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Research Article

A New Approach for Polymer-Free Coating with Paclitaxel Microparticles on Fully-Bioabsorbable Poly(l-Lactic Acid) Stent and Studies of Drug Release Behavior

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ABSTRACT

A first clinically-used, fully-bioabsorbable poly(L-lactic acid) (PLLA) stent was coated with microcrystals of paclitaxel (PTX), antiproliferative agent through seeding and/or crystal growing technique to investigate the drug properties on the stent surface and drug release behavior from the stent. PTX particles subject to only seeding process was peeled off after stent compression, while less PTX was coated on the stent subject to only crystal growth without seeding. PLLA stent with both processes could stably maintain an increased amount of PTX on its surface. The maximum amount of initial release (10 μg/cm2) was found in the sample only with seeding, which decreased to 3 μg/cm2 or below after one week. Also in the PLLA stent only with crystal growth, release amount decreased after one week. In the PLLA stent with seeding and crystal growth, formation of needle-like PTX crystals on the surface resulted in decreased initial release and lower solubility after hydration in phosphate buffered solution compared to other types of stent with different procedures. It is suggested that both seeding and crystal growth are required for stable application of PTX on fully-bioabsorbable PLLA stent and needle-like crystals containing water have lower aqueous solubility resulting in decreased PTX release.

Keywords: Polymer-free; Bioabsorbable; Stent; Paclitaxel; Microparticle; Drug release

INTRODUCTION

Bare metal stent (BMS) is a small, tubular, wire-mesh device which is mounted in a contracted form onto a catheter balloon, threaded to the narrowed site of the artery and expanded within the vessel. Once expanded, it permanently supports the vessel from the inside to maintain vessel patency. The use of BMS usually demonstrates favorable initial clinical results, for mid/long term outcomes however, re-narrowing of the treated vessel is commonly observed in 20-30% of patients. The cause of this re-narrowing is in-stent restenosis (ISR). ISR is defined as diameter stenosis of ≥50% in the stented segment of the vessel mainly due to excessive neointimal proliferation[1-3]. After the BMS's introduction in 1994, a lot of intense work had been done to address ISR caused by BMS and successfully led to the advent of drug-eluting stents (DES) in 2002. DESs have revolutionized endovascular intervention by dramatically reducing the ISR rates and the need for repeated intervention. First generation DESs using paclitaxel or sirolimus are more effective than BMS reducing the risk of restenosis and improving early results, regardless of their initial success, however, concerns about overall safety, especially late clinical adverse event, such as stent thrombosis (ST) are not resolved. To reduce the ST rate, second generation DESs using various drugs, such as evelolimus or zotarolimus have been introduced and further development and clinical studies are ongoing elsewhere focusing on long-term safety and improved efficacy[4].

In most DESs, BMS used as their platform is coated with polymer containing a therapeutic agent. Polymer serves as a carrier that controls drug release and enhances the mechanical and the chemical stability of the drug[5,6]. Though incidences of

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restenosis have been substantially reduced since the introduction of DES[7-10], late stent thrombosis (LST) or very late stent thrombosis (VLST) may occur after drug is released[11]. Residual non-absorbable polymer coatings used as drug carriers are supposed as the cause of LST and VLST. Thus, developments for drug carrier or drug delivery methods which do not induce any ST at later stage are required. Carrier-free or carrierless DES may be ideal to mimimize the use of polymers which display a disputed stability[12] and biocompatibility[13].

Paclitaxel (PTX) is an antineoplastic agent, made of rigid taxane rings and flexible side chains[14,15]. Though paclitaxel has been shown to markedly attenuate stent-induced intimal thickening[16,17] and efficacy of PTX eluting stent for prevention of restenosis is clinically proven in the field of endovascular intervention, the problems about VLST or long-term antiplatelet therapy still remain.

To control drug release, drug dissolution rate is essential. Generally, dissolution rate of a crystalline drug is largely different from that of an amorphous form[18]. Amorphous drug formulations tend to partially crystallize during storage or preparation, resulting in cracks on the coating or modification of the drug release profile[19,20]. As amorphous pharmaceuticals are markedly more soluble than their crystalline counterparts[18], their coatings require sophisticated coating techniques and controlled crystallization to control stability and release profile of the drug.

The objective of this work is to present PTX coating without using polymer carrier on fully-bioabsorbable, poly(L-lactic acid) (PLLA) stent, which has been already commercialized and the most promising platform in the field of endovascular intervention, and to investigate the drug release properties of the stent. As a coating methodology, surface crystallization using a seeding technique where microcrystalline PTX seeds are seeded and serve as crystal nuclei for further crystal growrth was employed[21,22]. Compared to a lot of previous researches to apply drug on metallic stent surfaces[23], the present work is a novel approach to apply the drug without using polymer carrier on the first clicically used fully-bioabsorbable stent which is intended to completely disappear from the body over time[24], thus does not require long term antiplatelet therapy associated with current DES using non-degradable polymer.

MATERIAL AND METHODS

Material

Heptane (Nacalai tesque, Kyoto, Japan) and ethanol (Nacalai tesque) were used as a poor solvent and as a good solvent, respectively. The bioabsorbable "REMEDY" PLLA stent (9 mm in diameter, Fig.1) was formed with PLLA monofilament of 0.24 mm diameter which was melt-spanned, extended and then thermally processed. The "REMEDY" stent was cut into a length of 12 mm and used as a substrate for the seeding procedure.

Seeding procedure

A 10 ml vial containing PTX and 6 ml of heptane was sonicated to diffuse PTX in a heptane solution. Then, the stent was put in the vial followed by further sonification to obtain uniform distribution of PTX in the solution for seeding.

Crystal growing procedure

PTX was put in a 5 ml vial and 2 ml of ethanol was added to it. The vial was sonicated to obtain complete dissolution of PTX and heptane was added to it. The seeded stent was immersed in the saturated solution to allow PTX crystals spontaneously grown.

Sample preparation

Samples with seeding only

PTX of 60 mg was placed in a 10 ml vial and 6 ml of heptane was added to it. The vial was sonicated to diffuse PTX in the heptane solution. The stent was put in the vial followed by additional sonication for two minutes to obtain uniform distribution of PTX in the solution. Then, the stent was removed from the solution and dried at 4 ºC or 25 ºC for 24 hours.

S1: Seeding + drying at 4 ºC

S2: Seeding + drying at 25 ºC

Samples with crystal growing with/without seeding

PTX of 80 mg was put in a 5 ml vial and 2 ml of ethanol was added to it. The vial was sonicated to obtain complete dissolution of PTX. Then, 2.5 ml of heptane was added to obtain saturated solution for immersion of seeded or non-seeded stents for 10 minutes. Then, the stents were removed from the solution and dried at 4 ºC or 25 ºC for 24 hours.

SC1: S1 + crystal growing + drying at 4 ºC

SC2: S1 + crystal growing + drying at 25 ºC

SC3: S2 + crystal growing + drying at 4 ºC

SC4: S2 + crystal growing + drying at 25 ºC

C1: Crystal growing + drying at 4 ºC

C2: Crystal growing + drying at 25 ºC

Stent compression study

For commercial use, stents are initially contracted and accommodated within a catheter which delivers the stent to the intended vessel site. Therefore, the stents of 9 mm in outer diameter used in the above coating studies were compressed and inserted in a catheter of 1.7 mm in inner diameter to investigate the effect of the compression on the PTX coating.

Surface evaluation

Each sample was gold-deposited by an ion coater (IB-2, Eiko Engineering, Tokyo, Japan) and studied using SEM (VE7800, KEYENCE, Osaka, Japan).

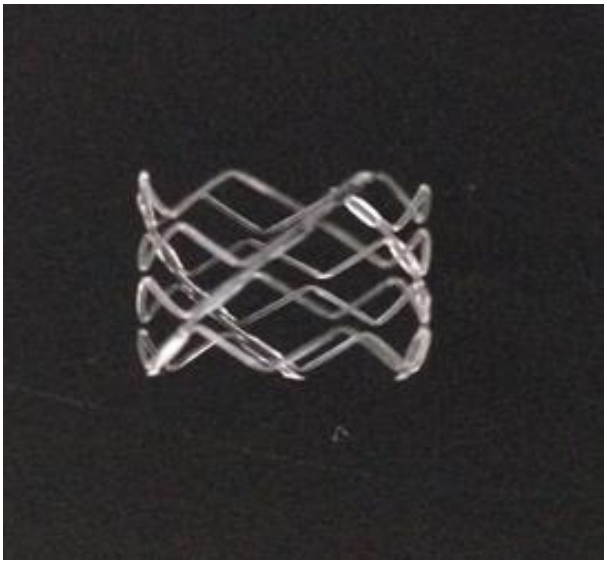

Fig. 1: The bioabsorbable stent made of PLLA.

Evaluation of drug release behavior

To evaluate drug release profiles from the stents containing PTX, samples were immersed in a phosphate buffered solution (pH7.4, Nacalai tesque) at 37 ºC for 12 weeks to monitor PTX amount released in the solution over time using HPLC (SCL-10A, SHIMADZU, Kyoto, Japan). In this release study, C-18 column at 50 ºC (COSMOSIL C18-MS-II, Nacalai tesque) and mixed eluent of acetonitrile/ ultrapure water (3/2, v/v) were used at a flow rate of 1.0 ml/min. and UV wave length of 227 nm. The mean of triplicate measurements for each type of sample was reported.

In addition, the stents were dissolved in 0.5 ml of acetonitrile (Nacalai tesque) and residual PTX on the stent surface after immersion in a phosphate buffered solution for 12 weeks was measured using HPLC. The initial amount of PTX was determined as the sum of the dissolved PTX in phosphate buffered solution and the residual PTX on the stent after 12 weeks.

RESULTS AND DISCUSSION

Surface evaluation study

Fig. 2(a), (b), (c) and (d) show SEM images of the stent samples with seeding only, seeding + crystal growing + drying 25 ºC or 4 ºC, or crystal growing only, respectively. Fig. 2(a) with seeding only shows microparticles which are considered to be PTX attaching on the surface. Needle crystals are found in the case of Fig. 2(b) and (c) with seeding + crystal growing + drying at 25 ºC and 4 ºC, respectively. These formations of needle crystals were nearly independent from temperatures after seeding or crystal growing. In addition, Fig. 2(d) with crystal growing only demonstrates that PTX spreads non-uniformly over the surface. It was found that after drying at a lower temperature, PTX tended to spread more uniformly.

Fig.3 shows the initial amount of PTX on the stent surface after application of different procedures. According to Fig. 3, in the

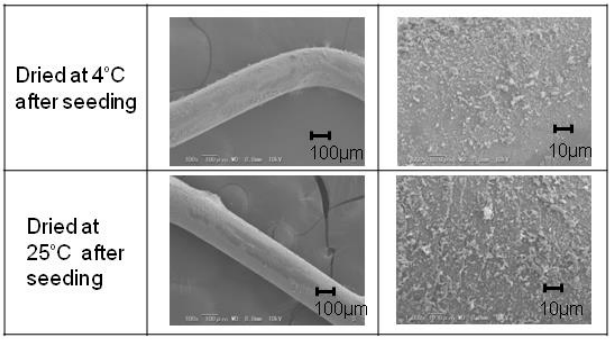

Fig. 2(a): SEM images of the stent surfaces after seeding.

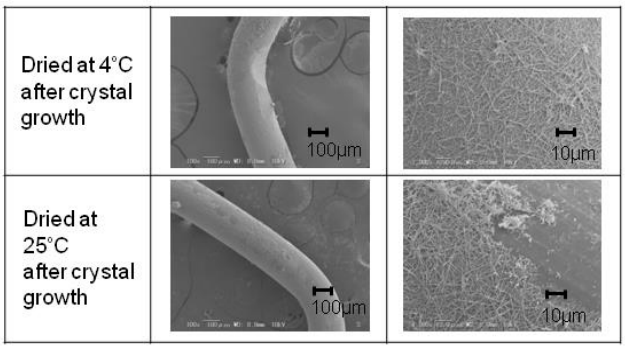

Fig. 2(b): SEM images of the stent surfaces after seeding + crystal growth + drying at 4^{O}C.

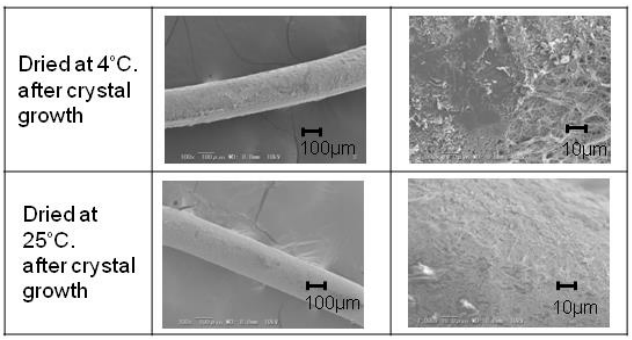

Fig. 2(c): SEM images of the stent surfaces after seeding + crystal growth + drying at 25^{O}C.

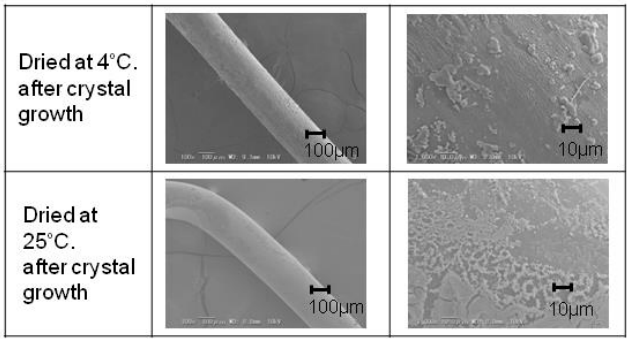

Fig. 2(d): SEM images of the stent surfaces after crystal growth.

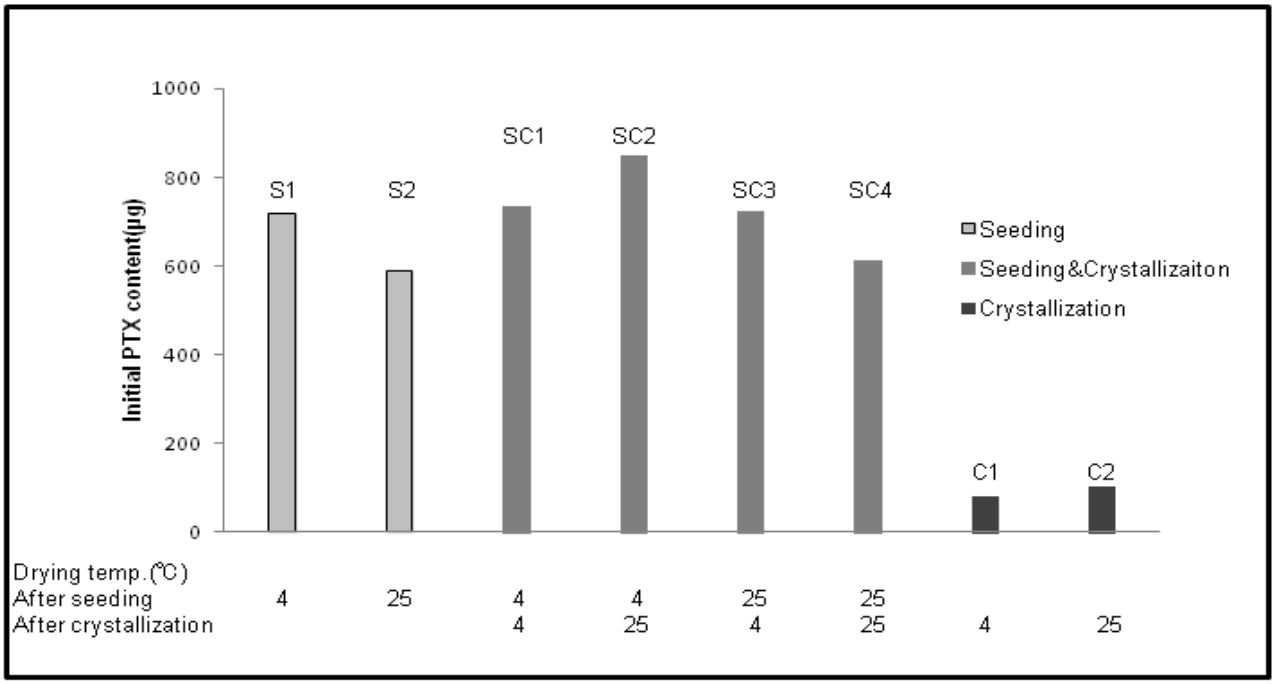

Fig. 3: Initial PTX contents of the stents prepared in various conditions.

samples with seeding only (S1 and S2), 600-700 μg of PTX particles are attached to the surface regardless of their drying temperatures. After the seeded PTX particles were grown, PTX amount slightly increased. In contrast, much less PTX was found on the surfaces of the stents without seeding irrespective of their drying temperatures. This is because crystal growth would be achieved only in the presence of seeded microcrystals, or microcrystals which were attached to the stent surface after spontaneous growth without seeded nuclei would be easily peeled off from the stent surface.

Impact of stent compression

According to the above results, no significant difference in PTX initial amount between samples with seeding only and samples with seeding + crystal growth was found, while the samples with only crystal growth had a minimum amount of PTX on their surfaces. Therefore, (S2) with seeding + drying at 25 ºC and

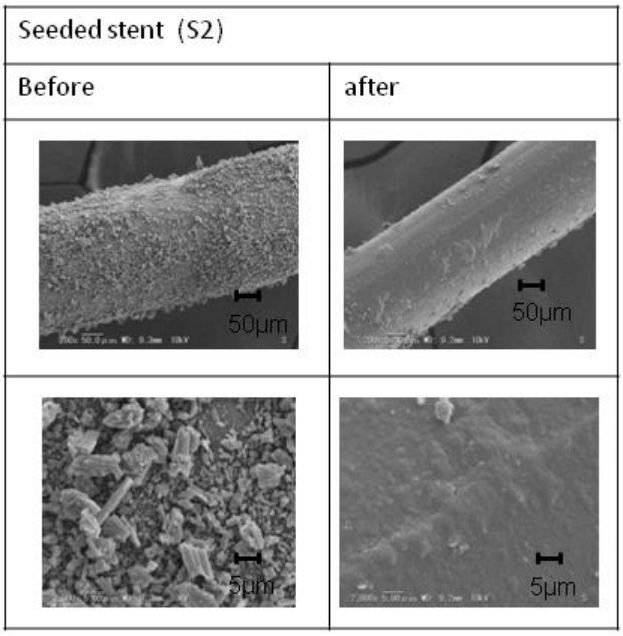

Fig. 4(a): SEM images of seeded stents before or after compression process.

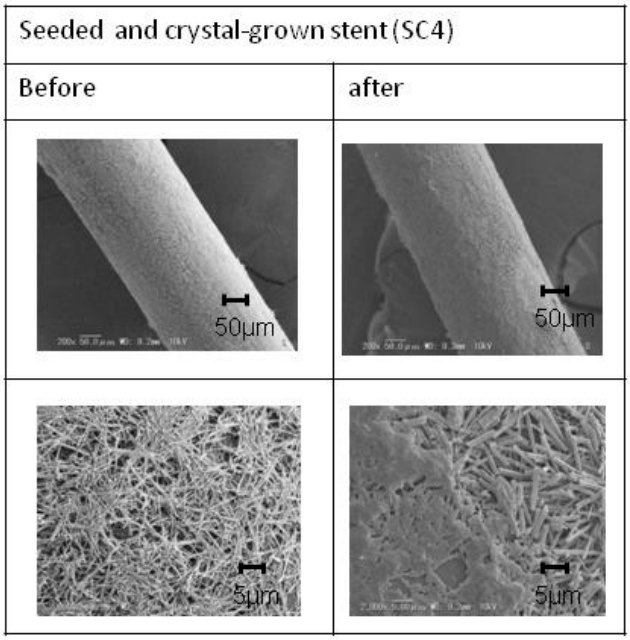

Fig. 4(b): SEM images of seeded + crystal-grown stents before or after compression process.

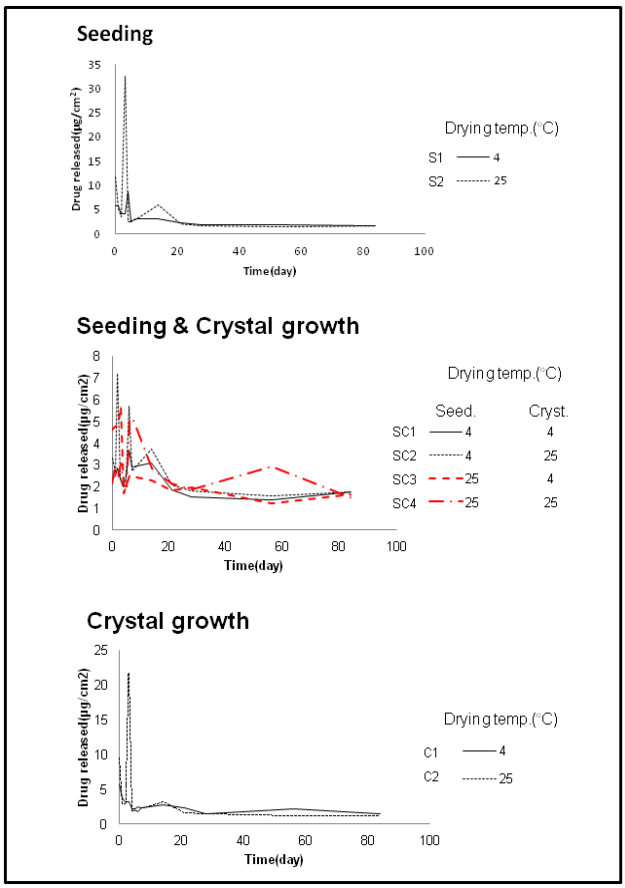

Fig.5 Drug release behavior from the stents prepared in various conditions.

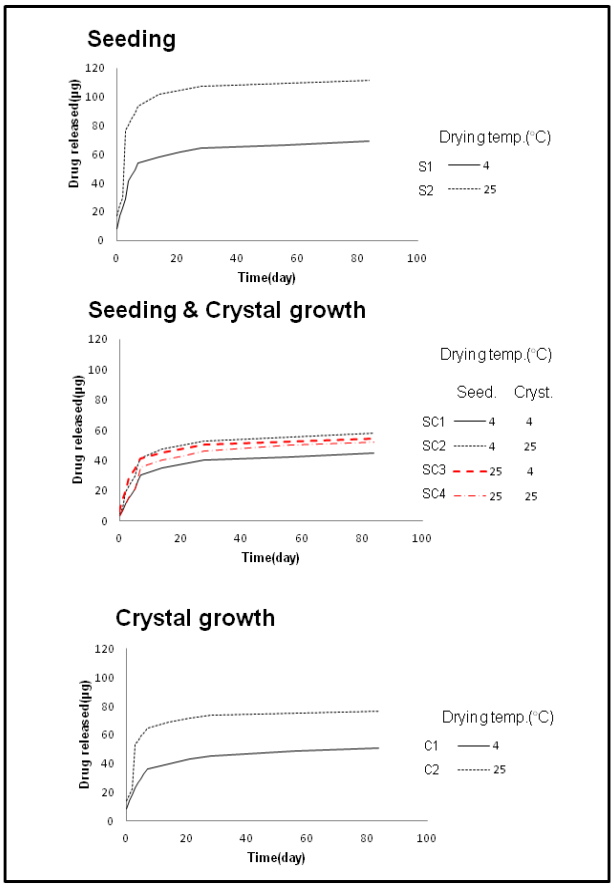

Fig.6 Cumulative drug release from the stents prepared in various conditions.

(SC4) with seeding + crystal growing + drying at 25 ºC were compressed to study PTX behavior on their surfaces. According to Fig. 4(a) showing the surface of the sample only with seeding, most of the PTX particles once attached to the stent surface has been peeled off after stent compression, on the contrary, the sample with seeding + crystal growth has needle crystals of PTX stably retained on its surface even after stent compression as shown in Fig. 4(b). Consequently, a stent applied only seeding procedure is not for practical use due to easy peeling of attached PTX.

Evaluation of drug release behavior

The amounts of PTX released in the phosphate buffered solution over time from the samples with seeding only or seeding + crystal growing were measured using HPLC and PTX amount per unit area of stent and cumulative PTX amount were determined (Fig. 5 and 6). In terms of PTX amount released per unit area of stent, the sample with seeding only + drying at 25 ºC and the sample with crystal growing only + drying at 25 ºC released approximately 10 μg/cm^2 of drug within the first one hour, thereafter however, release profiles of all samples including above two types are comparable releasing less than 3 μg/cm^2 of drug per unit. On day 3 after immersion, an increased amount of PTX was found with the above two types of stent, which seems to be due to PTX particles peeled off from the stent surface.

The study of solid-state characterization of PTX done by Liggin et al. demonstrated the existence of a dihydrated form of PTX which is stable in equilibrium with water at 37 ºC, but dehydrates at ＞45 ºC. They also confirmed that anhydrous PTX, PTX-2H_2O, and dehydrated PTX-2H_2O had different crystal structures[25].

Vella-Zarb et al. reported there were appreciable implications on physical properties of pure PTX due to the change in intermolecular interactions between the anhydrous and hydrated forms. Stronger or weaker hydrogen bonds in the drug can have a significant impact on its solubility. The additional hydrogen bonds imposed on the PTX structures by the presence of water serve as supplementary binding forces, which would potentially lower the solubility of the hydrates[26].

In literature, it is reported that aqueous solubility of PTX in anhydrous form is high, while it would decrease as PTX becomes hydrated. Thus, after day 3, decreased PTX releases were found due to PTX hydration, regardless of various initial PTX releases

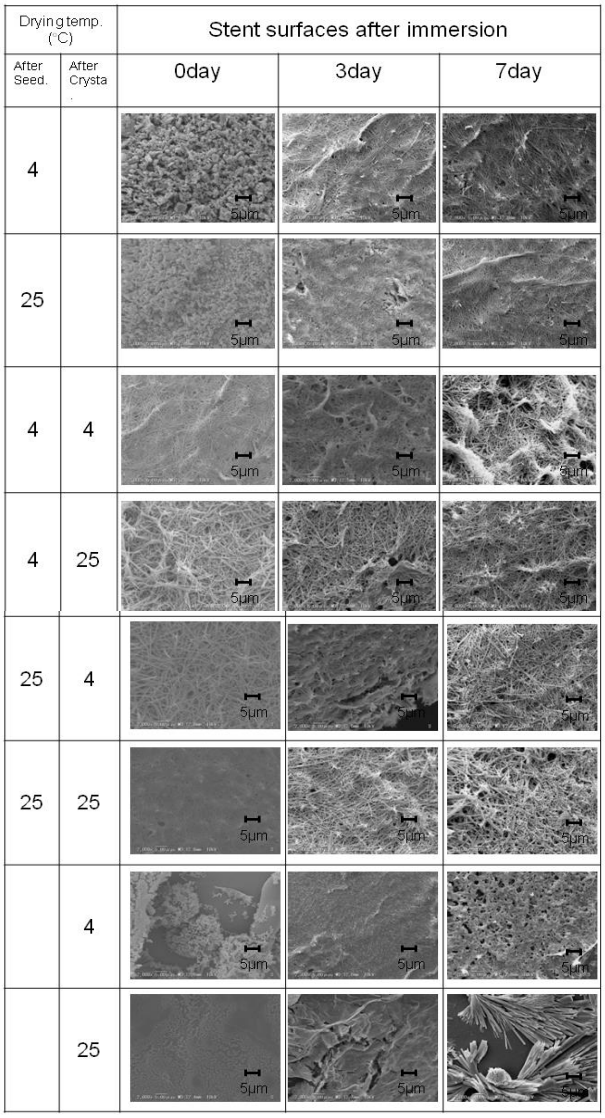

Fig.7 SEM images of the stents immersed in phosphate buffer solution for various periods of time.

for different samples depending on preparation procedures (seeding /crystal growing /drying).

Fig. 7 demonstrates additional observation for morphological change in PTX crystals on the stent surface immersed in phosphate buffered solution for the first seven days. For the stent applied only seeding, PTX microparticles observed before immersion in phosphate buffered solution were transformed to needle-shaped crystals after day 3 regardless of their drying temperatures. Therefore, it is assumed that initial release amounts from seeded anhydrate PTX could be high due to peeling of PTX microparticles from the stent surface or higher solubility of PTX, but PTX release would be lowered with the progress of PTX hydration over time. This is true in the stent with only crystal growing procedure, showing decreased PTX release due to formation of needle crystals in phosphate buffered solution over time, though amorphous or immature needle-like PTX particles were found on the stent surface before immersion in phosphate buffered solution.

CONCLUSION

PTX which has antiproliferative effect is applied on a fully-bioabsorbable stent made of PLLA monofilaments through a seeding procedure where microcrystalline PTX seeds are seeded and serves as crystal nuclei for further crystal growth.

PTX subject to only seeding process was peeled off after stent compression, while less PTX was coated on the stent subject to only crystal growth without seeding. PLLA stent with both seeding and crystal growing processes could stably maintain an increased amount of PTX on its surface. The maximum amount of initial release (10 μg/cm^2) was found in the sample only with seeding, which decreased to 3 μg/cm^2 or below after one week. Also in the PLLA stent only with crystal growth, release amount decreased after one week, indicating PTX was hydrated in phosphate buffered solution. In the PLLA stent with seeding and crystal growth, needle-like PTX crystals on the surface resulted in decreased initial release and lower solubility after hydration in phosphate buffered solution compared to other types of stent with different procedures. It is confirmed that both seeding and crystal growth are required for stable application of PTX on fully-bioabsorbable PLLA stent. Needle-like crystals containing water have lower aqueous solubility resulting in decreased PTX release. It is suggested that both seeding and crystal growing are required for stable application of PTX on a fully-bioabsorbable PLLA stent and that needle-like crystals containing crystallized water have lower aqueous solubility resulting in decreased PTX release.

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