

Effect of Sonication, Liquid Nitrogen Cooling During Crystal Growth and Heating Process on Crystal Growth of Paclitaxel Coated on a Fully-Bioabsorbable Poly (L-Lactic acid) Stent

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ABSTRACT

To achieve appropriate efficacy of paclitaxel (PTX) used in drug eluting stents, uniform PTX coating on the stent and increased initial release of PTX leading to immediate therapeutic level of the drug are required. The present study investigated whether either restriction of crystal growth through sonication and subsequent liquid nitrogen cooling, or formation of anhydrous PTX crystals through heating microcrystals after seeding and crystal growth could increase PTX initial release coated on a fully-bioabsorbable poly(L-lactic acid) stent. The sonication and subsequent liquid nitrogen cooling of the growing PTX crystals could prevent stable attachment of the crystals onto the stent surface. In addition, the initial PTX release from the stent applied sonication during the crystal growth process followed by heating showed five times as high as the stent without heating, which indicated the heating improved the PTX release from the stent at early phase. These results suggested that PTX was changed into an anhydrous soluble form.

INTRODUCTION

Endovascular interventions offer less invasive treatment options than conventional femoropopliteal bypass surgery, but their long-term clinical success is compromised by restenosis caused by neointimal hyperplasia. Restenosis rates of 40-65% at 1 year after superficial femoral artery (SFA) treatment by balloon angioplasty have been reported¹⁻³. Self-expanding, bare metal nitinol stents seem to improve patency compared with angioplasty, but the restenosis rates remain relatively high: 23-37% at 1 year and 46% at 2 years³⁻⁷. Drug-eluting stents, which are available all over the world, and drug-eluting balloons, which are available in Europe and Latin America, are established therapies for the treatment of coronary in-stent restenosis (ISR)⁸⁻¹⁰. In the previous studies, the potential for paclitaxel (PTX) to reduce neointimal formation and prevent restenosis in both coronary and peripheral arteries has been shown. Zilver-PTX (Cook Medical, Bloomington, USA) is a self-expandable, flexible nitinol stent coated with polymer-free

paclitaxel at 3 $\mu\text{g}/\text{mm}^2$ dose density. It is the first DES approved for use in the SFA. Zilver-PTX in 6 mm \times 20 mm size carries approximately 220 μg of paclitaxel, and that in 10 mm \times 80mm does approximately 880 μg ¹¹. The Zilver-PTX single-arm study investigated the performance of a paclitaxel-eluting stent in the SFA and the above-the knee arteries¹². This study had very broad inclusion criteria, which allowed for the treatment of patients with femoropopliteal ISR. Compared with most other reports of femoropopliteal ISR lesion treatment¹³⁻²⁰, treatment of ISR with paclitaxel-eluting stents resulted in higher midterm rates of primary patency. Numerous studies have shown that paclitaxel suppresses restenosis when applied only briefly at the time of injury²¹⁻²³, indicating no need for sustained drug release.

Our preceding studies demonstrated that both seeding and crystal growth were required for stable PTX coating on the fully-bioabsorbable poly (L-lactic-acid) stent. To achieve appropriate efficacy of paclitaxel (PTX), uniform PTX coating on the stent

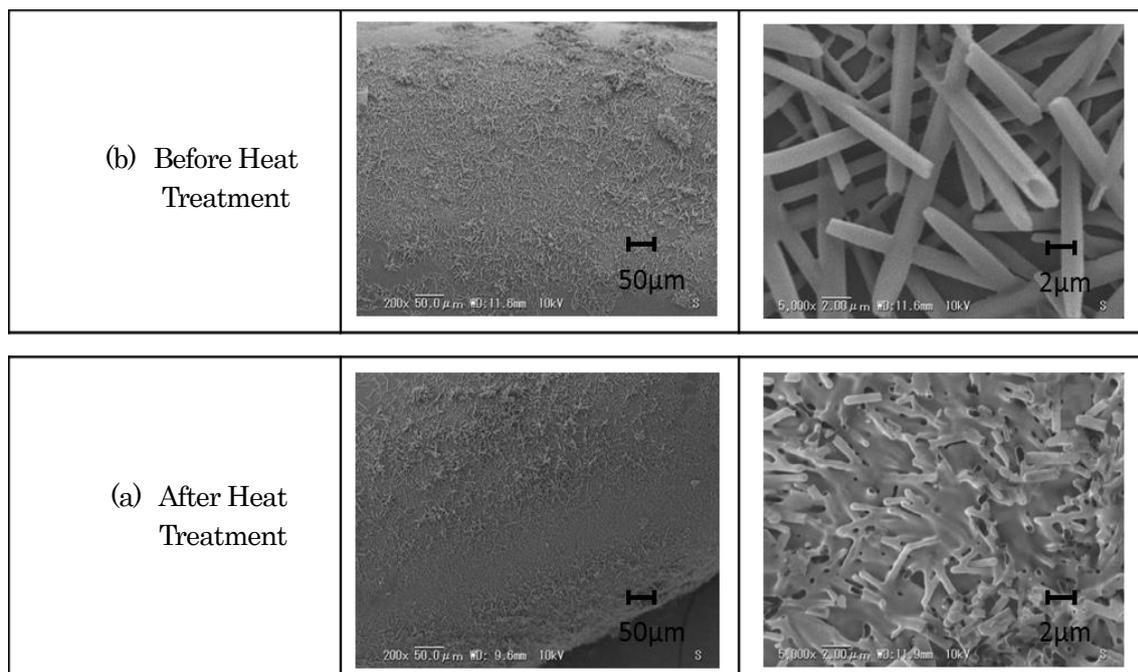


Figure 1: SEM photomicrographs of seeded and crystallized stents prepared in various conditions.

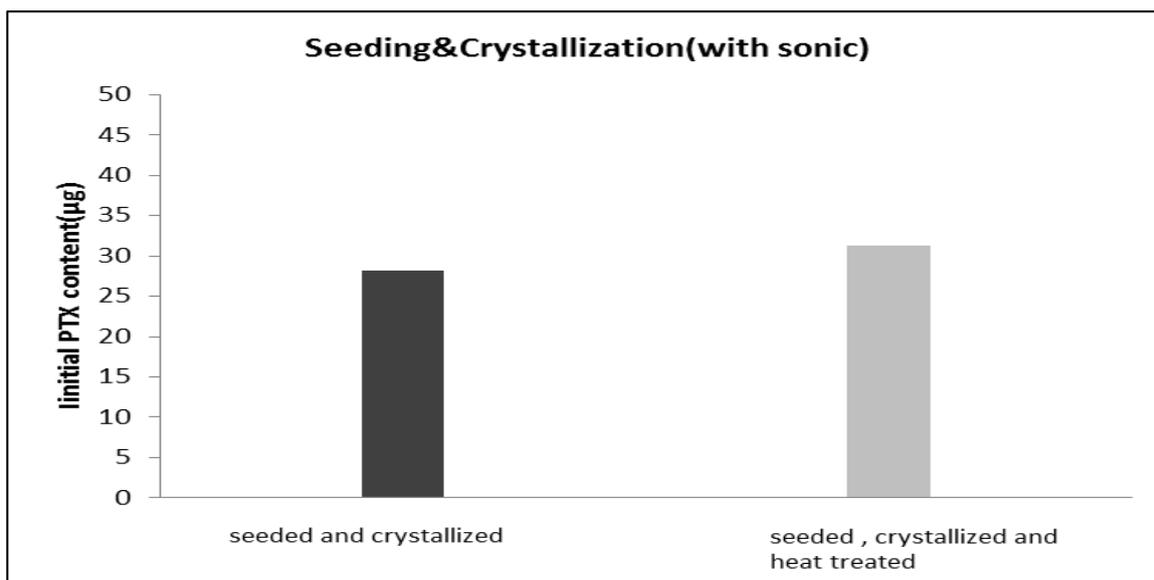


Figure 2: Initial PTX content of the stents prepared in various conditions.

surface and increased initial release of PTX leading to immediate therapeutic level of the drug are required. The present study investigated whether either restriction of crystal growth through sonication and subsequent liquid nitrogen cooling, or formation of anhydrous PTX crystals through heating microcrystals after seeding and crystal growth could increase PTX initial release coated on a fully-bioabsorbable poly(L-lactic acid) stent.

MATERIAL AND METHODS

Material

The poly(L-lactic acid) stent (9mm in diameter) was formed with polylactic monofilament of 0.24mm diameter which was melt-spun, extended, thermally processed and then cut into a length of 12mm. Paclitaxel (PTX) was used to coat the stent. Heptane (Nacalai tesque, Kyoto, Japan) and ethanol (Nacalai tesque) were used as a poor solvent and as a good solvent, respectively.

Seeding and crystal growing procedures

A 10 ml vial containing 30mg of PTX and 6ml of heptane was sonicated to diffuse PTX particles in the heptane solution. Then, the stent was put in the vial followed by a second sonication for

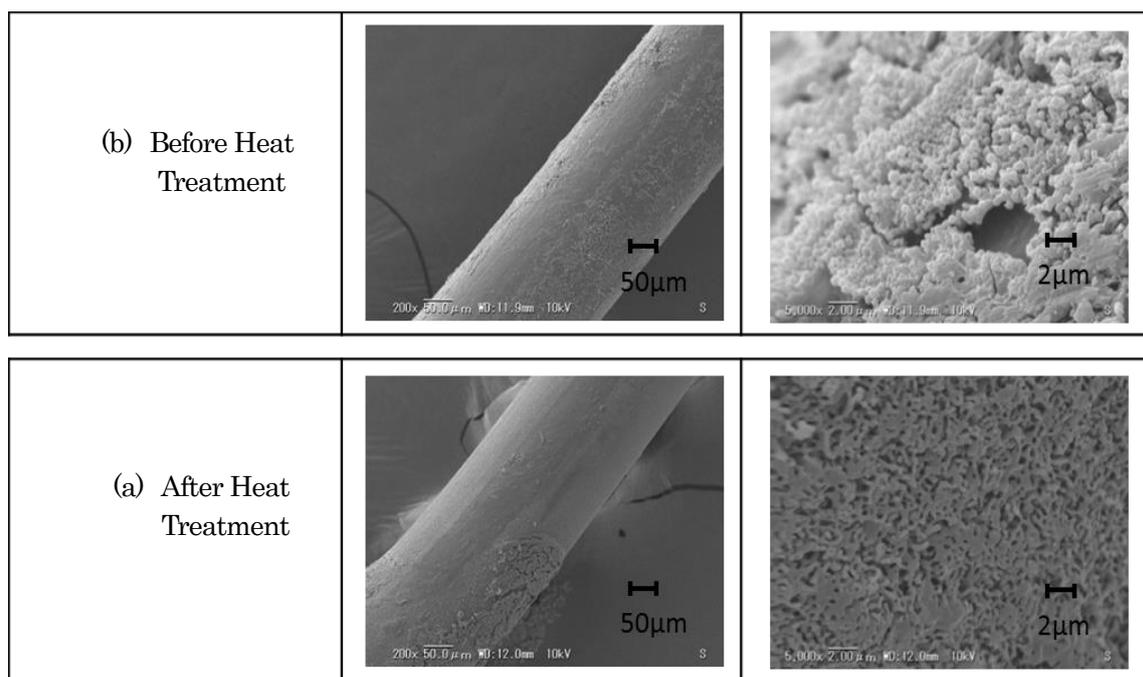


Figure 3: SEM photomicrographs of seeded and crystallized stents in various conditions.

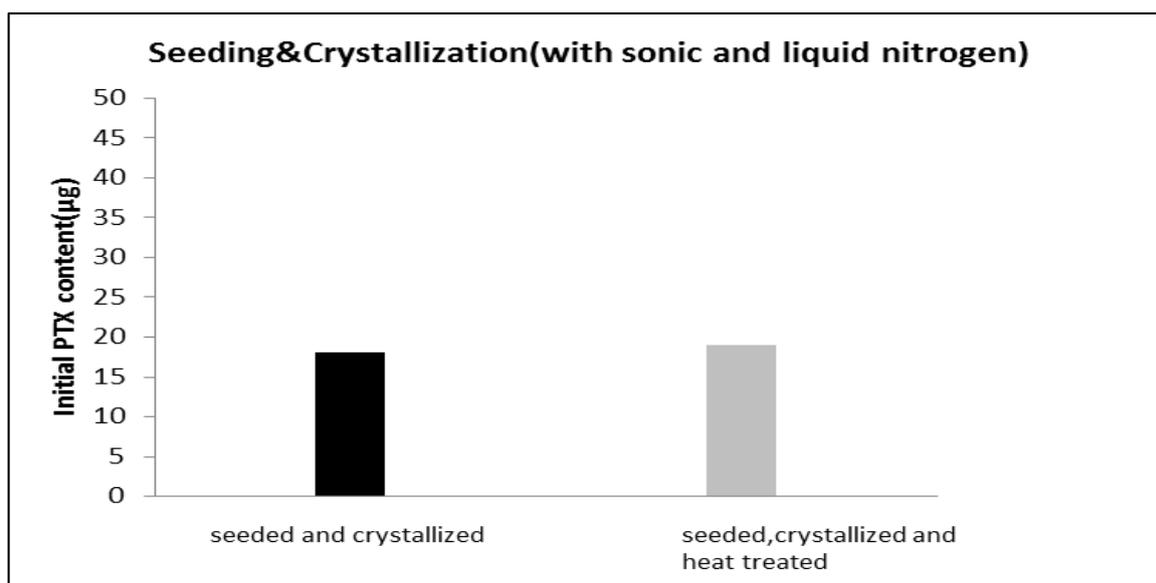


Figure 4: Initial PTX content of the stents prepared in various conditions

one minute for uniform distribution of PTX particles in the solution. The stent was removed from the sonicated solution and dried at 25°C for 24 hours to obtain seeded stent samples. To achieve crystal growth starting from the seeded crystals, PTX of 80mg 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, after addition of 2.5 ml of heptane, the seeded stent was immersed and sonicated in the resulted supersaturated solution for one minutes. The aim of the last sonication step on the seeded stent was to increase initial release amount of PTX by restricting

crystal growth of PTX microcrystals through ultrasonic. Finally, the stent was removed from the solution and dried at 25°C for 24 hours to achieve crystal growth of PTX microcrystals started from the seeded crystal nuclei.

Heating procedure

The stent after seeding and crystal growth of PTX microcrystals was heated in a oven (ADP200; Yamato Scientific, Tokyo, Japan) at 130°C for 5 minutes.

Seeding using liquid nitrogen and crystal growing procedures

PTX of 30 mg was placed in a 10 ml vial and 6 ml of heptane was

added to it. The vial was sonicated to diffuse PTX particles in the heptane solution. The stent was put in the vial followed by additional sonication for one minute to obtain uniform distribution of PTX in the solution.

Then, the stent was removed from the solution which was being sonicated and then immersed in the liquid nitrogen for two minutes to stabilize PTX particles for prevention of heterogeneous dissolution of PTX in the testing solution due to dipping of the residual PTX solution stayed on the stent surface. Finally, the stent was dried at 25°C for 24 hours to seed the crystal nuclei.

To achieve crystal growth starting from the seeded crystals, PTX of 80mg 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. After addition of 2.5 ml of heptane, the seeded stent was immersed and sonicated in the resulted supersaturated solution for one minutes, then removed from the solution and immersed in liquid nitrogen for two minutes to restrict further crystal growth due to residual supersaturated solution remained on the stent surface. Finally, the stent was dried at 25°C for 24 hours.

Heating procedure

The stent after seeding and crystal growth of PTX microcrystals was heated in the oven at 130°C for 5 minutes.

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).

Evaluation of drug release behavior

To evaluate drug release profiles from the stents containing PTX, samples were immersed in phosphate buffered solution (PBS, pH7.4, Nacalai tesque) at 37 °C 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°C (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.

In addition, the stent was dissolved in 0.5 ml of acetonitrile (Nacalai tesque) to measure residual PTX amount on the stent surface after immersion in the phosphate buffered solution for 12 weeks using HPLC. Initial amount of PTX was determined as the sum of the dissolved PTX in the phosphate buffered solution and the residual PTX amount on the stent surface measured after 12 weeks. The mean of triplicate measurements for each type of samples was reported.

RESULTS AND DISCUSSION

Surface evaluation

Fig. 1(a) and (b) show SEM images of the stent samples without heating and with heating, respectively. Fig. 1(a) without heating shows needle-shaped PTX microparticles on the surface. In the case of Fig. 1(b) with heating, it is demonstrated that grown PTX crystals starting from the seeded PTX nuclei seems to be dissolved on the surface.

Fig. 2 shows the initial amounts of PTX on the stent surface after application of different procedures. For an initial PTX content, 100-200 µg of PTX particles had been expected to attach to the stent surface before the experiment; however, the samples with/without heating demonstrated lower initial PTX contents, approximately 30 µg indicating that the sonication during the crystal growth process prevent stable attachment of PTX microcrystals on the stent surface.

According to Fig. 3(a) and (b) which show SEM images of the stent surfaces of samples with liquid nitrogen cooling, though small amount of needle crystals are found, majority of them are spherical considered to be immature PTX crystals. This indicated that the sonication during the crystal growth and/or liquid nitrogen cooling might prevent free growth of the PTX crystals. After the heat process however, dissolved and re-crystallized PTX microcrystals were found on the stent surface as shown in Fig. 3(b).

The initial PTX contents after different procedures are shown in Fig. 4. The PTX contents on the samples with seeding + crystal growth + liquid nitrogen cooling with/without heating were approximately 20 µg, lower than expected, which indicated the sonication during crystal growth process prevent the PTX crystals from being stabilized on the stent surface.

Evaluation of drug release behavior

As shown in Fig.5 and 6, the amounts of PTX released in phosphate buffered solution over time from the samples with seeding + crystal growth, seeding + crystal growth + heating were measured and PTX amount per unit area of stent and cumulative PTX amount were determined. The sample with heating process demonstrated much increased PTX release five times higher than that of the sample without heating. These results suggested that the heating process increased initial release of PTX from the stent. The study done by Liggins et al.²⁴ demonstrated that the maximum apparent solubility of anhydrous paclitaxel in water was 3.59 ± 0.41 µg/ml after 3 hours of dissolution at 37 °C and that of paclitaxel-2H₂O was 0.93 ± 0.14 µg/ml after 20 hours. In their study, the apparent solubilities of anhydrous paclitaxel and paclitaxel-2H₂O approached a single value near 1 µg/ml by 20 hours indicating anhydrous paclitaxel was more soluble than paclitaxel-2H₂O, but decreased over time.

In the present study, PTX was dehydrated and changed into a

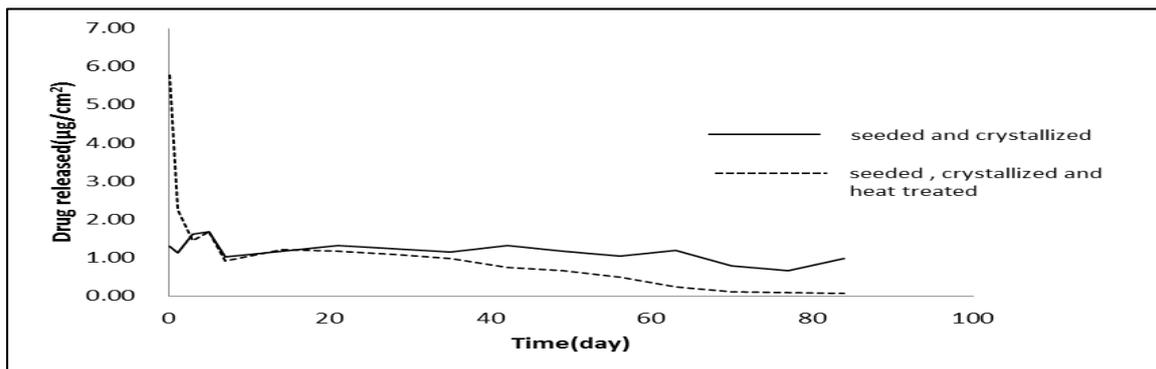


Figure 5: Drug release behavior from the stents prepared in various conditions.

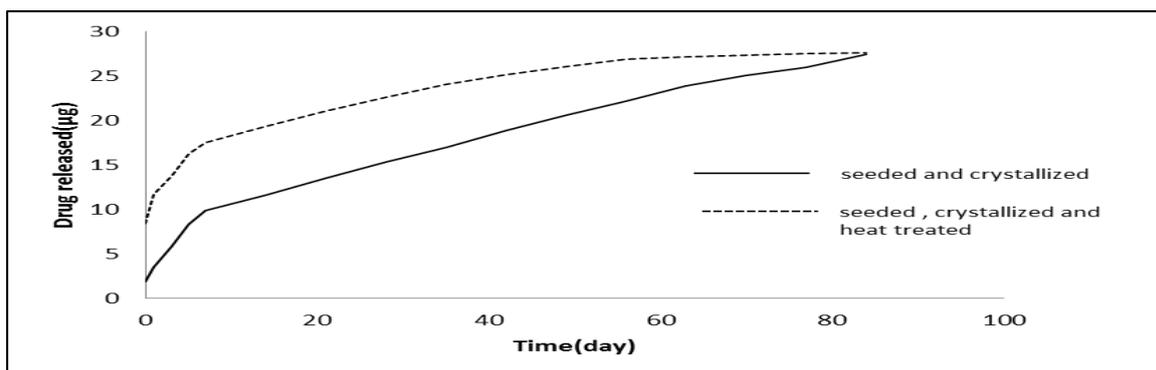


Figure 6: Cumulative drug release from the stents prepared in various conditions.

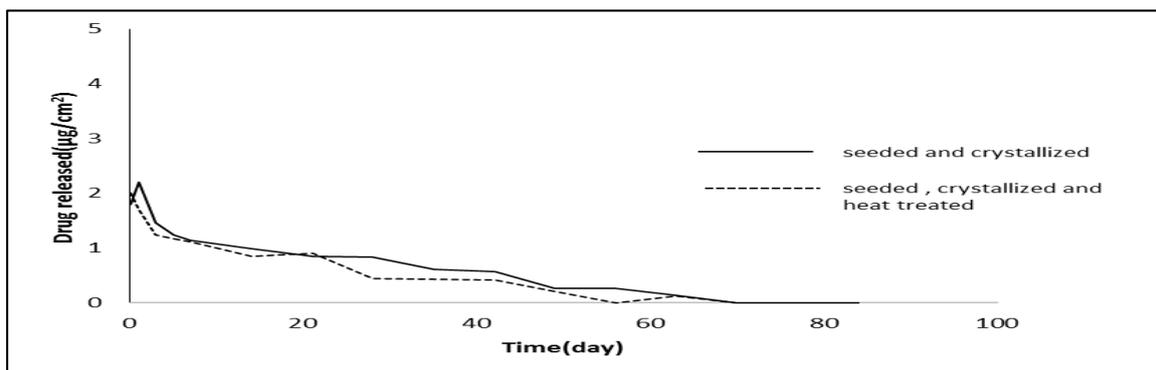


Figure 7: Drug release behavior from the stents prepared in various conditions.

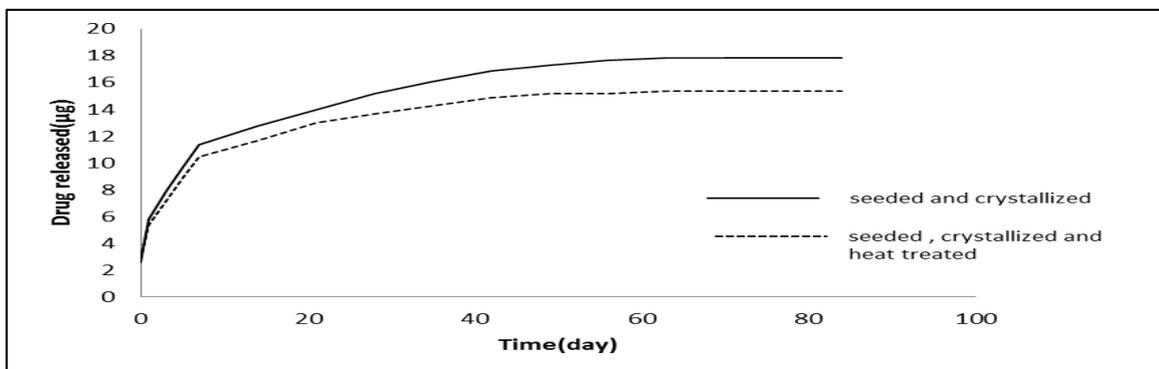


Figure 8: Cumulative drug release from the stents prepared in various conditions.

soluble anhydrous form due to the thermal process. After the anhydrate was immersed in the phosphate buffered solution, gradual water permeation led to the formation of the needle crystals containing water, resulted in the decreased PTX release over time.

Fig. 7 and 8 demonstrates the PTX amount released in the phosphate buffered solution from the stent applied seeding + liquid nitrogen cooling + crystal growth with/without heating along with PTX release amount /unit area and cumulative PTX release amount. In terms of the PTX release amount /unit area and the cumulative PTX release amount, there was no significant difference between the stent applied seeding + crystal growth with heating and the stent applied seeding + crystal growth without heating. In addition, marked difference in the initial PTX release was not found between them. The solubilities of needle-shaped PTX crystals and dissolved/re-crystallized PTX in the phosphate buffered solution were not remarkably different.

CONCLUSION

It was found that the sonication during PTX coating on the PLLA stent restricted stable attachment of PTX on the stent surface. The use of heating procedure on the needle PTX crystals with seeding + crystal growth could control the initial PTX amount.

The addition of liquid nitrogen cooling led to the formation of large amount of the immature PTX crystals, though very small amount of the needle crystals were also found. The process of sonication and/or liquid nitrogen cooling could hamper crystal growth of PTX particles, resulted in the unstable attachment of PTX on the stent surface. Although dissolution and re-crystallization of the PTX crystals on the stent surface were found after the liquid nitrogen cooling followed by heating process, the use of heating process was not able to increase the initial PTX release.

An animal study using Zilver PTX implanted in porcine peripheral arteries¹¹ showed that approximately 95% of the overall content of PTX was released at the intended site within 24 hours.

In an animal study where Zilver PTX stent was implanted in porcine peripheral arteries, the stent delivered approximately 95% of the total paclitaxel within 24 hours after deployment. Paclitaxel is highly hydrophobic and binds to proteins with high affinity, consequently can be efficiently absorbed by surrounding tissues and tightly bind to proteins within cells and the interstitium. Therefore, despite its rapid release, paclitaxel levels in the vessel wall were sustained¹¹.

In the present study, the largest initial release was found in the sample with seeding + crystal growth + heating; however, it was

only about 30% of the overall PTX content. The appropriate PTX content and release profile for improved efficacy in drug eluting stents remain unknown, requiring in-vivo evaluation for further investigation.

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