Crystalline paclitaxel coated DES with bioactive protective layer development

Shady Farah⁎,1, Abraham J. Domb⁎

Institute of Drug Research, School of Pharmacy-Faculty of Medicine, Center for Nanoscience and Nanotechnology and The Alex Grass Center for Drug Design and Synthesis, The Hebrew University of Jerusalem, 91120, Israel

A R T I C L E   I N F O

Keywords:
Drug eluting stents
Paclitaxel
Crystallization
Bioactive coating
Controlled multilayer release
Hyaluronic acid

A B S T R A C T

Drug eluting stents (DES) based on polymeric-carriers currently lead the market, however, reports on clinical complications encourage the development of safer and more effective DES. We recently reported on carrier-free DES based on rapamycin crystalline coating as a potential therapeutic solution. Here, we report for the first time surface crystallization of paclitaxel (PT) onto metallic stents. The physicochemical principles of crystallization and key process parameters were extensively studied for fabrication of controllable and homogeneous crystalline coatings on stent scaffolds. Stents loaded with nearly 100 μg PT were chosen as a potential therapeutic device with a multilayer coating of 4–7 μm thickness. In vitro PT release from these coated stents shows constant release for at least 28 days with 10% cumulatively released. The effect of fast dissolving top coating on the physical stability of the coated stent was determined. The top coating enhances the mechanical stability of the crystalline coating during deployment and expansion simulations. Also, incorporating PT in the protective top coating for developing bioactive top coating for multilayer controlled release purpose was intensively studied. This process has wide applications that can be further implemented for other drugs for effective local drug delivery from implantable medical devices.

1. Introduction

The introduction of drug-eluting stents (DES), i.e. metallic stents coated with drug loaded non-degradable/degradable polymers into clinical cardiology at the beginning of the new millennium can be considered a success story opening the gates for a new era in interventional cardiology [1]. As a recent consequence, this field has been dominated by DES [2–6], in which coronary arterial stenting with DES is a major therapy for the treatment of coronary arterial diseases [7]. Currently, four DES have been approved by the Food & Drug Administration for the U.S. market including: Cypher® stent (Cordis, Miami, FL), Taxus® stent (Boston Scientific, Inc., Natick, MA), Endeavour® stent (Medtronic Minneapolis, MN) and Xience® stent (Abbott Laboratories, Abbott Park, IL). Among these four approved DES, except for the Taxus® stent which is coated with anti-microtubule drug-paclitaxel (PT), all of the other DES are coated with either rapamycin-sirolimus (Cypher® stent) or its analogs zotarolimus (Endeavour® stent) and everolimus (Xience® stent) [8,9].

Various coating techniques, such as dip coating, spray coating and electropolymerization, are available for coating medical devices, i.e., DES [10–15]. In these DES, the coated therapeutic agent is applied along with a carrier responsible for several functionalities. It holds the drug mechanically, preserves chemical stability of the drug and most importantly regulates the release kinetics of the drug [16–18].

PT is a natural diterpenoid extracted from the bark of Taxus brevifolia, the Western yew [19,20], and is well-known as an antineoplastic drug that has been widely applied to treat various cancers, especially breast cancer and ovarian cancer [21–23]. PT is a highly lipophilic drug which can penetrate cell membranes easily and hence promotes rapid cellular uptake [24]. Several researchers have shown that PT reaches a cellular steady state concentration within 5 min [25]. PT disrupts the M-phase of the cell cycle and hence inhibits cell proliferation by enhancing extraordinarily stable microtubules. Microtubules are also building blocks of cytoskeleton that enable cell motion; thus, alteration to cytoskeleton property also means preventing cell migration. Since PT’s mode of action involves a cytoskeleton targeting therapy, its effect is long-lasting [2,7]. Its use in preventing coronary restenosis has been extensively investigated, since it is a potent inhibitor of all three phases of the restenosis process: smooth muscle cell proliferation, migration, and extracellular matrix formation [23,26]. PT is retained well in the
blood vessel wall for up to 3 days through specifically binding to its individual proteins after release from stents [8,27–29]. Moreover, new data have shown even relatively superior performance compared to other DES in preventing restenosis in high risk patients such as, having diabetes mellitus [8,28–32]. Several polymeric materials have been reported for controlling the PT release profile and enhancing localized drug delivery [33–41].

Despite DES performance compared with a bare metal stent, a cumulative increase in studies reporting safety concerns (i.e., late state thrombosis and inflammation due to DES coatings) [42–45]. Further findings demonstrating DES coating defects include cracking, flaking, and delaminating in commercially available stents pave the path for further investigations [46,47]. Accordingly, novel coatings are needed to overcome these issues. Polymer-free DES based crystalline drugs are considered among the most promising.

Crystallization of organic compounds is a routine process that should be carried out under strict control conditions [48,49]. Agglomeration and aggregation of crystals can be a significant phenomenon. One mechanism for agglomerate growth is attributed to growing nuclei colliding and becoming “cemented” together by continuing growth between two or more crystals. The addition of a large number of nuclei to original two-crystal agglomerates can readily occur by ongoing collisions, leading to very large agglomerates, which should generally be avoided [48]. Coating tiny implants such as cardiovascular stents requires a precise coating method that can follow the contour of each strut; such a control is used to manipulate the size of generated crystals on stent surfaces [50].

For release behavior, PT crystals have been reported to have a lower dissolution rate versus its amorphous state [19]. Utilizing this feature, we hypothesize that a crystalline coating can release the drug in a gradual manner without the need for a carrier controlling PT release rate. It is also possible to control the release kinetics of the drug by manipulating both crystal’s and crystalline coating morphology. Thus, the chemical stability of a crystallized drug is also superior compared to its amorphous form [51,52]. Crystalline coatings are, therefore, expected to have longer shelf life stability [48]. The surface layer of the drug crystals offers protection to the drug beneath the surface against degradation. An ideal example has been reported for PT that undergoes degradation after 48 h even when placed at room temperature conditions. However, when formulated as a nanocrystal stabilized with poloxamer 188, the drug shows excellent stability over a period of 4 years [53]. When the amorphous phase is in a metastable state, the drug tends to partially crystallize during storage, resulting in cracking and morphological changes that are expected to alter drug release properties. Such problems were recently reported for spray-coated DES [54].

Crystalline coating advantages we have examined in our recently reported development of carrier-free rapamycin eluting stent based crystalline rapamycin coating, as a potential replacement for polymer-rapamycin DES. Also we have reported a protective rapid dissolving polysaccharide top coating [55–57]. Here, we reports for the first time PT surface crystallization coating developments to generate a carrier-free crystalline PT coated stent. This new approach was applied on metallic stents and cylindrical tubes (cobalt-chromium). The process parameters were investigated in detail regarding the coating morphology, drug loading and crystal size, under different conditions and developed coatings. Also, here we examine the incorporation of PT into the top coating and release profiles to yield a bioactive protective top coating.

2. Material and methods

2.1. Materials

Stents and cylindrical tubes (CoCr, 15 mm length, batch M/Z: 45011011203, 4120200275-60) were obtained from Alvimedica, Turkey. Macrolane VRF20 (Hyaluronic acid (HA) aqueous solution) was purchased from Q-Med AB, Sweden. Paclitaxel (PT) was purchased from BiopolPharma (Lot No. GT-CH09). Tween 20 was purchased from Sigma-Aldrich, Israel. Ethyl acetate (AR) and n-Hexane (AR) were purchased from BIO LAB LTD. Sodium azide was purchased from MERCK, Darmstadt F. R. Germany. Sodium hydrogen phosphate, sodium phosphate monobasic, sodium carbonate (AR) and methanol (HPLC grade) were purchased from J. T. Baker, Holland. Shrinkable tubes (PVLF05J) came from Shrink Sleeve Ltd., UK. Glycerol was purchased from Biolab Ltd., Israel.

2.2. Methods

2.2.1. Stent PT surface crystallisation or amorphous coating

Stents with crystalline coatings of PT were prepared using our previously reported method after modification to fit drug solubility in both solvent/anti-solvent and mixture ratio optimization [55,57,58]. Briefly, the process is composed of two steps (seeding and crystallization). For seeding, 1.6 mg of fine powder PT was mixed in 4 ml of n-hexane using sonication until a homogeneous dispersion was formed. Stents were mounted onto shrinkable tubes and placed in the PT dispersion in hexane. These vials were then placed in the ultrasonic bath for 10 min at 30 °C to form a seeding layer. Stents were gently removed from the vials and allowed to dry at room temperature to yield a thin layer of 20 ± 5 μg/stent. The seeded stents were forwarded to the next crystallization step. For crystallization, 50 mg of PT was dissolved in 15 ml of ethyl acetate, and 65 ml n-hexane was slowly added to form homogeneous metastable solution at 25 °C. Stents were placed in this solution for 15 min for crystallization of PT on pre-seeded layers to form continues crystalline carpets. The coated stents were taken out of the crystallization chamber and dried in room air.

Amorphous coatings were prepared by spraying a drug solution using an ultrasonic spray-coating machine (SonoTek MediCoat Des 1000, USA) as previously reported [55,57]. Briefly, PT solution in ethyl acetate (1% w/v) was spray-coated at a flow rate of 0.2 ml/min; the ultrasonic generator power was set at 1.2 W. Spray-coated samples were tested for homogeneity by high resolution scanning electron microscopy (HR-SEM) and coating weight with microanalytical balance.

2.2.2. Protective top coating formulation

A 0.3% w/v aqueous solution of hyaluronic acid (HA) was used for making a protective top coating on crystallized PT. HA solution was prepared by dissolving 1 ml of Macrolane VRF20 (20 mg/ml HA) in 5.7 ml of double distilled water and then mixed vigorously on a stir plate for 3 days. 10% w/w glycerol was added to this solution as a plasticizer. The solution was used for coating stents by spray coating with SonoTek system (MediCoat Des 1000, USA) of a total sprayed volume of 0.1 ml solution, flow 0.2 ml/min and 10 watts power and 5 ml syringe (diameter 11.45 mm). Sprayed stents were dried overnight in an active hood.

A bioactive top coating containing PT was prepared by vigorously mixing on a stir plate for 30 min a PT methanolic solution diluted with HA aqueous solution in dropwise. Solution ratio DDW:methanol of 1:1 v/v was applied. Drug concentration was optimized for 10 mg/ml (methanol solution), and a HA-F formulation (0.3% HA + 10% w/w glycerol) was added drop wise to this solution to fit a final HA-F top coating: drug ratio 1:1. Then resulted transparent solution was sprayed onto the crystallized coating using a Sono-Tek spraying system with the former reported parameters. Drying process was reduced by 20 min in the active hood to avoid any undesired self-crystallization.

2.2.3. Therapeutic coatings analysis

2.2.3.1. Crystalline and amorphous coating integrity and crystallinity. During each stage prepared coatings were visualized microscopically for homogeneity. They were then weighted using a microanalytical balance. Crystalline and amorphous coatings were confirmed by X-ray powder diffraction measurements (D8 advance
diffractometer, Bruker, AXS, Germany with a goniometer radius of 217.5 nm, Gobel mirror parallel beam optics 2° Sollers slits and 0.2 mm receiving slit). Powder samples (collected from 10 stents) were placed on low background quartz sample holders. XRD patterns from 20° to 60° were recorded at room temperature using CuKα radiation (λ = 0.15418 nm) with the following measurement conditions: tube voltage of 40 kV, tube current of 40 mA, step mode with size of 0.02° and a counting time of 1 s per step. The instrumental broadening was determined using LaB6 powder (NIST SRM 660). Crystalline and amorphous coatings were collected from the stents using a scalpel. To avoid any possibility of self-crystallization of amorphous coatings on stents, both coatings amorphous and crystalline were prepared and immediately analyzed.

2.2.3.2. Coating thickness and roughness- Profilometry. The thickness of crystalline therapeutic coating was determined by a P-15 profilometer (KLA-Tencor Co., San Jose, CA). Specifically, the profile was recorded across a notch in the coating, which was manually scratched by a wooden stick. Sample analysis was performed on CoCr cylindrical tubes of the same composition as the stent, prepared similarly to the stent with PT crystalline coating.

2.2.3.3. Coating morphology, topography and crystals size. Coating morphology, topography and crystal size were studied with an HR-SEM. Stent samples were placed on a conductive carbon paper and were coated with gold to a thickness of about 10 nm using a sputtering deposition machine (Polarone E5100). Afterwards, they were imaged using a scanning electron microscope (FEI E-SEM Quanta 2000) at an acceleration voltage of 2–15 KV. Three stents with 3 images of each were used for collecting 10 random measurements per image for each studied parameter.

2.2.4. In vitro PT release from stents in PBS

A release study was conducted in phosphate buffered saline (PBS, pH 7.4, with 0.02% of sodium azide. Each stent was placed in a 2 ml medium) at 37 °C with and without 0.1% w/v Tween 20 for enhancing PT solubility. Sampling was carried out by replacement of 1.5 ml of the release medium with a fresh medium. Sampling points were 6 h, 1, 3, 5, 7 days and then weekly. PT concentration in samples was measured by reverse phase HPLC on C-18 column with a mobile phase consisting of water-methanol (10:90 v/v%). An isocratic mode was set at a flow rate of 1 ml/min, and a wave-length of 227 nm and 20 μl of samples was injected into an HPLC system (Waters, LC-Module-I). Calibration curves were prepared in concentration range of 0.05–10 μg/ml. PT concentration in different release samples was calculated using a prepared calibration curve. Bioactive top coatings releasing PT were studied in vitro using the former reported method.

Following release study completion stents were washed 3 times with 1 ml of double-deionized water before SEM imaging for avoiding undesired buffer salts trace. For mass balance analysis, remaining PT on
stents were extracted from stents either from crystalline or amorphous coated stents by incubation the stent in 1 ml methanol and stirred vigorously on an orbital shaker for 30 min. Then the obtained PT solution was filtered with 0.2 μm PTFE filters, and HPLC was analyzed.

2.2.5. Stent inflation

Stents with an initial diameter of 1.5 mm were mounted on a balloon catheter system (balloon-expandable stent system from Bxsonic) and inflated with air. The stents attained a diameter of 3 mm and a final pressure of 10 bar by using a 200 cc inflation device from Biometrics. Samples with or without top coating after inflation were microscopically examined and HPLC quantified using the method of 2.2.4.

3. Results and discussion

3.1. PT crystalline coating preparation

3.1.1. PT seeded stents preparation

Coating of PT crystals was performed in two steps: (1) seeding that serves as nucleation of spots, (2) followed by crystallization from a saturated solution. The particle size of the seeds was in the range of

<table>
<thead>
<tr>
<th>Crystallization process timea</th>
<th>Amount of drugb</th>
<th>Total drug crystallized (μgc)</th>
<th>Drug per (μg/mm²)d</th>
<th>Crystal size length, width (μme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Seeding</td>
<td>1.6 mg</td>
<td>5.0 ± 1.5</td>
<td>0.10 ± 0.03</td>
<td>0.5-1.5 μm max, 0.5-1.5 μm max</td>
</tr>
<tr>
<td>2  5 min</td>
<td>50.0 mg</td>
<td>15.3 ± 7.0</td>
<td>0.30 ± 0.14</td>
<td>3.5 μm, 0.3 μm</td>
</tr>
<tr>
<td>3  20 min</td>
<td>50.0 mg</td>
<td>87.0 ± 13.5</td>
<td>1.72 ± 0.27</td>
<td>13 μm, 2 μm</td>
</tr>
<tr>
<td>4  60 min</td>
<td>50.0 mg</td>
<td>213.2 ± 25.7</td>
<td>4.22 ± 0.51</td>
<td>22 μm, 3 μm</td>
</tr>
<tr>
<td>5  180 min</td>
<td>50.0 mg</td>
<td>450.9 ± 64.6</td>
<td>8.92 ± 1.28</td>
<td>30 μm, 5 μm</td>
</tr>
</tbody>
</table>

a Crystallization process timing after dipping the seeded stent into crystallization solution mixture.
b Seeding process was done as reported in 2.2.1 with seeding crystals of 500–1500 nm size. Amount of drug used in crystallization process at 25 °C where the drug was dissolved in 15 ml of ethyl acetate followed by adding 65 ml of n-hexane.
c Total weight increase per stent (15 mm), due to drug seeding or crystallization onto the stents struts.
d Total drug divided by available stent surface area for crystallization (50.53 mm²).
e Crystals size were calculated using SEM pictures, 3 pictures at different sites of stent (n = 3).
500–1500 nm. Low density seeded substrates were characterized, and a slight total weight increase of 5.0 ± 1.5 μg per stent was found. SEM analysis confirmed seeding crystal distribution throughout the outer stent strut surface (Fig. 1A–C).

PT surface crystallization onto the metal surface of stents following seeding was affected by a 15:65 v:v mixture of ethyl acetate and hexane respectively, with a PT concentration of 0.69 mg/ml. This crystallization solution was applied either on low or high density PT seeded stents under a constant temperature of 25 °C. Seeded stents were incubated for prolonged time to study crystal formation. Coating weight

Table 2
Improved and controlled Paclitaxel (PT) crystallization onto stents, process-time coating development study.

<table>
<thead>
<tr>
<th>Step</th>
<th>Process parameters&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total drug loading (μg)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seeding&lt;sup&gt;b&lt;/sup&gt; 10 min, Sonication, 30 °C</td>
<td>26 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>Crystallization&lt;sup&gt;c&lt;/sup&gt; 5 min, 25 °C</td>
<td>60 ± 2.3</td>
</tr>
<tr>
<td>3</td>
<td>Crystallization 10 min, 25 °C</td>
<td>79 ± 3.3</td>
</tr>
<tr>
<td>4</td>
<td>Crystallization 15 min, 25 °C</td>
<td>101 ± 1.4</td>
</tr>
<tr>
<td>5</td>
<td>Crystallization 20 min, 25 °C</td>
<td>135 ± 2.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Seeding process was done as reported in 2.2.1 with seeding crystals of 200–300 nm size.

<sup>b</sup> Crystallization process was done with Ethyl acetate:hexane 15:65 v:v ml respectively.

<sup>c</sup> Processing and temperature of the crystallization step.

<sup>d</sup> Total weight increase per stent (15 mm) due to drug seeding or crystallization onto the stents struts traced by microanalytical balance.

500–1500 nm. Low density seeded substrates were characterized, and a slight total weight increase of 5.0 ± 1.5 μg per stent was found. SEM analysis confirmed seeding crystal distribution throughout the outer stent strut surface (Fig. 1A–C).

PT surface crystallization onto the metal surface of stents following seeding was affected by a 15:65 v:v mixture of ethyl acetate and hexane respectively, with a PT concentration of 0.69 mg/ml. This crystallization solution was applied either on low or high density PT seeded stents under a constant temperature of 25 °C. Seeded stents were incubated for prolonged time to study crystal formation. Coating weight

---

Table 3
Process-time full crystalline coating development.

<table>
<thead>
<tr>
<th>Mag&lt;sup&gt;*&lt;/sup&gt;</th>
<th>(A) PT Seeded stent</th>
<th>(B) 5 min PT Crystallization</th>
<th>(C) 10 min PT Crystallization</th>
<th>(D) 15 min PT Crystallization</th>
<th>(E) 20 min PT Crystallization</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ii)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iii)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>*</sup>Representative scanning electron microscope images of: (A) Paclitaxel (PT) seeded stents with seeding crystals 200-300 nm: (i), (ii) and (iii) Magnifications 500 ×, 1000 × and 2000 × respectively. Homogenous and continuous PT seeding layer covering metal stent struts. Crystalline PT coating development after crystallization of PT in Ethyl acetate:hexane mixture, 15:65 v:v ml respectively for: (B) 5 min at 25 °C (i), (ii) and (iii) Magnifications 500 ×, 1000 × and 2000 × respectively. Incomplete crystals carpet was formed. (C) 10 min at 25 °C (i), (ii) and (iii) Magnifications 500 ×, 1000 × and 500 × respectively. Few areas on the stent struts with partially incomplete crystals carpet formation. (D) 15 min at 25 °C (i), (ii) and (iii) Magnifications 500 ×, 1000 × and 2000 × respectively. Homogenous and continuous crystalline PT carpet covering metal stent struts. (E) 20 min at 25 °C (i), (ii) and (iii) Magnifications 500 ×, 1000 × and 2000 × respectively. Homogenous and continuous crystalline PT carpet covering metal stent struts. The extended time or uncontrolled coatings process resulted with minor bulky “Stars/Snow balls” formation and were avoided in the further study.

---
and crystal size increased with crystallization time as determined by microanalytical balance and SEM (Table 1, Fig. 1D–F). Crystals of 22 × 3 μm were found after 60 min crystallization with complete crystalline coverage of the stent surface like “carpet” (Fig. 1F). The crystallization process exceeding 60 min resulted in broken crystal edges with a maximum crystal length of 30 μm (Fig. 1S). Extending crystallization time up to 180 min or an uncontrolled crystallization process (fast temperature drop) yielded crystalline coatings that partially separated from the surface of the stent struts. A form of multilayers of a crystalline carpet or crystalline bridges among struts was identified when time was extended. While fast temperature drop was found to induce rapid crystallization beyond the stent's metallic surface (Figs. 2S and 3S, respectively).

Seed crystal is a small piece of single crystal/polycrystal material from which a large crystal of the same material typically is to be grown. Low seeding density with non-continuous seeding layer is an easy way to follow individual crystals growing from the seed starting point. Crystal’s adhesion to surfaces have been reported to be driven by surface energy, nature of contacting between the condensed bodies, surface property and contact area [59,60]. Here, we have used SEM microscopy to follow the PT crystalline coating development onto the metallic surface. The crystalline coating at the early stage of development was found to establish an adhesion capability to the metallic surface and simultaneously inside crystalline coating in crisscross coherent interactions (Fig. 2). A potential explanation for the united crystalline strata formation is symmetrical directional crystals growth and enlargement as represented in Fig. 3.

Based on the aforementioned findings, multiple close sites growing at the same time are expected to yield a homogenous crystalline coating development in a shorter time-process. Accordingly, smaller crystal seeds and fine dispersion were used for further improving surface crystallization. The presence of an anti-solvent in the grinding mortar facilitated the formation of narrow size crystal seeds in the range of: 200–300 nm as confirmed by SEM analysis. Stable PT suspension was maintained, and a homogeneous fully covered seeding layer was achieved by extending the seeding process time to 10 min under sonication at 30 °C. A weight increase of 26 ± 1.7 μg per stent was found

<table>
<thead>
<tr>
<th>Coating</th>
<th>Process parameters</th>
<th>Total drug loading (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crystalline a b</td>
<td>15 min, 25 °C</td>
</tr>
<tr>
<td>2</td>
<td>Crystalline</td>
<td>15 min, 25 °C</td>
</tr>
<tr>
<td>3</td>
<td>Crystalline</td>
<td>15 min, 25 °C</td>
</tr>
<tr>
<td>4</td>
<td>Crystalline</td>
<td>15 min, 25 °C</td>
</tr>
<tr>
<td>5</td>
<td>Amorphous c</td>
<td>Spray coating, 1.2w, 1% Sol, 0.2 ml/min, 30 Sec</td>
</tr>
<tr>
<td>6</td>
<td>Amorphous</td>
<td>Spray coating, 1.2w, 1% Sol, 0.2 ml/min, 30 Sec</td>
</tr>
<tr>
<td>7</td>
<td>Amorphous</td>
<td>Spray coating, 1.2w, 1% Sol, 0.2 ml/min, 30 Sec</td>
</tr>
<tr>
<td>8</td>
<td>Amorphous</td>
<td>Spray coating, 1.2w, 1% Sol, 0.2 ml/min, 30 Sec</td>
</tr>
</tbody>
</table>

a Seeding process was done as reported in 2.2.1 with seeding crystals of 200–300 nm size.
b Crystallization process was done with Ethyl acetate:hexane 15:65 v:v ml respectively.
c Amorphous coating was prepared by spray coating, using Sono-Tek system with the following parameters: Generator power 1.2 w, 1% drug solution in ethyl acetate, flow rate 0.2 ml/min and 30 s coating process.
d Processing and temperature of the crystallization step.
e Total weight increase per stent (15 mm) as, due to drug crystallization/amorphous sprayed onto the stents struts traced by microanalytical balance.

Fig. 4. Representative scanning electron microscope images: (A), (B) and (C) Magnifications ×500, ×1000 and ×4000 respectively for 100 μg amorphous Paclitaxel (PT) coated stents prepared by spray coating of PT-ethyl acetate solution followed by fast evaporation. Semi-homogenous and continuous amorphous PT carpet covering metal stent's struts. (D), (E) and (F) Magnifications ×500, ×1000 and ×2000 respectively for 100 μg crystalline PT coated stents, PT 15 min crystallized at 25 °C, 15:65 v:v ml Ethyl acetate:hexane respectively.
A homogenous crystalline coating with a therapeutic dose of ~100 μg per stent with complete crystalline carpet coverage of stents was obtained after 15 min (Tables 2 and 3). Shorter crystallization time yielded partially incomplete carpet formation, while on the other hand extended time resulted in minimal undesired bulky crystals accumulation like “Stars/Snow balls” and were avoided in the following study.

Stents with a therapeutic dose of ~100 μg PT per stent was reported by 6 clinical trials conducted between 2003 and 2005 as the effective dose to address the medical need for cardiovascular PT eluting stents with a 1.0–3.1 μg/mm² loading per surface area [61,62]. Accordingly, in sections 3.4 and 3.5 we have focused on further characterization of the crystalline coatings with ~100 μg per stent and examine in vitro the release performance compared to its amorphous version of the same dose (Table 4 and Fig. 4).

3.2. PT coating thickness, roughness and XRD analysis

Crystalline coating thickness with a therapeutic dose of ~100 μg per stent was mounted on a cylindrical tube and analyzed for coating thickness by a profilometer. Profiles were recorded across a notch in the coating, which was manually scratched by a wooden stick. Three analyzed sites per cylinder were studied (n = 3). Crystalline coating thickness was found to range between 4.20 and 7.65 μm, and surface roughness 2.1 ± 1.4 μm. While at macroscopy level the crystalline carpet was found homogenously to fully cover the metallic surface (Table 3), roughness analysis indicated uneven surface in the micro-level. This surface roughness distribution was found to be maintained across the crystalline carpet attributed to rod-like crystals shape and crystalline coating strata. Stents with the therapeutic dose of ~100 μg PT per stent with either crystalline or amorphous PT coating were prepared and visualized (Table 4 and Fig. 4). The difference between the crystalline coating and amorphous coating was further studied using XRD analysis. Amorphous coatings were prepared by spraying followed by fast solvent evaporation (Figs. 4S and 5S). Amorphous coatings showed no peaks on XRD spectra, typical of amorphous PT (Figs. 5A, 4S) [63]. XRD spectrum of crystalline PT coating isolated from stents shows similar profile to the reported for pure PT crystals (Fig. 5B) [19,63]. Developed crystalline coating XRD spectra overlap the reported values for PT with unit cell parameters close to the theoretical values (a = 9.6520; b = 28.120; c = 33.538A°, found: a = 9.645; b = 28.093; c = 33.557A, Fig. 5C) [64].
crystallinity was 100% as calculated according to the method described by Shujun et al. 2005 [65]. These data proves the formation of PT crystalline coating, whereas spray coating of PT solution followed by fast solvent drying forms amorphous PT (Figs. 4S and 5S).

3.3. PT in vitro release

Following conformation, PT crystalline and amorphous coated stent crystallinity degree using X-ray analysis were studied for in vitro release behavior. In vitro release of PT from coatings of amorphous or crystalline coatings was studied under physiological conditions, PBS at 37 °C. Very slow release was found for both coatings, while the amorphous coatings exhibited higher initial release compared to crystalline coating (Fig. 6A). Adding 0.1% w/v Tween 20 to the medium to facilitate solubilization of PT exhibited a 5 fold difference in the release rate of PT from the crystalline vs. amorphous (Fig. 6B).

The difference in the release profiles between crystalline and amorphous coatings was found to be similar to our previous reports on crystalline rapamycin coatings [55,56]. Crystalline coatings in vivo were found to release slowly as similar to in vitro leading to a long-term

---

**Fig. 6.** Cumulative Paclitaxel (PT) release from ~100 μg crystalline/amorphous coatings determined by HPLC at 227 nm in: (A) PBS (B) PBS with 0.1% Tween 20.

---

**Fig. 7.** Representative scanning electron microscope images of Paclitaxel (PT) amorphous coated stents: (A) before release study, magnification 1000 x. Images (B) and (C) present section of the stent identified with self-crystallization sites after 28 days of release in PBS 37 °C, pH 7.4, + 0.1% w/v Tween 20, magnifications 300 x and 500 x, respectively. Most of the amorphous therapeutic coating was dissolved (green arrows), while a notable amount of clusters of the PT with semi-crystalline coating was found (red arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
antiproliferative efficacy [56]. Accordingly, these PT crystalline coatings are anticipated to show a slow release in vivo as similar as to in vitro with the therapeutic dose reported for PT loaded stents [61,62]. Moreover, SEM visualization of stents after in vitro release indicated crystal presence onto stents struts. 38% of PT loading was released from amorphous coatings after 24 h compared to 5.1% from crystalline coatings. The decrease in the release from the amorphous coating after a few days is a result of crystallization of PT which is in agreement with reports indicating the formation of PT 2H2O that is the most stable form in equilibrium with water at 37 °C [19,66]. These self-semi-crystalline regions were SEM documented after 28 days of the release (Fig. 7). Mass balance of the remaining drug on stents at the end of the study confirm 100% and 93% for crystalline and amorphous coating, respectively.

3.4. Challenges in the physical stability and application of protective coating

Stents with crystalline PT coating are subject to physical abrasion during deployment and inflation [46,47]. Unlike a polymeric based DES, the PT crystalline-coated stent is made of crystals that are rigid and fragile and may detach following stent insertion and inflation. Thus, it is important to protect the crystals during stent handling, crimping and deployment. We have recently reported a rapid dissolving biodegradable polysaccharide top coatings onto crystalline rapamycin as on stents [57].

To enhance physical stability and durability of crystalline coatings, we aimed to formulate a temporary flexible fast dissolving thin coating to absorb mechanical constrains through the stent shelf-life until deployment, which should dissolve or erode right after stent installation on site. A study of rapamycin coated stents indicate the use of a spray coating of 0.3% hyaluronic acid (HA) containing 10% w/w glycerol (HA-F) provides an excellent protective layer to crystalline coating of 0.3% hyaluronic acid (HA) containing 10% w/w glycerol on site. A study of rapamycin coated stents indicates the use of a spray deployment, which should dissolve or erode right after stent installation.

### Table 5

<table>
<thead>
<tr>
<th>Coating Num</th>
<th>Coating Code</th>
<th>Top coating Process</th>
<th>Total coat Loading (µg)</th>
<th>Paclitaxel (µg)</th>
<th>Hyaluronic Acid (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(HA:PT)-1 L-1</td>
<td>1 Cycle coating</td>
<td>50</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>2</td>
<td>(HA:PT)-1 L-2</td>
<td>1 Cycle coating</td>
<td>58</td>
<td>29.0</td>
<td>29.0</td>
</tr>
<tr>
<td>3</td>
<td>(HA:PT)-2 L-1</td>
<td>2 Cycle coating</td>
<td>120</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>4</td>
<td>(HA:PT)-2 L-2</td>
<td>2 Cycle coating</td>
<td>140</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td>5</td>
<td>(HA:PT)-3 L-1</td>
<td>3 Cycle coating</td>
<td>153</td>
<td>76.5</td>
<td>76.5</td>
</tr>
<tr>
<td>6</td>
<td>(HA:PT)-3 L-2</td>
<td>3 Cycle coating</td>
<td>168</td>
<td>84.0</td>
<td>84.0</td>
</tr>
<tr>
<td>7</td>
<td>(HA:PT)-4 L-1</td>
<td>4 Cycle coating</td>
<td>255</td>
<td>127.5</td>
<td>127.5</td>
</tr>
<tr>
<td>8</td>
<td>(HA:PT)-4 L-2</td>
<td>4 Cycle coating</td>
<td>307</td>
<td>153.5</td>
<td>153.5</td>
</tr>
<tr>
<td>9</td>
<td>(HA:PT)-OL-1</td>
<td>Continuous 300 µl coating</td>
<td>221</td>
<td>110.5</td>
<td>110.5</td>
</tr>
<tr>
<td>10</td>
<td>(HA:PT)-OL-2</td>
<td>Continuous 300 µl coating</td>
<td>198</td>
<td>99.0</td>
<td>99.0</td>
</tr>
</tbody>
</table>

*Abbreviations: HA-Hyaluronic acid, PT-Paclitaxel, L-Coating layer, OL-Overloading of therapeutic coating, 300 µl sprayed volume.

Each cycle of coating of 100 µl was sprayed with the following parameters: Generator power 10:00, sprayed volume 0.1 ml, flow 0.2 ml/min, rotation semi-automatic 60 rpm, while drying process was reduced for 20 min in the active hood. For the OL coatings, continuous coating process of all the sprayed volume.

Total weight increase per stent (15 mm) as, due to spray coating onto the stents struts traced by microanalytical balance.

Hyaluronic acid weight were included glycerol 10% w/w (HA-F).

4. Release of PT incorporated in HA-F top coating

The in vitro release of PT from HA top coating containing PT is given in Fig. 8A–C. A cumulative PT release (µg) from HA coatings in correlation to coating cycles was conducted, Table 5. The more cycles of coating, the more drug was released (Fig. 8A). Nevertheless, continuous PT release from these coatings over the study period was found, while 28–95% (25–55 µg) of drug loaded coatings, Table 5, were released in the first 72 h, where stents coated with minimum coatings cycles released the max percentage of PT loading (Fig. 8B-C). The remaining drugs on the stents were analyzed by HPLC for mass balance analysis and verified to range between 97 and 100%. Moreover, drug release kinetics were found regardless drug loading or coating cycle, indicating a Fickian diffusion mechanism along with HA top coating dissolving.

These data highlight the potential of achieving burst release for the first 2–3 days following stents deployment released from the top coating and to be followed by slow release from crystalline PT coating.
4. Summary and conclusions

A novel paclitaxel (PT) surface crystallization methodology to generate a carrier-free PT eluting stent with therapeutic dose was developed. PT crystals with a defined morphology and specific drug load were applied. Crystallization of PT after a well-controlled seeding step is the most suitable process for the preparation of this carrier-free PT eluting stent that is homogenous, continuous, uniform, and easy to control and prepare. PT was constantly released and traced up to 28 days. ~10% of the cargo was released from PT surface crystallized stents, showing the potential for controlled extended release for several months, while amorphous coated stents released almost ~7.5 times more in the first 24 h.

A protective top coating formulation was developed and applied to overcome the limited mechanical and physical properties of crystalline coatings in both crimping and stent inflation steps as found by HPLC. Due to fast solubility, the developed top coating does not affect the release profile of the crystalline coating guarantee, i.e., the safe displacement of the stent in its desired final destination. Nevertheless, we have examined bioactive top coatings for multilayer release purposes. They are stable and achievable by incorporating PT into the protective top coating. Burst release for the first 3 days following stent deployment from the top coating can be followed by slow release based crystalline PT coating to enhance the in vivo performance of DES in the long term. It can be anticipated that both the developed crystallization process and protective top coating can be further implemented for local drug delivery of other drugs and implanted devices coatings, either single or multiple layers releasing drugs for sequential release purposes.

Fig. 8. (A) Cumulative paclitaxel (PT) release (μg) from hyaluronic acid (HA) coatings formulated with 10% w/w glycerol (HA-F) studied in PBS 37 °C, pH 7.4, for 28 days, (B) cumulative PT release (μg) from HA-F coatings, focusing on the first 72 h and (C) cumulative PT release from HA-F coatings, PT releasing normalized to drug loading focusing on the first 72 h.