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Research Paper

The Application of Electrostatic Dry Powder Deposition Technology to Coat Drug-Eluting Stents

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Purpose. A novel methodology has been introduced to effectively coat intravascular stents with sirolimus-loaded polymeric microparticles.
Methods. Dry powders of the microparticulate formulation, consisting of non-erodible polymers, were produced by a supercritical, aerosol, solvent extraction system (ASES). A design of experiment (DOE) approach was conducted on the independent variables, such as organic/CO_2 phase volume ratio, polymer weight and stirring-rate, while regression analysis was utilized to interpret the influence of all operational parameters on the dependent variable of particle size. The dry powders, so formed, entered an electric field created by corona charging and were sprayed on the earthed metal stent. Furthermore, the thermal stability of sirolimus was investigated to define the optimum conditions for fusion to the metal surfaces.
Results. The electrostatic dry powder deposition technology (EDPDT) was used on the metal strut followed by fusion to produce uniform, reproducible and accurate coatings. The coated stents exhibited sustained release profiles over 25 days, similar to commercial products. EDPDT-coated stents displayed significant reduced platelet adhesion.
Conclusions. EDPDT appeared to be a robust accurate and reproducible technology to coat eluting stents.

KEY WORDS: coating; drug eluting stents; electrostatic deposition; platelet adhesion; supercritical fluids.

INTRODUCTION

Coronary stents have been recognized as the most effective, localized drug delivery systems for the treatment of vascular diseases in the last two decades (1). Drug-eluting stents (DES) are expandable, slotted metal tubes acting as a scaffold that provide structural support to blood vessels and deliver biologically-active agents directly to the target site (2). Stent implantation has been used in balloon angioplasty procedures to treat atherosclerosis due to irregularly distributed lipid deposits in the intima of the coronary arteries. The main function of DES is to prevent restenosis in which the vascular smooth muscle cells in the artery wall undergo hyper-proliferation. In addition, stents are designed to provide antic-inflammatory and anti-thrombogenic action.

There are several commercial stents (3,4) of different configurations, coatings and active agents clinically approved by the Food and Drug Administration (FDA). Many coating techniques and approaches have been used for DES to alter the dose, duration, release kinetics and clinical outcomes. These properties are critically important for coronary stents as the coating layer is in contact with both vascular tissues of the artery and blood flow. The coating materials are usually hydrophobic polymers (5), phosphorylcholine-containing polymers (6,7) triblock copolymers or heparin (8,9), which are biocompatible. Techniques such as dip-coating, (10) spray-coating (11), ink-jet technology (12), inorganic-coating (13), sputtering (14), plating (15), electrospinning (16) and layer-by-layer (17) coating technologies have been employed for laboratory and clinical applications. Stent coating has a significant impact on stent design affecting both angiographic and clinical outcomes (4,18). Polymer and drug surface uniformity, coating thickness, surface cracking and stent exposure to the artery could emerge as a result of insufficient coating. The latter case could generate unpredictable healing patterns due to protein interactions with the metal surface.

Several drug candidates, such as immune-suppressive agents, anti-inflammatory and cellular proliferation inhibitors have been employed for stent coating and evaluated in clinical trials. Sirolimus-eluting (Cypher®, Cordis) and paclitaxel-eluting stents (Taxus®, Boston Scientific) (2) have been extensively used for coronary angioplasty. Recently, the efficiency of both stent types was questioned due to adverse effects, including myocardial infraction and stent thrombosis, that led to life-threatening incidents (19,20). The Cypher® stent employs a combination of poly(ethylene-co-vinyl acetate) (PEVA) and poly(n-butyl methacrylate) (PBMA), whereas Taxus® uses poly(styrene-b-isobutylene-b-styrene) triblock copolymer as a coating material (2). The mixing ratio of PEVA/PBMA and sirolimus is 67% polymer and 33% drug.

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In the current study, we report for the first time a coating-technique using electrostatic dry powder deposition of sirolimus encapsulated polymeric PEVA/PBMA microparticles followed by fusion. The production of polymeric microparticles was based on a modified, supercritical aerosol solvent extraction system (ASES) (21) using a combination of supercritical N_2 and CO_2 fluids. Supercritical fluids were developed as an alternative approach to conventional emulsification techniques because these tools provided better particle size control, excluded the use of emulsifiers (mainly polyvinyl alcohol, PVA) and provided lower residual solvent concentration in the final product. The sirolimus release patterns and platelet adhesion of the developed stents were compared to the commercial Cypher® product.

MATERIALS & METHODS

MATERIALS

The PEVA, PBMA, Sirolimus and Cypher®-coated stents were kindly donated by Phoqus Pharmaceuticals Ltd., UK. The reagents used were High-Performance Liquid Chromatography (HPLC) grade dichloromethane (DCM), tetrahydrofuran (THF) and methyl alcohol (methanol) from Fisher Chemicals UK.

ASES Microspheres Preparation

ASES has been used widely in the pharmaceutical industry for particle micronization. The process used for the preparation of sirolimus polymeric microspheres involved a high pressure R250 CW reactor (Thar Technologies Inc. US). The process was adjusted accordingly in order to improve the micronization properties of non-crystalline polymers by using a combination of two gases, CO_2 and N_2, before adjusting the reaction conditions to create a supercritical fluid (SF). Briefly, the drug and polymers were dissolved in DCM that was sprayed through a nozzle (0.3 mm) into the supercritical mixture. Initially, the two gases were pumped continuously at a constant flow rate of 18 ml/min (N_2) and 12 ml/min (CO_2) into the high pressure vessel to reach the operating conditions of pressure (150 bars) and temperature (35°C). The flow rate was controlled by a metering valve located at the exit of the precipitation chamber.

Once the desired temperature and pressure had been achieved in the chamber, the organic solution containing the drug and the polymers was pumped at constant flow rates and sprayed into the chamber. After precipitation, DCM was flushed out by feeding the supercritical mixture at the operating pressure and temperature. The chamber was then gradually depressurized (15 min washing time), and the micronized powder was collected and stored in a freezer (−18°C) for future characterization. The powder was passed through an air jet mill (Hosokawa Micron Ltd., UK) to break any agglomerates prior to the coating process.

Further development of the microspheres produced was carried out by investigating process parameters such as CO_2–N_2/DCM ratios, drug polymer injection flow rates (ml/min) and polymer concentrations. The mean volume diameter of the particle size obtained was selected as the dependent variable. For this purpose, a three level fractional factorial response surface design was implemented (by D.o.E Fusion One, S–Matrix Corp., US).

Electrostatic Dry Powder Deposition Technology

The coating process was performed using a Sure Coat® manual spray gun system (Nordson Ltd. UK). The spray gun was composed of a powder funnel for spraying small powder amounts, a charging system, a cup gun, a venturi pump and a control unit. By regulating the operating parameters—coating time (4–10 s), voltage (50–90 kV, flow rate air (0.5–1.0 bar) and gun air (0.3 bar)—during the deposition process, a coated polymeric layer is created on the stent strut. Finally, the coated stent was placed between two infrared ceramic square flat elements (Ceramix Ireland Ltd.) to fuse the surrounding layer at different temperatures for 60 s.

The electrostatic dry powder deposition process (Fig. 1) applies powder coatings onto the metal substrate by spraying an electrically-charged cloud of fine powders. A voltage

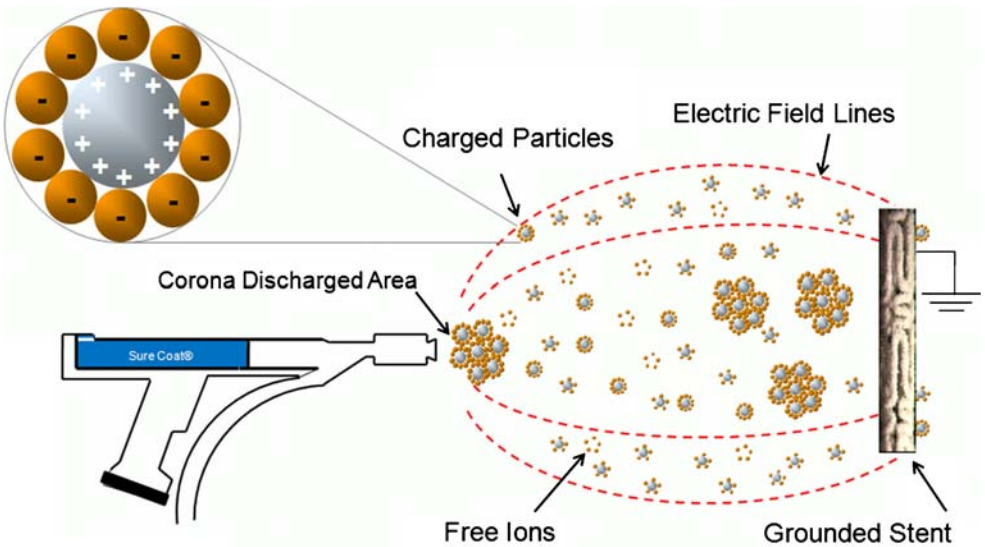

Fig. 1. Schematic diagram of the EDPDT process.

supplied by the control unit generates a high-strength electrostatic field between an electrode in the nozzle and the grounded item in front of the spray gun. The electrostatic field produces a corona discharge around the electrode. As the powder particles are sprayed past the electrode in the nozzle, they are electrostatically-charged under high voltage ranging from 50 to 90 kV and are attracted to the grounded item.

The atomizing air transports the powder particles from the feed funnel to the tip of a spray gun. At this point, the powder passes next to an electrode built to induce electrostatic charge to the powder particles rapidly. The spray pattern is controlled by the shape of the nozzle, the flow-rate air pressure, and the electrostatic field generated between the electrode and the grounded substrate.

Stent-Coating Process

Prior to coating, each stent was placed on a 0.2 mm stainless steel formed wire frame. The optimized operating parameters (coating time, voltage, and flow rate) were set through the control unit in order to initiate the deposition process. All the experiments were carried out with the relative humidity lower than 40% and at an average temperature of 20°C (±0.2°C). Once the powder had been applied, the stent was fused, and the sirolimus microparticles start "melting" to form a continuous, uniform film.

Differential Scanning Calorimetry

DSC measurements were made on a DSC 823E calorimeter (Mettler Toledo). Approximately 3–5 mg samples were accurately weighed into standard aluminium pans. An empty pan was used as reference. The samples were heated from −50°C to 210°C with scan rates 10°C/min in nitrogen atmosphere. All DSC curves were normalized to a sample mass of 1 g.

Particle Size Measurements

The particles were suspended in 5 ml water and sonicated for 30 s before particle size measurement. Mean volume diameters were measured using a laser diffractometer (Mastersizer 2000, Malvern, UK). The results are presented as the mean values of three samples.

Powder X-Ray Diffraction (XRPD)

Samples of pure and polymer encapsulated drug powders were evaluated using a Philips 1830/40 diffractometer. The samples were radiated using Ni filtered CuKa radiation operated at 40 kV and 30 mA. The sample was scanned from 5 to 30 of diffraction angle (2θ) at scanning speed of 1 2θ/s.

Nuclear Magnetic Resonance (NMR)

Both samples of sirolimus and heat-treated sirolimus were dissolved in deuterated chloroform (5 mg/ml) and ^{1}H NMR spectra were collected using a Jeol ECA, 500 MHz FT NMR Spectrometer, incorporating a 5 mm diameter inverse detect probe. ^{1}H NMR spectra were acquired using a single pulse experiment, with a relaxation delay of 2 seconds, the number of spectral accumulations was 512 scans, and the 90° pulse width was 7.8 μ seconds. Solutions were maintained at 25°C during data collection, and the resulting spectra were referenced with respect to tetramethylsilane (TMS). A D_2O shake was also carried out for the sirolimous sample using the same parameters as mentioned above.

Scanning Electron Microcopy

The morphology of the drug/polymer microspheres and coated stents were examined by Scanning Electron Microscopy (SEM). The samples were attached to aluminum stubs with double-sided adhesive carbon tape, then gold-coated and examined using a scanning electron microscope (JEOL 5200, SEM).

High Performance Liquid Chromatography and Gas Chromatography

Sirolimus quantities in all samples were determined by HPLC. An Agilent Technologies 1200 series with a quaternary pump, an autosampler and a detector used at 278 nm were used for HPLC assay (22). A Lichrocart 250-4-RP 18 5 μm (Merk) column was used with mobile phase 60:40 acetonitrile:water. The column was maintained thermostatically at 50°C. The flow rate was 1.0 mL/min and the injection volume 10 μl. Calibration curves were constructed using standard solutions of known concentrations (3–50 ng/mL). The Chemstation software calculated the peak area of each standard solution and sample automatically.

Chemical stability of fused sirolimus and the variation of the -α, -β and -γ isomeric forms was investigated using the same chromatographic conditions. For the purpose of these studies, five fused stents were placed in acetonitrile to extract sirolimus for further investigation.

To determine the amount of the residual solvents, a GC system (Agilent 6850 Series II) was used with a column PERMABOND® SE-54-HKW, 50 mm × 0.32 mm ID (MAcherey - Nagel). 50–60 mg of microspheres were weighed into 10-ml vials, closed airtight and analyzed by performing three extraction steps (injections) per sample. The method was adapted from Steckel *et al.* (23), using the following parameters: oven temperature was 160°C for 10 min, 160–225°C with 10°C/min, and 225°C for 30 s. The detector was set at 250°C and the gas was helium (0.9 bar).

In Vitro Sirolimus Release Studies

The sirolimus-loaded stents were immersed in test tubes containing 5 ml sterilized PBS (pH 7.4) (11). The tubes were then incubated in a water-bath shaker at 37°C at 120 rpm. The incubated PBS in each tube was collected and replaced with fresh buffer system everyday.

Platelet Adhesion

The hemocompatibility of the EDPDT-coated stent was obtained using an *in vitro* platelet adhesion assay. Fresh blood was drawn from healthy volunteers (120 mL), who were medication-free and used for radiolabeling of platelets with

^{111}In. The labeled platelets were resuspended in the Platelet Rich Plasma (PRP) to 2.5×10^6 platelets/mL. The coated (EDPDT/Cypher) and uncoated 316 L stainless steel substrates (n=3) were submerged with PRP at 37°C in an incubator for 90 min. After incubation, the samples were recovered and washed four times with saline. The samples were then fixed in 1.5% glutaraldehyde solution, and the number of platelets was determined using the gamma counter, taking into account the background, the decay and the radioactivity of blood samples used for calibration of the data.

RESULTS

ASES Microspheres Preparation

In the current study, ASES was chosen to produce sirolimus-encapsulated polymeric microparticles. This process was preferred to the conventional time-consuming emulsification methods to circumvent the use of emulsifier and complexity of solvent evaporation, washing steps and powder losses. ASES has been successfully used (24) for the production of microparticles in the range of 1–10 μm by employing the extraction properties of supercritical fluids. The ASES process consisted of two steps: a) CO_2 was first pumped inside a high pressure vessel to reach the operating conditions of pressure and temperature, and b) the organic solution, which contained the drug and the polymers, was sprayed through a nozzle into the supercritical bulk phase afterwards. At the end, the particles produced were collected on a filter at the bottom of the high pressure vessel. Carbon dioxide is the most commonly used supercritical fluid because of its relatively low critical parameters (T_c=31.1°C, P_c= 73.8 bar). The utilization of CO_2 as an anti-solvent relies on the usual negligible solubility of drug and polymers under supercritical conditions. However, in this case, the main obstacle was the interaction between supercritical CO_2 and the amorphous polymers PBMA and PEVA due to the high solubility of CO_2 in amorphous materials (25,26). As a result of this solubility, the polymers swell, because CO_2 acts as a plasticizer, which leads to the formation of porous and irregular shapes in the powdered product (data not shown). In order to address this problem, ASES was modified based on previous studies (26) with amorphous materials. A combination of CO_2 and N_2 (T_c=−147.1°C, P_c=33.9 bar) was employed to reduce the interaction between CO_2 and the polymers due to the lower relative solubilising power of N_2.

During the ASES process, the pressure was fixed at 150 bar and temperature at 35°C due to the low glass transition of the polymers. The initial sirolimus/polymer ratios were kept identical to the commercial product. The solvent chosen for ASES process was dichloromethane (DCM), which is miscible with the supercritical fluids and is extracted at the operational conditions.

Optimization of sirolimus-encapsulated polymeric microparticles was carried out by implementing a response surface fractional factorial experimental design (Table I). This experimental design is suitable for the exploration of quadratic response surfaces and constructs a second-order polynomial model, which helps optimise a process using a small number of experimental runs. The independent factors studied were the organic solution flow rate, total polymer concentrations and volume ratio of SF/DCM. The mean volume diameter and drug loading (%, DL) were the measured responses. All independent variables showed a significant influence, confirmed by ANOVA analysis (Table II), on the powder particle size obtained with probability values (p) less than 0.05. In contrast, polymer concentration and volume ratio presented significant influence on sirolimus DL. It can be seen from Figs. 2 and 3 that polymer concentration and volume ratio had significant influences on both response variables. As the polymer concentration increases, particle size increases above 50 μm, while drug loading decreases. The optimal total polymer concentration estimated from the response surface graphs lies in the range of 1.5–3 mg/ml. It can also be found that low or high CO_2/DCM volume ratios have a negative effect on the particle size and thus on DL too.

Table I. Fractional Factorial Experimental Design with Organic Solution Flow Rate, Polymer Concentration and the Volume Ratio of SF as Independent Variables and Particle Size (Mean Volume Diameter), Drug Loading Percentage as Response Variables

Injection rate (ml/min)	CO_2/DCM	PBMA-PEVA (mg/ml)	Particle size (μm)	Drug loading (%)
1	10	1,0	19,8	20,5
0,6	10	2,5	3,1	30,1
0,2	10	2,5	7,8	25,6
1	5	4,0	70,4	9,5
1	15	2,5	19,7	17,4
0,6	10	4,0	50,7	10,5
0,2	15	4,0	48,7	12,3
0,2	5	1,0	35,4	13,4
0,6	5	2,5	14,6	26,7
0,6	10	2,5	2,95	29,8
0,6	15	1,0	2,9	29,6

The optimum procedure was determined to be at 0.6 ml/min flow rate, 10 volume ratio and 2.5 mg/ml polymer concentration. The reproducibility of this procedure was good, since no obvious differences were observed in sirolimus content and loading efficiency of particles with diameters of 2.5–3.5 μm in six batches of microspheres (mean DL 29, 7% ± 0.96) prepared using the optimum procedure.

Sirolimus microparticles prepared by ASES have been assessed for DCM.

Microsphere Characterization

Microspheres prepared by ASES were discrete and spherical in shape, showing a smooth surface as determined by SEM of a representative sample (Fig. 4). Furthermore, DSC and XRPD studies were performed to characterize the drug status in the formulated microparticles. Each study was carried out for pure drug, pure polymer and drug-loaded microparticles. The thermogram (Fig. 5a, inset) obtained for pure sirolimus showed a melting endothermic peak at 188.63° C. In addition, sirolimus XRPD patterns (Fig. 6a) showed high-intensity diffraction peaks at 2θ values of 7.3°, 10.2°, 11.1°, 12.5°, 14.5°, 16.2°, 20.1°, 20.5° and 21.8°.

PBMA, which is a thermoplastic semi-crystalline material, showed a transition temperature (T_g) at 44.94°C and a melting point (T_m) at 171.66°C (Fig. 4b). The crystalline

Table II. Anova Table of the Independent Variables According to the Experimental Design

	Particle size		Sirolimus loading	
Variable	t -statistic	P -value	t -statistic	P -value
X1	58,447	0,0109	−6,779	0,0932
X2	−89,085	0,0071	13,291	0,0478
X3	271,204	0,0023	−55,671	0,0114
$(X1)^2$	119,616	0,0053	−34,641	0,0184
$(X2)^2$	59,194	0,0108	−7,526	0,0841
$(X3)^2$	339,525	0,0019	−68,908	0,0092
X1 * X2	39,324	0,0162	−26,879	0,0237
X1 * X3	18,924	0,0336	3,528	0,1758
X2 * X3	24,056	0,0264	−3,143	0,1961

X1: Injection rate (ml/min)
X2: CO2/DCM volume ratio
X3: [PBMA - PEVA] (mg/ml)
P: probability value
t: coefficient value

nature of PBMA was confirmed by the XRPD scans (Fig. 6b) with intensity diffraction peaks at 2θ values of 7.5°, 9.2°, 12.8° and 18.0°. Furthermore, the DSC thermogram (Fig. 5c) of sirolimus-loaded microparticles presented two characteristic endothermic peaks. The former, at 42.65°C, was attributed to the PBMA glass transition temperature, and the latter was a broaden peak at 185.4°C. The existence of this broaden peak close to the pure sirolimus melting point indicates that the encapsulated sirolimus is mainly at the amorphous state with a small crystalline content. The XRPD scan of the sirolimus-loaded microparticles (Fig. 6c) revealed diffraction peaks at 2θ values of 8.9°, 11.1° and 19.6°. By using the XRPD findings of Fig. 6 (a–b), the retained crystalline sirolimus in the polymeric microspheres was estimated between 4–5% (three different batches).

EDPDT and Fusion Process

The EDPDT tests were performed on bare stent struts using the previously prepared sirolimus polymeric micro-

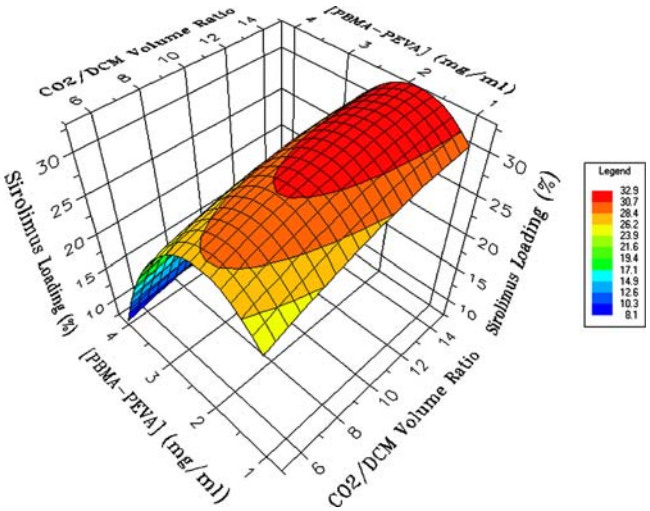

Fig. 2. Surface plot showing the influence of polymer concentration and CO_2/DCM volume ratio on sirolimus loading.

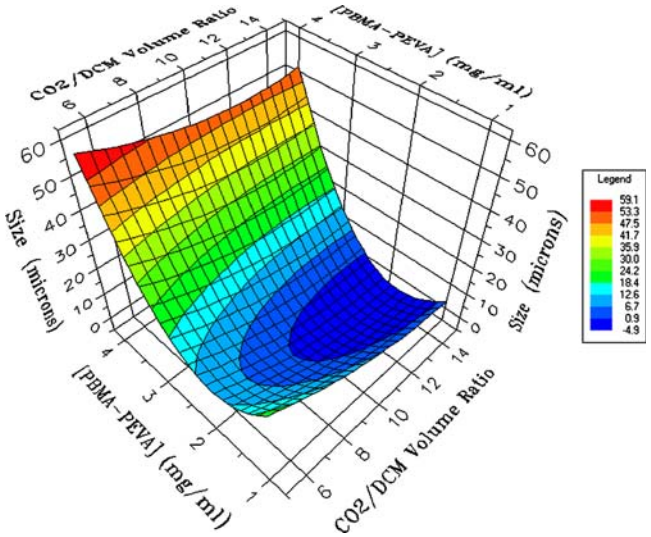

Fig. 3. Surface plot showing the influence of polymer concentration and CO_2/DCM volume ratio on particle size.

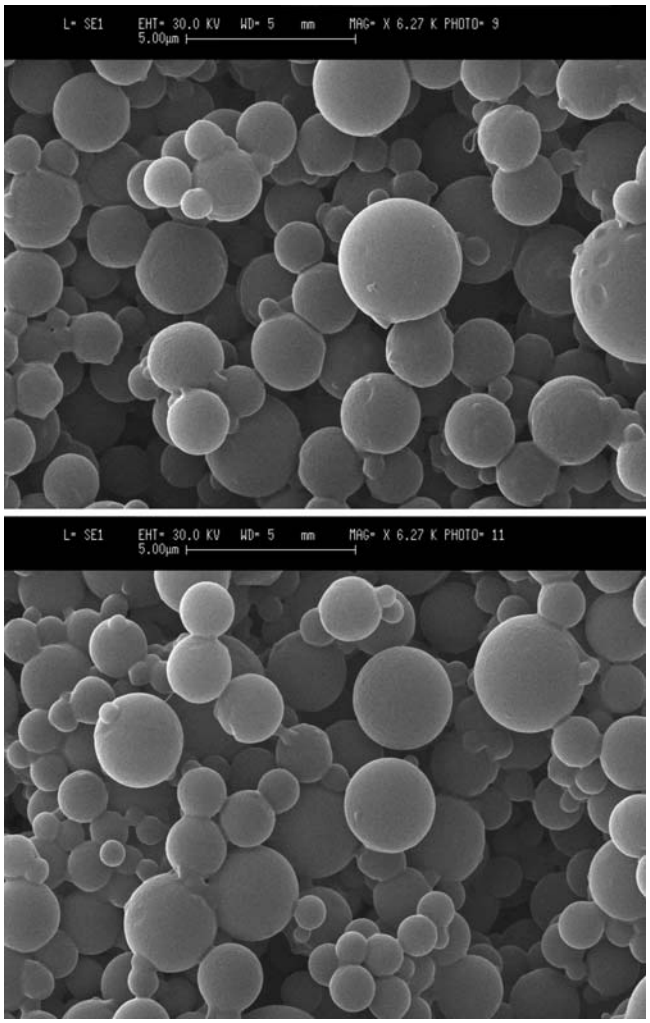

Fig. 4. SEM image of sirolimus polymeric particles ASES processed.

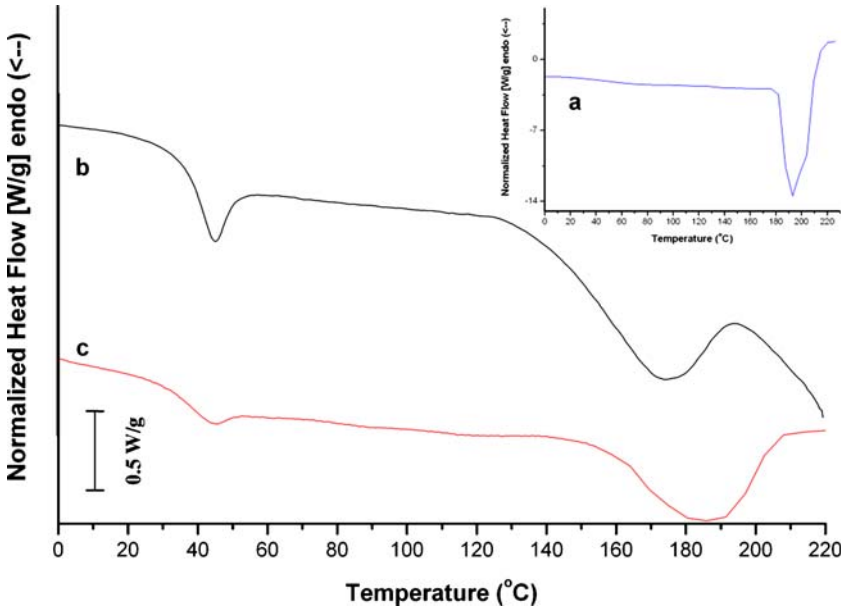

Fig. 5. DSC thermograms of **a** pure sirolimus powder, **b** PBMA and **c** sirolimus-loaded microparticles.

particles. The powder spray-gun, which uses a high voltage generator (0–100 kV), created a negative polarity potential on the powder particles through ion bombardment. Coating thickness (or weight) is controlled by the applied voltage to the charging media, exposure time to the cloud and the air flow rate fed to the spray-gun. The influence of the above variables on coat weight was investigated to identify the optimum process settings. As a result, the coating time was found to be 6 s, applied voltage 70 kV and flow rate at 5 L/min (0.3 bar). An increase of each operating parameter resulted in a significant development of coat thickness.

First, an increase in the applied voltage caused a considerable growth in coating thickness with an optimum range of 70–80 kV. Further voltage increase up to 90 kV created the opposite effect by reducing the transfer efficiency (back ionization phenomena). At 90 kV, a thick coating was developed in very short times (5 s), leading to a thinner coating with increased exposure times. An increase of the air flow rate accelerated powder deposition on the stent strut; however, flow rates over 7 m^3/h caused powder dispersion due to elutriation effects. The aforementioned EDPDT optimal settings were applied to load thirty uncoated Cypher® stents with 70 µg sirolimus per stent (or 245 µg of microparticles in total). The accurate powder weight of each stent was measured, and the average loading was found to be 247±10.1 µg, while loaded sirolimus, determined by HPLC, was 70±2.5 µg. The

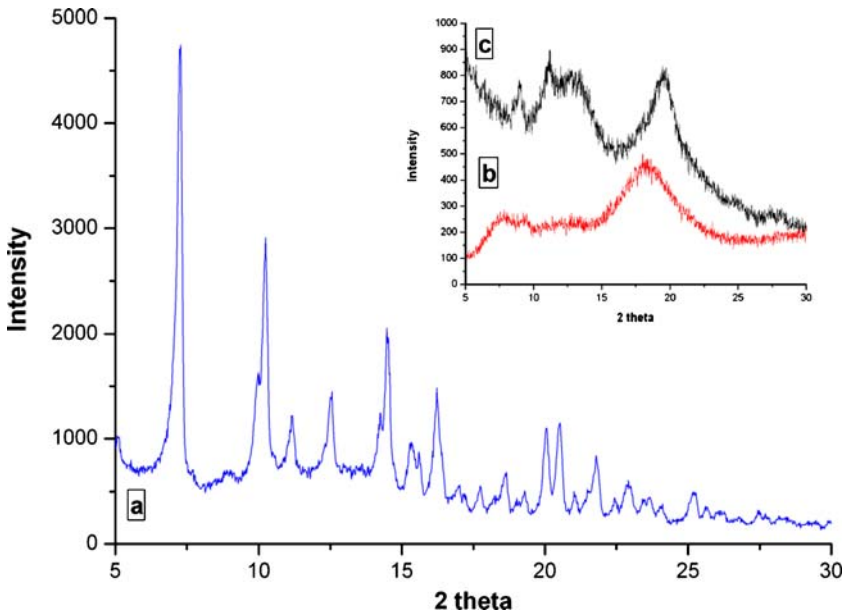

Fig. 6. XRPD patterns of **a** pure sirolimus powder, **b** PBMA and **c** sirolimus-loaded microparticles.

average drug weight represents 100% coating efficiency without drug wastage for the applied EDPDT process.

Subsequently, the coated stents were fused for 60 s at different fusion temperatures. Sirolimus (rapamycin) is a carboxylic lactone-lactam macrolide derived from an actinomycete (*Streptomyces hygroscopicus*), and the bulk powder consists of three isomeric forms (-α, -*β* and -*γ*). The chemical stability of the drug molecule appears to be sensitive to various factors such as pH (27) and organic solvents (28). Obviously, the fusion process of sirolimus polymeric microparticles using infrared radiation could have an impact on chemical stability and consequently alter the stent's immunosuppressive capability. Stents were fused, therefore, under various temperatures, and sirolimus chemical stability was investigated. These studies revealed the optimal temperature range for uniform and smooth coat surface to be obtained. Table III shows the initial degradation studies of several selected fusion temperatures of coated stents and of unfused bulk sirolimus powder. Surprisingly, no chemical degradation products were detected based on the HPLC analysis findings. However, transformation was observed among the various isomeric forms of sirolimus with varying relative percentage of the chromatographic peak areas.

Substantial sirolimus transformation was observed with elevated fusion temperatures above 100°C. For example form -*β* (86.2 %) underwent significant transformation to -α (11.2 %) and in smaller extent to -*γ* (2.6 %) form at 130°C, respectively. It can be seen that the optimal fusion range lies between 80–100°C as sirolimus transformation among the three isomeric forms within these temperatures is negligible.

Additional NRM studies were also carried out to determine whether sirolimus could be degraded due to the heat treatment. In this case, bulk sirolimus powder was fused for 1 min under identical conditions to the coated stents (100°C). Sirolimus decomposition studies have been reported in the literature (28,29) but not under heat stress. In these cases, sirolimus degradation has been related to the *trans* and *cis* variations. The ^{1}H NMR spectra of both sirolimous and heat-treated sirolimous have been recorded to determine if there are any decomposition products produced by the heat treating process (Fig. 7). The NMR spectral profiles for the sirolimous and heat-treated sample are almost identical, apart from the peaks labelled X (1.57 ppm) and Y (2.64 ppm) in Fig. 7. The signal at 1.57 ppm is attributed to a small amount of moisture in the sample, which all but disappears after the heat treating process. The peak at 2.64 ppm appears to decrease in intensity and broaden after the heating treating process. This signal is attributed to an exchangeable -OH group, which possibly shows a change in its dynamic equilibrium with solvent/water molecules, due to the loss of moisture after heating. These peak assignments have been further confirmed with respect to the results of the NMR D_2O shake experiment.

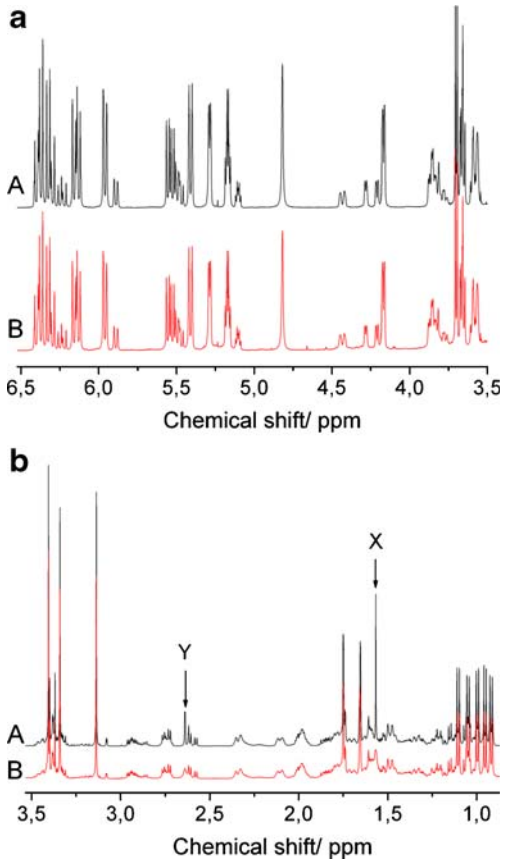

Fig. 7. Partial ^{1}H NMR spectra of **A** sirolimus and **B** heat-treated sirolimus.

Minor components can be seen in both the ^{1}H NMR spectra, which are attributed to a *cis/trans* amide equilibrium. It has been reported that when sirolimous is dissolved in chloroform, there is an equilibrium between the *cis/trans* amide isomers in a 80/20 ratio (28). This has been confirmed by our results, and, indeed, after the heat treating process, there appears to be no change in the ratio of the isomers.

In summary, there appears to be no degradation products after heat treating sirolimous, and thus, we can confirm the ≥95% purity of the heat-treated sample.

The surface morphology of coated stents was observed using SEM as shown in Fig. 8 (a–d). The fusion process led to smooth and uniform coating across the stent surface without any lumps or un-fused microparticles. In addition, the stent surface was fully coated without any fractures or bare metal surfaces seen under careful examination.

Table III. Typical Sirolimus HPLC Peaks after Stent Fusion at Various Temperatures

Surface temperature (°C)	% (-*β*)	% (-*γ*)	% (-α)	SD (±)
20–25	94.7	1.9	3.4	0.71
55–60	94.5	2.3	3.2	0.33
60–65	95.7	1.8	2.5	0.22
70–75	92.9	2.8	4.3	0.96
80–85	94.7	2.3	3.0	0.48
100–105	89.1	4.3	6.6	1.70
115–125	88.9	4.3	6.8	1.60
125–130	89.9	4.2	5.9	1.73
140–150	80.8	6.3	12.9	3.01

In Vitro Sirolimus Release Study

The *in vitro* release patterns of coated stents (n=3) using EDPDT were studied and compared with those of commer-

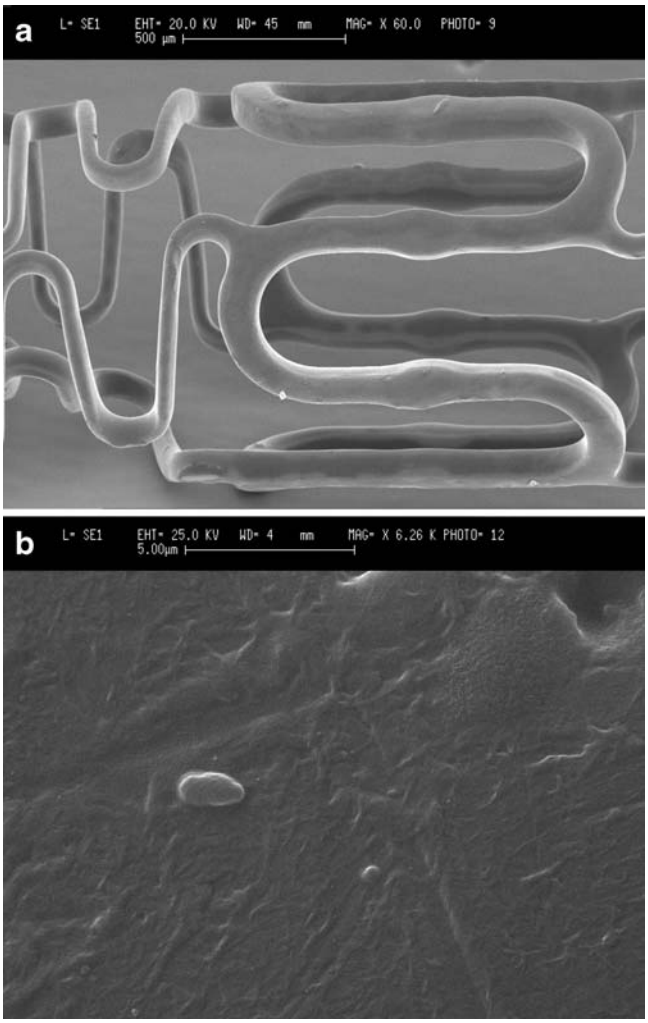

Fig. 8. SEM images **a** of a fused stent coated with EDPDT process and the surface **b** of a fused stent at 80°C.

cial Cypher® stents, respectively. Fig. 9 shows the release profile studied over a period of 30 days. In both cases, a sustained release manner was observed. Sirolimus showed a faster release pattern using the EDPDT coating process in comparison to the commercial product without any apparent statistical significant differences. A Mann-Whitney (two-tail P value) nonparametric test using GraphPad Instat® (GraphPad Software Inc.) was applied to examine whether there were significant differences among the sirolimus percents released at each time level. In addition, EDPDT, coated stents showed reproducible release patterns without substantial burst release at the beginning and slow sirolimus rates at the end stages.

Thickness Determination of Coated Stents

In order to estimate the coating thickness, stents were partly coated. The stent was affixed to a double-sided adhesive carbon tab mounted on to a pin-type aluminium stub. In addition, no conductive coating was applied prior to SEM analysis so as not to prejudice the measurement of the coating layer. A JEOL JSM 5310LV SEM was used in low vacuum mode at a pressure of 20 Pa. The image was taken using the backscattered electron detector, and collected and measured using Oxford Instruments ISIS 300 Autobeam software. There is a high backscattered electron contrast between the metal of the stent, which appears bright due to high mean atomic number, and the coating, which appears dark due to its low mean atomic number. Because the coating is atomically light and a fairly high accelerating voltage was used, it is possible to detect backscattered electrons from the metal of the stent beneath the coating. An area was chosen where the coating could be seen adjacent to the bare metal. The average measurements of 6215 nm (n=3) were made due to slight variations; the coating is therefore determined at approximately 6.2 µm (± 0.1 µm).

Residual Content in Sirolimus Microparticles

The use of dichloromethane to prepare microspheres by ASES led to very low residual contents of this solvent, as was expected due to the washing process. The analysed samples were collected immediately after the completion of the ASES process. The residual DCM was estimated 35 ppm (± 1.8 ppm) well below of the 600 ppm limit (from ICH Topic Q3 C(R3)). We anticipate that residual DCM further reduces through the fusion process due to the heat exposure.

Platelet Adhesion

The platelet adhesion of the EDPDT-coated stents was investigated and compared with those of Cypher® and bare 316 L stainless steel stents, respectively. The results illustrated in Fig. 10 showed that the uncoated 316 L stainless steel surface had increased platelet adhesion, while the EDPDT stents had decreased and negligible platelets comparable to Cypher®. The mean values for adherent ^{111}In-labeled platelets were $5.1\times10^5\pm1.5\times10^5$, $17.3\times10^3\pm3.5\times10^3$ and $21.2\times10^3\pm4.3\times10^3$ (mean±SD) for the control (316 L), Cypher® and EDPDT coated stent groups, respectively. A Kruskal-Wallis non-parametric test showed significant difference between Cypher®/EDPDT and the control metal stent but not between Cypher® and EDPDT.

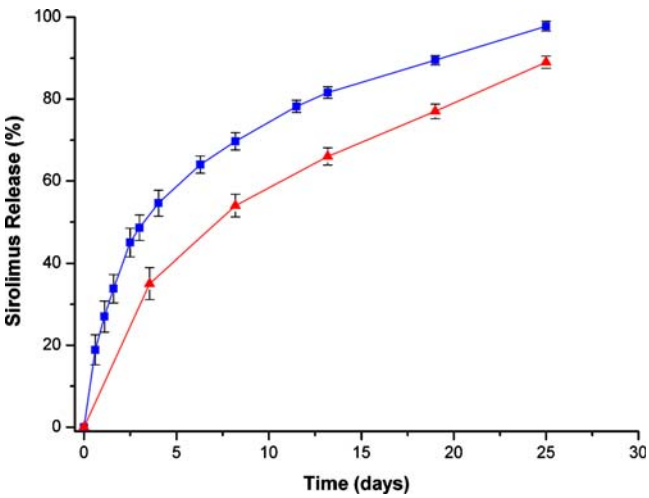

Fig. 9. Release profiles of EDPDT-coated (■) and Cypher® stents (▲), respectively (n=3).

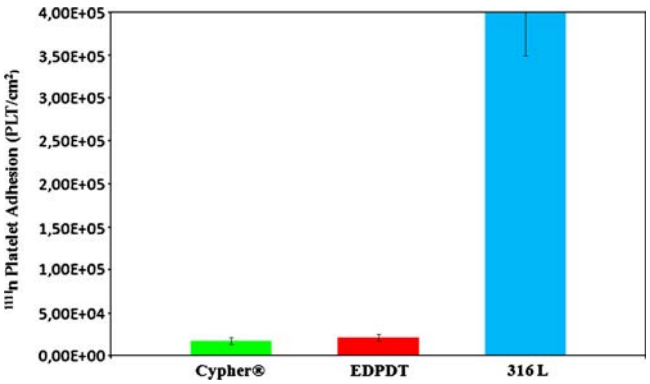

Fig. 10. Adherent ^{111}In-labeled platelet count on the stent surface. The displayed value is the mean ÁSD of the four individual averages; $p<0.01$, $n=3$.

DISCUSSION

We optimized coating stents with sirolimus-encapsulated polymeric microparticles using EDPDT followed by fusion of the coated particles on stent surface in this study. The aim of this study was two-fold. First, we wanted to improve the control of particle size and drug loading by modification of the solution-enhanced dispersion by supercritical fluids (SEDS) process. We improved the particle formation process by using a supercritical mixture (CO_2–N_2) to reduce polymer solubility within the extraction medium. In the ASES process, the solvent, which is miscible with the supercritical fluids, is extracted, while the drug and polymers, being insoluble, precipitate as microparticles. It has been found previously (26) that a CO_2–N_2 combination increases the dispersive effect and solidification rate of amorphous polymers, leading to smaller particle sizes. The PBMA and PEVA polymers used for microencapsulation have low glass transition temperatures, and, thus, moderate temperature/pressure settings were used (26).

Furthermore, it was essential to optimize process parameters in order to control particle size and DL of the microspheres produced. The application of a fractional factorial design assisted evaluation of the effects of process parameters. Polymer concentration had a significant impact for both variables. Increased polymer concentrations gave agglomerated irregular particles of 50–70 μm with very low DL. Particle size was also dependent on injection flow rate and DCM quantities. Increased flow rates or solvent volumes had a negative effect on particle size as well. Optimum particles produced by ASES had a mean volume diameter of 2.5–3.5 μm and high DL of 28–30% and were spherical in shape, with a smooth, non-porous surface. The main ASES advantage was the production of appropriate particles in a one-step process in the absence of emulsifier.

The optimized sirolimus polymeric microparticles with an average particle size of 3 μm were chosen for stent EDPDT coating. Microparticles of this size facilitate EDPDT process as they exhibit very good packing of the void spaces and create a dense coating layer. Coating trials with 3 μm sirolimus-loaded microparticles displayed enhanced coat uniformity and density compared to 20 μm powder material. This was due to particle size, particle shape, particle-size distribution and powder-flow properties.

Different stent coating approaches (10,11,30,31) have been used by various researchers to achieve efficient coating, drug loading and controlled release. Although these techniques provided consistent coating layers, they were unable to minimize coating material losses. EDPDT is advantageous, as unused powder can be easily recovered. In addition, EDPDT is a reproducible, accurate and easy-to-scale-up process by adjustment of operational parameters. The applied voltage, air gun flow and coating time are critical parameters that affect particle flow, particle charge and adhesion to the stent surface (32). Coat thickness could be controlled by regulating the above parameters to the desired levels. The applied voltage was found to be effective between 60–70 kV. Higher applied voltages caused back ionization phenomena that reduced transfer efficiency and coat thickness. Similarly, the exposure time showed rapid powder building on the strut surface at approximately 5–7 s, while reduced coat thickness was observed beyond 8 s.

The fusion process was applied for a period of 60 s through infrared radiation to create a polymeric film on the metal surface. Further studies at various temperatures indicated an optimum fusion temperature range of 80–100° C. A smooth, uniform coat was obtained at this temperature range, and no drug decomposition was observed. However, isomeric transformation between different sirolimus forms was noticed at elevated temperatures. Thus, the maximum fusion temperature was set at 100°C to avoid transformation that could possibly alter sirolimus immunosuppressive properties. In addition, ^{1}H NMR studies confirmed the absence of degraded sirolimus under heat treatment at the above temperature.

The release patterns of EDPDT-coated stents were slightly faster compared to the commercial Cypher® stents. The reason is due to the existence of a thin top-coat PBMA layer on the base coat of the commercial Cypher®. The absence of this layer on the EDPDT-coated stents resulted in faster diffusion of sirolimus through the polymeric layer. Nevertheless, the sustained and reproducible release patterns achieved by EDPDT could be further controlled by altering PBMA/PEVA ratios and sirolimus DL during the ASES process.

An *in vitro* model was employed to access the hemocompatibility effect of the EDPDT on stent performance. The results demonstrated that EDPDT has reduced platelet adhesion compared to the control uncoated stents and similar levels to the Cypher® stent. These findings are in good agreement with similar studies (33,34) suggesting that EDPDT can be effectively used for stent coating studies.

Finally, the EDPDT approach is not limited to the current delivery system, but it is applicable to different DESs active coatings. In the future, additional studies will be required to develop the layer uniformity further. Additionally, the manufacture of drug-encapsulated nanoparticles will provide thinner and easier-to-fuse coating layers.

CONCLUSIONS

Electrostatic dry powder deposition technology was used in this study to prepare coated stents with a smooth and uniform coating layer. The dry powder, microencapsulated sirolimus in PBMA-PEVA microparticles, was produced

using ASES, modified with a mixture of CO_2–N_2 supercritical fluids. Hemocompatibility studies showed negligible platelet adhesion on the coated surface. We believe that EDPDT can be utilized for high-precision stent coatings without any restrictions regarding the active substance or the polymers used.

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