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Notes

In vitro and *in vivo* Characterization of a Coronary Stent Coated with an Elastic Biodegradable Polymer for the Sustained Release of Paclitaxel

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Introduction

Even though drug eluting stent (DES) has archieved successful outcome reducing in-stent restenosis (ISR), treatments with early types of DES have resulted in several complications, including incomplete release of the incorporated drug, possible induction of an inflammatory and/or immune response by coating materials, and late angiographic stent thrombosis (LAST).[1]

To reduce current limitations, sustained release of antiproliferative agents and complete removal of the coating materials may be achieved by using biodegradable polymers. Therefore, many biodegradable polymers including poly(lactic-*co*-glycolic acid) (PLGA), poly(l-lactic acid) (PLLA), and polycaprolactone (PCL) were applied to coat stents with antiproliferative agents.[2-4] Although drug release kinetics from the biodegradable polymer coated stent is ideal for preventing ISR, some materials are very brittle and unable to support the shape of an expanded stent, which may cause fracture and fragmentation of the coating materials after stent expansion. In addition, complete degradation of some materials requires a few years, which can limit the rapid recovery of the artery.[5]

In order to prevent coating failure due to cracks upon expansion, we applied elastic, biodegradable, and biocompatible poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) as a coating material.[6] The objective of this study was to develop a new DES coated with PLCL containing paclitaxel. The morphology of the coated stents was examined after expansion of the stents. The *in vitro* and *in vivo* release profiles of the paclitaxel from the DES were studied, and prevention of luminal thickening was examined by application of DES in iliac arteries of rabbit model.

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Experimental

PLCL with medical grade (L-lactic acid : ε-caprolactone = 25:75, inherent viscosity: 0.7 dL/g, Lactel, CA, USA) was dissolved in tetrahydrofuran (THF) at 1, 2, and 5 wt% (PLCL-1, PLCL-2, and PLCL-3), respectively, and each solution was mixed with paclitaxel (0.5 wt%). 316L stainless steel stents (13 mm in length) (Vasmed technologies limited, United Arab Emirates) were immersed in mixture solutions for one minute. The polymer coated stents were dried under ambient conditions for 12 h and subsequently in a dry oven at 50 °C for three days.

The surface of PLCL/paclitaxel coated stents was analyzed before and after expansion of the stents by scanning electron microscope (SEM, JEOL, Tokyo, Japan).

To analyze *in vitro* paclitaxel release profiles from the stents, the coated stents were immersed in 5 mL phosphate buffered saline (PBS, pH 7.4) containing ethanol (9:1, v/v) as described in other study, and then shaken in an orbital incubator (37 °C, 150 rpm) to release paclitaxel for 50 days.[7] At predetermined time points, the amount of the released paclitaxel was quantified using an ultraviolet-visible spectrophotometer (Shimadzu model UV-2101 PC, Kyoto, Japan) at 227 nm.

In order to measure the released amounts of paclitaxel *in vivo*, the PLCL/paclitaxel coated stents (PLCL-2) were implanted in iliac arteries in rabbits. Implanted stents were removed with surrounded vessels at 15 and 30 days post-surgery. The remaining paclitaxel was extracted with chloroform and ethanol from the vessel tissues and the stent, respectively, and was quantified using high pressure liquid chromatography (HPLC) (Young lin instrument, Seoul, Korea) equipped with a 4.6×250 mm ZORBAX Eclipse C-18 column (particle size: 5 μm, Agilent, USA). The sample running conditions consisted of a mobile phase composition of 25:75 (v/v) water and acetonitrile with 0.05% trifluoroacetic acid at a flow rate of 0.2 mL/min.

After implantation of the PLCL/paclitaxel-coated stent for 90 days in the iliac arteries in rabbits, blockage of stented vessels was confirmed using angiography. Rabbit iliac arteries with stents were carefully removed from the rabbits and rinsed with physiologic saline. After fixation with 10% formaldehyde, the iliac arteries were dehydrated in alcohol baths and embedded within a poly(methyl methacrylate) (PMMA) polymer resin. Specimens were sectioned in 5 μm intervals using

microtome with a tungsten carbide knife. Each sample was stained with hematoxylin and eosin (H&E).

Results and Discussion

The application of biodegradable polymers using PLGA and PLLA as coating materials to stents has demonstrated the ability to control the drug release rate for a sustained period of time and the possibility of resolving the issue of LAST. However, the mechanical properties of PLGA and PLLA are brittle and thereby caused subsequent peeling and fragmentation of coated polymers after stent expansion, which may elicit acute thrombosis and cytotoxic effects. Therefore, the use of an elastic biodegradable polymer as a DES coating material may be beneficial to minimize these side-reactions upon expansion of stents at the site of implantation. Although these polymers have been widely used to facilitate tissue regeneration requiring elastic strain (cardiovascular, muscular, and skin tissue), they have never been utilized as a stent-coating material. Herein, we developed a paclitaxel-incorporated PLCL-coated stent using a deep-coating method, and concentrations of PLCL solution were varied at a constant paclitaxel concentration. PLCL-coated surface morphology was analyzed using SEM.

As the concentration of the polymer solution increased, an increase in the roughness of the polymer-coated surface was evident, and an unequal deposition of polymeric film on the stent was observed only on the surface with the 5 wt% of polymer concentration (Figure 1). On contrary to previous report that DES coated with PLGA showed peelings of the coating material at the bending ends of the stent,[8] the PLCL-coated stent showed consistent and uniform surface morphology without major deformation, peelings, and cracks after expansion due to its elasticity.

The PLCL-coated stents loaded with 0.5 wt% paclitaxel were then used to examine their *in vitro* and *in vivo* release characteristics. As shown in Figure 2(A), up to 96% of paclitaxel from PLCL-1 (1 wt% PLCL solution) was released (total loading: 62.71±2.98 μg) over 50 days of *in vitro* release experimentation, whereas Taxus express2 as a control group showed that 40% of the total loaded paclitaxel was released during the same period of the release study. The rate of the paclitaxel release was dependent on the polymer concentration; the release rate of paclitaxel was the lowest at higher polymer concentration (PLCL-3), and a faster release was observed as the polymer concentration decreased. The paclitaxel releasing rates from the PLCL-coated stents at all

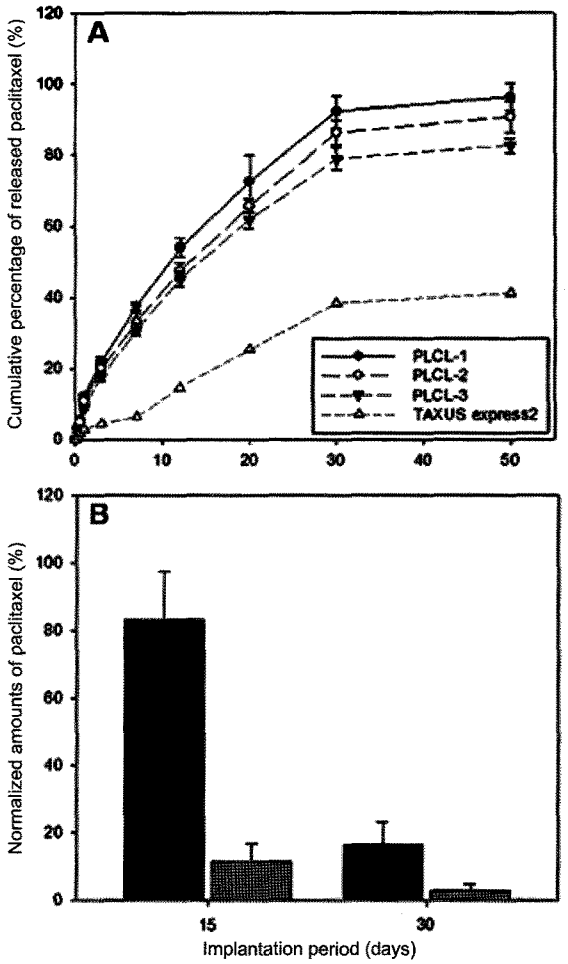

Figure 2. *in vitro* and *in vivo* paclitaxel release profiles. A. an *in vitro* released amount relative to the total loading drug. PLCL-1 (●): coated with 1 wt% PLCL, PLCL-2 (○): coated with 2 wt% PLCL, PLCL-3 (▼): coated with 5 wt% PLCL, and Taxus express2 as a control (△). Each PLCL coated stent contained 0.5 wt% paclitaxel. B. an *in vivo* paclitaxel release profile at 15 and 30 days. Black bar represents the remaining paclitaxel in the stent and the white bar denotes the detected paclitaxel in the vessel tissue.

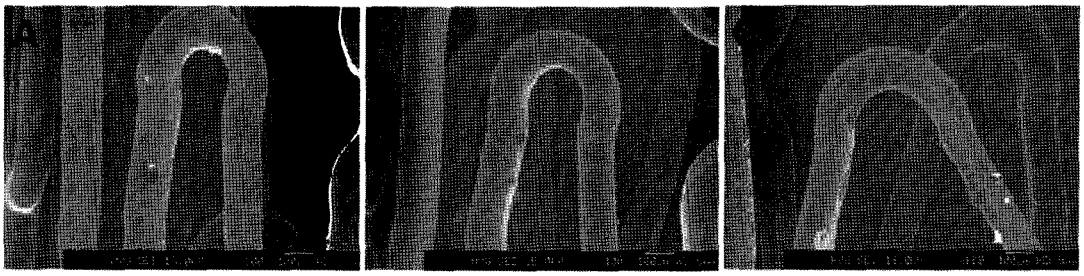

Figure 1. Scanning electron microscopy (SEM) of paclitaxel-incorporated PLCL-coated stents after expansion. (A) 1 wt% PLCL coated stent, (B) 2 wt% PLCL coated stent, and (C) 5 wt% PLCL coated stent.

polymer concentrations were relatively faster than that from Taxus express2. For example, paclitaxel from PLCL-2 and PLCL-3 was released up to 55.95±5.65 μg (90% relative to the total loading of 61.73±6.34 μg), and 55.62±4.12 μg (82% relative to the total loading of 67.33±8.41 μg) after 50 days of incubation, respectively. Most importantly, the release of paclitaxel showed no-burst, indicating that PLCL/paclitaxel coating is effective for controlling the drug release rate. It has been known that re-proliferation of smooth muscle cells can be problematic over the period of a month when the drug is no longer present around the implanted stents. Since it may cause ISR, the sustained release of anti-proliferative agents over a period of time, particularly longer than a month, should be required for the complete reduction of ISR. However, most of biodegradable polymer-coated stents reached the maximum release within less than a month, which may be ineffective for controlling late ISR.[9,10] Our results suggest that the initial amounts of paclitaxel released amount from the PLCL-coated stents is not only sufficient to mitigate a sudden proliferation of smooth muscle cells, but may be effective for reducing re-proliferation of the smooth muscle cells over 50 days.

In *in vivo* release profiles, the amount of extracted paclitaxel from vessel tissue was 40 μg (83.3% of the originally incorporated amount of 48 μg), and the remaining paclitaxel in the stent was 5 μg (11.4%), suggesting that 3 μg of paclitaxel appeared to be eluted to the blood or metabolized at day 15. At day 30, 8 μg (16.7%) of paclitaxel was detected in vessel tissue, and 1.2 μg (2.7%) of paclitaxel was detected in the stent.

These results indicate that approximately 34 μg of paclitaxel impregnated within the PLCL coating was released and eluted to the blood or metabolized (Figure 2(B)). In comparison with the result of *in vitro* paclitaxel release, the paclitaxel was released faster *in vivo*. This may be due to the presence of many hydrophobic components in tissue fluids, which may facilitate the diffusion of hydrophobic paclitaxel from the stent as compared with the *in vitro* experimental condition. In addition, the degradation of the PLCL caused by hydrolysis and enzymatic cleavage may be faster *in vivo*, which may have synergistically affected the enhanced drug release. It is also possible that direct contact between the cells and the stent may influence the additive effect on the release rate of paclitaxel.

Implanted DES was observed by angiography in both left and right iliac arteries where DES was implanted (Figure 3(A)). After injection of a contrast agent, all blood vessels around the iliac artery were clearly observed, and formation of thrombus and ISR on DES implanted position weren't observed from all animals (Figure 3(B)).

For the histological analysis results, an overgrowth of the neointimal layer was observed to evaluate the efficacy of new DES. As shown in Figure 3(C) and 3(D), neointimal hyperplasia and a thickening of the vessel wall were observed in bare metal stent (Figure 3(E)), while PLCL/paclitaxel coated stent showed little neointima formation (Figure 3(F)).

In addition, re-endothelialization is critical for successful treatment after DES implantation. Even though we did not directly observe re-endothelialized stent vessel, most of stent struts were surrounded with cells after 90 days of implantation indicating successful progress of re-endotheilalization (Figure 3(D)). Therefore, slow degradation PLCL hardly affected re-endothelialization from our *in vivo* study.

Currently available DESs in the clinical field are loaded with approximately 100 μg of paclitaxel per stent. Since paclitaxel is known as an anti-cancer agent, and an increase in the local concentration of paclitaxel may be cytotoxic, determination of the appropriate loading amounts and an optimized delivery system are important to ensure safe treatment. In this study, we reduced the loading amount of paclitaxel from 100 μg to between approximately 48 and 67 μg, and their complete release was achieved from both *in vitro* and *in vivo* conditions using the elastic biodegradable polymer. In addition, histological analysis revealed that the PLCL/paclitaxel coated stent reduced neointimal hyperplasia for 90 days and we did not observe the recruitment of inflammatory cells around the implants as shown in Figure 4.

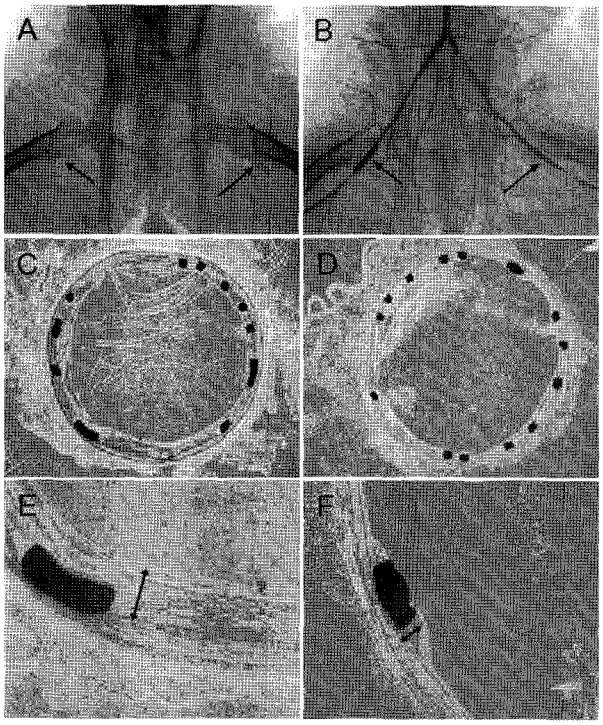

Figure 3. Angiographic and histological images of the stented vessel after 90 days of implantation. (A) implanted stent under angiography. Black arrows indicate implanted stent. (B) no blockages were observed after injection of a contrast agent. (C) and (E) are images from the bare metal stent implantation group under the magnification of 10x and 100x, respectively. (D) and (F) are images from the PLCL/paclitaxel-coated stent implantation group. Black arrows exhibit overgrowth of the neointimal layer.

Taken together, these results indicate that a PLCL/paclitaxel-coated stent loaded with a lower amount of paclitaxel may be a promising candidate to replace current DES.

Conclusions

In conclusion, we developed a DES with PLCL and paclitaxel. After expansion with a balloon, there was no evidence of cracks or peelings in the PLCL/paclitaxel coated stent. Paclitaxel was released *in vitro* up to 96% relative to the total loading weight over 50 days in a PLCL concentration-dependent manner and eluted *in vivo* up to 97.3% for 30 days. From the rabbit iliac artery implantation study, the PLCL/paclitaxel-coated stent successfully reduced the formation of neointimal hyperplasia and thrombosis without causing inflammatory reaction after 90 days of implantation. These results suggested that PLCL/paclitaxel-coated stents may be used as an alternative DES system.

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