1. Introduction

Coronary artery disease is mainly caused by atherosclerosis and is known to be a leading cause of death in modern society. The most common strategy for cardiovascular disease treatment is the use of vascular stents[1,2]. Drug eluting stents (DESs), which are useful for remarkably reducing the stenosis rate, have been developed and have led to a new phase of interventional cardiology in the past decade[3,4]. However, DESs have vital limitations when used for treating coronary atherosclerotic diseases, such as recurrent stenosis and late stent thrombosis. Recent studies have indicated that DESs leads to complications such as stent thrombosis, sensitive response to polymers, poor adherence between the stent and the arterial wall, and, even worse, aneurysms[5,6].

Various synthetic polymers, such as fluoropolymer, poly(glycolide), and polylactides have been used to fabricate DES because of their bio-degradability and ability to control drug release[7,8]. However, synthetic polymer-based DES can cause adverse effects such as inflammation and thrombosis[9,10]. Moreover, polymer degradation may cause the formation of fragments, which can lead to emboli[11,12]. Other methods for generating intelligent biocompatible polymeric interfaces are needed to overcome the numerous side effects of stent implantation[6,13].

Dextran is a biodegradable and biocompatible polysaccharide predominantly consisting of 1,6-glucosidic linkage[14]. As a biodegradable polymer, it is very attractive because of its colloidal biocompatibility and hence is widely used in commercial drug formulations[15]. Many studies have focused on the Biosafety and effectiveness of dextran sulfate (DS) and diethylaminoethyl dextran-dextran (DEAE-DS) for hybridization experiments and because of their anti-viral and anti-inflammatory characteristics. Further, DS and DEAE-DS with PEM react with enzymes and cells and can be used for the stabilization of proteins[16].

Synthetic polymer-based drug-eluting stents may cause inflammations. Therefore, various natural polymers have been suggested as alternatives to synthetic polymers for drug coating. Dextran, a natural biopolymer, is easily obtained by polyelectrolyte multilayer (PEM) interaction, and it inhibits smooth muscle cell (SMC) proliferation. The aim of this study was to fabricate a dextran layer on a bare metal stent (BMS) and to evaluate the layer’s bio-physical properties.

The polyelectrolyte multilayer (PEM) interaction of polycations and polyanions has emerged as an efficient, versatile, and simple technique for generating intelligent biocompatible polymeric interfaces. Fabrication and Evaluation of Polyelectrolyte Complexes of Dextran Derivatives for Drug Coating of Coronary Stents

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Abstract

The aim of this study was to fabricate a dextran polyelectrolyte multi-layer on a bare metal stent (BMS) and to evaluate bio-physical properties of the layer. Diethylaminoethyl-dextran (DEAE-D) as a polycation and dextran sulfate (DS) as a polyanion were successively coated on the bare metal stent by a well-known layer-by-layer procedure. The morphology of the stent surface and its cell adhesion were studied after each coating step by scanning electron microscopy. The stent showed more blotched and slightly rougher morphology after dextran-DS coating. The contact angle of the DEAE-DS group (39.5 ± 0.15°) was significantly higher than that of the BMS group (45.16 ± 0.08°), indicating the improvement of hydrophilic. The SMC proliferation inhibition in the DEAE-DS-coated stent group (20.9 ± 0.04%) was stronger than that in the control group (21.7 ± 0.10% in DS-coated group only). The DEAE-DS coating is desired for stent coating materials with bio-compatibility and anti-restenosis effect.

Keywords: Dual drug-eluting stent, Electrolyte coating, Dextran, Nature polymer, Anti-restenosis
to create biologically active surfaces. The method relies on the sequential charge inversion of a polymeric surface followed by successive immersions of this surface in solutions of oppositely charged polyelectrolytes[17]. The PEM technique has been used to coat bioactive molecules, such as drugs, enzymes, DNA, or proteins, on various substrates[18]. This process can be easily applied to a wide variety of substrates and involves the incorporation of bioactive molecules in multilayers during the polyelectrolyte deposition process, yielding drug-releasing interfaces[19].

In this study, biocompatible DEAE-DS and DS were used to confirm the physical properties and cell effects of the stent surface.

## 2. Material and Methods

2.1. Preparation of bare metal stent

Previously, we reported a customized bare metal stent (BMS) (designated as the CNUH stent). It showed excellent flexibility and biocompatibility and moderate foreshortening and recoil under Food and Drug Administration (FDA) guidelines[20,21]. Therefore, it was utilized as the platform for dextran derivative coating. Briefly, a cobalt-chromium alloy (Co-Cr alloy, 3.0 × 18.0 mm², L605) was used as the stent material, as this alloy has been reported to be the most suitable in terms of biocompatibility[22]. Fabrication of the BMS with the Co-Cr alloy was performed using a laser cutter (Rofin, Starcut, Hamburg, Germany). Thereafter, the BMS was exposed to an acidic atmosphere (50% H₂SO₄) for 1 h to remove the burr. Heat treatment and a polish were performed to restore the mechanical properties. The cleaned BMS was heated in a vacuum oven at 60 °C for 2 h to evaporate the residual water.

2.2. Polyelectrolyte multilayer coating on bare metal stent

The polycation was DEAE-DS and the polyanion was DS, both purchased from Sigma-Aldrich, UK, and with a mass of 500 kDa (Figure 1). Polyelectrolyte solution of DS was deposited on the cleaned stent to create negative charges on its surface: the stent was first immersed in a 1 M sodium hydroxide (NaOH) solution heated at 90 °C for 30 min and then in a 1 M hydrogen chloride (HCl) solution at 34 °C room temperature for 10 min. Polyelectrolyte solutions of DEAE-DS were prepared at a concentration of 5 mg/mL in a 0.15 M NaCl solution at physiological pH. The stent was alternately dipped in baths containing each solution for 10 min[23].

2.3. Scanning electron microscopy

The micromorphology of DS-coated and DEAE-DS-coated stents was observed by scanning electron microscopy (Mini-SEM, SNE-300M, SEC Co. Ltd., Korea) at an acceleration voltage of 5 kV. The samples were dried overnight and sputter-coated using gold prior to SEM observations.

2.4. Contact angle measurements

Contact angles were obtained by the static contact angle measurements of deionized water droplets. Contact angles of the cobalt-chromium surface coated with different numbers of dextran layers were measured using a contact angle system by titrating a 1 µL droplet of deionized water on the sample surfaces (bioactive coatings of endovascular stents based on polyelectrolyte multilayers - Benjamin Thierry).

2.5. In vitro cell proliferation

The in vitro cytotoxicity of DS and DEAE-DS-coated stent was assessed by the 2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay. Briefly, 1 × 10⁴ smooth muscle cells (SMCs) were seeded on BMS, DS-coated stent and DAEA-DS coated stent in 96-well plates and allowed to attach for 24 h. The cells were incubated with DS-coated stent and DEAE-DS-coated stent for 24 h at 37 °C. The culture plate was incubated at 37 °C in a humidified atmosphere of 5% CO₂. Cell proliferation was evaluated by the XTT assay using an EZ-cytox cell-viability assay kit (Daeil Lab Service Co., Seoul, Korea). Briefly, 40 µL of the EZ-cytox reagent was added to each well of a 24-well culture plate. By the action of mitochondrial dehydrogenases, XTT was metabolized to form a formazan dye that could be spectrophotometrically assayed by measuring the absorbance at 450 nm using a microplate reader (Bio-Tek Instruments, Winooski, VT). The amount of formazan salt corresponded to the number of viable cells in each well (n = 5). The experiments were performed as independent duplicates.

2.6. Immunochemistry

Cells were seeded at a density of 1 × 10⁴ cells/well onto non-coated glass cover slips (20 mm) (Thermo-Scientific) in 35-mm culture dishes and quiesced by serum deprivation for 24 h. The cover slips were sterilized in industrial methylated spirit and washed twice in phosphate buffer saline (PBS) prior to culturing. The culture media were then replaced by a medium containing 10% fetal bovine serum and cultured again for 48 h. The cells were washed twice in PBS and fixed for 15 min at room temperature with 3.7% paraformaldehyde prepared in PBS, washed twice with PBS again, and then permeabilized by room-temperature exposure to a 0.1% Triton X-100/PBS solution for 15 min. The cells were washed, blocked for 1 h with a PBS solution containing

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**Figure 1. Chemical structures of DEAE-dextran (DEAE-DS) (A) and dextran sulfate (DS) (B).**
5% bovine serum albumin and 1% tween before treatment with specific primary antibodies. The secondary antibodies used were Alexa Fluor 488 goat anti-rabbit (Invitrogen). Nuclei were stained with 4,6-diamidino-2-phenylindole at a concentration of 2 µL/mL in PBS at room temperature for 10 min. Fluorescent images were acquired using an Olympus DP 50 fluorescent microscope with the appropriate excitation and emission spectra at 4 ×, 10 ×, 20 ×, and 60 × magnifications. Non-specific labeling was assessed following the secondary antibody treatment.

2.7. Statical analyses
All data presented are the mean SD. A one-way analysis of variance (ANOVA) was performed and statistically significant differences were defined as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

3. Results & Discussion

3.1. Surface morphology observations
Stent thrombosis remain an important concern after stent implantation. Polymers used to encapsulate these anti-proliferative drugs have been associated with DES thrombosis. Natural polymers better resemble living tissue than synthetic polymers due to their high water absorption; furthermore, this high water absorption contributes to their bio-compatibility[24]. Dextran is highly biocompatible and has been used as a drug coating. In this study, DS and DEAE-DS (Figure 1) were successively coated onto the CNUH stent as BMS by PEM.

The stent surface morphology was investigated by SEM. The stent showed more blotted and slightly rougher morphology after dextran-DS coating (Figure 2B and C) compared to the BMS group (Figure 2A and B). It has been confirmed that the physical properties of the surface are different by the PEM coating. By applying PEM coating to BMS, it is expected to obtain different results physically and histologically.

3.2. Hydrophilic and water-absorption properties
The effect of the hydrophilic properties of DADE-DS coated stent was examined using water contact angle analysis. The first step in the PEM coating procedure was the adsorption of the PEM layer onto the substrate to yield a positively charged surface; this surface was then placed in contact with a solution of the polyanion DS and a solution of the polycation DEAE-DS. Figure 3 shows the contact angle of BMS, DS-coated stent, and DEAE-DS coated stent. The contact angle of the DEAE-DS group was significantly higher than that of the BMS group ($39.5 \pm 0.15$ vs. $72.7 \pm 0.02$, $n = 10$, $p = 0.002$, Figure 3), indicating the improvement of hydrophilic.

Contact angle measurements showed that these hydrophilic materials decreased the bacterial adherence, which was related to the hydrophobicity of the bacteria. It has been reported that certain hydrophilic materials cause a decrease in bacterial adherence, probably because of the hydrophobicity of the bacteria[25,26]. Dextran is hydrophilic on its first contact with water, becoming very hydrophilic on prolonged water contact, suggesting water uptake. Hydrophobic surfaces tend to absorb a larger amount of proteins that hydrophilic ones. As a result, dextran is beneficial for decreasing protein absorption compared to other more hydrophobic materials such as synthetic polymers[27]. These results indicate that coating with dextran is a biocompatible method for surface modification.

3.3. Cell attachment and morphology
In this study, cell attachment and morphology were analyzed by fluorescence microscopy to understand the interactions of cells with a substrate. The comparison of the number of cells grown on different surfaces and the drug effect of these surfaces was analyzed. The fluorescence microscopic images indicated that both DS and DEAE-DS offered favorable conditions for SMC inhibition. Figure 4 shows the typical fluorescence microscopic images of vascular SMCs. Cell adhesion was morphologically analyzed to prove the SMC inhibition effect of DS and DEAE-DS. As shown in Figure 4, the DEAE-DS-coated stent confirmed that adhesion of SMC was suppressed. In addition, DEAE-DS-coated stent inhibited cell adhesion rather than BMS. DEAE-DS-coated stent confirmed the SMC adhesion inhibitory effect more than BMS and DS-coated stent.

3.4. Suppression efficiency of smooth muscle cell proliferation
The XTT assay showed that SMC inhibition for 1 h was always followed by a decrease in the XTT after 24 h of exposure to DS and DEAE-DS-coated stent. This result was consistent with a previous report that dextran suppresses SMC growth on the stent surface[28]. Suppression of SMC proliferation is a very important aspect of improving the performance of a stent. The XTT assay was performed to...
investigate the inhibitory effect of dextran on SMC proliferation. The SMC proliferation inhibition in the dextran-PEM coating group was stronger than that in the control group (20.9 ± 0.04% in DEAE-dextran and 21.7 ± 0.10% in DS, n = 10, p = 0.002, Figure 5).

4. Conclusion

This study has demonstrated the feasibility of coating Co-Cr alloy stent with DEAE-DS via PEM technique in DS and DEAE-DS. The coating is uniformly covered all surfaces of the stent and slightly rougher morphology after DEAE-DS coating. DEAE-DS coated stent showed the lower water contact angle, meaning it can be hydrophilic. Also, DEAE-DS-coated stent inhibited cell adhesion and SMC proliferation rather than BMS. For future, we will evaluate in-stent thrombosis until reendothelialization.

These results indicate that coating with dextran is a biocompatible method for surface modification. Contact angle measurements showed that these hydrophilic materials decreased the cell adherence. Suppression of SMC proliferation is a very important aspect of improving the performance of a stent. The proposed DAEA-DS by PEM coatings technique holds great potential in clinical applications for stent implantation to treat damaged blood vessels. This suggests that DAEA-DS by PEM coatings technique can change the surface properties of various materials.

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REFERENCES

1. S. Tsimikas, Drug-eluting stents and late adverse clinical outcomes


