Curcumin-Loaded PLGA Nanoparticles Coating onto Metal Stent by Electrophoretic Deposition Techniques

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Restenosis after percutaneous coronary intervention (PCI) continues to be a serious problem in clinical cardiology. To solve this problem, drug eluting stents (DES) with antiproliferative agents have been developed. Variable local drug delivery systems in the context of stenting require the development of stent manufacture, drug pharmacology and coating technology. We have worked on a system that integrates electrophoretic deposition (EPD) technology with the polymeric nanoparticles in DES for local drug delivery and a controlled release system. The surface morphology and drug loading amount of DES by EPD have been investigated under different operational conditions, such as operation time, voltage and the composition of media. We prepared poly-D,L-lactide-co-glycolic acid (PLGA) nanoparticles embedded with curcumin, which was done by a modified spontaneous emulsification method and used polyacrylic acid (PAA) as a surfactant because its carboxylic group contribute negative charge to the surface of CPNPs (−53.5 ± 5.8 mV). In the process of 'trial and error' endeavors, we found that it is easy to control the drug loading amount deposited onto the stent while keeping uniform surface morphology. Accordingly, stent coating by EPD has a wide application to the modification of DES using various kinds of nanoparticles and drugs.

Key Words : Curcumin, PLGA, Nanoparticles, Stent, Electrophoretic deposition

Introduction

Coronary stents are the biggest advance in the treatment of obstructive coronary artery disease since the advent of balloon angioplasty. However, restenosis after angioplasty and stenting occurs followed by the accumulation of vascular smooth muscle cell (SMCs) in response to stent-induced inflammatory reactions and remains a significant clinical challenge. In the past, a lot of efforts have been devoted to manufacturing mechanical devices and to the search for drugs to prevent restenosis.[1] As a result of many unceasing "trial-and error" endeavors, drug eluting stents (DES), which are coated stents capable of releasing single or multiple bioactive agents for inhibiting neointimal hyperplasia into the blood stream and surrounding tissues, are now regarded as a potential solution for restenosis.[3,4] Applying a local drug delivery concept, DES is a promising approach to the treatment of both stent-induced geometric remodeling and neointimal hyperplasia. Many researchers have used polymeric nanoparticles as drug carriers of DES for a programmable and sustained release. However, one of the challenges is to develop new techniques for the uniform coating of nanoparticles on the three dimensional stent to overcome the damage caused by the luminal environment of the blood vessel. Thus this report describes the formation of drug loaded polymeric nanoparticles capable of controlled drug release by a novel coating method using electrophoretic deposition (EPD) for loading nanoparticles onto the stent.

EPD represents an important alternative coating technology, due to its high reproducibility, rapid coating process, and the low cost of the process. These represent clear advantages over dipping and spray coating methods, which are general methods for stent coating. EPD involves the formation of an electrical layer around particles suspended in a fluid medium, which causes motion in the existence of a spatially uniform field. Under the influence of the electric field, the charged particles move towards the oppositely charged electrode and are deposited onto the substrates, forming a relatively dense and homogeneous compact particles.[5-7]

Over the past few decades, there have been considerable interests in developing biodegradable nanoparticles as effective drug delivery carriers. Various polymers have been used in drug delivery research that can effectively deliver the drug to a target site and increase the therapeutic effect, while minimizing any side effects.[8,9] Curcumin (1,7-Bis(4-hydroxy-3-methoxy phenyl)-1-6-heptadiene-3,5-dione) is a naturally occurring yellow compound found in the rhizome of the plant *Curcuma longa*. It has been found to be safe and effective in many clinical situations. There are many studies reporting on the toxicology, pharmacokinetics and biologically effective dosage of curcumin in humans. Extensive investigations indicate that curcumin reduces blood cholesterol, prevents LDL oxidation, inhibits platelet aggregation, suppresses thrombosis and prevents certain cancers.[11-15] There are no reports investigating DES coated by the EPD method, and specifically those using Curcumin-loaded PLGA nanoparticles (CPNPs). This study was undertaken to work on developing a system that integrates EPD with the coating of bare metal stent with CPNPs.

Materials and Methods

Materials. Poly (D,L-lactide-co-glycolic acid) (PLGA, lactide:glycolide = 50:50, Mw 30,000) was supplied by Boehringer Ingelheim (Ingelhdim, Germany). The number indicates the monomer ratio of mol % between lactide verses glycolide in the copolymer. Polyacrylic acid (PAA, Av, Mw ~5,000, 50 wt % in solution in water), the surfactant, was purchased from Aldrich Chemical company (USA). Curcumin was purchased from Sigma chemical company (USA). Bare metal stent was purchased from Humed (republic of Korea). Absolute grade Ethanol was purchased from MERCK (USA). HPLC grade Acetone was purchased from Fisher Scientifics (USA). All other reagents were of analytical grade and used without further purification.

Preparation of curcumin-loaded PLGA nanoparticles. Curcumin-loaded PLGA nanoparticles (CPNPs) were prepared according to a modified version of the spontaneous emulsification method. In brief, a given amount of curcumin and PLGA were dissolved in 1 mL acetone. The resulting solution was poured drop by drop into 10 mL aqueous solution containing 0.2% v/v PAA and was kept under bath sonication for 2 min. The emulsion was evaporated for 30 min with magnetic stirring to remove the organic solvent. After emulsification, this solution was diluted in ionized water in order to determine size and zeta potential values. The CPNPs were diluted in media that consisted of ionized water and absolute ethanol for EPD.

Chacracterization of curcumin loaded PLGA nanoparticles and morphology of coated stent with CPNPs. The size and zeta potential values of CPNPs were determined by the Malvern Zetasizer 3000HAs system (Malvern Instruments, Worcestershire, UK) at 25 °C at an angle of 90° using PCS 1.61 software. For the measurement, freshly prepared particles were appropriately diluted in deionized water.

The surface morphology of the CPNPs and CPNPs-coated stent was studied by scanning electron microscope (SEM, JSM 640-A. Jeol Ltd, Tokyo, Japan). The coated stent was dried overnight in a chamber containing P_2O_5 after EPD and mounted on an aluminum stub, sputter coated with 100 layer of platinum (Cressington 108, Jeol, Ltd, Tokyo, Japan) and viewed under SEM.

Quantification of the loaded drug value on stent. The amount of entrapped curcumin in CPNPs and quantification of the loaded value of curcumin on stent was determined by a high-performance liquid chromatograph (HPLC; Agilent 1100 Series, USA)equipped with a C8 analytic column (4.6 × 250 mm, particle size 5 μm, ThermoQuest, BDS, Runcorn, UK). The flow rate of the mobile phase (60% methanol and 40% deionized water by v/v), delivered by an HPLC pump (TCP, P-100, Riviera Beach, FL, USA), was 1 mL/min at 60 ± 2 °C. The eluent was monitored with an UV detector at 420 nm. Under these conditions, curcumin was eluted at 8 min .

Electrophoretic deposition. The stent was coated with CPNPs by EPD. EPD was achieved via the forced motion of charged particles under an applied voltage towards an electrode of the opposite charge, followed by the coagulation of the particles to form a deposit. The electrodes used in the present work were a stainless steel (316L) plate and a stainless steel (316L) stent. They were arranged in a 5 mm apart parallel configuration. Both electrodes were immersed in a container holding a liquid medium containing CPNPs, prepared by the mixture of ethanol and deionized water. The portion of ethanol in the mixture of ethanol and deionized water was tested in an increasing ratio such as 0%, 10%, 20%, 30%, 40% and 50%. The prepared CPNPs solution was diluted using the mixture of ethanol and deionized water. The concentrations of CPNPs in solution were 0.02%, 0.050%, 0.075% and 0.100%. EPD was carried out under various conditions of voltage and time. We applied voltages of 5 V, 9 V and 13 V and set EPD times were set to 10 min, 20 min, 30 min, 40 min and 60 min. The concentration of CPNPs means wt % value of total weight sum of polymer and drugs used for CPNPs preparation in media.

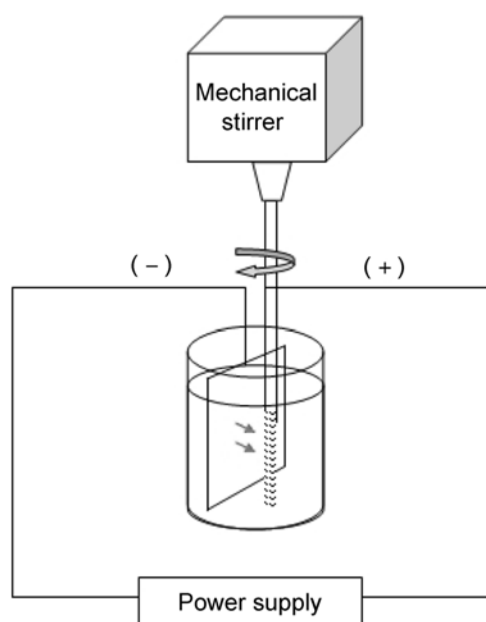

Figure 1. Schematic of coating apparatus: Electrophoretic depositon (EPD).

The stainless steel plate, as the anode, was connected to the negatively charged terminal of a power supply. The stent, as the cathode, was connected to the positively charged terminal of a power supply. Since the stent has cylindrical structure, it was constantly rotated to give a uniform CPNPs coating. The EPD was carried out in an ice bath in order to diminish the heat generated during the process. The schematics of the EPD experiments are given in Figure 1.

Results and Discussion

Preparation and characterization of curcumin loaded PLGA nanoparticles. The size of CPNPs was shown to be 276.0 ± 3.4 nm by dynamic laser light scattering. The zeta potential of CPNPs was measured to be −53.5 ± 5.8 mV. The

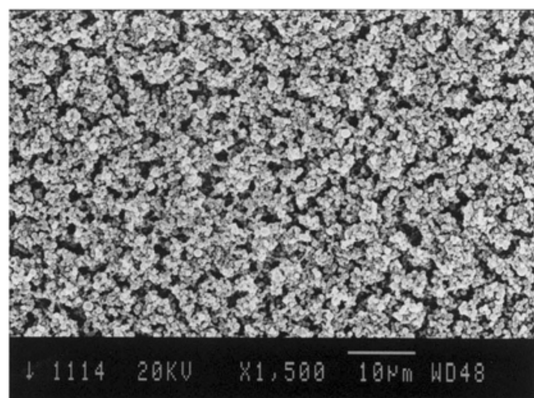

Figure 2. SEM images of curcumin-PLGA-NPs.

surface morphology of CPNPs was observed by SEM, which is shown in Figure 2. Aliphatic polyesters such as poly-(lactide) and its copolymers with glycolic acid have received considerable interests because they are biodegradable and biocompatible synthetic polymers which degrade to lactides and glycolic acids *in vivo*, which are subsequently eliminated as carbon dioxide and water via the Krebs cycle. These polymers is degraded by simple hydrolysis of the ester linkages; a process which is both acid and base catalyzed as well as enzymatically catalysed.[16] Due to its biocompatibility and biodegradability, PLGA could be a good candidate for the use in engineered particles for drug detoxification.

Polymeric nanoparticles offer some specific advantages; for instance, nanoparticles help to increase the stability of drugs and possess useful controlled release properties.[17,18] Nanoparticles generally vary in size from 10 to 1000 nm. It has been reported that microparticles (5-10 μm) give rise to inflammatory reactions causing fibrosis, but nanoparticles (200-500 nm) usually cause little or no focal inflammation.[19,20] Therefore, CPNPs (276.0 ± 3.4 nm) do not cause inflammatory response or any other condition that is likely to be problematic. Drug loaded PLGA NPs can be prepared by various methods. The drug is dissolved, entrapped, encapsulated or attached to a NP matrix, which depends upon the method of preparation.[8,10] The carboxyl group of PLGA could account for the negative potential of 53.5 ± 5.8 mV. Moreover, the carboxyl group of PAA, a surfactant, could play an important role in enhancing the negative potential of CPNPs. However, CPNPs aggregated after modified spontaneous emulsification which could not easily disperse in media because of the high viscosity of PAA. Therefore we diluted the CPNPs solution with a mixture of ethanol and deionized water instead of the usual washing process after CPNPs formation. The remaining PAA was dissolved in the media and had no effect on EPD because it was diluted more than thirtyfold at the maximum.

There are many kinds of drugs having an anti-restenosis effect.[1,21] We selected curcumin as it has been reported to suppress proliferation of a variety of cells, down-regulates transcription factors NF-kb, AP1, and Egr-1, and inhibits the expression of COX-2, NOS, MMP-9, TNF and cell adhesion molecules.[11-14] However, curcumin has poor solubility in methylene chloride (MC), used in the solvent evaporation method, but good solubility in acetone. For that reason, we modified the spontaneous emulsification method. Usually, in the spontaneous emulsification method, the polymer and drugs are dissolved or dispersed in an organic solvent and then emulsified into an aqueous solution containing a surfactant. In our experiment, we chose acetone as a water-miscible organic solvent that can dissolve PLGA and curcumin, and PAA as a surfactant or stabilizer due to its carboxyl group. As previously recommended, this carboxyl group also helps to enhance the negative charge of CPNPs and plays an important role in EPD. In order to decrease the concentration of a surfactant in the process of EPD, we diluted CPNPs solution in a liquid medium solution. SEM particle images taken after dilution exhibit a very clear shape compared to that of CPNPs without dilution, in which we can find blurred images due to the remaining PAA around CPNPs.

Stent coating with CPNPs by electrophoretic deposition. In order to control the drug loading amounts, we experimented by varying the conditions of EPD. EPD performed at a potential higher than 1 V led to water decomposition, where hydrogen absorption onto the surface of anode (stainless steel, substrates) occurred, resulting in heterogenous deposition. In addition, a high electric field was necessary to induce substantial particle motion.[22,23]

Concentrations of the mixture of ethanol and deionized water were varied from 10% to 50% ethanol. Usually, EPD is performed at 50% of EtOH media because there is no generation of O_2 and H_2 gas by water decomposition. Unfortunately, because CPNPs almost melt in 50% EtOH, we tried to find the appropriate concentration of the media. The amount of curcumin deposited onto stent under various media conditions is indicated in Table 1, and Figure 3 shows the surface morphology of CPNPs loaded stents under different media condition. The voltage was fixed at 9 V for 30 min during EPD and a concentration of 0.075% of CPNPs in the media. The amount of curcumin deposited onto stent in 50% EtOH media is about 0.71 μg in comparison to 491 μg in 100% deionized water. The higher the concentration of EtOH, the lower the amount of curcumin, and in particular, it dramatically decreased at more than 30% EtOH. In 100% deionized water, however, we observed a hollow hemisphere shape on the surface of stent after EPD, as shown through Figure 3. In our results, 10% ethanol

Table 1. Total amount of drug onto stent on condition with various media, 9 V, 30 min and 0.075% CPNPs (μg/stent)

% of ethanol	Num. 1	Num. 2	Num. 3	Num. 4	Num. 5	Average	Standard deviation
0%	481.17	502.60	491.89	515.24	467.76	491.73	18.42
10%	246.72	265.71	269.40	260.70	266.00	261.71	8.93
20%	234.58	210.22	231.23	269.98	187.97	226.80	30.53
30%	26.21	29.11	29.75	28.09	24.89	27.61	2.03
40%	2.87	2.79	2.35	3.00	3.28	2.86	0.34
50%	0.60	0.89	0.51	0.77	0.77	0.71	0.15

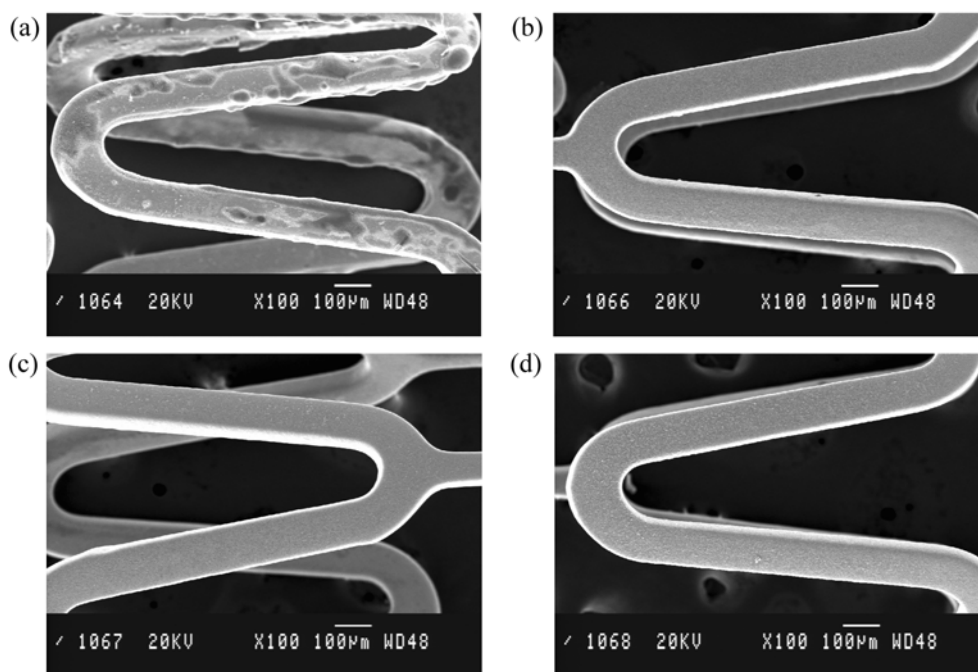

Figure 3. SEM image of curcumin-PLGA-NPs coated stent with various media conditions, 9 V and 0.075% CPNPs (a) 100% deionized water (b) 10% ethanol and 90% deionized water (c) 30% ethanol and 70% deionized water (d) 50% ethanol and 50% deionized water.

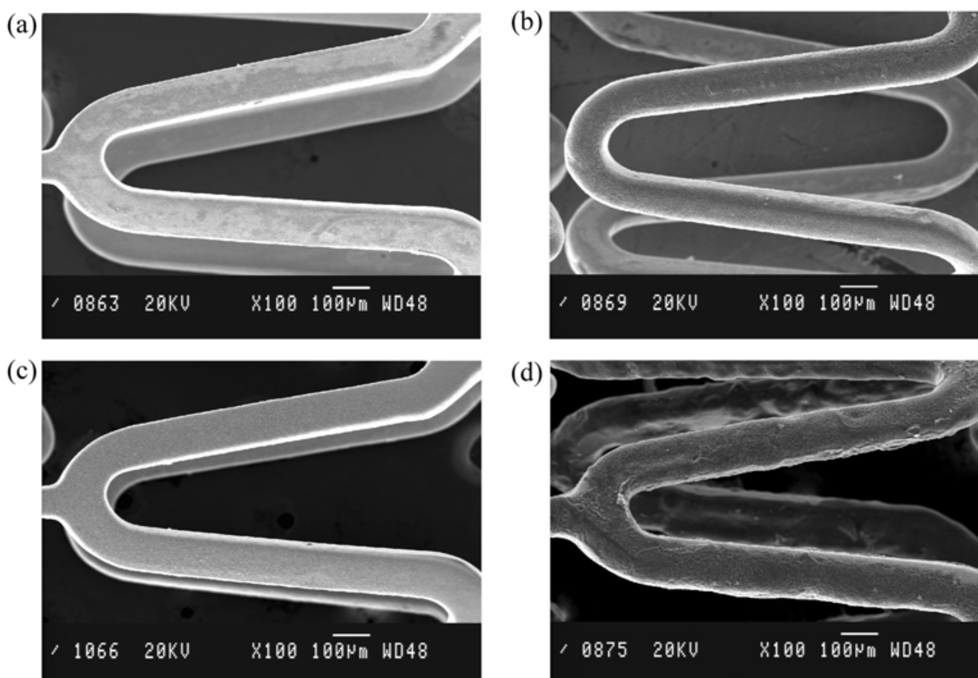

Figure 4. SEM image of curcumin-PLGA-NPs coated stent on condition with various curcumin-PLGA-NPs concentrations, mixture of 10% ethanol and deionized water and 9 V: (a) 0.025% (b) 0.050% (c) 0.075% (d) 0.100%

mixture was the best choice for EPD from the point of surface morphology and drug loading amount.

Next, we changed the concentration of CPNPs solution within fixed conditions of 9 V in 10% EtOH for 30 min. The concentration of CPNPs solution varied from 0.025% to 0.100%. The amount of curcumin deposited onto stent in various concentrations of CPNPs solution is shown in Table 2, and Figure 4 represents the surface morphology of stent after EPD. Increased concentration of CPNPs resulted in an increased amount of loaded curcumin. However, CPNPs were clumped at 0.100% CPNPs solution. SEM images showed a uniform and flat surface morphology of stent after EPD, except for the case of 0.100% CPNPs. Table 3 and Figure 5 indicate that the amount of curcumin increases at

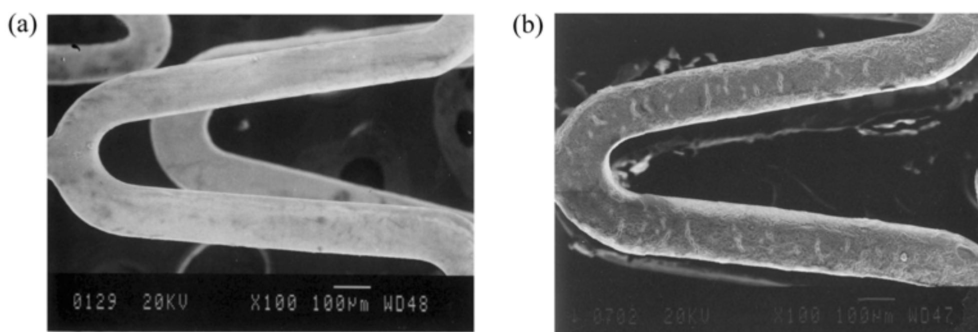

Figure 5. SEM image of curcumin-PLGA-NPs coated stent on condition with various Voltage, mixture of 10% ethanol and deionized water and 0.075% curcumin-PLGA-NPs: (a) 5 V (b) 13V

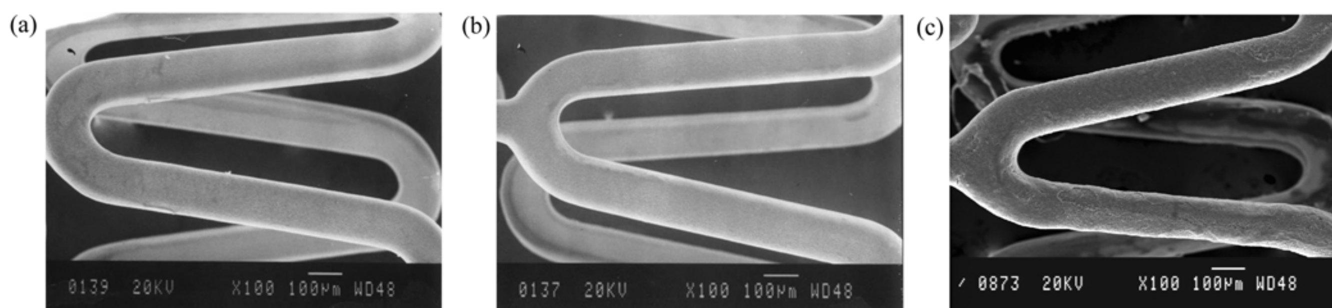

Figure 6. SEM image of curcumin-PLGA-NPs coated stent on condition with various coating time, mixture of 10% ethanol and deionized water and 0.075% curcumin-PLGA-NPs: (a) 10 min (b) 20 min (c) 60 min

Table 2. Total amount of drug onto stent on condition with various CPNPs concentration, the mixture of 10% ethanol and deionized water, 9 V and 30 min (μg/stent)

% of CPNPs	Num. 1	Num. 2	Num. 3	Num. 4	Num. 5	Average	Standard deviation
0.025%	23.29	25.28	23.19	22.62	26.04	24.08	1.49
0.050%	133.03	175.35	146.75	166.53	189.57	162.25	22.53
0.075%	246.72	265.71	269.40	260.70	266.00	261.71	8.93
0.100%	362.98	484.50	489.39	467.70	381.72	437.26	60.16

Table 3. Total amount of drug onto stent on condition with various Voltage, media of 10% ethanol and 90% deionized water, 30 min and 0.075% curcumin-PLGA-NPs (μg/stent)

Voltage	Num. 1	Num. 2	Num. 3	Num. 4	Num. 5	Average	Standard deviation
5 V	30.91	27.01	25.50	30.11	30.29	28.76	2.37
9 V	246.72	265.71	269.40	260.70	266.00	261.71	8.93
13 V	464.88	480.60	480.22	421.98	478.44	465.22	25.03

higher voltages, and there are empty spaces in stent under 5 V. Under various time conditions, many CPNPs remained on the stent for just 10 min under EPD (Fig. 5), but irregular surfaces were found in the case of 60 min after EPD because CPNPs coagulated.

From these various tests, we found that EPD is a commendable coating method for controlling the amount of drug loading on the stent and creating uniform surface morphology.

Table 4. Total amount of drug onto stent on condition with various coating time, on condition with various coating time, mixture of 10% ethanol and deionized water and 0.075% curcumin-PLGA-NPs (μg/stent)

Time	Num. 1	Num. 2	Num. 3	Num. 4	Num. 5	Average	Standard deviation
10 min	79.25	0.36	61.64	67.95	73.98	70.64	6.59
20 min	216.09	204.28	219.88	196.73	196.73	206.74	10.80
30 min	246.72	265.71	269.40	260.70	266.00	261.71	8.93
40 min	384.03	347.52	351.97	400.10	411.33	378.99	28.45
60 min	430.80	421.92	405.99	424.74	455.77	427.84	18.10

Conclusions

In summary, EPD is a very reasonable and flexible method for coating CPNPs onto a stent. We demonstrated that the amount of drug deposited onto the stent can be controlled by using EPD, and also found a method for forming curcumin-PLGA NPs. In this study, we chose curcumin-PLGA NPs as a suitable candidate, but it is possible to apply these methods to many kinds of NPs having negative or positive charges. Further experiments and clinical studies are needed to control the pattern of drug release and determine the release kinetics and the effects under realistic biological conditions.

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