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New Coating Method for Sustained Drug Release: Surface Modification of ePTFE Grafts by inner coating PLGA

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Expanded polytetrafluoroethylene (ePTFE) grafts have been used as vascular access for many patients suffering from end stage renal disease. However, the vascular graft can cause significant clinical problems such as stenosis or thrombosis. For this reason, many studies have been performed to make drug eluting graft, but initial burst is major problem in almost drug eluting systems. Therefore we used biodegradable polymer to reduce initial burst and make sustained drug delivery. The ePTFE grafts were dipped into a paclitaxel-dissolved solution and then PLGA-dissolved solution was passed through the lumen of ePTFE. We analyzed whether the dose of paclitaxel is enough and the loading amount of PLGA on ePTFE graft increases according to the coating solution's concentration. Scanning electron microscope (SEM) images of various concentration of PLGA showed that the porous surface of graft was more packed with PLGA by tetrahydrofuran solution dissolved PLGA. In addition, *in vitro* release profiles of Ptx-PLGA graft demonstrated that early burst was gradually decreased as increasing the concentration of PLGA. These results suggest that PLGA coating of Ptx loaded graft can retard drug release, it is useful tool to control drug release of medical devices.

Key Words : Hemodialysis, Vascular access, ePTFE graft, Paclitaxel, Biodegradable polymer

Introduction

Many patients suffering from end stage renal disease usually receive hemodialysis three times a week. To perform the hemodialysis, the secure of vascular access is important. Most common forms of vascular access are the native arteriovenous fistulas (AVF) and arteriovenous graft (AVG) using expanded polytetrafluoroethylene (ePTFE) graft.[1] The ePTFE is made under high temperature and pressure of polyterafluoroethylene and they have suitable mechanical and biocompatible properties.[2] Although the vascular graft access has many advantages such as short maturation time, multiple access site and high blood flow rates, it may induce clinical problems[3] such as thrombosis associated with stenosis of anastomosis site or vein.[4] Smooth muscle cells (SMCs) mainly forms the neointima which contributes of blockages in arteriovenous shunts at the central to both vein failure and restenosis.[5,6] To reduce this problem, we have developed the drug delivery system that drug eluting graft can reduce venous stenosis or thrombosis in our previous study.[2,7,8] Paclitaxel (Ptx) is microtubule-stabilizing agent and also has popent antiproliferative and antimigratory activity.[9,10] Many studies have shown that paclitaxel have effect in cancer such as tumoricidal activity against several human neoplasms, including non-small lung, ovarian, breast, brain cancer[11] and in other various fields.[12] Also there are many studies to make appropriate graft eluting drug, but these drug eluting systems have limits such as initial burst or low coating amount of drug coated graft. This burst release of drug could induces various side effects and cytotoxicity. Therefore, in our study, we used the biodegradable polymer, Poly (D,L-lactide-*co*-glycolic acid) (PLGA) for long term release of drug. Because PLGA slowly degrades into natural metabolites as poly lactic and glycolic acids in living system, it is called biodegradable polymer[13] and approved by the Food and Drug administration (FDA).[14] The sustained drug delivery drug by using PLGA have been thought useful strategy due to their outstanding biocompatibility and biodegradability.[15] In addition, because of long clinical experience of it, PLGA is most widely used among the various available biodegradable polymers for sustained drug delivery.[16] We devised the drug eluting ePTFE graft containing enough drug as well as releasing drug without initial burst.

Experimantal

Materials. Paclitaxel was procured from Samyang Genex co. (South Korea) and Poly (D,L-lactide-co-glycolic acid) (PLGA, lactide:glycolide = 50:50, MW 30,000) was purchased from Evonik Ind. (Darmstadt, Germany). The ePTEF graft was supplied by Bard Peripheral Vascular, Inc. (Arizona, USA). HPLC-grade acetone and acetonitrile were obtained from J.T Baker (USA). Tween 20 was purchased from Hayashi Pure Chemical Ind., Ltd. (Japan). Tetrahydrofuran (THF) was obtained from DAEJUNG Ind (Korea). Phosphate-buffered saline (PBS) was supplied by Lonza (USA). All other reagents were of analytical grade.

Paclitaxel-PLGA loaded Graft preparation. Paclitaxel (Ptx) was dissolved in acetone and ePTFE vascular graft was dipped into Ptx solution for 30 minutes at 37 ºC hybrization

incubator. The Ptx loaded graft was dried and maintained in a vacuum overnight. After then, the vascular graft was also coated with PLGA dissolved-tetrahydrofuran solution along the inside by peristaltic pump. To coat the both upper and under surface of the vascular graft, we put it on the 30º slope. The ptx-PLGA loaded graft was dried with nitrogen gas for 5min and maintained in a vacuum overnight.

Analyze the Coating Amount. To detect quantitative of PLGA, the Ptx-PLGA graft was analyzed by attenuated total reflection-FTIR (ATR-FTIR 300E, Jasco). we calculated calibration curve by the peak ratio of PLGA and PTFE. PLGA and PTFE show the spectral peak of ester, 1750 cm^{-1} and C-F bond, 1200 cm^{-1} each. Paclitaxel was detected by the high-performance liquid chromatography (HPLC; Aglient 1100 series, USA) analysis and we used a mobile phase of water/acetonitrile (50/50, v/v) under isocratic conditions at a flow rate of 0.8 mL/min, a 4.6 × 150-mm C18 reverse phase column. The UW detector was set at 227 nm and retention time of paclitaxel was 9.5 min.

Surface Morphology. The surface morphological change of Ptx-PLGA loaded graft was analyzed by scanning electron microscopy (SEM, JEOL JSM-840A, Japan). A fragment of coated graft was mounted in an aluminum stub and coated with a platinum layer (Cressington 108, JEOL Ltd, Japan) and viewed on SEM.

***In vitro* Drug Release Studies.** For the *in vitro* release studies, we used a solution of phosphate-buffered saline (PBS, pH 7.4) containing 0.05% (w/v) Tween 20. We also designed a sinker like iron mesh to stay graft in solution, because the ePTFE graft floats the release medium. We cut the coated graft about 1cm to put into the sinker and soaked the sinker containing paclitaxel-PLGA coated graft in 30 mL release solution. And the sinker incubated in a 37 ºC/20 rpm hybrization incubator, FINEPCR (Seoul, South Korea) for 5 days. We moved the sinker into new fresh release solution at each designated time. To analyze the existing the paclitaxel, we put the graft in cornical tube including acetone and incubated for 2 h and then the solutions were analyzed by HPLC.

Physical Characterization. The vascular grafts coated Ptx-PLGA were prepared as about 5 cm long segments.[17] The physical properties of graft are tested by LLOYD instrument (LRX*plus* series, UK) and we used 1 kN loadcell. We set preload value 0.200N, speed 100.0 mm/min. The graft was put the gauge and the length is 30 mm. Young's modulus is determined by calculating the initial slope of the stress-strain curves.

Results and Discussion

Loading Amount of Ptx-PLGA Loaded Graft. The ePTFE graft was first coated with Ptx and then PLGA dissolved solutions. We used tetrahydrofuran (THF) as solution to cover graft with PLGA. THF not only dissolves well the high concentration of PLGA but does not leak out the graft when the THF containing PLGA was flowed into inner surface of graft coated with Ptx. Therefore THF is suitable solvent to coat PLGA on the surface of vascular graft. To determine the final concentration of paclitaxel on the graft, we analyzed the amount of Ptx before and after PLGA coating. First, ePTFE graft was dipped into 0.5, 1, and 2 mg/mL Ptx solutions. After vacuum overnight, the 30 mg/mL PLGA solution was flowed into graft containing ptx. As shown Figure 1(a), paclitaxel residues increased depending on the concentration of Ptx solutions. As the same method, we tested tendency in paclitaxel residues and PLGA solutions. We used 2 mg/mL Ptx solution and PLGA concentration was 30, 50, and 70 mg/mL each solutions. The Ptx amount increased according to increase the concentration of PLGA (Figure 1(b)). Because PLGA of high concentration is difficult to penetrate deeply in the graft, paclitaxel coated inner graft couldn't wash out with PLGA solutions. It means that the most Ptx remains on the graft and we can control the coating amount of drug by concentration of Ptx solution.

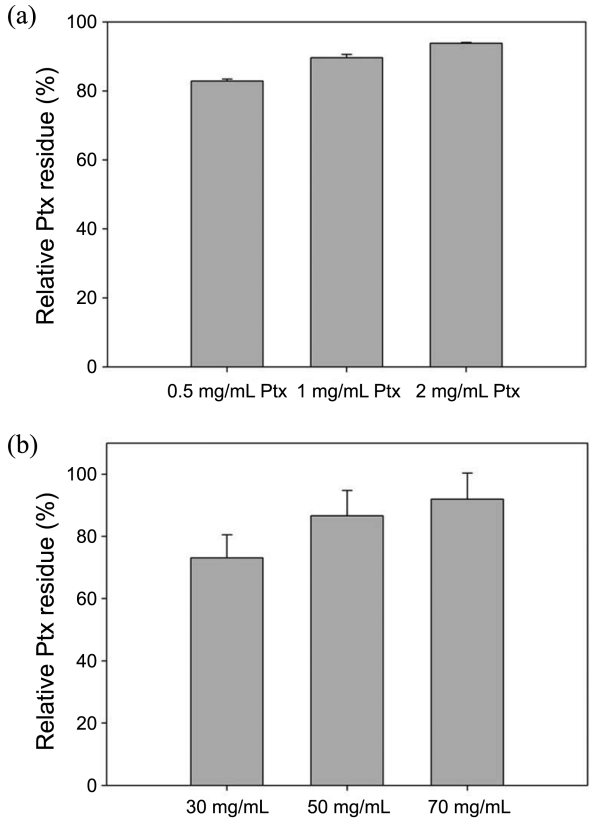

Figure 1. Remaining amount of graft coated Ptx-PLGA according to the different concentration of Ptx and PLGA solution.

We also confirmed PLGA coating qualitatively and quantitatively by ATR-FTIR. As shown Figure 2(a), the graft coated with only paclitaxel has the 1200 cm^{-1} spectral peak of ester bond PTFE and when the graft was coated with PLGA, they have both paclitaxel peak and the PLGA spectral peak of C-F bond 1750 cm^{-1}. The quantitative analysis shows that as PLGA solution's concentration increases, the amount of coated PLGA is increased (Table 1).

Characterization of Surface Morphology. To check whether aggregation is formed on the graft inner surface due to high concentration of PLGA, the Ptx-PLGA loaded graft was observed by SEM (Fig. 3). As our previous study

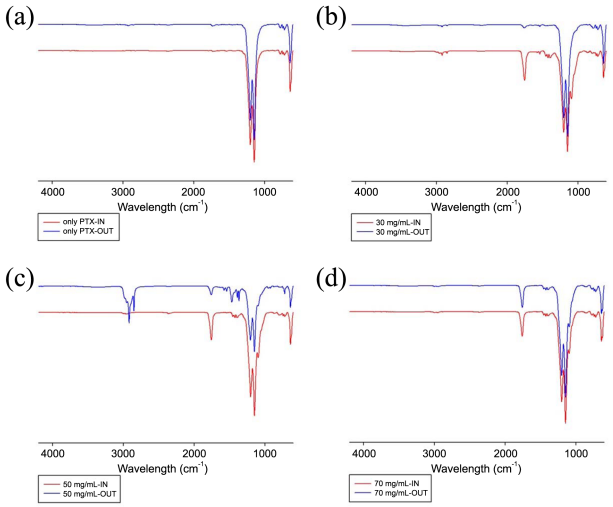

Figure 2. ATR-FTIR spectra. Ptx solution's concentration was 2 mg/mL. (a) was Ptx only, (b), (c), and (d) was Ptx-PLGA grafts. PLGA solution was 30 mg/mL (a), 50 mg/mL (b), 70 mg/mL (c), and 100 mg/mL (d).

Table 1. PLGA coating amount of Ptx-only and Ptx-PLGA graft

PLGA solution concentration	Amount of coated PLGA (mg/cm^3)
30 mg/mL	1.00
50 mg/mL	1.44
70 mg/mL	1.70

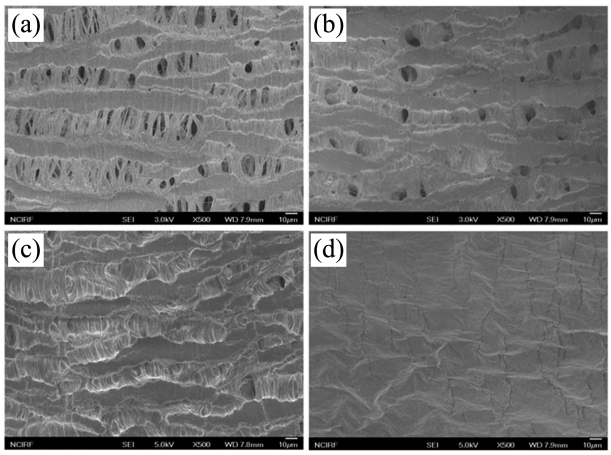

Figure 3. SEM images of the Ptx-PLGA loaded graft. All the grafts were coated with 2 mg/mL Ptx, PLGA solution was (a) 30 mg/mL, (b) 50 mg/mL, (c) 70 mg/mL, and (d) 100 mg/mL.

revealed, the graft morphology by paclitaxel coating was not changed.[2,7,8] When PLGA was coated on graft with 2 mg/mL paclitaxel solution, the pore of graft is gradually filed with PLGA as the polymer concentration increase. When the Ptx loaded graft was coated with 70 mg/mL PLGA, the almost every pore of graft was packed. These results are correlated with the release profiles of paclitaxel. It means that the release of paclitaxel is inhibited by packing of PLGA at the porous ePTFE graft surface.

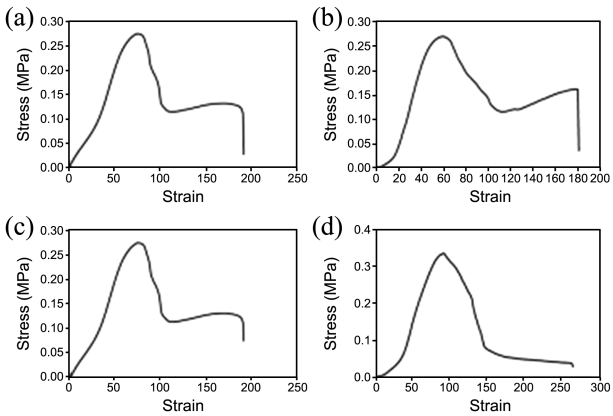

Figure 4. Stress-strain curves of Ptx-PLGA graft. All the grafts were coated with 2 mg/mL Ptx. (b), (c), and (d) were coated with PLGA 30, 50, and 70 mg/mL solutions each.

Table 2. Mechanical properties of Ptx-only and Ptx-PLGA graft

Substrate	Young's Modulus (MPa)	Tensile strength (MPa)
PTX only	16.22 ± 3.3	0.40 ± 0.07
PTX + 30 mg/mL PLGA	19.257 ± 3.8	0.34 ± 0.04
PTX + 50 mg/mL PLGA	24.31 ± 8.2	0.38 ± 0.03
PTX + 70 mg/mL PLGA	13.65 ± 3.5	0.34 ± 0.03

Analysis Mechanical Properties of Graft. The ePTFE graft has suitable properties as medical device because expanded polytetrafluoroethylene is stable and also it has proper mechanical strength. We tested the physical properties of graft coated PLGA to confirm whether there are any changes by PLGA or not. We calculated Young's modulus by analyzing Stress-strain curves. As shown Table 2, Young's modulus and tensile strength make a little difference, but it is hard that the concentration of PLGA solution makes difference with Ptx-only coated graft. These results mean that PLGA coating appears to have little effect on mechanical

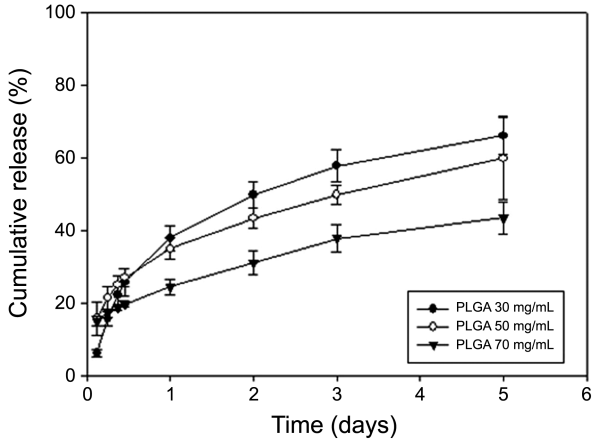

Figure 5. *In vitro* release profiles of paclitaxel from different PLGA coating amount. All the grafts were coated with 2 mg/mL Ptx and 30 mg/mL (○), 50 mg/mL (●), and 70 mg/mL (▼) PLGA.

properties.

***In Vitro* Release Profiles of Paclitaxel from Ptx-PLGA in the Graft.** The figure shows *in vitro* release profiles of paclitaxel from Ptx-PLGA loaded grafts in PBS containing 0.05% tween 20 for 5 days. The graft was coated with 2 mg/mL paclitaxel and 30, 50, 70 mg/mL PLGA each. As we expected, the release amount of drug decreased as more PLGA concentration increased. While the graft coated with 30 mg/mL released paclitaxel about 65% at 5 days, the graft coated with 70 mg/mL released only about 40%. Further, the initial burst of drug significantly decreased in comparison paclitaxel only coated graft compared to our previous study.[18] Therefore we can control release pattern of paclitaxel by PLGA of appropriate concentration. Also we can reduce problems caused initial burst of drug, this Ptx-PLGA coating system is useful tool of drug delivery method.

Conclusion

In this study, we tried to develop the coating method to reduce initial burst and release drug gradually compared to control group.[18] This method which is Ptx dip coating and PLGA inner coating has appropriate properties because the lumen of porous graft is packed with PLGA without aggregation. Therefore, this system can deliver the drug locally with low systematic side effect for long term. Furthermore, *In vivo* studies such as animal experiments and clinical studies should be performed to determine the therapeutic effect under realistic biological conditions.

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