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Coating and pharmacokinetic evaluation of air spray coated drug coated balloons

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Abstract

Purpose—Drug coated balloons (DCB) are becoming the standard-care treatment for peripheral arterial disease (PAD). DCB use excipients to transfer and retain anti-proliferative drugs, such as paclitaxel. Excipients thus play a vital role in the design and function of DCB, however methods to coat balloons with excipients and anti-proliferative drugs remain unknown. The goal of this study was to thus develop an approach to coat and evaluate DCB for various excipients.

Methods—An air sprayer method was developed to deposit paclitaxel and various excipients onto non-coated commercially available angioplasty balloons. The coating of the angioplasty balloons was evaluated for drug deposition and coating efficiency using high performance liquid chromatography tandem mass spectrometry. Drug transfer and retention of the coated angioplasty balloons into arterial segments were evaluated *ex vivo* using harvested pig arteries in a pulsatile flow bioreactor.

Results—The air sprayer method successfully delivered varying excipients including bovine serum albumin (BSA), urea and iohexol. The air spray method was configured to coat four angioplasty balloons simultaneously with paclitaxel and iohexol with an average paclitaxel load of $4.0 \pm 0.70 \mu\text{g}/\text{mm}^2$. The intra-day (within) and inter-day (between) coating precisions, defined as relative standard deviation (RSD), was 17.2 % and 15.5%, respectively. *Ex vivo* deployment of iohexol-paclitaxel DCB yielded an arterial paclitaxel concentration of $123.4 \pm 44.68 \text{ ng}/\text{mg}$ (n=3) at 1 hour, $126.7 \pm 25.27 \text{ ng}/\text{mg}$ (n=3) at 1 day, and $12.9 \pm 12.88 \text{ ng}/\text{mg}$ (n=3) at 7 days.

Conclusions—This work provides proof-of-concept of a quick, inexpensive approach to coat commercially available angioplasty balloons with paclitaxel and various excipients.

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Compliance with Ethical Standards

Conflict of Interest: Saami K. Yazdani serves on the Scientific Advisory Board of Advanced Catheter Therapies and Toray Industries and has received grant support from Advanced Catheter Therapies, Toray Industries and Lutonix, Inc., and is a shareholder in Advanced Catheter Therapies. Other co-authors have no conflict of interest to report.

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Keywords

drug coated balloon; coating; pharmacokinetics; iohexol; paclitaxel

Introduction

Peripheral arterial disease (PAD) affects 8.5 million Americans and costs the economy an estimated 4.37 billion annually.^{1, 2} Standard treatment of PAD has traditionally included balloon angioplasty and stenting, however, success of such devices is limited at best. Balloon angioplasty results in high incidence of restenosis post-treatment, up to 54%.^{3–8} Stents are also limited by high rates of restenosis, commonly as a result of stent fracture.⁹ Though stents are accepted as the standard care treatment for coronary artery disease, stents in the periphery are subjected to biomechanical stresses such as flexion and extension/contraction which result in stent fracture.¹⁰ Incidence of stent fracture varies greatly in the literature with fracture rates ranging from 2% to 65% in peripheral segments.¹¹ Stent fracture in the superficial femoral artery can occur in up to 65% of cases whereas, in the femoropopliteal segment, fracture incidence is 24.5%.⁹ Primary reasons for variability in fracture rate are differences in biomechanical forces at regions of stent placement,¹² multiple stent placement and overlapping stents.¹¹ Long lesions requiring more than one stent, such as those below-the-knee (BTK) (mean lesion length 130–170 mm), are indicated with balloon angioplasty with stenting as a bail-out strategy.^{13, 14} Consequently, new technology, such as the drug coated balloon (DCB), is seen as the next generation technology for treatment of PAD.

DCB employ an anti-proliferative drug coating, typically paclitaxel, to combat restenosis. DCB act by transferring drug from a coated angioplasty balloon to the vessel lumen. DCB require a quick, two-minute transfer of the drug load to the artery. With such a short time to interact with the target lesion, excipients or drug carriers can be used to enhance drug delivery and retention. Aided by excipients, drug transfer is mediated by multiple aspects including mechanical transfer, excipient and/or drug solvation, and excipient mediated binding. Excipients are the key for enhancing drug solubility, drug transfer and drug uptake.^{15–20} DCB thus require excipients, either as a single or multi-layered approach, to achieve the optimal set of conditions to sustain therapeutic paclitaxel levels for the duration of the restenotic cascade.

There are a variety of excipients used today such as iopromide, urea, dimethyl sulfoxide (DMSO), shellac, polysorbate and sorbitol, butyryl trihexyl citrate (BTHC), and polyethylene glycol (PEG).^{15, 19–24} Coatings can be composed of several excipients and can even be applied through layering approaches acting to protect drug loss during tracking, to modulate drug burst, and to provide controlled drug release.^{25–27} Just as the selection of excipients is a determinant of pharmacokinetics, coating deposition also plays a role in the functionality of the drug transport and release. Coating methods include dipping, air spraying, ultrasonic spraying, micropipetting, and others.²⁸ Each of these techniques is capable of yielding different coating morphologies and/or drug elution properties. For example, dipping techniques yield smooth surfaces but deposition at balloon folds is highly variable.²⁸ Ultrasonic air spraying can yield surface micro-cracks affecting coating stability

and drug elution as a result of increased contact of media between coating and balloon surface.²⁸ These coating methods and their effect on pharmacokinetics however are not well described in literature.

The goal of this manuscript was thus to develop a method to coat surfaces and angioplasty balloons with paclitaxel and a variety of excipients. Subsequently, the coating stability and transfer of drug was evaluated *ex vivo*. To our knowledge, this is the first detailed, validated description of coating deposition techniques for DCB. Application of these methods will aid in discovery and development of new DCB, potentially leading to improved drug delivery and retention.

Methods

Drug/excipient preparation

Paclitaxel (LC Laboratories, Woburn, MA) was dissolved by sonication in absolute ethanol [200-Proof] (Fisher Scientific, Hampton, NH) with a final concentration of 40 mg/mL and stored at -20 °C. Urea (Fisher Scientific, Hampton, NH) was dissolved in water to a final concentration of 1000 mg/mL. Bovine Serum Albumin (BSA) (Sigma-Aldrich, St. Louis, MO) was dissolved in water to a final concentration of 400 mg/mL.

Air spray coating

Circular, polymer coverslips (Thermo Fisher Scientific, Waltham, MA) were mounted onto inverted petri dishes using silicone sealant (Dow Corning, Midland, MI). Underneath a ventilated workstation with back draft air flow (TBJ, Inc., Chambersburg, PA), 2.5 mL of each drug and excipient (detailed above) and iohexol (OMNIPAQUE™, GE Healthcare, Little Chalfont, UK) alone were sprayed onto the coverslips. A Master Airbrush (TCP Global, San Diego, CA) air sprayer was used for coating. The system consisted of a TC-20 air compressor, pressure regulator, and G22 multi-purpose high performance airbrush with a one third ounce fluid cup and 0.3 mm tip. Pressure regulator was set to 50 psi. The spray tip was spaced 3 inches from the spraying surface. The spray trigger was cocked halfway setting the width of the airstream. The spray nozzle was held at 3 inches from the spraying surface and circulated in a counterclockwise matter to achieve an even coating. Coated coverslips were then dried for a minimum of one hour at room temperature. Air spraying was performed under a chemical fume hood with backdraft airflow and standard personal protective equipment.

Up to four commercially available balloon catheters 6×30 mm (Abbott Vascular, Abbott Park, IL) were mounted upright on a permanent stand with tape (Figure 1A). Four spray locations were spaced equally around the mounted balloon(s) three inches outwards from the center of the mounted balloon(s) (Figure 1B). One fourth of the total volume to be coated on the balloon(s) was sprayed at each spray location to achieve circumferential coating. To coat, the air spray gun was positioned at the spray location, the spray trigger was cocked halfway, and the spray gun was moved up and down to achieve an even coating (Video 1). Layered excipient-drug-excipient coatings were prepared for testing. The airbrush was cleaned after

each change of solution. There was no dry time between layers however, after the final coat, the balloon was allowed to dry at room temperature for a minimum of one hour.

Drug Deposition

To assess drug load, paclitaxel alone was coated on three balloon catheters using the described spraying method. Following a one-hour dry time, the balloons were then removed from the coating stand, cut from the catheter and sonicated for 30 minutes in ethanol to retrieve paclitaxel from the balloon surface. The balloons were removed following sonication and paclitaxel quantified via high performance liquid chromatography tandem mass spectrometry (HPLC-MS).²⁹ Transfer efficiency was defined as the percentage of drug transferred from the airbrush cup to the balloon. Transfer efficiency was used to calculate the volume of paclitaxel needed to achieve the desired $4 \mu\text{g}/\text{mm}^2$.

Precision of air spray coating method

Consistency of drug load and inter- and intra-spray precision was evaluated by repeated coatings of three different balloons for three different trials. Precision of air spray coating was described by calculating repeatability, variance of the daily mean and within-laboratory precision.³⁰

Scanning electron microscopy (SEM)

Coverslips were removed from the petri dish with a scalpel. Iohexol-paclitaxel coated balloon segments and commercially available Sequent Please (B. Braun Melsungen AG, Berlin, Germany) were carefully cut from the catheter with scissors. Samples were mounted on studs and sputter coated with gold palladium. Using a Quanta 250 with xT Microscope Control software (FEI, Hillsboro, OR) samples were visualized under SEM.

Ex vivo deployment

The *ex vivo* bioreactor system consisted of a flow reservoir, pump, vessel housing compartment, and a distal flow constrictor.³¹ The pressure was monitored via a catheter pressure transducer (Millar Instruments, Houston, TX). Flow was monitored by an ultrasonic flow meter that was positioned prior to the bioreactor. A custom LabVIEW program generated pulsatile waveform and controlled and monitored the mechanical conditions (flow and pressure) within the bioreactor system.³¹ The bioreactor medium consisted of Dulbecco's modified eagle's medium containing 10% fetal bovine serum and 1% antibiotics-antimitotics. The test section consisted of porcine carotid arteries harvested from large pigs (250–350 lbs) from a local abattoir and transferred in sterile phosphate buffered saline (PBS) with 1% antibiotic-antimitotic (Gibco, Grand Island, NY, 14072). Vessels were then additionally rinsed in sterile PBS in a culture hood. Each vessel was trimmed of excess fat, connective tissue, and fascia, cut into segments of approximately 8 cm, and placed into the bioreactor system. Within the bioreactor, the diameter of each vessel was evaluated by ultrasound. Local drug delivery was performed with iohexol-paclitaxel DCB. Vessels were denuded prior to DCB deployment with standard balloon angioplasty. Delivery pressure was determined by the manufacturers' specification for the determined vessel size. DCB were deployed for two minutes. Treated segments were visualized and marked within the

bioreactor. At 1 hour, 1 day and 7 days post-deployment, treated vessel segments (n=3 for each time point) were excised, flash frozen and pharmacokinetic analyses were performed.

Residual paclitaxel

Following deployment of DCB into the ex vivo artery, residual paclitaxel, paclitaxel remaining attached to the balloon, was quantified. Deployed DCB were removed from the vessel, sonicated for 30 minutes in ethanol, and evaluated for residual paclitaxel by HPLC-MS.

Paclitaxel quantification

A previously described HPLC-MS method was used for quantification of paclitaxel from balloon extracted samples in ethanol²⁹. The setup consisted of an Accela HPLC Autosampler, Accela 600 LC Pump, and a LTQ Velos Orbitrap Mass Spectrometry with Electrospray Ionization (Thermo Scientific, Waltham, MA). Paclitaxel standards were prepared in starting mobile phase conditions (acetonitrile - water + formic acid 0.1% (55:45, v/v)) and 5 ng/ μ L docetaxel (Tocris Bioscience, Bristol, United Kingdom) was used as an internal standard. The calibration range of paclitaxel was from 0.01 to 1.25 ng/ μ L. A Symmetry C18, 100 Å, 3.5 μ m, 100 $\times$ 2.1 mm column was used for separation (Waters Corporation, Milford, MA). Operated in positive ion mode paclitaxel was detected at 853.9 m/z and internal standard, docetaxel was detected at 807.9 m/z. Quantification of paclitaxel from arterial samples was performed as described previously.^{31, 32} Briefly, arterial samples were excised, flash frozen, stored at -80°C and shipped on dry ice to the iC42 Bioanalytical Laboratory (Aurora, CO, USA). Arteries were homogenized in phosphate buffered, extracted with water-methanol mixture, and quantified via HPLC-MS/MS. One hundred μ L of the samples was injected onto a 4.6 $\times$ 12.5 mm 5 μ m extraction column (Eclipse XDB C-8, Agilent Technologies, Palo Alto, CA). Tissue calibration curves were prepared using homogenized untreated pig artery tissues and had the following concentrations: 0, 0.5, 1, 2.5, 5, 10, 25, 50, 100 ng/mL; and the quality control samples run during study sample analysis had the following concentrations: 0.75, 7.5 and 75 ng/mL. Paclitaxel tissue concentrations were calculated based on the peak area ratios of Paclitaxel and its internal standard.

Statistical Analysis

Results are reported as mean $\pm$ standard deviation. Data were compared with analysis of variance (ANOVA) using GraphPad Prism 7 (GraphPad Software, La Jolla, CA). Comparison of quantitative data of multiple groups was performed by Tukey's multiple comparisons post hoc test. Significance is reported as $p < 0.05$.

Results

The coating setup could accommodate the coating of up to four balloons at one time. Balloons were coated successfully while remaining in the folded state throughout the coating process (Figure 2). Balloons were coated simultaneously with iohexol, paclitaxel and iohexol in layers. A total of 2 mL iohexol (1 mL per each layer) and 48 mg of paclitaxel [40 mg/mL in ethanol] was coated on each 6 $\times$ 30 mm balloon. Following coating, the

balloons were lightly handled and paclitaxel transfer was quantified to be 356.8 μg resulting in a transfer efficiency of 0.75%. To achieve the desired dose of 4 $\mu\text{g}/\text{mm}^2$, transfer efficiency was used to calculate the quantity of paclitaxel required to pass through the air sprayer. For balloons sized 6 $\times$ 30 mm, 7.069 mL of paclitaxel [40 mg/mL] was sprayed using the above described spraying method. Average drug load was $4.0 \pm 0.70 \mu\text{g}/\text{mm}^2$.

To assess the precision of the air spraying method, nine total balloons were coated. Three balloons were coated together at three different times (trials) and total drug content of each balloon was evaluated (Table 1). There was no significant difference in drug load between balloons within the same trial (trial 1: $p=0.3121$; trial 2: $p=0.3095$; trial 3: $p=0.3112$). There was no significant difference in drug load between trials (trial 1 = $3.477 \pm 0.5119 \mu\text{g}/\text{mm}^2$, trial 2 = $4.323 \pm 0.755 \mu\text{g}/\text{mm}^2$, trial 3 = $4.323 \pm 0.6231 \mu\text{g}/\text{mm}^2$; $p=0.2500$). Intra- and inter-spray precision (also known as coefficient of variance) were 17.2 %RSD and 15.5 ± 1.64 %RSD, respectively. Repeatability was calculated to be $0.636 \mu\text{g}/\text{mm}^2$, variance of the daily means was $0.240 \mu\text{g}/\text{mm}^2$ and the within-laboratory precision was $0.714 \mu\text{g}/\text{mm}^2$.

Visualization of coatings under SEM showed distinct morphological differences between coatings. BSA demonstrated a very smooth surface with significant cracking and fracture. Iohexol yielded a relatively smooth surface coating with some porosity and mild cracking of the coating. Conversely, urea represented a highly porous, non-uniform surface with some patterned, crystalline-like structures throughout. All coating morphologies aside from urea showed high uniformity of coating (Figure 3). Paclitaxel demonstrated crystallization with various crystal sizes (Figure 4A). Upon layering, the surface morphology appeared to take on the characteristics of the top layer of coating as demonstrated by layering of iohexol and paclitaxel respectively (Figure 4B). Paclitaxel coatings alone or on top of iohexol had a comparable surface morphology however the paclitaxel loaded on top of the iohexol exhibited cracking and fracture as a result of the iohexol.

When the same coating (iohexol, paclitaxel, iohexol) was applied to the balloon surface, the coating morphology was very similar to the morphology on the paclitaxel-iohexol coated coverslip with a very smooth surface with some cracking and fracture of coating (Figure 5A). The SeQuent Please balloon showed a rougher surface profile as compared to the iohexol-paclitaxel coated balloon while demonstrating circumferential coverage (Figure 5B).

Iohexol-paclitaxel DCB were successfully deployed *ex vivo*. Arterial diameter was determined using ultrasound and DCB were deployed using the manufacturers' guidelines for pressure to achieve the desired, 10–20% overstretch (Figure 6). The average flow rate in our *ex vivo* pulsatile condition was approximately 99 ml/min, corresponding to an average Reynolds number of 367. The pressure ranged between 80 to 120 mmHg. The iohexol-paclitaxel DCB successfully transferred paclitaxel from balloon surface to the artery as paclitaxel levels were measurable in all treated arteries. Paclitaxel levels 1 hour post deployment were $123.4 \pm 44.68 \text{ ng/mg}$ ($n=3$), $126.7 \pm 25.23 \text{ ng/mg}$ ($n=3$) at 1 day, and $12.9 \pm 12.88 \text{ ng/mg}$ ($n=3$) at 7 days. Significant decrease in arterial paclitaxel levels was observed at 7 days as compared to 1 hour ($p=0.0022$) and 1 day ($p<0.0001$).

Following deployment of iohexol-paclitaxel balloons *ex vivo*, balloon catheters showed minimal visual coating remaining on the balloon. Balloons were evaluated for residual paclitaxel remaining on the balloon by HPLC-MS. Residual paclitaxel following *ex vivo* deployment was $6.02 \pm 1.66\%$ (n=3) of the original paclitaxel dose.

Discussion

This study describes a method to coat and evaluate drug coated balloons with paclitaxel and various excipients. The described multi-angle air spraying method demonstrated successful deposition of paclitaxel and excipients including BSA, urea, and iohexol, in a single and multi-layered approach. The results show that DCB drug loading can effectively be controlled with the proposed method resulting in average drug load of $4.0 \pm 0.70 \mu\text{g}/\text{mm}^2$ and inter- and intra-spray precision of 17.2 %RSD and $15.5 \pm 1.64 \%$ RSD, respectively. Morphology of coating demonstrated uniform, circumferential coating. Deployment of the coated DCB in an *ex vivo* pulsatile bioreactor demonstrated therapeutic levels of paclitaxel up to 7 days post-deployment, with insignificant difference in arterial paclitaxel between 1 hour ($123.4 \pm 44.68 \text{ ng}/\text{mg}$ (n=3)) and 1 day ($126.7 \pm 25.27 \text{ ng}/\text{mg}$ (n=3), $p=0.1252$) and a significant drop-off between 1 day and 7 day ($12.9 \pm 12.88 \text{ ng}/\text{mg}$ (n=3), $p=0.0022$). To our knowledge, this study describes for the first time a detailed, validated and cost effective approach to coat angioplasty balloons with paclitaxel and test the feasibility of novel excipients for DCB coatings. These methods can be extrapolated to assess a wide range of excipients and subsequently lead to improved excipients of paclitaxel and improved clinical outcomes.

Previous literature on coating methods of drug coated balloons evaluates either coating morphology or arterial pharmacokinetic profile, but not both.^{28, 33–36} The literature describes (at varying degrees of detail) various coating methodologies including dipping, micropipetting, and air spraying. Dipping was one of the first methods used to apply hydrophilic coatings on angioplasty balloons for ease of tracking.³⁷ When evaluated as a method for DCB coating, dipping resulted in poor coating uniformity with increased deposition of coating at the folds.²⁸ Deposition via dipping also differs with solution viscosity and drug concentration.²⁸ Hence, reproducibility is low with dip coating. For these reasons, the most widely used method in DCB literature is air spraying (with or without ultrasonic or electrospray atomization), however, these methods are not rigorously described.²⁸ Ultrasonic air spraying yields highly uniform coatings with low efficiency of drug transfer, reported at 56% in one study.³⁸ Ultrasonic air spraying also affects drug concentration and leads to solvent evaporation resulting in increased drug concentration, decreased solvent deposition, and increased drug crystallinity.^{28, 39} Air spraying was chosen as the method of deposition in this work as a result of the limitations of dipping and micropipetting. Air spraying has a broader range of options and is the most reliably controlled. Our method shows reliable deposition with inter- and intra-spray precisions of 17.2 %RSD and $15.5 \pm 1.64 \%$ RSD, respectively.

Commercial methods of coating balloons remain undisclosed as companies aim to protect their intellectual property. However, there are some commercially available coating systems which use an ultrasonic spray to deposit coating on angioplasty balloons. The cost of these

types of equipment are very high and realistically unattainable for the standard research laboratory. In addition to the high costs associated with the coating process, the cost of folding balloons following coating multiplies the expenses associated with developing DCBs.

The described air spraying coating method is effective in drug/excipient deposition, however, the transfer efficiency is very low (0.75%) making it wasteful and potentially costly for high priced drugs or drug carriers. While using an air sprayer with a hollow cone nozzle, the spray cone is not uniform therefore it is necessary to move the sprayer during coating in order to achieve a uniform coated surface. The method is as controlled as possible in terms of spray angles, spray distance, and movement of the spray gun yielding 17.2 %RSD intra(within)-spray (trial 1 = $3.477 \pm 0.5119 \mu\text{g}/\text{mm}^2$; $p=0.3121$, trial 2 = $4.323 \pm 0.755 \mu\text{g}/\text{mm}^2$; $p=0.3095$, trial 3 = $4.323 \pm 0.6231 \mu\text{g}/\text{mm}^2$; $p=0.3112$) and 15.5 %RSD inter(between)-spray ($p=0.2500$). Variance in the coating deposition is however, inevitable as the spray gun is controlled by the human hand. Another drawback is that the spray coater gun can only handle low viscosity liquids. Higher viscosity liquids begin to clog the air spray nozzle leading to droplet formation and inconsistent coatings.

The developed method allows for layered coating approaches in a simple fashion in that the spray nozzle requires only a quick wash-out of the reservoir with no priming of the liquid required.²⁷ Layering approaches can serve many functions where each layer can provide different functions such as adherence, protection, coating stability, and drug solvation. Layering on a stent can determine release rate as a function of layer composition(s).⁴⁰ Adherence coatings are applied to promote adherence of the coating to the balloons surface.²⁷ Protective layers are applied on top of drug layers to protect from loss of drug during balloon insertion through the sheath, exposure to blood flow, and tracking to the target lesion.^{25–27, 40} Drug concentration, composition, and layering approaches can be combined in nearly infinite combinations. This highlights the need for methods of coating and evaluation of paclitaxel coated balloons. Here, common paclitaxel excipients including urea, iohexol bovine serum albumin were evaluated for coating deposition/morphology through the described air spray coating method.^{22, 41, 42} Iohexol was used in place of iopromide in this study due to its relative availability and comparable chemical properties and systemic effects.

Stable, uniform coatings are gravely important in ensuring transfer of drug to the target lesion during delivery. The method of coating iohexol-paclitaxel yields a coating comparable to the SeQuent Please DCB (Fig 6). As opposed to dip coating, we visualized no accumulation of coating at the folds with air spraying, displaying uniform coverage. Our iohexol-paclitaxel balloon was subjected to several adversities as experienced clinically with DCB deployment including introduction through the sheath and loss of coating under flow. Loss of coating through the sheath can be a major issue as insertion point imposes high shear forces on the balloon coating. A recently approved DCB in Europe, Ranger™ (Boston Scientific Marlborough, MA), is accompanied with a loading tool which aides in sheath introduction by widening the sheath lumen and allowing the coating to pass with less friction/shear. To determine the coating stability of our air sprayed DCB, we introduced our iohexol-paclitaxel coated balloon into a 6 French Terumo Glidesheath™ Basic (Terumo

Corporation, Tokyo, Japan) and visually assessed the coating. Our coating shows no visible coating loss upon sheath introduction. Additionally, we visualized the stability of our coating under flow in a steady flow loop in which the coating visibly remained on the catheter for up to 8 minutes. This time frame overcompensates for clinical tracking time with a goal of tracking time less than one minute.⁴³

In vitro and bench-top test are critical to the evaluation of DCB as an initial assessment of efficacy. Once validated here, coated DCB can then be extended to *in vivo* models and further parameters such as restenosis, inflammation, and systemic effects can be evaluated. Modeling physiological pulsatile flow and temperature and looking at pharmacokinetics in the native porcine artery, our model demonstrates a viable model for testing pharmacokinetics and various excipients before costly preclinical testing. Through this model we can evaluate many characteristics of DCBs such as coating stability, arterial transfer and arterial retention. Drug deposition with the coated iohexol-paclitaxel balloon is well within the therapeutic range of paclitaxel at 123.4 ± 44.68 ng/mg (n=3) for 1 hour. These levels are sustained in the *ex vivo* model at 126.7 ± 25.27 ng/mg (n=3) for 1 day and 12.9 ± 12.88 ng/mg (n=3) for 7 days. In comparing these levels to other commercially available DCB in the United States, the Lutonix balloon exhibits a drug level of 58.8 ± 54.2 ng/mg (n=24)⁴⁴ at one hour in a preclinical porcine model with a significant drop off at 1 day to 4.4 ± 6.9 ng/mg (n=16).⁴⁴ In. Pact also exhibits significant drop off with levels of 35.43 ng/mg⁴⁵ immediately following deployment and 7 ng/mg⁴⁶ at 1 day. The iohexol-paclitaxel balloon sustains therapeutic levels up to 7 days (12.9 ± 12.88 ng/mg) whereas the Lutonix balloon is shown to drop to 1.0 ± 0.9 ng/mg⁴⁴ at 7 days in a preclinical porcine model. These data demonstrate the ability of our coating to sustain paclitaxel levels over time where other DCBs have demonstrated significant drop-off at the same time points.

Pharmacokinetics has become the gold standard to evaluate and predict the success of DCBs. The minimum therapeutic level of paclitaxel is reported in the range between 0.01 and 1 ng/mg.^{47, 48} For DCB applications, these levels need to be maintained up to 28 days, the time frame of restenosis.⁴⁹ This has proven to be a difficulty in the development of DCBs with first generation DCBs retaining less than 10% of the total drug load 1 hour post deployment.^{15, 16} Improved excipients are still needed to create more durable drug coatings and longer-lasting pharmacokinetics. By altering excipients, drug retention can be elongated and lead to further inhibition of restenosis.^{17–20} In addition to excipients, drug state (amorphous vs. crystalline) has shown to play an important role in drug retention where amorphous drug releases more quickly than crystalline or semi-crystalline forms.^{50, 51} Deposition of crystalline/semi-crystalline paclitaxel is aided by air spraying methods leading to improved pharmacokinetic profile.²⁸

The DCB coated here are limited in the lack of coating within the folds of the balloon. As previously mentioned, the cost of balloon pleating is high. Although our coating does not extend to the balloon folds, the coated portion of the balloon still allows us to assess coating stability and pharmacokinetics although the arterial drug distribution is not circumferentially uniform. Tracking models which consist of turns to mimic clinical tracking were not evaluated here. These models can aide in assessing coating stability under more clinically relevant circumstances. In assessing coating stability and pharmacokinetics under flow, our

circulating media does not contain blood/blood components. Therefore, some effects such as coating solvation and arterial transfer/retention may be affected. Paclitaxel is known to bind to circulating blood components.⁵² A limitation of our *ex vivo* model is that circulating paclitaxel does not actively filter from the circulation as it would *in vivo*. Although our *ex vivo* arteries are subjected to pulsatile flow, they differ in flow rates and pressure curves from *in vivo* and clinical conditions. Additionally, pharmacokinetics was evaluated in healthy and not diseased arteries, which has shown to alter pharmacokinetic measurements.^{53, 54} In addition, no off-the shelf products were evaluated for a side-by-side comparison. The *ex vivo* model needs to be compared to *in vivo* counterparts to determine the efficacy in comparison of pharmacokinetic profiles.

Conclusion

This study provides a fast and cost-effective method to coat and evaluate the feasibility of novel drug coated balloons. Angioplasty balloons were successfully coated with a mixture of paclitaxel and iohexol. The air sprayed, coated balloons demonstrate uniform coating characteristics similar to commercially available DCB. Paclitaxel transfer and retention were successfully demonstrated up to 7 days in an *ex vivo* model utilizing native swine tissue under physiological flow conditions. Further studies are warranted to demonstrate the safety and efficacy profile of the air sprayed drug coated balloons in a relevant *in vivo* model. Overall, this method can be utilized to develop novel drug coated balloons to more effectively improve paclitaxel retention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Setup of mounted balloons for air spray coating. A) Four balloons were mounted vertically in offset positions. An air sprayer was then utilized to coat balloons with drug solution (side view). B) To ensure consistent coating, the four mounted balloons were spray coated at 4 directions starting at 0° , followed by 90° , 180° and 270° (Top view). C) At all coating locations (1–4) all four balloons are exposed to the spray area and no balloons are covered by the others.

Figure 2.
Angioplasty balloon before and after coating. A) Uncoated balloon, B) Iohexol-paclitaxel coated balloon.

Figure 3.
Scanning electron micrograph of excipient coatings onto coverslips. A) Urea [1000 mg/mL], B) BSA [400 mg/mL], C) Iohexol.

Figure 4. Scanning electron micrograph of paclitaxel coatings onto coverslips. A) Paclitaxel [40 mg/mL], B) iohexol followed by paclitaxel [40 mg/mL].

Figure 5.
Scanning electron micrograph of paclitaxel coated balloons. A) iohexol-paclitaxel coating B)
SeQuent Please DCB.

Figure 6.

Artery mounted in bioreactor to simulate arterial deployment. (A) Inner artery is affixed to plastic fittings via suture. An outer, Sylgard sheath is affixed with o-rings. The artery is bathed in cell culture media inside and between the artery and Sylgard sheath. (B and C) Diameter measurement of explanted arteries. (B) An ultrasound probe can be directly placed on the outer sleeve of the bioreactor housing to measure the diameter. (C) Ultrasound image showing the inner diameter of the explanted artery (markers outlined in red). Recorded graphs of (D) flow rate and (E) pressure within the bioreactor system.

Table 1

Drug load of DCB coated at three different trials. Data presented as mean $\pm$ standard deviation.

	Average drug load ($\mu\text{g}/\text{mm}^2$)	%RSD	p-value
Trial 1 (n=3)	3.477 ± 0.5119	14.7	0.3121
Trial 2 (n=3)	4.323 ± 0.755	17.4	0.3095
Trial 3 (n=3)	4.323 ± 0.6231	17.4	0.3112