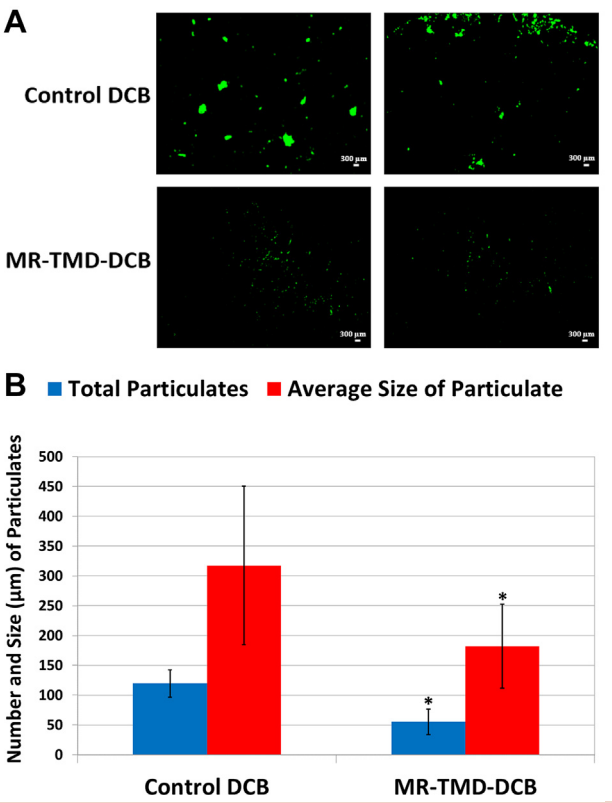

Fig 3. Particulate testing of multiple-release Tailored Medical Devices drug-coated balloon (*MR-TMD-DCB*). **A,** Images of the particulates shed from the MR-TMD-DCB collected on filter paper. The particulates were artificially colored *green* for improved visualization. **B,** Quantitative analysis of the number of total particulates and average size of the particulate collected from three balloons. The results from the control drug-coated balloon (*DCB*; commercially available DCB) are provided from Anderson et al[8] *Statistical significance at $P < .05$ in comparison to control DCB.

Drug released and uptake into explanted arteries. The drug released from the MR-TMD-DCB after insertion into the in vitro peripheral artery flow model is provided in the Table. The total drug loaded on the MR-TMD-DCB was 6.8 ± 0.7 μg/mm^2. During the 30-second insertion period, the initial loss was 15% of the total drug loaded. The drug taken up into the first artery was 707 ± 109 ng/mg, which is 15% of the total drug loaded onto the balloon.

Table. In vitro drug release and drug uptake into explanted porcine arteries using multiple-release Tailored Medical Devices drug-coated balloons (MR-TMD-DCBs) within the in vitro peripheral artery flow model

	Total drug loaded	30-Second initial loss	Drug in first artery	30-Second intermediate loss	Drug in second artery	Residual drug on balloon
Drug amounts	6.8 ± 0.7 μg/mm2	0.99 ± 0.1 μg/mm2	707 ± 109 ng/mg	1.8 ± 0.4 μg/mm2	783 ± 306 ng/mg	1.5 ± 0.6 μg/mm2
Drug released from balloon, %		15 ± 3	15 ± 3	27 ± 6	17 ± 6	22 ± 9

Values are the mean ± standard deviation of drug released from four MR-TMD-DCBs. The remaining drug not shown was lost in the phosphate-buffered saline solution during the inflation periods.

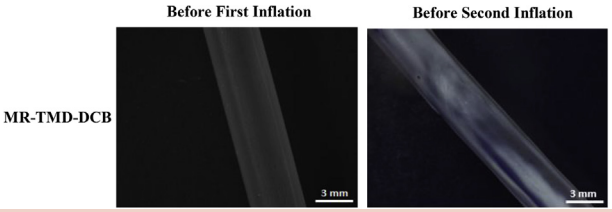

Fig 4. Coating distribution on the multiple-release Tailored Medical Devices drug-coated balloon (*MR-TMD-DCB*) before each inflation. Images of the coating distributed on the MR-TMD-DCB before the first inflation and second inflation during passage through the in vitro peripheral artery flow model.

After the first inflation in the artery, 27% of the total drug loaded was lost during the 30-second intermediate transit period. The drug taken up into the second artery was 783 ± 306 ng/mg, which is 17% of the total drug loaded onto the balloon. After the two inflations, 22% of the total drug loaded onto the balloon remained. These results show that the MR-TMD-DCB can successfully deliver similar amounts of drug during two separate inflation periods within the in vitro peripheral artery flow model.

Coating distribution on MR-TMD-DCB. The images of the coatings on the MR-TMD-DCB before the first and second inflations in the explanted arteries are provided in Fig 4. Before the first inflation period, the coating on the MR-TMD-DCB is distributed evenly throughout the balloon surfaces. Before the second inflation period, the coating appears to be mostly uniformly distributed throughout the MR-TMD-DCB surface; however, there are some locations where the coating appears to be thin. These results suggest that a homogeneous layer of drug coating is available on the MR-TMD-DCB surface to be transferred into the tissue during the first and second inflation periods.

In vivo drug retention at days 1 and 7 using MR-TMD-DCB

Fig 5 shows the amount of drug retained in the arteries after 1 day and 7 days of the MR-TMD-DCB treatment with superimposed points for each measure. A negligible amount of drug was measured in the control arteries and blood samples at each of the time points (<1 ng/mg). Equivalent drug levels were present between the first and second treatment arteries at day 1 ($P = .0044$) and day 7 ($P = .0006$). At day 1, the first treatment artery contained 63 ± 19 ng/mg and the second treatment artery contained 59 ±19 ng/mg of drug. At day 7, the first treatment artery contained 9 ± 6 ng/mg and the second treatment artery contained 10 ± 6 ng/mg. After the two treatments in the swine, 19% ± 6% of the total drug loaded remained on the MR-TMD-DCB. According to previous literature, the drug levels retained in both treatment arteries at days 1 and 7 were clinically relevant to inhibit smooth muscle cell growth.[15] Also, the drug levels in both treatment artery sites using the MR-TMD-DCB were above the drug levels at day 1 and day 7 in our previous study using the SR-TMD-DCB (20 ng/mg at day 1 and 5 ng/mg at day 7).[8] These results demonstrate the feasibility of the MR-TMD-DCB to treat two different artery sites using a single DCB and to obtain equivalent and clinically relevant amounts of drug at each of the treatment sites for at least 7 days.

DISCUSSION

The current DCB technology is limited to treatment of a single lesion. However, long, diffuse, and multiple lesions are present in 30% to 40% of DCB procedures.[9-13] As shown in Supplementary Fig 1 (online only), many complicated peripheral artery disease cases cannot be effectively treated using a single-release balloon. Even in the simplest case, multiple short lesions close together (case I, Supplementary Fig 1, online only) require the use of multiple DCBs or a single long DCB. However, patients treated with longer DCBs have significantly worse 1-year primary patency rates compared with patients treated with shorter DCBs.[16,17] One of the reasons behind the limited success in longer lesions could be that the blood vessels in legs vary largely in diameter and the degree of stenosis. This can make DCB treatment of these lesions not only inefficient but also unsafe as different DCB inflation pressures may be desirable at different ends of the arteries. Therefore, when the multiple lesions are long or the gap between the two lesions is >3 cm, at least two DCBs are used, increasing the chances for complications such as arterial dissection, arterial perforation, and

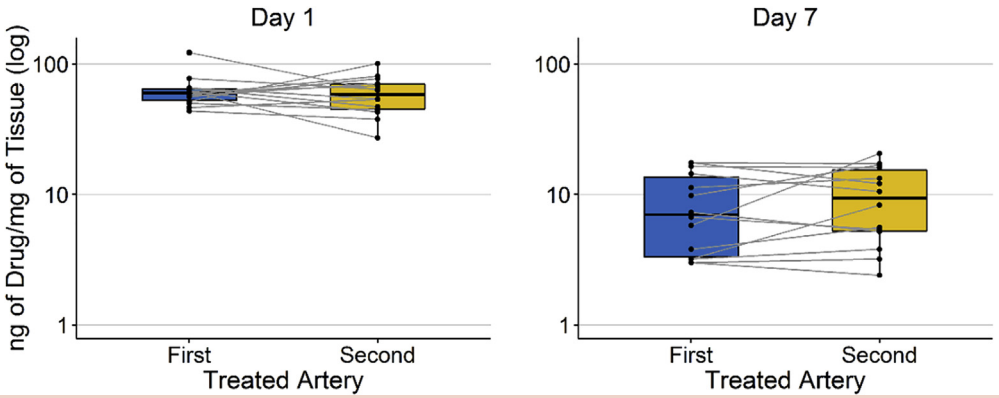

Fig 5. In vivo drug retention after 1 day and 7 days of the multiple-release Tailored Medical Devices drug-coated balloon (MR-TMD-DCB) treatment—the amount of drug retained in two different treatment artery sites at days 1 and 7 after performing subsequent inflation using a single MR-TMD-DCB. The amount of drug is represented as a box plot of paired observations with superimposed points for each measure using 14 MR-TMD-DCBs at each time point. Equivalence was defined as ±50% of the first treatment artery. A 90% confidence interval was used to test for significance using the paired two one-sided test method. (From Anderson JA, Lamichhane S, Vierhout T, Sherman A, Engebretson D, Pohlson K, et al. In vitro particulate and in vivo drug retention study of a novel polyethylene oxide formulation for drug-coated balloons. J Vasc Surg 2018; 67:1537-45. Reproduced with permission of Elsevier).

access site pain. Also, with the DCBs costing the hospital approximately $1500 per balloon[18,19] and the procedure being time-consuming, the marketplace needs a single DCB that can effectively treat multiple arterial locations to decrease cost and time of these procedures with the current emphasis on reducing the high cost of insuring the health of our population. This has led to the idea of developing a multiple-release DCB using PEO as the platform to safely and effectively treat the lesions with a single DCB. The concept of obtaining an accurate release of the desired amount of drug to treat multiple arteries from a single DCB has never been applied, and no previous studies have reported the use of such balloons to our knowledge.

In this study, we first optimized an MR-TMD-DCB platform and inflation times as shown in Supplementary Figs 5 and 6 (online only). A bilayer coating strategy approach was used whereby the top inert layer with no drug acts to prevent drug loss during the initial transit period, and then hydration of the inner layer is allowed to release the drug at the desired treatment sites. The MR-TMD-DCBs were then prepared in a certified cGMP facility and characterized for coating morphology, thermal and chemical changes, particulate shedding, and in vitro drug uptake. The coating showed that the properties were similar to our previous SR-TMD-DCB[8]; specifically, the MR-TMD-DCB coating was both smooth and uniform with molecularly dispersed PAT in amorphous form. Also, the safety of the coating was maintained as significantly fewer particulates were shed from the MR-TMD-DCB compared with a commercially available DCB. An in vitro peripheral artery flow model was designed to test drug uptake in two explanted porcine arteries using the MR-TMD-DCB. The MR-TMD-DCB allowed a similar amount of PAT to be taken up in the two arteries with the use of a single DCB. Therefore, the MR-TMD-DCB was able to safely and effectively deliver equivalent drug into two separate arteries using flow models.

The MR-TMD-DCB treatment successfully resulted in an equivalent amount of drug into two arterial locations at day 1 in the porcine model. Also, the drug amounts in both of the treatment arteries (63 ± 19 ng/mg in the first treatment artery and 59 ± 19 ng/mg in the second treatment artery) were above our previously obtained amounts at day 1 using the SR-TMD-DCB.[8] The drug was retained up to day 7 in the clinically relevant range in both treatment arteries (9 ± 6 ng/mg in the first treatment artery and 10 ± 6 ng/mg in the second treatment artery) compared with a commercially available DCB and previous studies.[8,20,21] As demonstrated in 28 treatments in swine, the MR-TMD-DCB has the potential to deliver a desired therapeutic amount of drug into two separate arterial segments without compromising the safety of the coating. Further modifications in the coating design and drug-polymer ratios of the formulation along with the addition of drug/inert layers can be done to make these DCBs suitable for the treatment of more than two arterial locations.

One limitation of this study is that the MR-TMD-DCB was not manufactured and packaged in an industrial setting. The folding/pleating and terminal sterilization of the device may alter the properties of the coating. Future studies include characterizing the MR-TMD-DCB after ethylene oxide sterilization and crimping of the device. A second limitation of this study is the lack of a commercially available DCB for a control. In our next study, we look to compare our MR-TMD-DCB directly with a commercially available DCB in clinically relevant long-term neointimal hyperplasia animal models.

CONCLUSIONS

A multiple-release DCB was developed to treat two different arterial segments. The MR-TMD-DCB delivered similar amounts of drug in two separate arteries in an in vitro peripheral artery flow model while shedding a negligible number of particulates. The MR-TMD-DCB was deployed at two arterial segments in a porcine model. An equivalent and clinically relevant amount of drug was obtained in the two arterial segments at both day 1 and day 7. Thus, this study demonstrated the preclinical feasibility of our multiple-release DCB (MR-TMD-DCB) to treat multiple arterial segments using a single DCB.

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AUTHOR CONTRIBUTIONS

Conception and design: JA, SL, TR, KP, RE, DE, PK
Analysis and interpretation: JA, SL, KF, TR, KP, RE, DE, PK
Data collection: JA, SL, KF
Writing the article: JA, SL
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Final approval of the article: JA, SL, KF, TR, KP, RE, DE, PK
Statistical analysis: JA, SL
Obtained funding: JA, SL, TR, KP, RE, DE, PK
Overall responsibility: JA

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SUPPLEMENTARY METHODS (online only).

Sterility and endotoxin testing of multiple-release Tailored Medical Devices drug-coated balloons (MR-TMD-DCBs)

The MR-TMD-DCBs were tested for sterility and endotoxin levels after preparation in the Current Good Manufacturing Practices (cGMP) facility. The sterility of the prepared MR-TMD-DCBs was performed by following United States Pharmacopeia Chapter <71> and Chapter <85> for sterility testing and bacterial endotoxin testing, respectively. For sterility testing, the MR-TMD-DCBs were directly inoculated into either soybean-casein digest medium (Millipore Sigma, Burlington, Mass) or fluid thioglycolate medium (Millipore Sigma) for 14 days to test for the presence of aerobes and fungi or anaerobes, respectively. The turbidity of the medium was used to indicate the presence of microbes. For endotoxin testing, an Endosafe PTS Kinetic Reader (Charles River, Charleston, SC) was used to measure the endotoxin levels from the prepared MR-TMD-DCBs. After dissolving the MR-TMD-DCBs in endotoxin-free water for 24 hours at 37°C, 25 μL of the solution was inserted into the Endosafe Reader. The endotoxin levels (endotoxin unit [EU] per milliliter) were then read from the instrument. For both sterility and endotoxin testing, at least four samples were used from each batch of MR-TMD-DCBs prepared, and a negative control (medium or water only) and positive control (MR-TMD-DCB prepared in unsterile environment) were used for comparison purposes.

Coating formulation preparation for MR-TMD-DCBs

Preliminary studies were performed to determine the optimized coating strategy. In this study, the two-layer coating strategy was chosen. The solution used to coat the MR-TMD-DCBs was prepared as previously described.[1,2] Briefly, polyethylene oxide (PEO; 10%, w/v) was dissolved in deionized water and allowed to stir for 6 hours. In parallel, paclitaxel (PAT; 40%, w/w) was sonicated in ethanol solution for 60 minutes. Then, PAT solution was added dropwise to the polymer solution, and the drug-polymer mixture was allowed to stir for an additional 18 hours. The 5%, w/v PEO coating solution was prepared in the same way but with no added PAT solution. Thus prepared solutions were used to coat the MR-TMD-DCBs.

MR-TMD-DCB preparation

The dip coating method was used to prepare the MR-TMD-DCBs as described previously.[1,2] Initially, angioplasty balloon catheters (Admiral Xtreme, 5-7 mm × 20-40 mm; Medtronic, Santa Rosa, Calif) were inflated using air to a pressure of 4 to 6 atm. Then, the balloon was dipped in the prepared drug-polymer solution (PEO, 10%, w/v; and PAT, 40%, w/w) for 1 minute and transferred to an oven at 50°C for 30 minutes. For the second coating, the balloon was dipped again into the polymer-only solution (PEO, 5%, w/v) for 5 seconds followed by the transfer into the oven for 15 minutes at 50°C. The schematic representation of the MR-TMD-DCB is provided in Supplementary Fig 3. Thus prepared MR-TMD-DCBs were used in this study.

Characterization of MR-TMD-DCB coating

The MR-TMD-DCBs were characterized for their surface morphology, thermal properties, and chemical composition using scanning electron microscopy, differential scanning calorimetry, and Fourier transform infrared (FTIR) spectroscopy, respectively. A Quanta 450 scanning electron microscope (FEI, Hillsboro, Ore) was used to image the coating on the balloon in the deflated state, inflated state, and cross sections of the coating. To determine the thickness of the coatings, five separate segments of the balloon were used to collect at least 25 measurements as previously described.[2]

A Q200 differential scanning calorimeter (TA Instruments, New Castle, Del) was used to determine the degree of crystallinity of PAT in the MR-TMD-DCB as we previously carried out.[1] A Nicolet 6700 FTIR spectrometer (Thermo Scientific, Waltham, Mass) equipped with attenuated total reflection accessory was used to characterize the chemical properties of the coating on the MR-TMD-DCB.

For differential scanning calorimetry and FTIR characterizations, the powder forms of PEO and PAT both alone and as a physical mixture were used as controls. The physical mixtures were prepared by crushing the two powders together in the same total concentrations as used for developing the MR-TMD-DCB (15% w/v of PEO, 40% w/w of PAT). Then, the mixture was distributed evenly using a vortex for 1 minute. The MR-TMD-DCB was also characterized to determine any thermal or chemical changes after incorporation of the coating onto the balloon.

Testing of MR-TMD-DCB in an in vitro model using explanted arteries

In an in vitro peripheral artery flow model (Supplementary Fig 4, online only) with explanted porcine carotid arteries (received from John Morrell & Co, Sioux Falls, SDak), drug uptake was determined into two separate arteries using the MR-TMD-DCB (5 mm × 40 mm) immediately after drug-coated balloon (DCB) inflation. Initially, the deflated DCB was inserted through a 7F sheath into 6.4-mm inner diameter Masterflex tubing (Cole-Parmer, Vernon Hills, Ill) using standard vascular procedures. The balloon was then passed 650 mm through a stream of phosphate-buffered saline (PBS; warmed at 37°C; flow rate of 200 mL/min) for 30 seconds to simulate the transit period to the arterial site (30-second initial loss). The balloon was then inserted into the first explanted artery submerged in 40 mL of 37°C PBS solution and inflated for 30 seconds using a pressure of 10 to 12 atm. After the inflation period, the balloon was deflated and the flow of PBS solution was passed over the balloon and collected for an additional 30 seconds to simulate the transit period to the second

treatment site (30-second intermediate loss). The balloon was then inserted into the second artery submerged in 40 mL of 37°C PBS solution and inflated for 3 minutes using a pressure of 10 to 12 atm. Finally, the balloon was deflated, and the residual drug remaining on the balloon was sonicated for 1 minute in a water:ethanol (50:50: v/v) solution. To remove PAT physically adsorbed on the containers, 1 to 10 mL of ethanol was added to each of the PBS-containing solutions.[1] The amount of PAT in the solutions as well as in the arteries was measured through high-performance liquid chromatography as we previously described.[3] At least four MR-TMD-DCBs were used to measure the drug release and drug uptake.

Coating distribution of MR-TMD-DCBs before each inflation in the explanted arteries in the in vitro peripheral artery flow model

The coating distribution on the MR-TMD-DCB before each inflation in the explanted artery was determined through images taken from an Olympus SZX10 stereo microscope equipped with Olympus DP22 camera (Olympus America, Center Valley, Pa). The balloon was removed from the in vitro model and imaged after the 30-second initial loss and 30-second intermediate loss periods for the coating integrity before the first and second inflations, respectively. At least three MR-TMD-DCBs were used, and the representative image of the coating on the balloon was chosen.

Preliminary in vivo porcine model

Preliminary in vivo studies were performed to determine the optimized coating strategy, balloon sizes, and inflation times to use. The in vivo protocol and informed consent were approved by the Institutional Animal Care and Use Committee and the Institutional Review Board, and the animal care complied with the *Guide for the Care and Use of Laboratory Animals* (Protocol No. 121-10-19D). The swine (female domestic farm pig; average body weight, 50-60 kg) used in this study were purchased from Heine Swine (Gibbon, Minn) and housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. The MR-TMD-DCBs were developed in the University of South Dakota cGMP cleanroom as described in the Methods section. The swine were fasted for a minimum of 8 hours before surgery without withholding water. Fasting, health, and age were controlled across groups. Initially, the swine were anesthetized by an intramuscular injection of atropine sulfate (1/120 grain; Med Pharmex, Pomona, Calif), xylazine (100 mg/mL; ProLabs, St. Joseph, Mo), and Telazol (tiletamine HCl and zolazepam HCl, 100 mg/mL; Zoetis, Kalamazoo, Mich). The animals were then intubated, and anesthesia was maintained with 2% to 3% isoflurane (Piramal, Bethlehem, Pa) in 100% oxygen with mechanical ventilation (Ohmeda 7800; Datex-Ohmeda, Madison, Wisc). The swine were placed on a surgical table in a supine position with their hair clipped in the groin bilaterally. The animals were prepared and draped using standard sterile technique. Supportive intravenous fluids at a rate of 10 mL/kg/h and supplemental heat for body warming were provided. Throughout surgery, the swine were monitored for the following parameters: respiratory rate (15-18 respirations/min), body temperature (99°F-102°F), heart rate (90-120 beats/min), and oxygen saturation as measured by pulse oximetry (95%-100%) using Datascope Spectrum (Mindray, Mahwah, NJ) capnography and Ohmeda Excel 210 anesthesia machine.

Optimization of coating strategy. A two-layer (first layer: PEO 10%, w/v and PAT 40%, w/w; second layer: PEO 5%, w/v) and four-layer (first layer: PEO 10%, w/v and PAT 25%, w/w; second layer: PEO 5%, w/v; third layer: PEO 10%, w/v and PAT 10%, w/w; fourth layer: PEO 5%, w/v) coating strategy both had satisfactory in vitro results in terms of coating morphology, thermal properties, chemical composition, drug loading, and drug delivery in the in vitro peripheral artery flow model. Therefore, the two different coating strategies were chosen for the preliminary in vivo procedures.

Treatment of porcine arteries for coating strategy optimization. Treatment of the arteries was carried out as described in the Methods section. However, MR-TMD-DCB sizes of 7 mm × 40 mm and inflation times of 30 seconds and 3 minutes for the first and second inflations, respectively, were used for this initial trial. One animal was used for each two-layer and four-layer MR-TMD-DCB, and the arteries on the swine were harvested after day 1. Therefore, because two treatments can be performed in each animal (left and right external arteries), two treatments were used for each balloon type at the time point of day 1. The amount of PAT taken up into the walls of the arteries was evaluated as described in the Methods section.

Treatment of porcine arteries using two-layer MR-TMD-DCB with optimized inflation times. Treatment of the porcine arteries was carried out as described in the preceding paragraph. However, only the two-layer MR-TMD-DCB was used with optimized inflation times of 1 minute and 2 minutes for the first and second inflations, respectively. Also, balloon sizes of 7 mm × 20 mm were used instead to make harvesting of the artery more accurate. For this trial, one animal was used at each day-1 and day-7 time point after treatment with the MR-TMD-DCB. Therefore, two treatments were used at each time point with the optimized inflation times (1 minute and 2 minutes).

SUPPLEMENTARY RESULTS

Sterile testing of MR-TMD-DCBs

The sterility and endotoxin results of the MR-TMD-DCBs prepared in the cGMP facility are provided in Supplementary Fig 2 (online only) and the Supplementary Table (online only), respectively. For

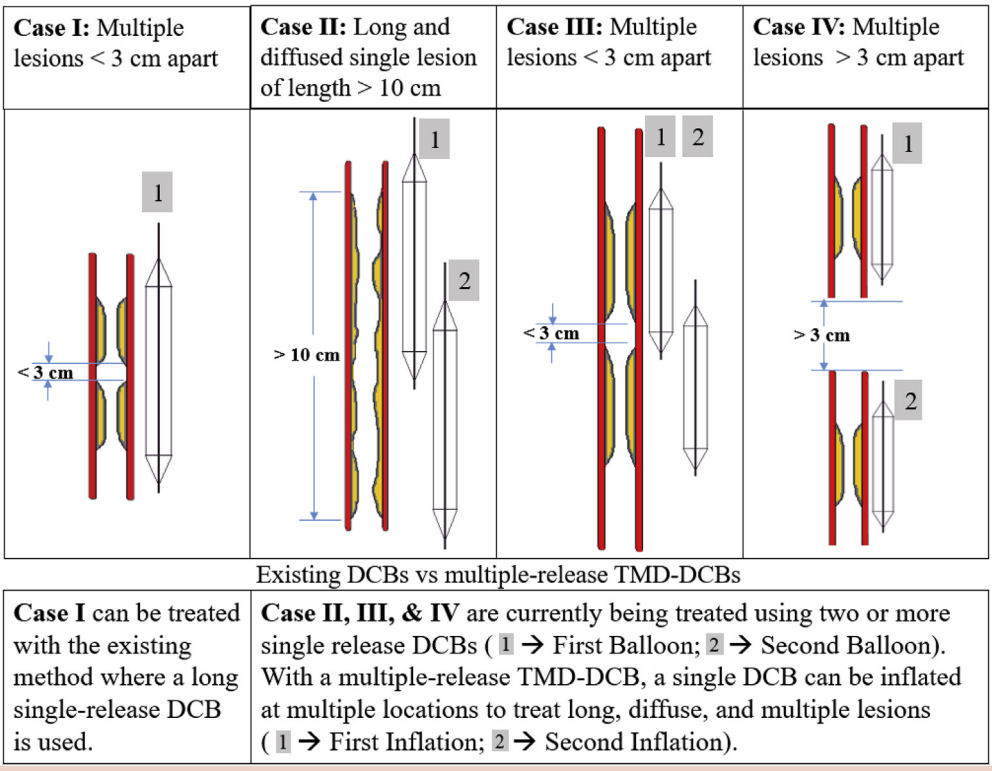
sterility testing, there was no presence of microbial growth in either medium for the negative control (medium only) at day 14 (Supplementary Fig 2, online only). However, the positive control (MR-TMD-DCB prepared in an unsterile environment) showed growth in both types of medium as indicated by thick turbidity at day 14 (Supplementary Fig 2, online only). These results show that both controls validate the study. From the images, the MR-TMD-DCBs prepared in the cGMP facility (samples) did not show any presence of microbial growth in either medium by day 14 (Supplementary Fig 2, online only). The slightly cloudy solution in the cGMP-prepared MR-TMD-DCB samples is believed to be due to dissolving of the coating as the solution did not change in turbidity from day 1 to day 14.

For endotoxin testing, the negative control (water only) produced a result of <0.1 EU/mL (Supplementary Table, online only). This shows that the endotoxin-free water used to dissolve the samples does not contain endotoxins. The positive control (MR-TMD-DCB prepared in an unsterile environment) produced results of 0.115 and <0.1 EU/mL for batch 1 and 2, respectively (Supplementary Table, online only). These values are below the Food and Drug Administration limit of 0.5 EU/mL for products that directly or indirectly contact the cardiovascular system.[4] As shown, all four samples in each batch (MR-TMD-DCBs prepared in cGMP facility) were below the Food and Drug Administration limit of 0.5 EU/mL with all values of <0.1 EU/mL. These results indicate that the MR-TMD-DCBs prepared in the cGMP facility are safe to use in the animal studies.

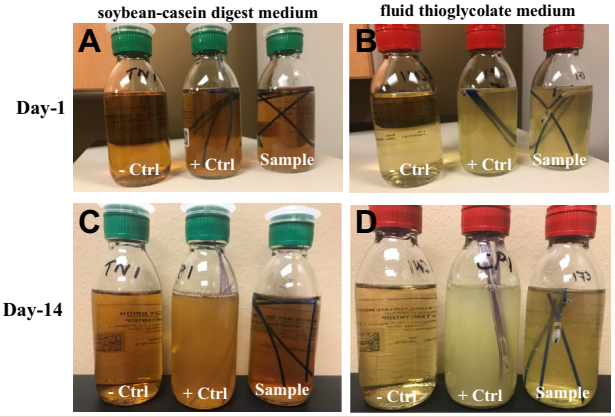
In vivo porcine model

In vivo coating optimization using preliminary inflation times. Supplementary Fig 5 (online only) shows the drug levels in the arteries after 1 day of the two-layer and four-layer MR-TMD-DCB treatments using inflation times of 30 seconds and 3 minutes for first and second inflations, respectively. No drug was found in the nontreated control artery after using either of the MR-TMD-DCBs. For the two-layer MR-TMD-DCB, the first treatment artery contained approximately 10 times less drug (38 ng/mg) than the second treatment artery (394 ng/mg). For the four-layer MR-TMD-DCB, the first treatment artery contained approximately two times as much drug (102 ng/mg) as the second treatment artery (50 ng/mg). These results suggest that the drug levels in all of the treatment tissues using both multiple-release balloon types were above the drug amount using the single-release Tailored Medical Devices DCB (20 ng/mg) obtained at day 1 in our previous study.[2] The two-layer MR-TMD-DCB coating strategy was chosen for future studies on the basis of a thinner coating (~45 μm vs ~100 μm), shorter preparation time (~1 hour vs ~2 hours), and higher total drug delivered in the tissue at day 1 (~430 ng/mg vs ~150 ng/mg). However, the inconsistent drug amounts obtained between each inflation were believed to be due to overlapping of the balloons at the artery locations or the inflation times used.

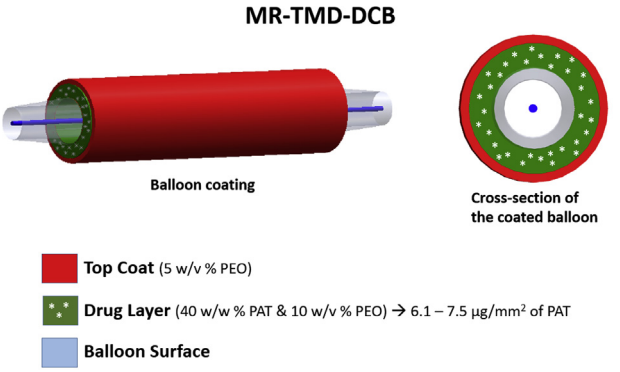
In vivo drug levels after day 1 using optimized balloon and inflation times. To deliver a more consistent amount of drug in the two artery locations, smaller balloon lengths (7 mm × 20 mm) and inflation times of 1 minute and 2 minutes were used for the first and second inflations, respectively. The amount of drug in the two artery locations after day 1 and day 7 of treatment using the two-layer MR-TMD-DCB is provided in Supplementary Fig 6 (online only). At the day-1 time point, the drug retained in the artery was not significantly different between the first treatment artery (24 ng/mg) and the second treatment artery (21 ng/mg). Also, both of these arteries were above the drug level of 20 ng/mg obtained at the day-1 time point using the single-release Tailored Medical Devices DCB.[2] At day 7, the drug amounts were 5.1 ± 0.8 ng/mg and 6.3 ± 0.8 ng/mg for the first and second treatment arteries, respectively. According to previous literature, the drug levels retained in the tissues at both the 1-day and 7-day time points were clinically relevant to inhibit the growth of smooth muscle cells.[5] These results suggest that the MR-TMD-DCB with the optimized inflation times was successfully able to treat two arteries using a single balloon and to obtain consistent and clinically relevant amounts of drug at both locations up until day 7. Therefore, the coating strategy (two-layer), balloon sizes (20-mm length), and inflation times (1 minute and 2 minutes for first and second inflations, respectively) were used for the animal studies in the manuscript.



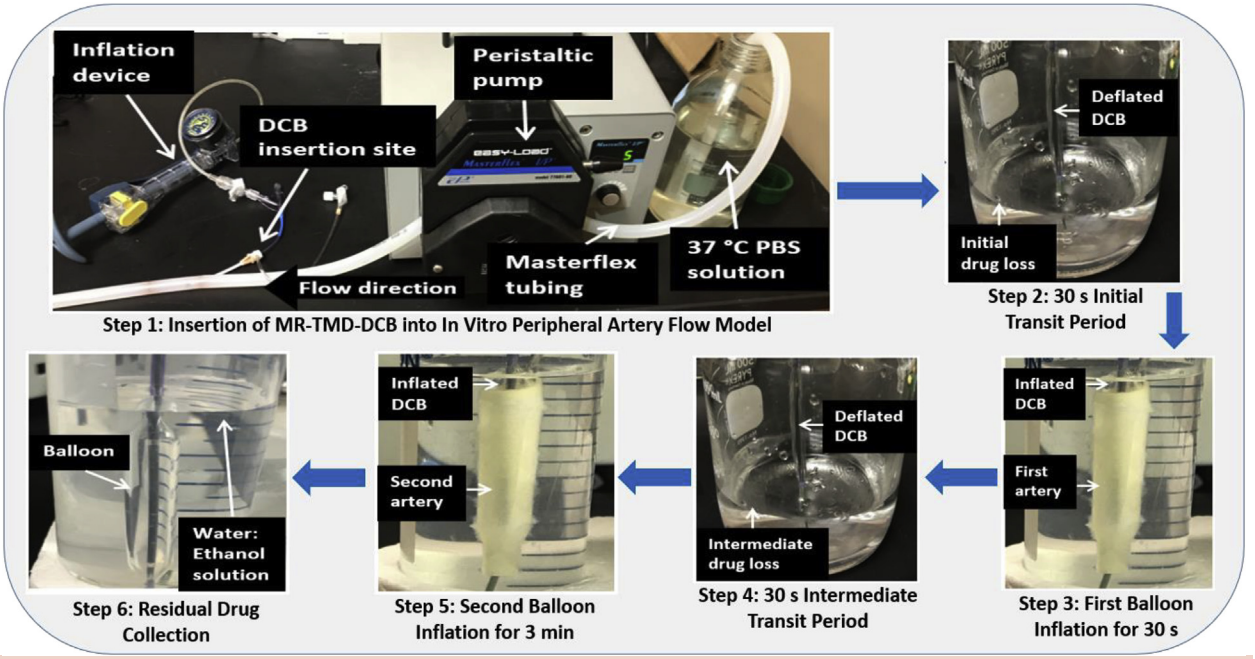
Supplementary Fig 1 (online only). Treatment of long, diffuse, and multiple lesions—the current treatment of multiple lesions close together (case I or case III), long and diffuse lesions (case II), and multiple lesions far apart from one another (case IV). The multiple-release Tailored Medical Devices drug-coated balloon (*TMD-DCB*) can be used to reduce the total number of balloons in these cases, which can decrease cost, time, and complications of procedures. *DCB*, Drug-coated balloon.



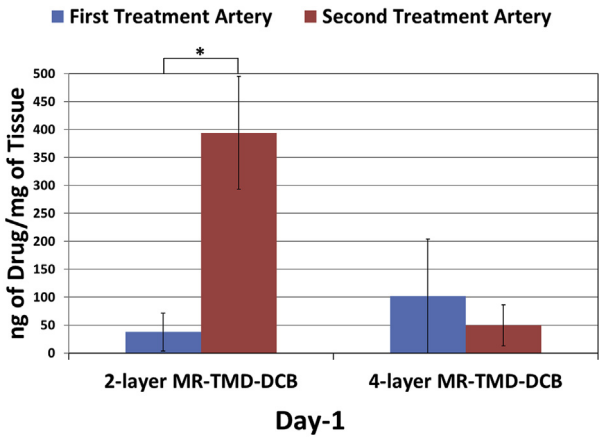
Supplementary Fig 2 (online only). Sterility testing of multiple-release Tailored Medical Devices drug-coated balloon (MR-TMD-DCB). The representative images are of the negative control (*Ctrl*; medium only), positive control (MR-TMD-DCB prepared in unsterile environment), and samples (MR-TMD-DCB prepared in sterile Current Good Manufacturing Practices [cGMP] facility) immersed in media to test the presence of microbes up until day 14. The specimens were inoculated in soybean-casein digest medium (**A,** day 1; **C,** day 14) to test for the presence of aerobes and fungi or were incubated in fluid thioglycolate medium (**B,** day 1; **D,** day 14) to test for the presence of anaerobes.



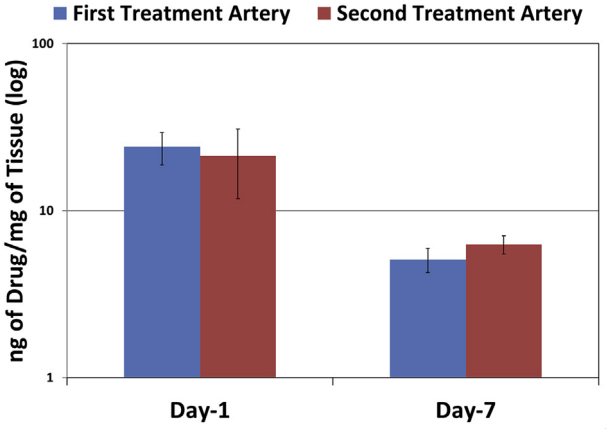
Supplementary Fig 3 (online only). Schematic representation of the multiple-release Tailored Medical Devices drug-coated balloon (*MR-TMD-DCB*) used to treat two different arterial sites. A bilayer coating approach was used to give more control over the release kinetics of the drug; the top coat with no drug acts to prevent drug loss during the initial transit period, and then hydration of the inner drug layer is allowed to release the drug at the desired treatment sites. *PAT,* Paclitaxel; *PEO,* polyethylene oxide.



Supplementary Fig 4 (online only). In vitro peripheral artery flow model. The multiple-release Tailored Medical Devices drug-coated balloons (*MR-TMD-DCBs*) were inserted into the system using standard vascular procedures, and the drug was collected and measured at predetermined time points (step 1). Initially, 37°C phosphate-buffered saline (*PBS*) solution was allowed to pass over the deflated drug-coated balloon (*DCB*) for 30 seconds during the initial transit period (step 2). The DCB was inflated in the first explanted artery for 30 seconds while immersed in warmed PBS (Step 3). The DCB was then deflated and removed from the first artery while PBS solution passed over the DCB for an additional 30-second intermediate transit period (step 4). The DCB was inserted into the second artery and inflated for 3 minutes (step 5). The balloon was finally removed, and the residual drug was collected in a water:ethanol solution (step 6).



Supplementary Fig 5 (online only). Drug levels at day 1 using two-layer and four-layer multiple-release Tailored Medical Devices drug-coated balloon (*MR-TMD-DCB*) with preliminary inflation times—the amount of drug retained in two different arteries after day 1 using the two-layer and four-layer MR-TMD-DCBs. The balloons were inflated in the first treatment artery for 30 seconds and the second treatment artery for 3 minutes. The amount of drug is represented as mean ± standard deviation using two treatments for each balloon type. *Statistical significance at $P < .05$.



Supplementary Fig 6 (online only). Drug retention after 1 day and 7 days using optimized balloon and inflation times—the amount of drug retained in two different arteries after days 1 and 7 using the two-layer multiple-release Tailored Medical Devices drug-coated balloon (MR-TMD-DCB) with optimized inflation times. The balloon was inflated in the first treatment artery for 1 minute and the second treatment artery for 2 minutes. The amount of drug is represented as mean ± standard deviation using two MR-TMD-DCB treatments at each time point.

Supplementary Table (online only). Endotoxin levels of the negative control (water only), positive control (multiple-release Tailored Medical Devices drug-coated balloons [*MR-TMD-DCBs*] prepared in unsterile environment), and samples (MR-TMD-DCBs prepared in the Current Good Manufacturing Practices [cGMP] facility)

Sample	Batch No.	EU/mL
Negative control	1	<0.1
Positive control	1	0.115
Samples (4 different MR-TMD-DCBs)	1	<0.1 ± 0.0
Negative control	2	<0.1
Positive control	2	<0.1
Samples (4 different MR-TMD-DCBs)	2	<0.1 ± 0.0

EU, Endotoxin unit.

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