A Novel Deposition Method of PLGA Nanoparticles on Coronary Stents

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Received March 5, 2009, Accepted March 23, 2009

Bare metal stents which were used to treat coronary artery disease have several biochemical problems. Polymer-based drug-eluting stents (DES) have opened up a new paradigm in the treatment of in-stent restenosis. Many studies and research programmes have proved that DES can prevent restenosis. In our study, paclitaxel-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles have been deposited along the three dimensional scaffold of coronary stents by a method using self-assembling properties of colloidal particles. We found that the nanoparticles were deposited uniformly and closely packed. The amount of paclitaxel was easily controlled by the drug content of the nanoparticles and the deposition count.

Key Words: PLGA, Paclitaxel, Stent, Nanoparticle deposition

Introduction

Recently drug-eluting stents (DES)[1,2] are the major devices to treat coronary artery disease (CAD).[3] These stents, however, have several problems: drug loss during the operations, fast drug release in the arteries, side effects which are caused by polymer bases, and so on.[4,5] Many research groups have been developing new drug-eluting systems to overcome these drawbacks.

Research of degradable/nondegradable polymer bases is important in containing and delivering drug molecules.[2,4,6] Polymer bases were recently employed to control the drug release rates and achieved good results that minimized the drug loss during the operations. Any polymer, however, is also an alien material and can itself be the cause of several biochemical side-effects like abnormal irritation, inflammation, blood protein-adsorption and thrombosis.[4,6] Therefore, the biocompatibility of a polymer is one of the key points to take into consideration, in addition to having no toxicity, good mechanical characteristics and the ability to provide controlled release of drug molecules. Poly (lactic-co-glycolic acid) (PLGA) is one of the biocompatible polymers. The monomers which are generated after degradation are not harmful to a living body so PLGA has been used in various medicinal applications.[7,8]

In this study, we introduced polymer nanoparticles (NPs) for paclitaxel,[9,10] an anti-cancer drug delivery system.[11] Microparticles have been also used for drug carriers. They have, however, a smaller surface/volume ratio which is important to adsorb than that of nanoparticles and therefore microparticles are not appropriate for deposition on stents. Various methods have been developed to encapsulate drug molecules in polymer particles[12–15] and we used an emulsion-solvent evaporation method.

Materials and Methods

Materials. Poly (lactic-co-glycolic acid) [PLGA (RESOMER® RG 504 H), 50:50, Mw 48,000, i.v. 0.45 ~ 0.60 dL/g in chloroform] was purchased from Boehringer Ingelheim (Germany). Paclitaxel was obtained from Samyang Genex Co. (ROK). Polyvinyl alcohol (PVA, Mw 30,000 ~ 70,000) was obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Bare metal stents were purchased from Humed (ROK). The organic solvent dichloromethane/methylene chloride (MC) was 'Baker Analyzed' HPLC solvent. Distilled water produced by Millipore (Millipore Corporation) was used throughout. All other reagents were of analytical grade and were used without further purification.

Preparation of PLGA Nanoparticles Entrapping Paclitaxel. Paclitaxel and PLGA were dissolved in methylene chloride (oil phase) and the organic solution was poured in 0.2% (w/v) polyvinyl alcohol (PVA) aqueous solution (water phase). An ultrasonic processor (VCX 600 Watt Vibracell; Sonic & Materials, Danbury, CT) formed an o/w emulsion by an set at 40% amplitude for 2 minutes with a pulse (on: 5 seconds, off: 2 seconds). The resulting emulsion was stirred for 3 hours at room temperature under reduced pressure to evaporate the methylene chloride. After removing the volatile solvent, the resulting aqueous suspension particles were centrifuged and re-suspended in distilled water prior to freezedrying.

Particle Characteristics Analysis and Morphology Observation. PLGA nanoparticles were re-dispersed in distilled water. Their mean particle size was measured by a Malvern Zetasizer 3000HS system (Malvern Instruments Ltd., Worcestershire, U. K.). The obtained homogeneous suspension was analyzed to determine the volume mean diameter, size distribution and polydispersity. The measured values were presented as the average value of 5 runs. The sampling time was set to automatic.

The morphological examinations of PLGA nanoparticles and nanoparticle layers on the stents were achieved by scanning electron microscope (SEM, JSM 840-A, Japan). A droplet of the suspension was placed on a slide glass (35 × 25 mm) and dried at room temperature under reduced pressure to get a uniform arrangement of the particles. Samples of the above and of nanoparticle-coated stents were platinum coated using a sputter coater.

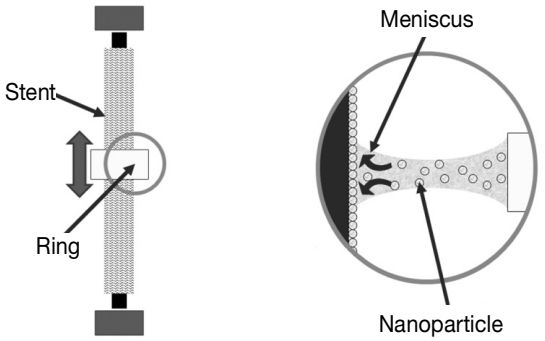

Figure 1. Schematic of the RST coating apparatus.

RST Method Procedure. We developed a nanoparticle-coating protocol and named it the RST (Ring-shaped Surface Tension) method. The schematics of the tools are given in Figure 1. A stent was immobilized and a ring was set on the stent. Droplets of the concentrated suspension were injected between the stent and the ring. Capillarity led to the formation of a meniscus in which the suspension was held. Nanoparticles in the suspension were deposited onto the surface of the stent at the wedge where the meniscus met the surface when the ring was moved up and down over the stent.[16] The remaining suspension was removed by centrifugation. The product was then dried in a desiccator.

HPLC Procedure. The amounts of paclitaxel in the nanoparticles and that on a stent were determined by a reversed phase high-performance liquid chromatography (RP-HPLC) method. Paclitaxel-loaded nanoparticles were dissolved in organic solvent and applied to an HPLC system (Agilent 1100 Series, USA). Separation was achieved by using a reversed-phase column (300Extend-C18, 4.6 × 150 mm, Agilent, USA) thermostated at 40 ^{o}C and with the flow rate of the mobile phase set at 1.0 mL/min. The mobile phase composition was 60:40 (v/v) of water and acetonitrile, and a UV detector set at 242 nm. Analysis of drug mass on a stent was performed under the same conditions of HPLC as explained above. Each of the stents, which had been coated under different coating conditions, was placed in a tube containing organic solvent and shaken. Paclitaxel concentrations in the organic solution were determined by HPLC.

Results and Discussion

Characteristics of Paclitaxel-loaded PLGA Nanoparticles. The PLGA nanoparticles were spherical in shape. Their mean particle size was estimated and turned out to be 311.9 nm in diameter with a narrow distribution (Figure 2). We were able to prepare several degrees of the drug contents by modulating the paclitaxel/PLGA ratio (data not shown). The surface morphology of paclitaxel-loaded PLGA nanoparticles was observed by SEM (Figure 3). The optimized conditions for stent deposition were achieved by dissolving 45 mg of paclitaxel and 100 mg of PLGA in 2 mL of methylene chloride and emulsifying the mixture in 20 mL of 0.2% (w/v) PVA aqueous solution. The HPLC analysis results showed that 1 mg of the nanoparticles which were prepared under these conditions contained 144 ± 13 μg (mean ± SD) of paclitaxel. The encapsulation efficiency

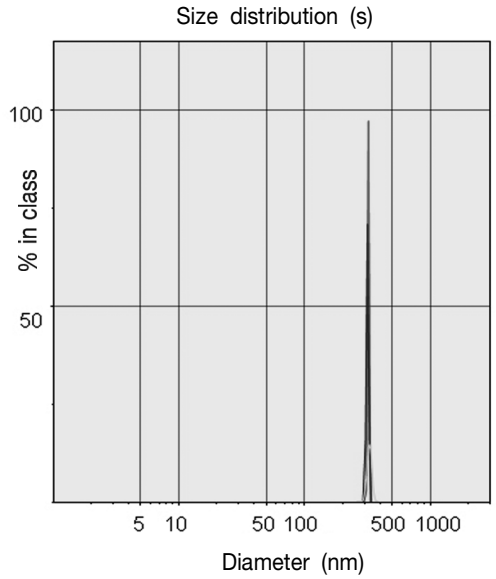

Figure 2. Size distribution of PLGA nanoparticles.

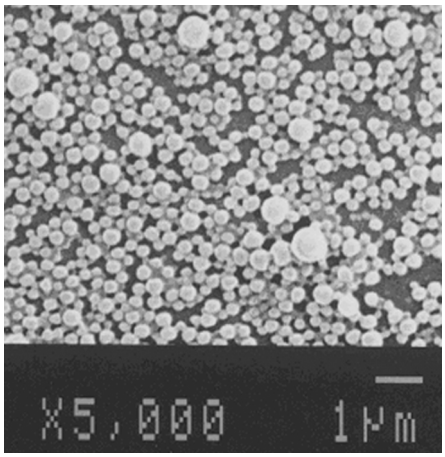

Figure 3. SEM image of PLGA nanoparticles.

of PLGA nanoparticles was about 59% if the PVA molecules were not washed out at all.[17]

Deposition of Paclitaxel-loaded Nanoparticles on Stent by RST Method. The surfaces of the stents were observed with SEM after nanoparticle deposition. Our goals were that the nanoparticle layers would be regularly distributed and not choke the gaps between the struts of the stents. The SEM images (Figure 4) showed that the RST method was able to deposit nanoparticles over the surface of the stents uniformly. The thickness of the nanoparticle layers expanded according to the count of the deposit. The regularity of the layers did not decline while the RST procedure was performed several times.

The merit of the RST method is that we can regulate the drug mass on the stents using several options (Figure 5). We found that there were three factors which could decide the drug mass on a stent: the drug content of the nanoparticles, the concentration of the nanoparticle suspensions and the count of the deposit. The optimized conditions for a hundred microgram-loaded stent were that the drug content of the nanoparticles was 46%, the concentration of the nanoparticle suspen-

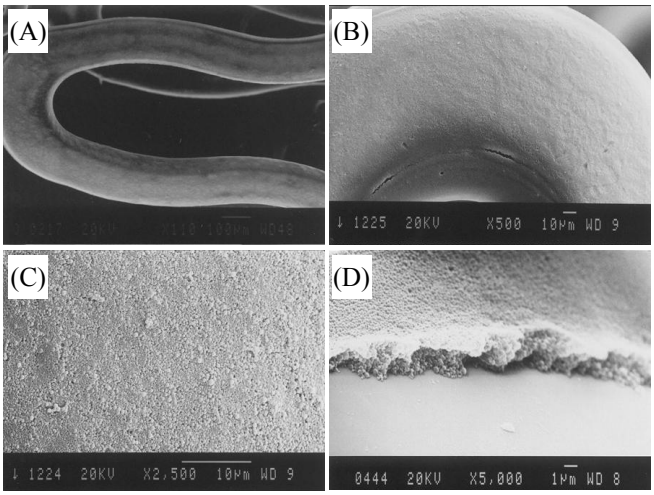

Figure 4. SEM images of PLGA nanoparticle-coated stents. (A) × 110, (B) × 500, (C) × 2,500, (D) × 5,000 (a cross section of a nanoparticle layer).

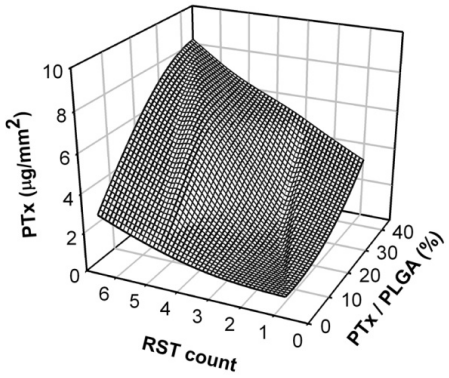

Figure 5. Total amount of paclitaxel on nanoparticle-coated stents.

sion was 19.3% (w/v) and the count of the deposit was 6 or 7. The drug mass of 6-count deposited stents was a little lower than the 7-count ones but the difference was not statistically significant. The average drug mass was 7.66 ± 0.76 μg/mm^2 (mean ± SEM, n = 16).

The powerful advantage of the RST method is that nanoparticle layers which were already deposited do not crumble away even though the RST method was executed several times. It may be due to polymer chain-entanglement between the interfaces of the nanoparticles. The polymer nanoparticle is similar to a wad of cotton although the surface looks like that of a sleek billiard ball in SEM images. It consists of numerous polymer chains which are entangled with each other in a complicated manner. Some of them even protrude to the outside like fuzz. The polymer chains of two particles become tangled together and the particles are physically connected when the polymer nanoparticle comes into contact with the other one. That is the reason how deposited layers of nanoparticles can sustain the structures during additional deposit protocols. These factors make it possible to control the drug mass deposited on stents easily.

Conclusions

We obtained PLGA nanoparticle-coated stents by the novel deposition method. The SEM images proved that the nanoparticles could be coated on stents and the layers of the nanoparticles had the physical strength to sustain the layer structures. The amount of paclitaxel was easily controlled through varying the drug content of the nanoparticles, the concentration of the nanoparticle suspension and the count of deposition.

Acknowledgments. The authors acknowledge the KOSEF S&T graduate scholarship (J. Joo and S. H. Nam).

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