의학박사 학위논문

견종 경동맥을 이용한 자가 팽창성, 생분해성 폴리다이옥사논 스텐트의 생체적합성 연구

Biocompatibility of self-expanding, biodegradable polydioxanone stents in normal canine carotid arteries

2021년 2월

서울대학교 대학원

의학과 뇌신경과학 전공

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지도교수 오 창 완

이 논문을 의학박사 학위 논문으로 제출함

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ABSTRACT

Biocompatibility of self-expanding, biodegradable polydioxanone stents in normal canine carotid arteries

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Introduction

We report a preclinical study of polydioxanone (PDO) self-expanding, biodegradable, intracranial vascular stents for biocompatibility after experimental placement in canine carotid arteries and compare phosphorylcholine-coated and non-coated PDO stents.

Materials and Methods

All experiments were approved by the committee of animal research. *In vitro* PDO filament degradation and platelet adhesion tests were performed. A total of 12 PDO stents and 12 phosphorylcholine-coated PDO stents were implanted in normal canine carotid arteries of 3.0- to 4.0-mm diameter in six dogs. The dogs were divided into three groups with 2 dogs per group. Each group was sacrificed at 4, 8, and 12 weeks after stent placement, respectively. Stent

patency was assessed by angiography followed by a microscopic histological examination of the dissected carotid arteries.

Results

Stent deployment was technically successful in all dogs without procedure-related complications. On angiographic analysis, in-stent stenosis (9.48 ± 7.13%) was documented in both stent groups at 4 weeks after implantation. Fifty percent of implanted carotid arteries were completely occluded with increased in-stent stenosis (15.80 ± 12.32%) in the other patent vessel at 8 weeks. Total occlusion at 12 weeks was observed in all implanted vessels. PDO filaments began to demonstrate a loss of 5% of their original mass by 4 weeks. PDO filaments started to degrade at 4 weeks and by 16 weeks reached about 50% of their original weight by using *in vitro* degradation examination. On histologic examination, the degradation of PDO stents started at 4-8 week and at 12 week periods, fragmentation of the braid was observed. According to the degradation of PDO polymer, PDO stents evoked extensive inflammatory responses at 8 and 12 week periods. Inflammatory scores (0-4) were 1.63 ± 0.71 at 4 weeks, 1.79 ± 0.83 at 8 weeks (p=0.360) and 2.33 ± 0.96 at 12 weeks (p=0.008). Although the phosphorylcholine-coated PDO stent showed statistical significance in platelet adhesion inhibition on *in vitro* platelet adhesions test compared with non-coated PDO stent, there was no different *in vivo* biocompatibility, such as luminal thrombosis formation and endothelialization between both stent groups. And, lower yet still severe inflammatory responses were observed for the phosphorylcholine-coated PDO stent group. Luminal thrombosis formation and histomorphometric stenosis also increased with increasing time intervals.

Conclusion

The PDO stent induced an inflammatory reaction within the carotid artery with subsequent neo-intimal thickening. In 4-week follow-up period before polymer degradation, the vessel patency was preserved with mild in-stent stenosis. However, the degree of stenosis progressed according to the time interval and finally vessel occlusion was occurred. The observed tissue response may be attributable to the degradation of the polymer, which induced an inflammatory response, resulting in the occlusion of the arteries. The phosphorylcholine-coating did not show any difference compared with the non-coated PDO stent.

Keywords: biocompatibility, biodegradable vascular stent, canine carotid artery, phosphorylcholine, polydioxanone

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INTRODUCTION

Intracranial stents have revolutionized the treatment of intracranial aneurysms by significantly increasing the success rate of coil embolization and lowering the rate of recanalization. However, the presence of a permanent metallic stent in the vasculature may impose long-term mechanical stress, leading to low-grade injury and persistent inflammation.[1] In addition, the permanent metal struts can inhibit re-intervention, interfere with normal vasomotion, and prevent the normal healing response of the vessel.[2, 3] Biodegradable polymeric stents may deliver an ideal solution by providing acute luminal patency while completely absorbing after endothelialization and vascular remodeling have occurred.

Biodegradable polymeric intravascular stents are promising devices for blood vessels that are narrowed or occluded by disease without long-term thrombosis and restenosis, as often reported in permanent metal stents.[4-7] These materials have shown good *in vitro* biocompatibility[8-10] and have been widely used in the body as sutures, orthopedic tissue fixation devices, and drug delivery systems.[11] Biodegradable polymers have also shown good biocompatibility in applications outside the vasculature such as airway, hepatobiliary, intestine, and urinary systems.[12] Additionally, the mechanical strength and degradation profile of these polymers can be tailored by selecting different monomers and monomer compositions.[13] Finally, biodegradable polymers degrade via hydrolysis into metabolites that can be safely eliminated from the body through citric acid cycles.[14]

However, it is unknown whether tissue compatibility data established from

in vitro or small animal model studies adequately reflect intravascular compatibility. Some studies have found a strong inflammatory response to polymers placed in the vasculature: Van der Giessen and colleagues implanted five degradable polymers into the vasculature as strips on coil wire stents. The inflammatory response to the polymers was graded as severe and resulted in the loss of lumen patency.[15] Others demonstrated that low molecular weight poly-L-lactic acid (L-PLA) was poorly tolerated in the vasculature with an inflammatory response leading to luminal stenosis and destruction of normal vascular architecture.[16] L-PLA stents were also found to be more inflammatory relative to stainless steel stents and resulted in decreased luminal patency in porcine carotid arteries[17] and other vessel beds.[18-19]

In contrast, other studies have demonstrated that biodegradable polymers and polymeric coatings are well tolerated by the vasculature.[1, 20, 21] Peng *et al* concluded that a poly-lactic-co-glycolic acid (PLGA)-coated stent did not induce a more severe reaction than a bare-metal stent after three or twelve months follow-up in porcine coronary arteries.[21] In human coronary arteries, Tamai *et al* found that biodegradable stents were feasible, safe, and effective.[22] Thus, there is significant disagreement in the existing literature.

Polydioxanone (PDO) monofilaments, which were certified by the FDA as safe,[23] were used in this study as raw materials for braiding biodegradable stents. The PDO stent is expected to support lumen patency for more than two months but not beyond six months; thus PDO presents an ideal alternative for fabricating absorbable stents because its degradation time is approximately 180 days.[24] Also, PDO was selected due to its degradation into low-toxicity monomers and reported biocompatibility.[25-27] PDO sutures were shown to elicit a lower inflammatory response than Vicryl (PLGA) and Dexon (polyglycolic acid, PGA).[28] In the vasculature, PDO-polypropylene copolymers have been tested as vascular grafts in a canine model, and did not result in an unacceptable

host response or thrombosis.[29]

In addition, the surface modification with phosphorylcholine prevents protein absorption and platelet adhesion on the metal stents, which will result in a decrease of re-stenosis, an acceleration of endothelialization of intravascular metallic stents, and an improvement in the performance of stents.[30] In this study, dip-coating technology was utilized to modify PDO polymer surfaces with 2-methacryloyloxyethyl phosphorylcholine to produce a phosphorylcholine mimetic cell membrane surface. Drug-eluting stents with phosphorylcholine-coated surfaces have been commercially available for over 10 years.[31] The success of phosphorylcholine-coated coronary stents inspired researches to explore other implantable materials. While biodegradable stents are becoming more common in cardiovascular interventions, biodegradable stents are not yet established in neurovascular interventions. The covalent attachment of phosphorylcholine to the implant wires has been shown to reduce thrombus formation in *in vitro*,[32, 33] *ex vivo*,[34] and *in vivo*[35] studies. Also, a phosphorylcholine surface treatment flow diverter for intracranial aneurysm, which could mitigate device material related thromboembolic complications, is now commercially available.

Despite the promising results of an acceptable inflammatory reaction with biodegradable stents, there are no biodegradable stents for neurovascular use on the market. Aiming to advance the development of neurovascular biodegradable stents, our study presents the biocompatibility of the PDO biodegradable, self-expanding stent. In this study, we assessed follow-up radiographic and histologic characteristics and the effect of the phosphorylcholine-coated PDO stent compared with non-coated PDO stents in a canine carotid model.

MATERIALS AND METHODS

Device preparation

PDO polymers were obtained as sutures (0.100 mm = 100 μm PDO fibers; Ethicon, Inc. obtained as PDO II 5-0 suture). The polymers were woven into a braided structure with 8 ends in a closed-looped ends model by Taewoong KOREA (Figure 1) at a braiding tension of 70 grams. One closed-end loop nitinol thread was incorporated to improve radiographic visibility and radial force (extensile force for self-expanding). All braids were annealed under tension. The PDO was annealed at 118°C for 30 minutes. The dimensions of the stent were 6.0 mm diameter and 20 mm in length. The stents were cleaned, inspected, and mounted onto a delivery system.

Both phosphorylcholine-coated and non-coated PDO were used in the study. The phosphorylcholine coating applied to the PDO stent consisted of a copolymer of 2-methacryloyloxyethyl phosphorylcholine. The stents were dip coated from a solution of the polymer in ethanol to provide a coating that was approximately 50 nm thick.

PDO stent characterization

The PDO stent characteristics were measured, including pixels per inch (PPI) and radial force. The characteristics were compared with a low profile visualized intraluminal support junior stent (LVIS Jr. Microvention, Tustin, CA, USA) with the same braided design. For the radial force test, we used the model TTR2 tensile testing machine (Blockwise, Tempe, AZ, USA).

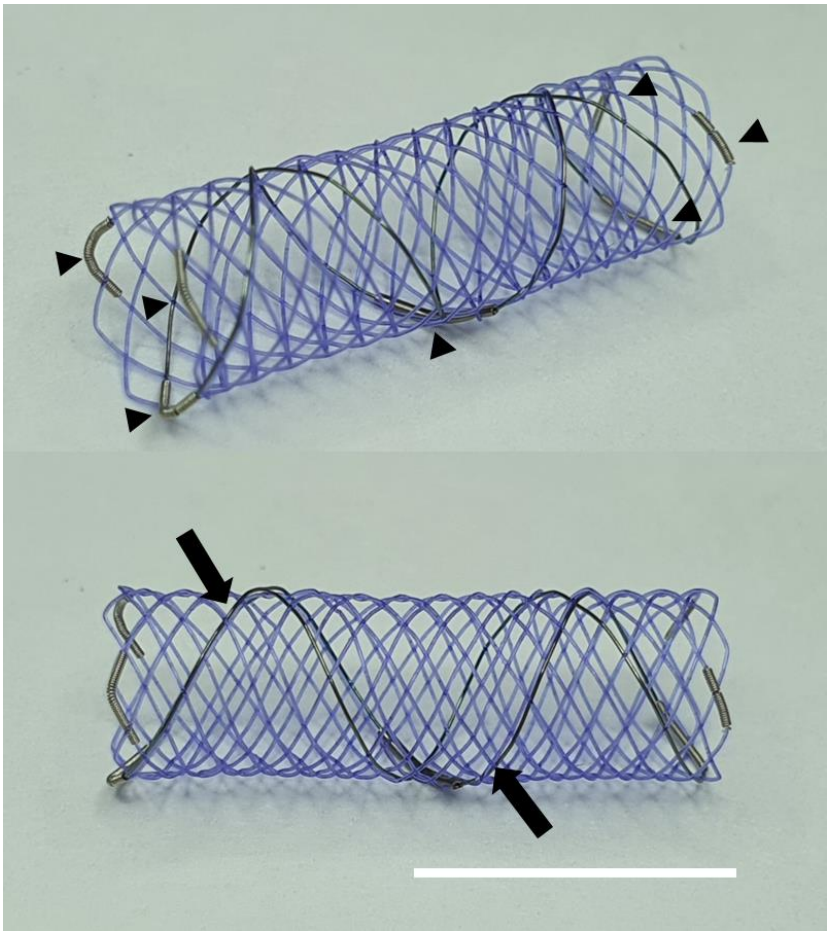

Figure 1

Photograph of the biodegradable polydioxanone (PDO) self-expanding braided stent in an unconstrained status used in this study. The stent was braided with a continuous suture of 5-0 PDO (100 μm PDO fibers) with 8 ends in the closed-looped ends model. One closed-end loop nitinol thread (100 μm) was incorporated to improve radiographic visibility and radial force (arrows). The dimensions of the stent were 6.0 mm diameter and 20 mm in length. The stent has seven radiopaque markers (3 for each end, 1 midline, arrow-heads) for high visibility during fluoroscopy. Scale bar, 1cm.

In vitro PDO filament degradation studies

In vitro degradation was tested by placing the 20 cm PDO 5-0 suture filament in 37℃ phosphate buffer saline (PBS, 0.1 mol/L, pH 7.4) on day 0. Filaments were removed periodically, dried in the vacuum oven for 2 hours at room temperature, and then the mass and tension strength were measured. The filaments were then returned to PBS to continue the assay; measurements were continued until the braids lost integrity (i.e., fragmentation of the braid was observed).

In vitro platelet adhesion test

Twenty microliter platelet-rich plasma (PRP, 1 x 10^8cell/ml) was dropped onto each sample surface and kept for 2 hours at 37℃ temperature. Non-coated PDO, phosphorylcholine-coated PDO and heparin-coated PDO polymer wires were evaluated. The samples were gently washed three times with PBS and then dipped into a 2.5% glutaraldehyde solution for 30 min to fix the platelets.

Animal preparation

Experiments were performed in beagle dogs (weight, 25-30 kg) fed on a normal diet. Experiments were performed according to the regulations of the animal care committee of the Seoul National University and K-bio health (Osong Medical Innovation Foundation, Korea) in accordance with standard guidelines. To prevent or reduce the occurrence of thrombotic events, animals received aspirin (325 mg) and clopidogrel (150 mg) for two days prior to the procedure, then 81mg and 75mg daily, respectively, for the duration of the study.

Stent implantation

After an overnight fast, animals were sedated and underwent intra-tracheal intubation. The dogs were connected to a ventilator and anesthesia was maintained with gas anesthesia. Arterial access was obtained via cut a down the femoral artery. A 7-F introduction sheath was placed and advanced into the artery. Heparin (50-200 IU/kg, intra-venous) was administered after the placement of the introducer sheath to prolong activated clotting time. Angiographic images of both common carotid arteries were obtained with contrast media to identify the proper location for the target deployment site with a target vessel size ranging between 3.0 and 4.0 mm. The implant/delivery system assembly was advanced over the 0.034-inch guidewire to the determined location in the carotid artery. Based on the selected vessel size, each stent was deployed to a target vessel. The post-dilation with a 4.0mm balloon was employed to ensure a consistent 25% over expansion among all implants. Intra-arterial tirofiban (0.4μg/kg/min) was administered via delivery system assembly during the procedure. Two stents (PDO and phosphorylcholine-coated PDO stents, respectively) per carotid artery were implanted alternatively (Figure 5a). In total, 24 stents (12 phosphorylcholine-coated, 12 non-coated) were implanted into 6 animals. After repeat angiography of the stented carotid arteries to confirm patency, the arteriotomy was repaired.

Follow-up angiography and necropsy

The catheterization procedure for follow-up angiography at 4, 8, and 12 weeks (two dogs in each time period) was similar to the procedure described above. Following angiography, the animals were euthanized by intravenous administration of potassium chloride. The carotid arteries were dissected,

leaving sufficient vessel proximal and distal to the stented portions. The stented arteries were perfused with Ringer's solution until they were cleared of blood, then perfusion fixed with 10% neutral buffered formalin. The vessels were placed in a fixative until further histological analysis.

Angiographic analysis

The fluoroscopic output from the stent implantation (pre-stent angiography and post-stent angiography) and at final angiography was recorded in a digital format. The diameter of stenosis [1 - (MLD/RVD)] x 100, where MLD is the minimal luminal diameter and RVD is a calculation of the reference diameter at the same point, was measured.

Histologic examination

The stented arteries were embedded in methyl methacrylate and the subsequent blocks were cut into six segments (Figure 2). Graded ethanol and a resin mixture were used for the dehydration and infiltration of resin block sections. These sections were then grounded and polished on the EXAKT micro grinder to a thickness of 15 μm and stained with hematoxylin and eosin (H & E). Graded ethanol and a xylene mixture were used for the dehydration and infiltration of paraffin sections. The blocks were embedded in paraffin wax and then sliced at a 3-4 μm thickness. H & E staining and Movat pentachrome staining were performed on these sections.

For stented artery sections, both semi-quantitative scores and quantitative scores were used to assess the biological response of vascular tissue to the braid-loaded stents. The samples were evaluated for the following: inflammatory (fibrosis) response, luminal thrombus formation, and the amount

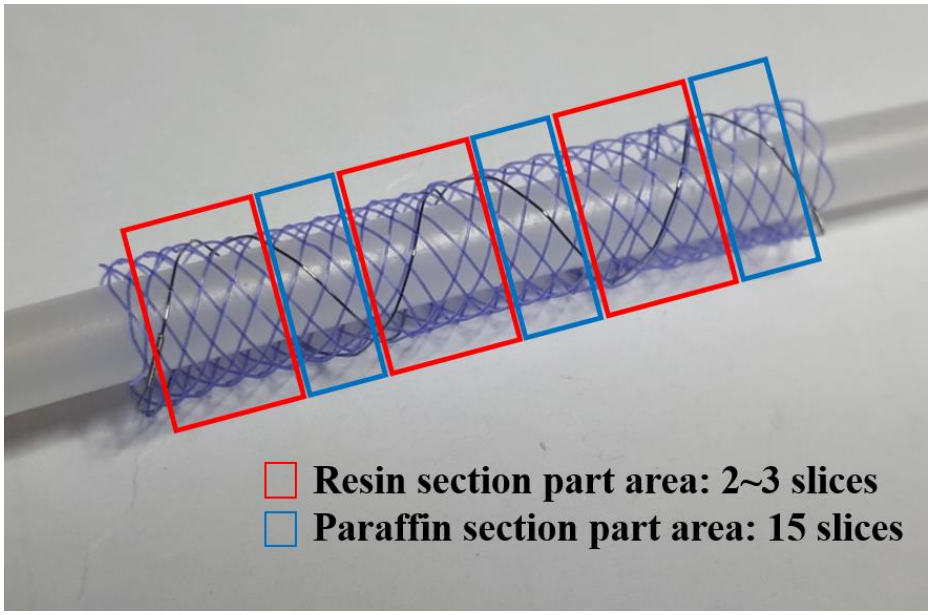

Figure 2

Schematic examples of preparation for histologic analysis.

of degradation according to the scoring system defined in Table 1, adapted from Hedberg *et al*.[36] Since the primary interest was tissue response to the polymers, parameters were quantified only in the area surrounding the polymer fibers or fiber particulates. All scoring was performed by a histopathologist blinded to the identification of the groups.

A quantitative examination for the degree of stenosis was performed on H & E stained sections of stented arteries. Images were captured using light microscopy and measurements were made using quantitative morphometric computer-assisted methods (IP Lab, BioVision Technologies). For each section, the operator delineated the internal elastic lamina (IEL) and the luminal border. Intimal thickness [Luminal area of IEL (μm^2) - Luminal area of luminal border (μm^2)] and area of stenosis [1- (Luminal area/IEL area) x 100] were measured.

Statistical assessment

Statistical significance was evaluated by Mann-Whitney U-test with $p<0.05$ considered as significant.

Table 1

The semi-quantitative histologic scoring system used for the evaluation of stented arteries. Adapted and modified from Hedberg *et al.*[36]

Inflammation Score	
0	No inflammation, 0-3 inflammatory cells, adjacent to strut
1	Minimal response, 4-10 inflammatory cells, adjacent to strut
2	Mild response, >10 inflammatory cells, adjacent to strut
3	Moderate response, >10 inflammatory cells, effaces surrounding tissue
4	Severe response, 2 or more struts associated granulomatous inflammatory reactions
Endothelial injury	
0	No injury to internal elastic lamina (IEL)
1	IEL lacerated
2	IEL and media lacerated
3	External elastic lamina lacerated
Luminal Thrombus	
0	No luminal thrombus
1	(Minimal) Occupies ~ <5% of the luminal area
2	(Mild) Occupies ~ 5-35% of the luminal area
3	(Moderate) Occupies ~ 35-70% of the luminal area
4	(Severe) Occupies ~ >70% of the luminal area
Endothelialization	
0	Endothelium absent over >75% of circumference
1	Endothelium absent over 25-75% of circumference
2	Endothelium absent over <25% of circumference
3	Complete endothelial covering
Polymer Degradation	
5	Complete degradation of polymer
4	Decrease in polymer area
3	Excessive fragmentation (throughout polymer scaffold)
2	Minimal fragmentation with immune cells entering polymer
1	Intact polymer (stained with dye)
0	Intact polymer (not stained with dye)

RESULTS

PDO stent characterization and in vitro degradation studies

The profiles of PDO and LVIS Jr. stents are shown in Table 2. With the same braided design, the PDO polymer stent showed lower radial force compared with the metal braided stent [3.922 Newton (N) vs. 6.145 N]. The PDO filaments underwent testing to measure degradation *in vitro*. Figure 3 demonstrates the percentage of mass loss per unit of time and tension strength for PDO filaments. PDO filaments began to demonstrate a loss of 5% of their original mass by 4 weeks. PDO filaments started to degrade at 4 weeks and by 16 weeks reached about 50% of their original weight. Tensile strength started to decrease at an early period and lost complete tensile strength at 12 weeks. There were no differences between non-coated PDO and phosphorylcholine-coated PDO filaments.

In vitro platelet adhesion test

The average number of adhered platelets per mm^2 was 1340.55 ± 411.43 cells in the non-coated PDO stent, 1067.56 ± 318.07 cells in the heparin-coated PDO stent, and 172.24 ± 76.32 cells in the phosphorylcholine-coated PDO (Figure 4). Only the phosphorylcholine-coated PDO stent showed statistical significance in platelet adhesion inhibition.

Table 2

The profiles of PDO stent and LVIS Jr. stent

	PDO stent	LVIS Jr.
Dimension (diameter x length)	6 x 20 mm	3.5 x 22 mm
Pixels per inch (PPI)	23 (8 cells/0.345 inches)	46 (16 cells/0.351 inches)
Wire thickness	PDO 0.100 mm	Nitinol 0.062 mm
Radial Force (Newton)	3.922 N	6.145 N
Radial Force per 1cm	1.961 N/cm	2.973 N/cm

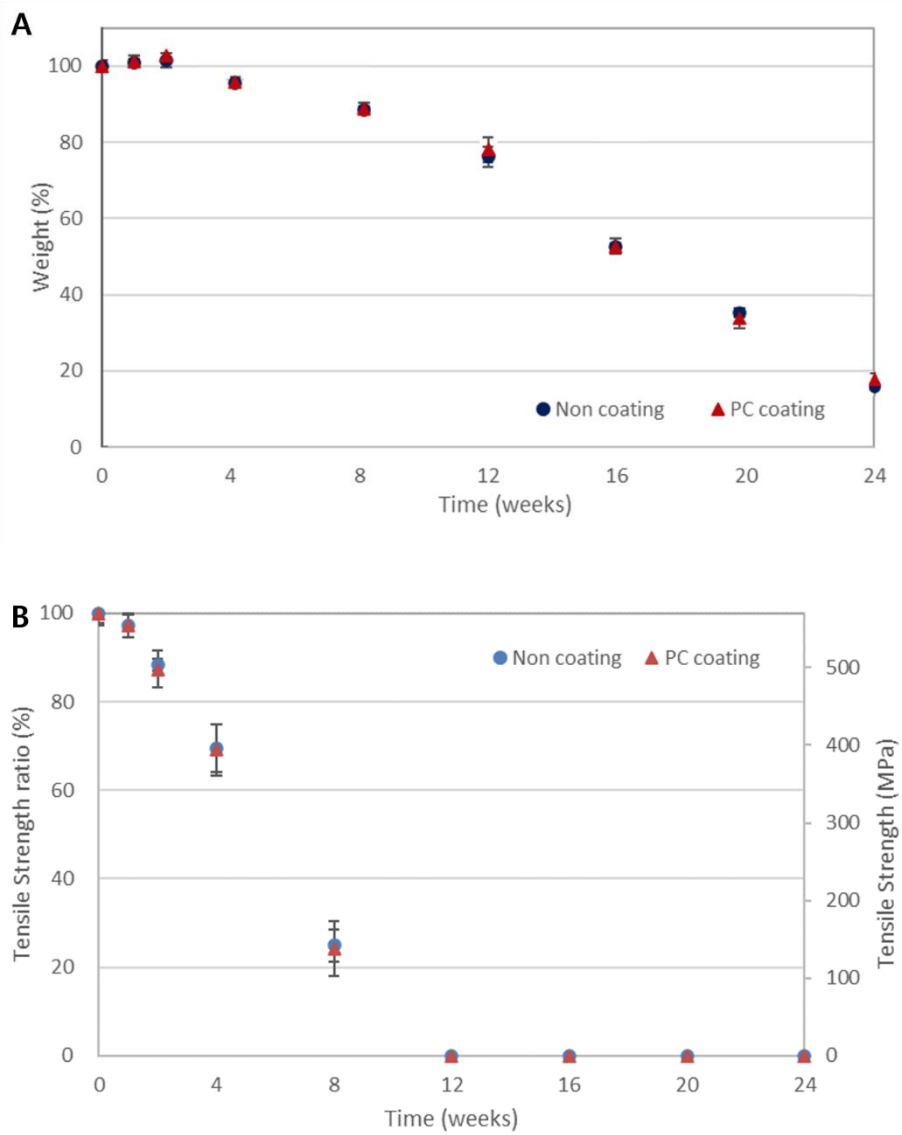

Figure 3

(A) *In vitro* degradation of polydioxanone (PDO), non-coated (blue circles), and phosphorylcholine-coated (red triangles). The results of the degradation test showed a decrease in weight by 25% at 12 weeks. (B) *In vitro* tensile strength ratio (%) of PDO. Error bars, standard deviation.

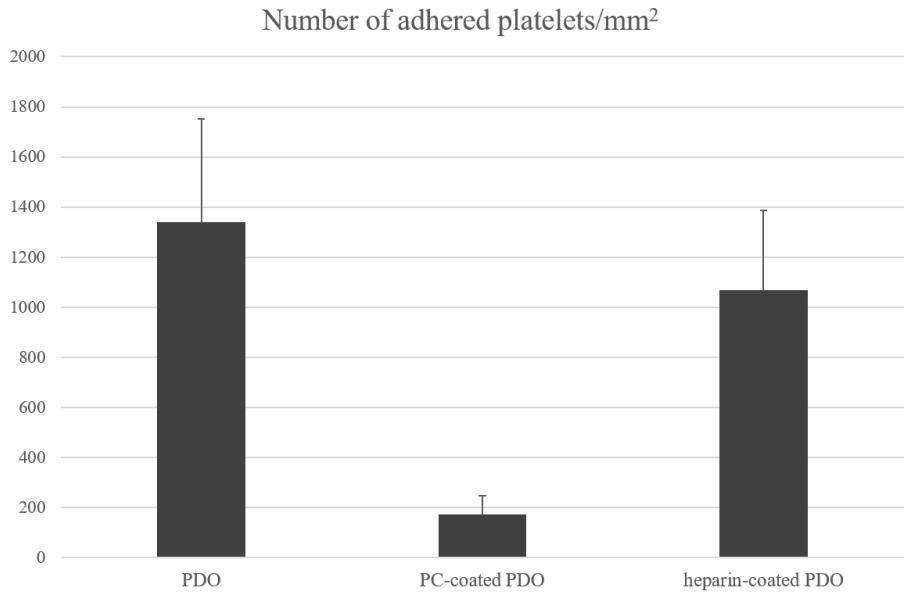

Figure 4

In vitro platelet adhesion test. The platelet aggregation was significantly reduced ($p < 0.05$) in the phosphorylcholine (PC)-coated polydioxanone (PDO) stent. There was no difference in platelet adhesion inhibition in the heparin-coated PDO stent.

In vivo stent implantation

A total of 12 phosphorylcholine-coated PDO and 12 non-coated PDO stents were successfully deployed into the predetermined carotid artery. In three cases (one phosphorylcholine-coated PDO and two non-coated PDO stents), a small thrombus formation occurred but the flow was maintained over the procedure. Angiography after implantation showed that all carotid arteries were patent, with no signs of intraluminal defects. (Figure 5-7)

Angiography follow-up

All animals survived the follow-up period without adverse events. Representative angiograms are shown in Figures 5-7 for each group immediately after implantation, and at 4, 8, and 12 weeks. Angiography demonstrated that all vessels were fully patent at 4 weeks. However, late stent occlusion of both carotid arteries was angiographically demonstrated at 8 and 12 weeks. The arterial patency rate at 8 weeks was 50% and 0% at 12 weeks. The distal carotid artery reconstitution was observed via the posterior communicating artery collateral from the vertebral artery in occlusion cases. In the patent early period follow-up, the angiography showed an eccentric lumen reduction at the site of stent implantation. Quantitative vascular analysis (Figure 8) showed low angiographic stenosis at the 4-week follow-up point ($9.48 \pm 7.13\%$ stenosis). At 8 weeks follow-up, the stenosis degree increased to $17.80 \pm 9.83\%$. However, no statistical difference was noted between the 4- and 8-week stenosis results. Angiographic stenosis was slightly higher for the non-coated PDO stent relative to the phosphorylcholine-coated PDO stent in the same time period.

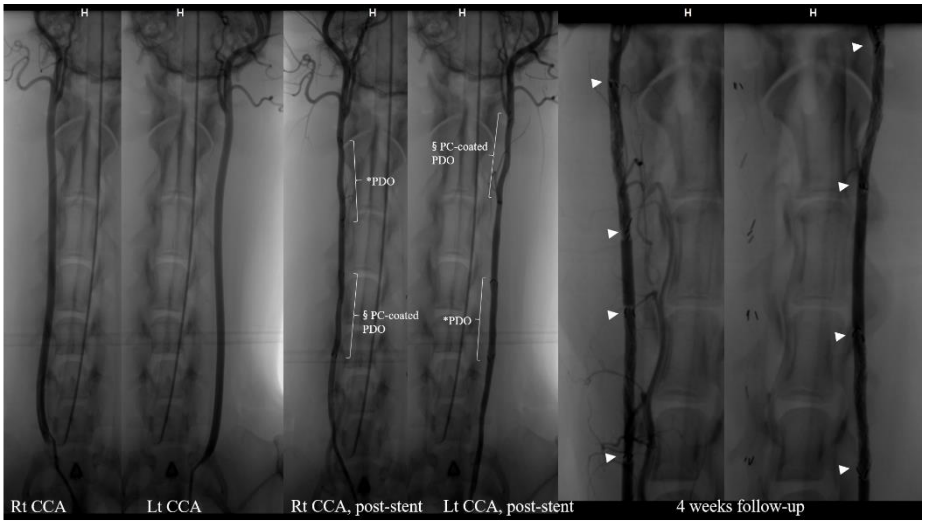

Figure 5

Both common carotid artery angiography (first and second column), immediate post-implantation (post-stent) angiography (third and fourth column) and follow-up angiography at 4 weeks (fifth and sixth column, the white arrows indicate the ends of the stents). Immediate angiography after stent implantation showed some degree of vasospasm. However, no in-stent thrombosis was observed. Although there was mild degree of in-stent stenosis, the vessel patency was preserved at 4 weeks follow-up.

PC = phosphorylcholine, PDO = polydioxanone

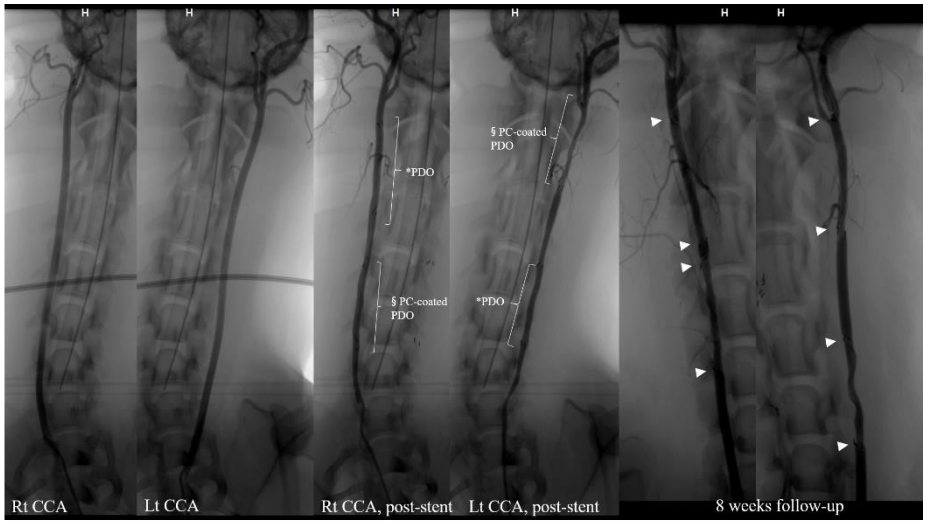

Figure 6

Both common carotid artery (CCA) angiography (first and second column), immediate post-implantation angiography (third and fourth column), and follow-up angiography at 8 weeks. On immediate angiography after stent implantation, a mild degree of vasospasm was observed (left CCA). However, the vessel patent was preserved. At 8 weeks follow-up, the degree of stenosis was more prominent compared with 4 weeks follow-up.

PC = phosphorylcholine, PDO = polydioxanone

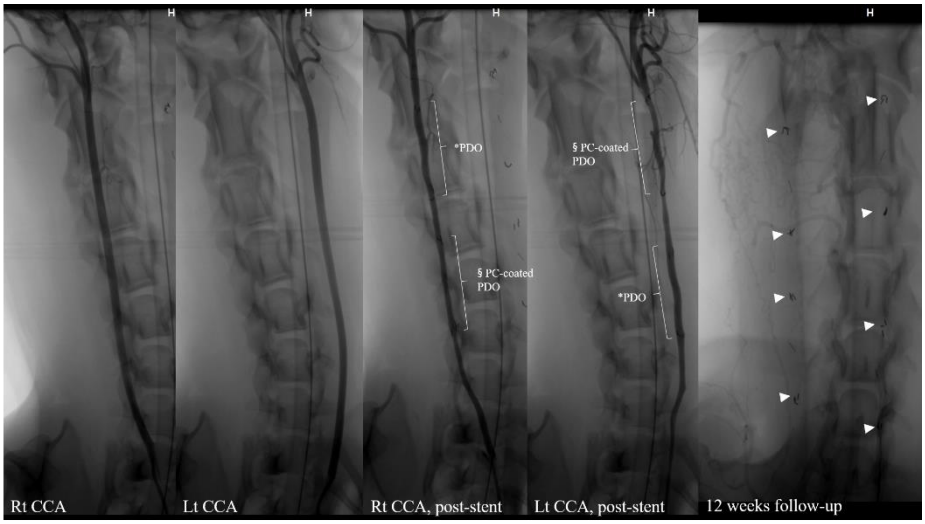

Figure 7

Both common carotid artery (CCA) angiography (first and second column), immediate post-implantation angiography (third and fourth column) and follow-up angiography at 12 weeks. On immediate angiography after stent implantation, some degree of vasospasm was observed (right and left distal CCAs). However, the vessel patent was preserved. At 12 weeks follow-up, 100% of the implanted vessels lost their patency. The distal CCAs were reconstituted via vertebro-basilar collaterals.

PC = phosphorylcholine, PDO = polydioxanone

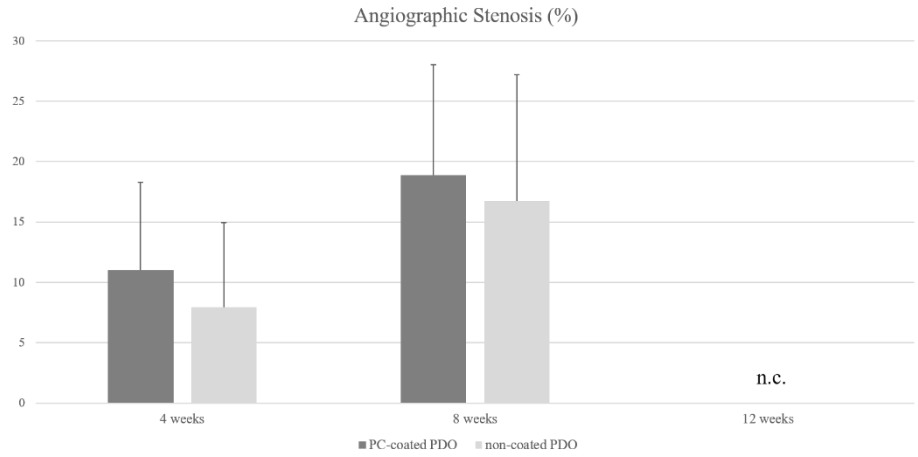

Figure 8

The percentage diameter stenosis was calculated for polydioxanone stents at 4 and 8 weeks in patent carotid arteries. Angiographic stenosis was increased between 4 and 8 weeks follow-up. However, there were no statistical differences in the percent diameter stenosis. n.c. = not checked due to total occlusion.

Histologic analysis

Inflammation, endothelial cell injury, luminal thrombosis, and endothelialization (neo-intimal formation) were scored for all implants at 4, 8, and 12 weeks (Figure 9). The inflammatory score was the sum of the factors, including the number of inflammatory cells (polymorphonuclear cells, lymphocytes, plasma cells, and macrophages), necrosis, neovascularization, fibrosis, and fatty infiltration. The number of inflammatory cells surrounding the struts was counted and scored for proximal, middle, and distal sections of each implant. Figure 10 depicts representative H & E and pentachrome images of each group at 4, 8, and 12 weeks. The average inflammatory score within each time period is presented in Figure 9A. Inflammation was largely confined to the neo-intima for all groups, as no medial or adventitial inflammation was observed (Figure 11). The non-coated PDO polymer had a slightly higher inflammatory response (score 1.92 ± 0.67) at 4 weeks compared to phosphorylcholine-coated PDO polymers (score 1.33 ± 0.65) without statistical significance. At 8 weeks a thin, organized neo-intima was observed, with increased inflammatory sequelae (score 1.79 ± 0.83, p=0.360). An increased inflammatory response to PDO polymers was observed at 12 weeks with statistical significance (score 2.33 ± 0.96, p=0.008). We observed that the more robust inflammatory response corresponded to an increase in the number of foreign body inflammatory cells.

Endothelial cell injury, luminal thrombosis, and endothelialization demonstrated the same results shown in the inflammatory response according to the follow-up periods. The endothelial injury levels were scored according to the integrity of the IEL. All devices demonstrated minimal or mild injury levels at 4 and 8 weeks (Figure 10B). The injury score was increased at 90 days (p=0.003), demonstrating further injury of IEL. Luminal thrombosis formation

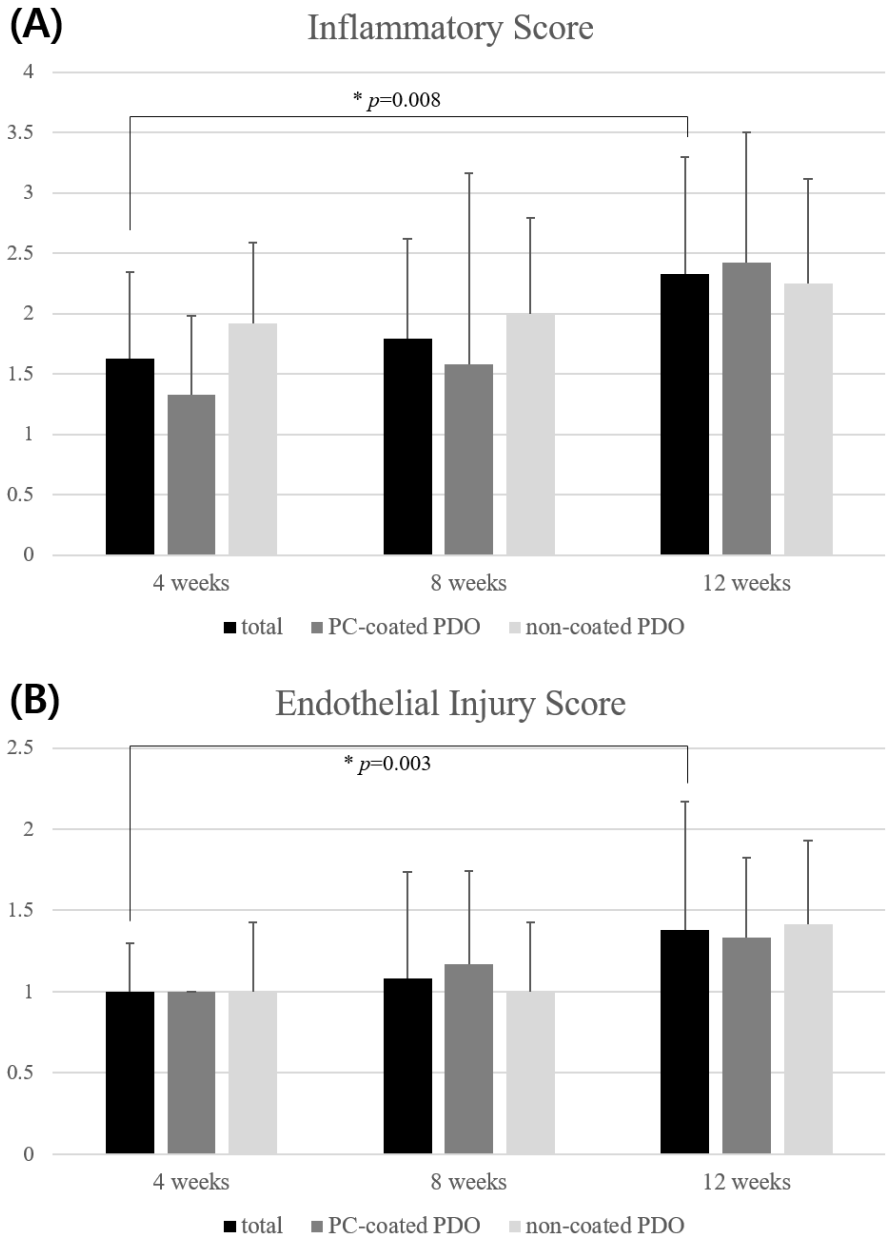

Figure 9A, B

Semi-quantitative histologic analysis. Inflammatory score (A) and endothelial cell injury score (B) were measured. All parameters were significantly increased ($p < 0.005$) between 4 and 12 weeks in polydioxanone stents.

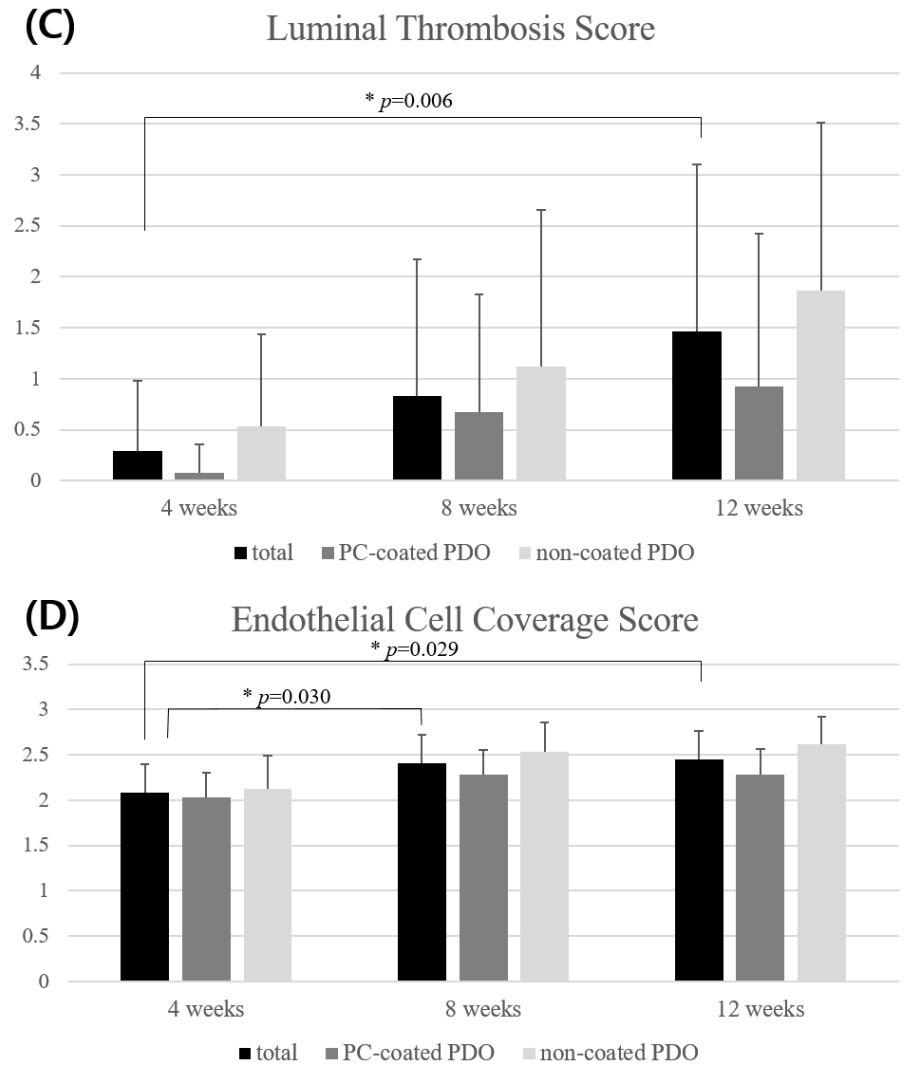

Figure 9C, D

Semi-quantitative histologic analysis. Luminal thrombosis formation (C) and endothelial cell coverage score (D) were measured. All parameters were significantly increased ($p < 0.005$) between 4 and 12 weeks in polydioxanone stents.

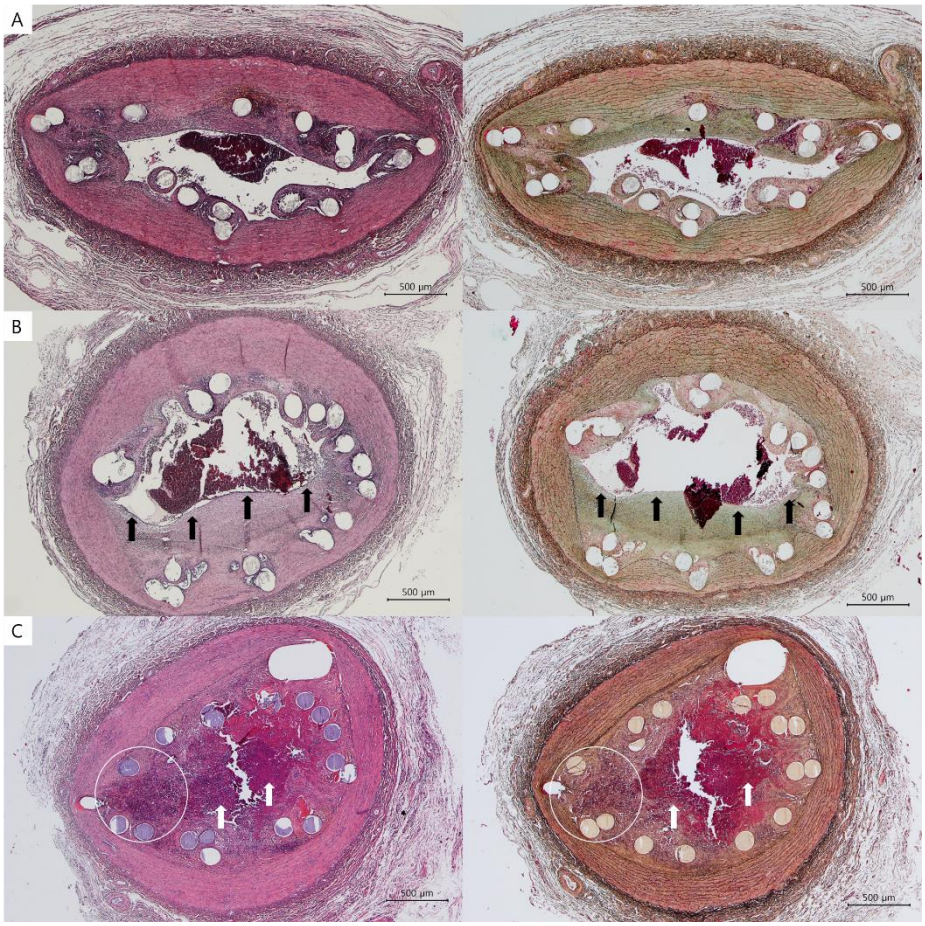

Figure 10

Representative H & E (left column) and pentachrome (right column) stain micrographs of explanted carotid arteries containing polydioxanone stents at 4 weeks (A), 8 weeks (B), and 12 weeks (C). The asymmetric luminal narrowing with neo-intimal hyperplasia (B, black arrows) is presented. At 12 weeks, a thrombus (white arrows) associated with marked inflammatory cell infiltration (C, white circles) induced vessel occlusion. Magnification x40.

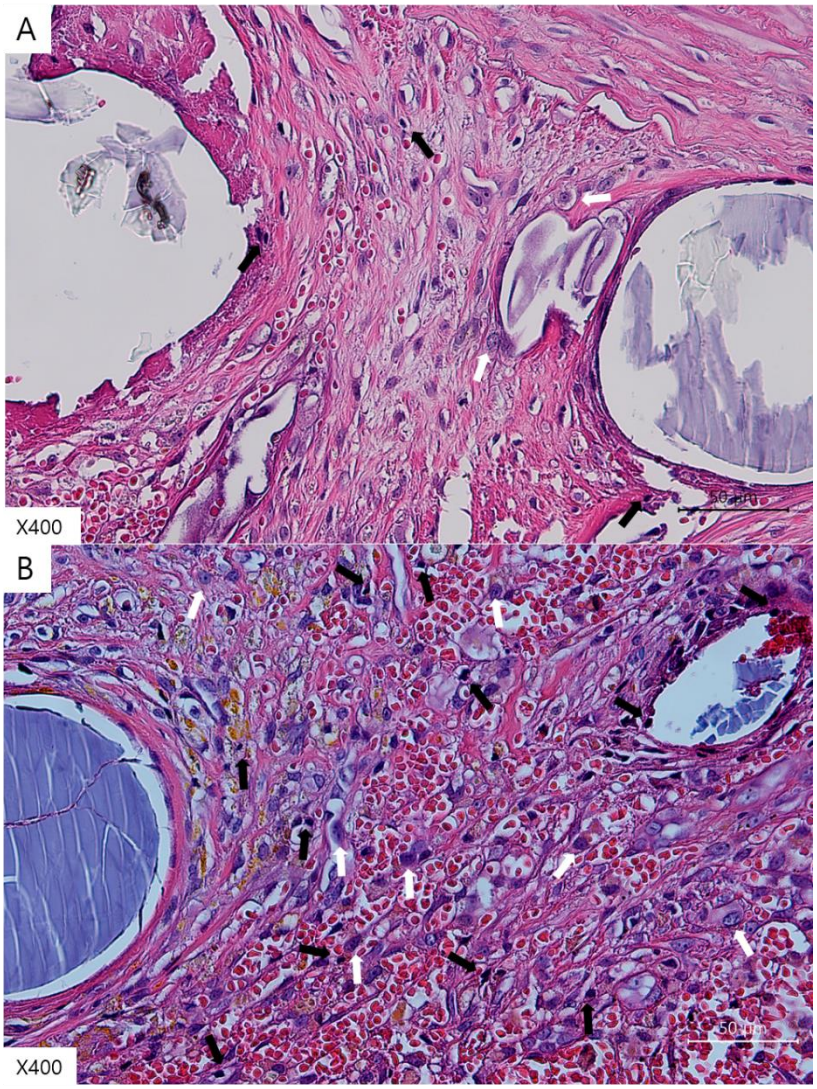

Figure 11A, B

Representative H & E stain micrographs are presented. (A) 4 weeks follow-up and (B) 12 weeks follow-up. The polydioxanone polymer at 12 weeks induced an immense inflammatory cell infiltrations into the neo-intima around the polymer (black arrows = lymphocytes, white arrows = macrophages).

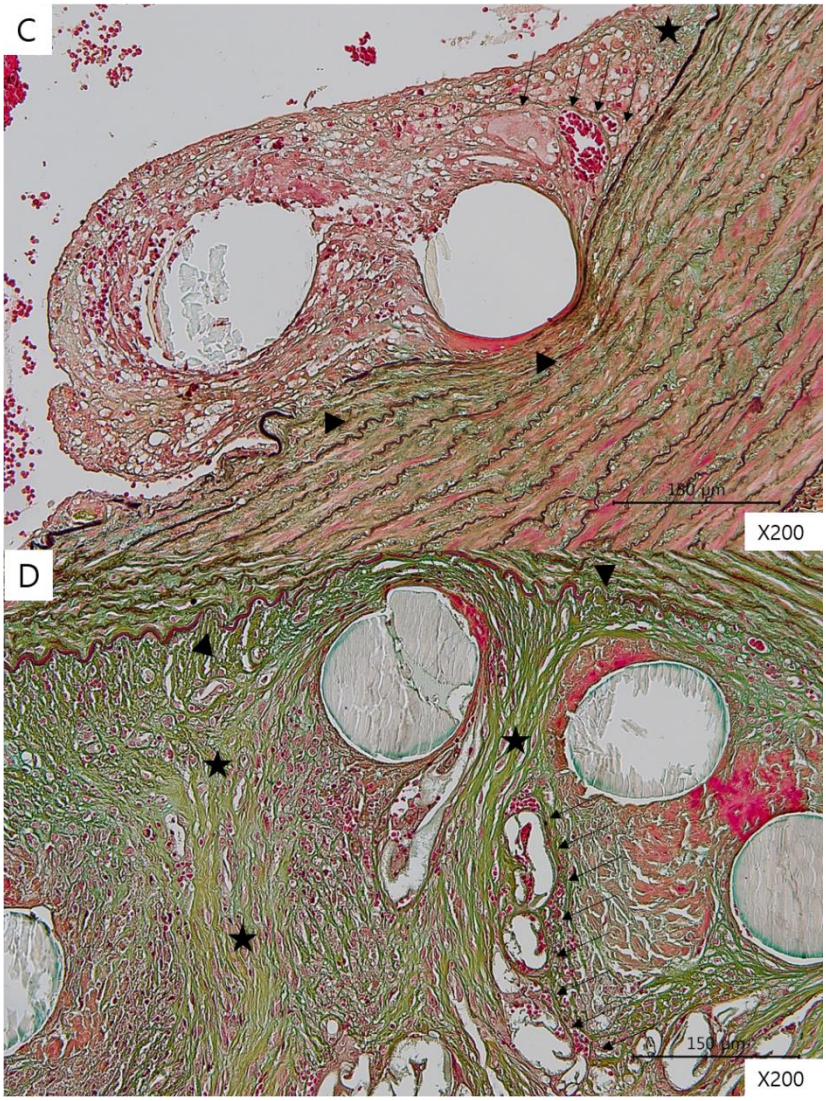

Figure 11C, D

Representative pentachrome stain micrographs are presented. (C) 4 weeks follow-up and (D) 12 weeks follow-up. The polydioxanone polymer induced endothelial cell injury (disruption of internal elastic lamina, black arrow-head). The reactive fibrosis (black stars) and neovascularization (small arrows) are demonstrated.

also showed the same tendency (Figure 9C). Figure 9D demonstrates nearly full endothelialization of PDO stents at 4, 8, and 12 weeks, respectively. Endothelialization was near complete in the early stage (4 weeks) of over >75% endothelial coverage of circumference.

Polymer absorption is presented in Figure 12. Minimal fragmentation of the PDO strut with intact polymer stained with dye was observed at 4 weeks. At 8 weeks, the fragmentation of the PDO strut increased with immune cells entering the polymer. Excessive fragmentation of the PDO strut was observed by 12 weeks.

All patent vessels demonstrated concentric stenosis with moderate to severe neo-intimal formation. Increased stenosis was observed according to increasing follow-up intervals (Figures 10 and 13). The mean percent diameter stenosis at 4 weeks was 26.46 ± 7.38% and 23.08 ± 7.97% for non-coated PDO stents and phosphorylcholine-coated PDO stents, respectively. At 8 weeks in the patent vessels, the percent diameter stenosis was 45.46 ± 31.62% for non-coated PDO stents and 44.80 ± 31.62% for phosphorylcholine-coated PDO stents. The area of stenosis and neo-intimal thickness were not significantly increased (p=0.128) between 4 and 8 weeks in both non-coated PDO and phosphorylcholine-coated PDO stents.

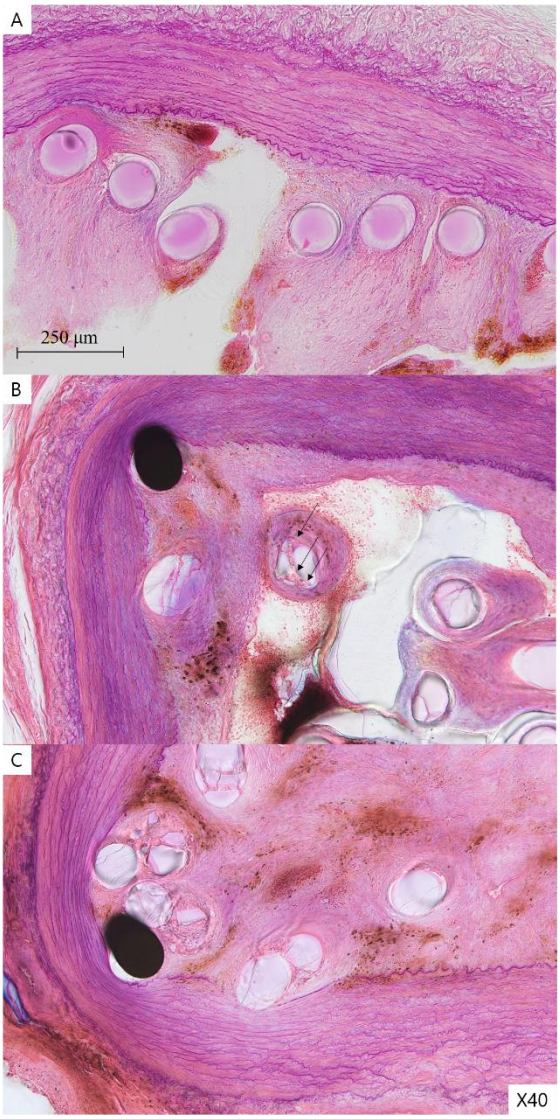

Figure 12

Representative H & E stained images are presented for degradation the degree of polydioxanone polymers. (A) At 4 weeks, most polymers were intact (stained with dye) with minimal fragmentation. (B) At 8 weeks, the polymer showed moderate fragmentation with immune cells entering (arrows). (C) At 12 weeks, more excessive fragmentation of the polymers was observed.

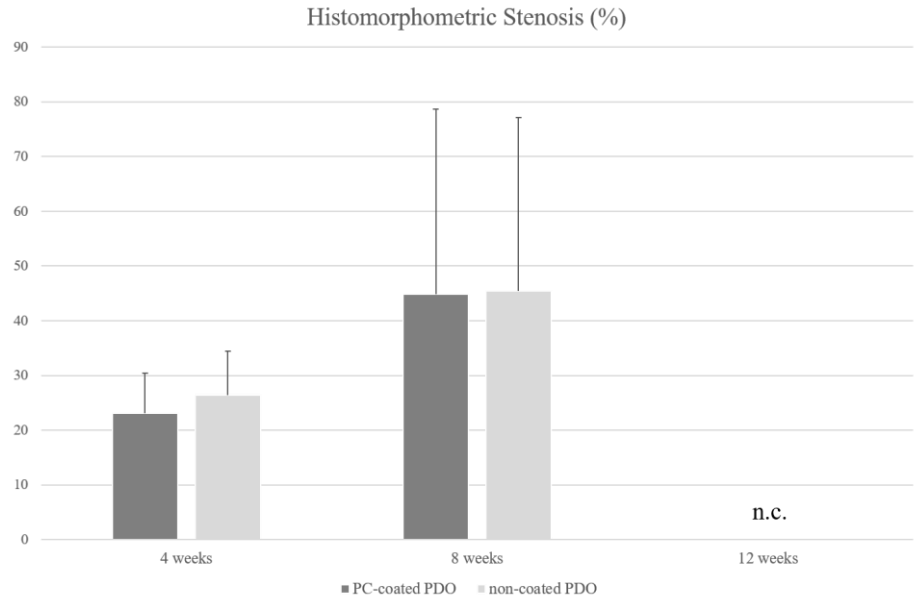

Figure 13

Histomorphometric percentage area stenosis was calculated for polydioxanone stents at 4 and 8 weeks in patent carotid arteries. The area stenosis was increased between 4 and 8 weeks follow-up. However, there were no statistical differences in the percent area stenosis. n.c. = not checked due to total occlusion.

DISCUSSION

The main objective of this study was to determine the biocompatibility of the PDO stent over a period of up to 12 weeks. The main study results are summarized as follows: (1) after an early follow-up period (at 4 weeks, before polymer degradation), the PDO stents were associated with a minimal to mild inflammatory and proliferative response. Although there was mild degree of in-stent stenosis, the vessel patency was preserved; (2) in the late period associated with polymer biodegradation (after 4 weeks), implants were complicated by thrombotic vessel occlusion with further inflammatory and proliferative responses; (3) this response was observed with both non-coated PDO and phosphorylcholine-coated PDO stents. The requirement for blood compatibility was added to the requirements for mechanical performance, absence of toxic reactions, and long-term maintenance in this intra-vascular environment. In the vessel environment in which complex and integrated cellular and humoral systems exclude the foreign body from being incorporated into the vascular wall, it is not surprising that PDO polymer implants in the carotid artery elicit a more severe reaction. Also, the recipient vessel diameter may influence the success or failure of patency. In vessels <4 mm in diameter, all synthetic materials fail in the vascular graft.[37] Although this report is not associated with the stent cases, our results in the small-diameter carotid circulation extend this effect of recipient vessel diameter.

Biodegradable polymeric intravascular stents have shown significant improvement since their introduction, and several biodegradable polymeric stents are undergoing study in clinical trials, including Igaki-Tamai (Kyoto Medical, MA),[38] REVA stent (Boston Scientific, Natick, MA),[39] and Ideal stent

(Bioabsorbable Therapeutics Inc.).[3] The biocompatibility of biodegradable polymeric materials has been the subject of extensive study,[13, 40] but direct evaluation in the vascular space has been less common. In this study, we sought to evaluate the biological response of the PDO polymer stent. As a member of the polyester family, L-PLA has been used as a stent in the coronary vasculature in preclinical and clinical studies. The L-PLA stent was shown to have an acceptable vascular response in a porcine model[17] and in a small human study.[41] Though these results are promising, the degradation time of the L-PLA stent is more than two years.[41] In humans, vascular healing in response to stent placement is typically complete by one year[42] and once the healing occurs, the presence of a stent is no longer required. Thus, the degradation time of two years is not only unnecessary but also may carry the same risk factors as permanent metallic stents. Consequently, a stent with a shorter lifespan may be more advantageous. PDO monofilaments were used in this study as raw materials for braiding biodegradable stents. The PDO stent is expected to support lumen patency for more than two months but not beyond six months; thus PDO presents an ideal alternative as a biodegradable stent because its degradation time is approximately 180 days.[24]

More specifically, several studies have demonstrated that biodegradable polymers with rapid degradation time frames relative to L-PLA elicited high inflammatory responses in the vasculature.[14, 16, 43-45] This has also been demonstrated for other rapidly degrading materials such as poly-organo-phosphazine,[46] and polyhydroxybutyrate.[47] Several studies have reported that the in-stent stenosis process peaks at the early degradation periods and rarely reaches its peak thereafter.[48, 49] Although the PDO polymer did not result in vessel occlusion and was not associated with inflammatory granulomas and a sign of vascular incompatibility, a significant inflammatory response was

observed with all implanted polymer stents. In all cases, this consisted of a chronic inflammatory reaction with an acute component and a persistent foreign-body response.[50] Our *in vitro* degradation measurements data demonstrated that PDO braids began the degradation process at 4 weeks and by 12 weeks had lost 25% of their mass. Similarly, we observed that more than half of the braid struts demonstrated cellular infiltration by 8 weeks and were fragmented and cracked in almost all PDO struts by 12 weeks. According to the degradation of PDO polymers, our data showed moderate to severe neo-intimal formation in the canine carotid vessels. In most cases, a substantial part of the overall inflammatory and proliferative response may have been aggravated by the degradation of the PDO polymer stents. A role for initial vessel injury of stent implantation and stretch injury of the post-ballooning cannot be excluded, but it seems more likely that the presence of progressive stenosis with delayed occlusion was evidence of an inflammatory and proliferative reaction and vessel occlusion. The possibility that this acute damage adds to the final outcome should be substantiated by acute experiments in future studies testing the intravascular biocompatibility of other synthetic polymers.

In-stent restenosis always impedes the application of intravascular stents. Approximately one-third of the patients who accept percutaneous transluminal with bare metal stents in coronary stenosis have suffered from in-stent stenosis[51] which resulted in recurrent angina and minor/major heart attack. Additionally, for intravascular stents (compared to coronary artery), in-stent restenosis is still an issue and rates remain high.[52-54] The modification methods have evolved from early simple physical polishing to current chemical modification, and even to the immobilization of biological molecules[55] such as coating or surface grafting of phosphorylcholine moieties. Phosphorylcholine is a zwitterion moiety that can benefit from its reduced protein, platelet, and

cell adhesion *in vitro*.[56-59] Therefore, the modification of intravascular stents with phosphorylcholine can lead to anti-thrombotic effects. This reduction in inflammation and luminal gain has been noted before with everolimus-eluting biodegradable PLA stents in the porcine and human coronary arteries.[41, 60] In humans, intravascular ultrasound data from the ABSORB trial showed a significant increase in the minimal luminal area, mean luminal area and volume at 6 months and 2 years with a corresponding decrease in plaque area and volume.[41] In our preliminary study, we also found a different degree of platelet adhesion after stent placement. Acute thrombus formation is one of the most dangerous events during interventional therapy. In particular, there are still concerns about acute thrombus formation and massive inflammatory reaction early after stent deployment in biodegradable polymer stents, including PDO in intravascular usage. Concerning the safety of intravascular stents, the platelet adhesion on the surface of stent materials must be dealt with. We evaluated the platelet adhesion on our samples *in vitro* using fresh PRP. The results were different from the *in vitro* platelet adhesion test which showed a significant decreased in the amount of platelet adhesion in phosphorylcholine-coated PDO polymers. Here, there was no significant difference between coated and non-coated stents in radiologic and histologic examinations. With our preliminary results, we used intra-arterial tirofiban as a loading dose.

Unlike intramuscular or subcutaneous space, standardized biocompatibility screening protocols do not exist for the vasculature. Due to differences in material, animal model, implantation vessel bed, device conformation, and assessment time points, a direct comparison cannot be made between this study and previously reported data. However, the degree of neo-intimal formation in our study is consistent with the data obtained from other studies performed in the carotid vessels.[17, 61-64] For instance, Bunger *et al*. implanted a PDO stent in

the porcine carotid artery and found similar neo-intimal hyperplasia to PDO struts despite differences in device conformations, molecular weight, and time points.[17]

Following stent implantation, histologic parameters of completed vessel wall healing are endothelialization, and the progression of granulation tissue to a mature neo-intima. In a previous study, data suggested that a heparin coating delayed re-endothelialization at four weeks such that the neo-intimal thickening was also affected.[65] The phosphorylcholine might be expected to delay the rate of re-endothelialization since it inhibits the deposition of plasma proteins onto the stent surface.[66] The absence of a protein layer may make the stent surface delayed for endothelial growth. In our study, the endothelial covering at all time periods was similar in both non-coated and phosphorylcholine-coated groups. Therefore, the phosphorylcholine coating did not delay the early wound healing response.

Previous material biocompatibility studies have looked at thin polymer layers coated on metallic stents[16, 67] or asymmetric material strips.[15] In addition, some studies have had difficulties decoupling material response and device design.[17, 21] In this work, we presented a self-expanding platform for studying and comparing the compatibility of materials in the vascular space. However, similar to other polymers, PDO is inferior to metallic stents in terms of its functional mechanical characteristics and has a relatively higher stent recoil degree than metallic stents. The possibility of improving the mechanical properties of absorbable polymeric stents via thermal treatment has been proposed in other contexts.[68, 69] However, our study allowed for the evaluation of a pure polymeric material similar in material mass to a biodegradable polymeric stent with minimal application of metallic wire (single strain in Figure 1). In addition, post-stent balloon angioplasty encouraged the wall

composition of the PDO stents. We believe our method provides a more accurate platform for assessment of the biocompatibility of fully degradable vascular stents. We used the canine carotid artery, which was easily accessed through minimally invasive intervention and was large enough to test multiple devices in the same animal; thus, allowing for comparisons between materials within the same animal. The response in the carotid arteries may not translate directly to other vessel beds. Differences in inflammatory and re-stenotic responses between various vascular beds have been demonstrated both in humans and other animal models.[70, 71] However, our results suggest that the carotid vascular space is viable for material screening.

There are several limitations to this study. (1) We tested materials with a range of degradation times at 4, 8, and 12 weeks. Short-term studies are required to fully evaluate the biological response to the absorption of these polymers. (2) We did not include a control such as a bare-metal stent or L-PLA polymer that has been successfully tested in animal models and in humans. (3) In the canine model of oversized stent implantation, the degree of vascular injury that accompanies stent implantation induces a variable amount of inflammation, and neo-intimal growth.

CONCLUSION

The biocompatibility of PDO polymers was evaluated in the canine vasculature at quantities relevant to biodegradable stents. In our animal model, the PDO stents showed marked inflammatory and neo-intimal hyperplasia according to the degree of biodegradation after implantation in the canine carotid arteries. In 4-week follow-up period before polymer degradation, the vessel patency was preserved with mild in-stent stenosis. However, the degree of stenosis progressed according to the time interval and finally vessel occlusion was occurred. Vessel patency was not maintained and a faster-degrading PDO showed a non-favorable host response with the incidence of acute stent thrombosis. Phosphorylcholine-coated PDO stent showed a marked platelet adhesion inhibition on *in vitro* test, however, did not show difference in luminal thrombosis formation and endothelialization after *in vivo* implantation comparted with non-coated PDO stent. During the subsequent process of wound healing, the phosphorylcholine-coated and non-coated PDO stents elicited a similar tissue response.

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국문 초록

서론: 저자는 자가 팽창성, 생분해성 폴리다이옥사논 스텐트의 생체적합성을 확인하기 위하여, 견종 경동맥에 스텐트 삽입 후 영상의학적, 조직학적 검사를 시행하였다. 또한, 추가적으로 포스폴린콜린 코팅한 스텐트와 비교하고자 하였다.

대상 및 방법: 모든 동물 실험은 동물 실험 위원회의 승인 하에 진행하였다. 총 여섯 마리의 개(비글)의 3㎜에서 4㎜ 크기의 양측 경동맥에 총 12개의 폴리다이옥사논 스텐트와 12개의 포스폴린콜린 코팅한 폴리다이옥사논 스텐트를 삽입하였다. 개는 각각 두 마리씩 스텐트 삽입 후 4주, 8주, 12주에 혈관조영검사 및 조직검사를 시행하였다.

결과: 24개의 스텐트는 모두 합병증 없이 삽입하였다. 혈관 조영 검사 상에서 스텐트 삽입 후 4주 경과 후, 스텐트 내 협착은 9.48 ± 7.13% 보였으며, 8주 경과 시에는 15.80 ± 12.32%의 협착 소견이 보였다. 하지만, 8주 경과한 경동맥의 50%와 12주 경과한 경동맥은 완전 폐색된 상태였다. 체외에서 시행한 폴리다이옥사논 섬유 분해 실험에서, 4주에 분해되기 시작하여 16주에는 초기 질량의 50%의 감소를 보였다. 조직학적 검사 상에서 폴리다이옥사논 스텐트는 4에서 8주 사이에 분해하기 시작하였으며, 12주에는 작은 분절로 분열된 소견이 관찰되었다. 또한, 폴리다이옥사논 고분자의 분열에 따라, 8주에서 12주 추적 검사에서 스텐트 주변의 심한 염증성 반응을 동반하였다. 염증 수치 (0-4)는 4주에 1.63 ± 0.71, 8주에 1.79 ± 0.83, 12주에 2.33 ± 0.96으로 점차

증가하였으며, 12주에는 4주에 비해 통계적으로 유의하게 증가하였다. 체외 혈소판 응집 실험에서는 포스폴린콜린 코팅한 스텐트에서 혈소판 응집이 통계적으로 유의하게 감소하였으나, 생체 실험에서는 혈관내 혈전 생성이나 혈관 내피 생성 등에서 코팅하지 않은 스텐트와 차이점을 보이지 않았다. 또한, 포스폴린콜린 코팅한 스텐트에서는 염증 수치가 낮게 관찰되기는 하였으나, 통계적 의의는 보이지 않았다. 혈관내 혈전 생성 및 조직학적 협착도 염증 수치와 비슷하게 시간 경과에 따라 증가하는 소견을 보였다.

결론: 이 동물 실험에서 폴리다이옥사논 스텐트는 분해됨에 따라 혈관 내에서 심한 염증 반응과 혈관 내막의 과증식을 유발하였다. 생분해가 활발하게 일어나기 전의 4주 경과에서는 경도의 스텐트내 협착은 관찰되나 혈관 개방성은 유지되었다. 하지만, 시간 경과에 따라 혈관 협착이 증가하였으며, 이러한 염증 반응 및 혈관 내막의 증식으로 최종적으로 혈관의 완전 폐색이 유발되었다. 포스폴린콜린 코팅한 스텐트도 같은 반응을 보였다.

주요어: 생체적합성, 생분해성 혈관 스텐트, 견종 경동맥, 폴리다이옥사논, 포스폴린콜린

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