RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Development of a Novel Drug Eluting Stents Consists of an Abluminal and Luminal Coating Layer Dual Therapy System

Jun-Kyu Park [a, b], Jeong Ho Lee [e], Jae-Woon Nah [a], Han Ki Kim [b], Kyung Seob Lim [c], In-Ho Bae [c], Myung Ho Jeong [c], Afeesh Rajan Unnithan [d] ,Cheol Sang Kim [d, e*], Chan-Hee Park [e*]

[a]*Department of Polymer Science and Engineering, Sunchon National University, Sunchon, Republic of Korea*

[b]*Bioalpha Co. Ltd, Seongnam, Republic of Korea*

[c]*Cardiovascular convergence Research Center, Chonnam National University Hospital, Gwangju, Republic of Korea*

[d]*Bionanosystem Engineering Department, Graduate School, Chonbuk National University, Jeonju561-756, Republic of Korea*

[e]*Division of Mechanical Design Engineering, Chonbuk National University, Jeonju561-756, Republic of Korea*

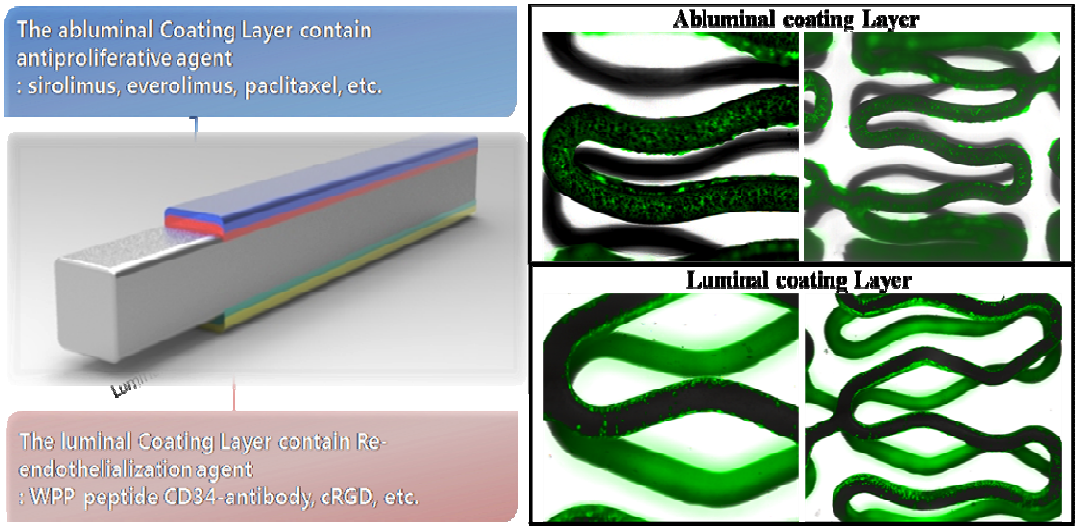
*****Corresponding Authors:

Chan-Hee Park (biochan@jbnu.ac.kr **)**

Cheol Sang Kim (chskim@jbnu.ac.kr)

Tel: +82 63 270 4284, Fax: +82 63 270 2460

Table of contents



Abstract

The aim of this study was to develop the dual drug-coated stent using bi-directional coating system. The sirolimus (SRL) was coated to abluminal area of stent for preventing restenosis. And the WKYMVm, a peptide for endothelial homing, was coated to luminal area for stent for enhancing endothelialization. To verify the bidirectional coating of materials, various morphological analysis were carried out by using optical microscope, scanning electron microscope, and fluorescence microscope. The release velocities of the drugs coated to luminal and abluminal surface of stent were sustained investigated by using instrument mimicking body circulation system. The proliferation of smooth muscle cell was inhibited by SRL. Whereas the proliferation of human umbilical vein endothelial cell was enhanced by WKYMVm. This study demonstrated that it is feasible to separate coating layer of stent strut with new coating technology for the bi-directional function of drugs.

Keywords: Stent coating; Drug-eluting stent; Dual coating; Coronary stent; Abluminal; Luminal

1. Introduction

Cardiovascular disease is a leading cause of death globally and the ratio increases each year in a dramatic way [1]. Coronary artery stenosis can be treated with coronary artery bypass surgery or angioplasty [2]. But, treatment using angioplasty can cause restenosis (recurrence of stenosis) within 6 months after the procedure [3]. Restenosis is believed to include of vascular smooth muscle cells. Over the last decade, coronary stents have revolutionized the treatment of cardiovascular disease [4]. Stents have been commonly used to prevent restenosis [5]. Stents have generally open tubular structures and can be either balloon expandable or self-expandable.

Stents have become increasingly important to restore the function of cardiovascular system. Stent based local drug release at the site of cardiovascular injury via a polymer coated stent is an attractive therapeutic method to achieve an effective local concentration of the drug for a designed period of time [6]. However, stents may stimulate foreign body reactions that result in thrombosis or restenosis. Residues of the coated polymers left after the complete elution of drug can trigger significant thrombogenic risk and are accountable for the subsequent hypersensitivity and inflammation [7]. To avoid these complications, a variety of coating strategies on stent were applied, such as different coating methods and variations in coating materials like with drug, protein, antibody, growth factor etc. Such method has been proposed in this literature to overcome the drawbacks associated with stent coating.

Instead of completely disqualifying the use of polymeric coatings on stents, the best way to increase the overall hemo-compatibility of the stent is by enriching the stent surface by a coating with inert or bioactive components. Non-thrombogenic coatings that may aid re-endothelialization were an encouraging alternative to conventional drug-eluting stents. Stent thrombus formation is mostly caused by stent surface hydrophobicity, morphology, roughness,

and electrical charge which are triggered by the adsorption of plasma proteins [8]. Low-molecular weight heparin (LMWH) immobilized stent surface were coated with sirolimus for improved thrombo-resistance. Drug-eluting stent (DES) with LMWH layer was showed 90% reduction in platelet deposition during the in vitro test [9].

The first generation anti-proliferative coated stents, paclitaxel and sirolimus (SRL), were inhibited the vascular smooth muscle cell (SMC) proliferation via cell cycle targeting [10]. SRL arrest the cell cycle at the G1 phase because mTOR transduction disruption. Sirolimus was found to be a potent and immunosuppressive agent against SMC, and blocks cell division that can lead to an arrest in the G1 interface. The antiproliferative effect of SRL has been used to prevent restenosis in DES [11, 12].

Phosphorylcholine (PC), a naturally occurring lipid group for biocompatibility in blood, is a part of the coating used for the zotarolimus eluting Endeavor® stents. However, PC-coated stents have not shown promising results despite of the favorable characteristics such as biocompatibility, non-allergenicity as well as lower inflammatory response. In fact, except stents coated with anti-proliferative drugs, there has been no significant clinical success in the area of favorable stent coatings [13]. In a study, Anti-human CD133-antibody and glycoprotein expressed on endothelial-regeneration cells, were covalently coated stent surface. This stent just achieved with effective binding of CD133-positive cells in vitro [14]. In fact, performances of DES (Drug Eluting Stents) need to increase direct cellular effect in re-endothelialization and vessel wall healing.

WKYMVm (Trp-Lys-Met-Val-D-Met-NH_2) (WPP) is synthetic peptide that angiogenesis of endothelial cells proliferation, migration, and formation such as active healing processing. To overcome the cardiovascular system, therapeutic compounds such as RLS and WPP were efficiently delivered to the cardiovascular system. The safety and

efficacy of such an approach critically depends on the delicate combination of drug, polymer, and kinetics of release [15].

In this study, we introduced stents having abluminal and luminal surface coated with different coating materials containing drug to avoid restenosis and enhance the re-endothelialization (Figure 1). Here, we present a novel ultrasonic spraying dual coating system (fabricated by own system) in which two different drug solutions are used for making abluminal and luminal surface coated stent using two spray nozzle with separate drug solution syringe pumps. Our set-up is different than the reported manual ultrasonic spray system set-ups because we use a robot-controlled movable multi-armed system. We evaluated the present set-up by checking the changed surface of coated stent and their effect (dual coated stent) on cumulative amount of drug release.

2. Results and discussion

2.1. Identification of coating method on abluminal and luminal surface of the stent strut

We prepared a DES using an ultrasonic spray coating method with our newly developed device in our schematic coating design (Figure 1). The OM images shows that luminal stent coating has a bright violet color on the luminal surface of the stent (Figure 2, a-1 and a-2), while abluminal surface had not a bright color. On the contrary, abluminal coating stent showed bright color on the abluminal surface of the stent without luminal coating layers (Figure 2, b-1 and b-2). The exchangeable drug positioning of polymer and drug and simultaneous coating to luminal and abluminal surface were investigated by DES with abluminal coating (DAC), DES with luminal coating (DLC), and DES with abluminal and luminal coatings (DALC), respectively. As shown in Figure 3, the conventional morphology of metal and polymer were observed in abluminal and luminal area of stent, respectively. It

showed that coating layer was separated between luminal and abluminal on stent surface according to process. Figure 4 shows the fluorescence microscope (FM) images of abluminal or luminal coated layer. The images also showed the uniform dispersion of FITC labeled fluorescent WPP. These results confirmed that stent has been coated only on the luminal surface not on the abluminal surface of the stent. On the contrary, abluminal coated stent show fluorescence on the abluminal surface of the stent compared to the luminal surface of stent.

General DES with circumferential coating has been shown to delay re-endothelialization and induced in-stent thrombosis during arterial repair [16]. To overcome this limitation, control over positioning of drug coating to luminal or abluminal is required. According to this study, in-stent restenosis and thrombosis may be prevented by coating an abluminal surface of a stent strut with a drug for preventing restenosis and coating a luminal surface of the stent strut with an endothelialization accelerating drug. Figure 5a shows that the coating thickness of abluminal coating layer (9.5±0.95 μm) and coating thickness of luminal coating layer (4.4±0.82 μm). The concentration of drug and thickness were higher in abluminal area when compared to luminal area. This caused because of different coating method along with the coating position of drug. For the abluminal coating, top-to-bottom coating system was employed. Whereas, for the luminal coating, nozzle was located at beside of stent and drug was sprayed through reflect plate. Therefore, more drug loss to inner cavity side was happened when performing luminal coating. Figure 5b-1 showed atomic force microscopy (AFM) image of surface roughness on luminal layer (Ra: 6,593 nm) and Figure 5b-2 showed AFM image of surface roughness on abluminal layer (Ra: 15.888 nm). The value of abluminal surface roughness was lower than luminal surface.

2.2. Determination of drug loading and in vitro release

Referring to the stent surface, the SRL loading per mm^2 stent surface was 1.4±0.03 μg in abluminal coating layer, and the WPP loading per mm^2 stent surface was 1.0±0.05 μg in luminal coating layer. The in vitro drug release kinetics was also determined by HPLC for 32 days at regular intervals.

The release profile of WPP and SRL from DLC (Figure 6a), DAC (Figure 6b), and DALC (Figure 6c) are shown in Figure 6. The drug release was faster in luminal area compared to abluminal area. An initial burst release of WPP release within the first day more than 30% was observed in the luminal coating layer, whereas no apparent burst release of SRL was observed. Only slow release observed within the first day from abluminal coating layer of DES. After 8 days, the cumulative release percentage of WPP release from stent was approximately 80% and was continuous for 32 days. In contrast, the cumulative percentage of SRL released from stent was significantly slow. In vitro drug release from DES exhibited broadly a sustained release during the test period.

Hydrophilic peptide of WPP readily distributes and conveys in tissue, but is rapidly cleared in the absence of tissue. Hydrophobic SRL, on the other hand, is more often subject to associative or dissociative resulting in sustained localization close to the delivery source. This drug binding slowed transport, leading to high concentrations of drug localized near the site of application. This excessive tissue SRL concentration, in fact, exceeds the driving concentration. In contrast, a hydrophilic drug such as WPP would have lower tissue levels than those of the driving medium, resulting in a low volume of arterial distribution. Hydrophobic drug displayed more heterogeneous local tissue concentration than hydrophilic drug, but achieved higher mean concentrations. Hydrophobic drugs also tended to remain closer to the intima following luminal release, while hydrophilic drugs achieved higher

concentrations in deeper layers [17].

The commercially available sirolimus-eluting stent releases its drug in a similar pattern over several week and the stent is completely free against sirolimus within 45 ~ 60 days [11]. The drug release of DLA, DAC, and DALC were slowly released for 32 days. The release velocity of SRL and WPP in DLA was similar to DALC. These results suggest that the coating method for distinguishing the luminal and abluminal does not affect the drug release and the coating thickness of each other. The drug and polymer were mixed simply with identical solvent and coated to surface. Thus, as the coating thickness increased gradually, so the amount of drug and polymer was increased. In addition, drug release velocity was delayed with increasing of drug amount as well (Figure 5a).

2.3. Assay of smooth muscle cell and endothelial cell proliferation

The results showed that HUVEC cell viability after 7 days of DAC, DLC and DALC were 80%, 170% and 153%, respectively. The groups with WPP increased cell proliferation (Figure 7). During the treatment of DALC in SMC, the cell viability has no change when compared to the DLC treatment group. The WPP has absolutely no effect on SMC viability. The in vitro experiments indicated that SRL inhibited SMC proliferation in a concentration-dependent manner, SRL (< 0.1 mM). The WPP did not affect the SMC proliferation compared with control. But the WPP affect the HUVEC proliferation. The WPP was also indicated a potent and highly efficacious agent at HUVEC cell. The DLAC showed inhibition towards SMC proliferation and proliferation of HUVCE. The results would be beneficial in decreasing restenosis and healing improvement of stent covered endothelial cell in the process of vascular wall healing.

2.4. *In vivo* study

Figure 8 shows the lumen surface of rabbit iliac artery at 14 days and the results showed that the DLC, DAC and DALC were covered the surface of stent. An enhancement of strut coverage ratio in the DLAC group (77.5%) was higher than that of DAC group (50.5%). However, the coverage ratio of DLAC group is quite similar to DLC group (78.1%). It doesn't show fibrin deposition on the stent for all groups without thrombosis. This results might suggest to conclude that luminal surface with WPP accelerate the artery for healing process. When compared with other stents, the stent coverage ratio was reported to be 53.5 % for bare metal stent (BMS) in rabbit iliac artery for 28 days [18]. PTCA containing DES for maintaining an expanded vessel wall and continuous drug eluting for suppressing the proliferation of neointima layers were done more effectively by suppressing proliferation and movement of smooth muscle cells. However, for arterial healing, a surgery using a DES may induce more serious disorders than BMS. Furthermore, since endothelialization occurs slowly, DES induces in-stent thrombosis more frequently than BMS [10]. According to the result, arterial healing showed obvious difference on luminal surface.

3. Experimental

3.1. Materials

Poly [D, L-lactide-co-glycolide] (PLGA, 50:50, Mw= 48 kDA) was purchased from EVONIK (UK) Sirolimus was purchased LC Laboratories (USA) and WKYMVmpeptide (WPP) was purchased Anygen (Korea). Nile red, phosphate-buffer saline (PBS), 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) and Molecular porous dialysis membrane bags (MWCO: 3,500 Da) were Sigma-Aldrich (st. Louise, Mo, USA). All other chemicals and solvents were analytical or reagent and used without further purifications.

3.2. Novel ultrasonic coating system development

Novel ultrasonic coating system was carried out using the set-up we have designed, which is shown in Figure 9. It was composed of two separate ultrasonic nozzles with diameters of 0.51 mm (CERATORQ Corp. KOREA), two 10 mL plastic syringes, and robot system that moves laterally controlled by LabVIEW 2013 program (National Instruments, USA). The whole setup was placed in a sealed chamber with automatic exhaust system, dehumidifier and heating system.

The ultrasonic coating was carried out at 24ºC and 35% humidity with a closed chamber. The most important feature of our setup is the ultrasonic spray for inside of stent coating (Figure 10) which can easily be controlled via a robot system. A robotic system having a linear servo motor (TPC for one axis linear motor) to enable micro step control was used. The robot can be controlled through a computer PCI bus controller (National Instruments PCI-7390) using the LabVIEW 2013 program interface. We can precisely control the robot's acceleration, speed and stroke distance. The system consists of noise filter in front of the power supply for stability. To control the stroke position, we provided a limit photo sensor to the robot. Furthermore, we developed a terminal block for the easy connection of the robot to the PCI-7390 board (National Instruments, USA).

3.3. Novel method for coating on abluminal and luminal surface of the stent strut

To identify coating surface, we prepared coating solution with Nile red. Briefly, coating method is as follows. PLGA (20 mg) was dissolved in 5 mL tetrahydrofuran (THF) and nile red solution was added (1 mg) and stirred for 1 hour. Bare metal stent (BMS, Co-Cr L605 alloy) was coated ultrasonic spray method using our coating machine (Model, Korea).

The coating solution was applied to operate for flow rated of 50 µl/min while BMS was placed on a mandrel that is attached to a rotating shaft. To remove residual solvent in stent, coated stent was vacuum-dried for 24 hour. The whole procedure of stent coating is explained in Figure 8. First move to fixed coating jig following the pin provided with stent. After inserting into the fixture, the supply pin is removed. Then the needle type ultrasonic nozzle is moved to the inside of stent, after coating the inside of the Stent.

3.4. Identification of coating method on abluminal and luminal surface of the stent strut

The surface morphology was observed by Optical microscope (OM, Xi-CAM, Bestec Vision, Korea), Scanning electron microscopy (SEM, SNE-1500M, SEC Co., Ltd, Korea), and fluorescence microscope (FM, JULI, NanoEnTek, korea). SEM sample was sputter coated with gold for 1.5 min to achieve a coating of approximately 15 nm. To investigate the homogeneity and identification of coating on abluminal and luminal surfaces FM were used. The distribution of the coating layer with fluorescent marker nile red was detected using an excitation wavelength of 488 nm. The green nile red was identified with 530 nm and 10x and 20x objective, The overlay of the fluorescence and the transmission light was recorded.

3.5. Preparation of drug eluting stent of abluminal and luminal coating (DALC)

A schematic representation of the coating system is proposed in Figure 1. The coating solution on luminal surface of the stent strut was prepared 2.0 wt% PC and 0.5 wt% WPP in Methanol. BMS with a length of 18 mm and a diameter of 1.6 mm were fix mandrel and the solution was sprayed on to the rotating stent at a distance of 0.5 cm. After coating, the residual Methanol was removed by drying under vacuum for 24 hour.

The coating solutions of abluminal surface of the stent strut were prepared 5.0 wt% PLGA and 0.5 wt% sirolimus in THF. BMS with a length of 18 mm and a diameter of 1.6

mm were fix mandrel and the solution was sprayed on to the rotating stent at a distance of 3 cm. After coating, the residual THF was removed by drying under vacuum for 24 hour.

3.6. Coating evaluation

The surface morphologies of coated stent were observed by OM and SEM. Samples of SEM were sputter coated with gold for 1.5 min to achieve a coating of approximately 15 nm. Coated stents were also inspected in the FM. Coating thickness was measured by Reflection spectrometry (RS, F40, Filmetrics, Inc., USA). The topography and the roughness of the surfaces were measured using atomic force microscopy (AFM, XE-100, Park System, Korea) under a tapping mode at room temperature.

3.7. Determination of drug loading and in vitro release

To determine the drug loading of the coated stents, standard curve was obtained from various concentrations of SRL and WPP by high pressure liquid chromatography (HPLC, Nanospace SI-2, Shiseido, Japan). The coating stent was expanded into silicon tube by balloon (3.0 × 20 mm) with 10 mmHg of pressure, and then both ends of silicon tubes were dip in 5 mL of 10% ACN/PBS pH 4.5 solutions at 37℃.

Release measurements were performed using a peristaltic pump at 37℃ for 32 days in a set up similar to body's blood circulation system. The solution in collected release was detected at 278 nm (SRL) and 330 nm (WPP) using a HPLC. The chromatographic separation of SRL and WPP was accomplished using 50 x 4.6 mm, Phenomenx Kuna C18 – 3 μm reverse phase analytical column. The mobile phase consisted of methanol, water, formic acid of 90:10:0.02 v/v%. The measurements were carried out in triplicate.

3.8. Assay of smooth muscle cell and endothelial cell proliferation

Smooth muscle cells (SMC) and human umbilical vein endothelial cells (HUVEC) were purchased from the American Type Culture collection and grown in DMEM and EGM-2 supplemented with 10% FBS and 1% penicillin/streptomycin sulfate. Cells were maintained under 37℃, 5% CO_2 up to passage 3 and used at the predetermined concentration. The combination effect of SRL and WPP on cell proliferation was determined using the MTT cell proliferation assay. Cells were seeded at a density of 5×10^4 per well in 96-well plates using 100 μL of DMEM supplemented in a CO_2 incubator for 24 hour. After that coating stent containing sirolimus and WPP were added. After 7 days of incubation, MTT was added to the 96 wells. The absorbance was measured at 560 nm using a microtiter plate reader (Thermomax microplate reader, Molecular Devices).

3.9. *In vivo* study

The study was performed with the approval of the ethics committee of Chonam National University Hospital for the research. Since implantation of stent was performed by expanding of balloon, stent was located to intima of artery. The animal experiment on New Zealand white rabbits has been described previously [18]. The DLC, DAC and DALC were implanted in the left or right iliac arteries for 14 days. The stent was deployed by inflating the balloon catheter to nominal pressure (artery ratio: 1.1~1.3). The animals were sacrificed with 5 ml of potassium chloride intracarotid artery. Iliac arteries on the stent were removed carefully and fixed in 10% neutral buffered overnight. The arteries were step-sectioned and were processed for SEM.

3.10. Statistical analysis

Statistical analysis was performed with the aid of the commercially available software (SPSS Version 11.0, Chicago, IL). The results are expressed as the mean ± standard deviation (SD) from three experiments. A value of * $p < 0.05$ was considered statistically significant.

4. Conclusion

In summary, this study demonstrated that it is feasible to have separate coating layer of stent strut with new coating technology designed to increase stent endothelialization. This technology may promote vascular healing using PTCA. We showed that drug release pattern from abluminal and luminal coating layer results in inhibition of SMC proliferation and proliferation of endothelial cells. In future perspective, we are planning to have animal experiment with BMS, DLC, DAC, DLAC, and circumferential DES to evaluate the preclinical evaluation.

Notes

The authors declare no competing financial interest.

Acknowledgments

This research was supported by a grant from the technology innovation Development Project funded by the Korean Small and Medium Business Administration. (Project no. S2083465)

References

1 A. N. DeMaria, *J Am Coll Cardiol*, 2013, **61**, 1205.

2 D. W. Park, K. B. Seung, Y. H. Kim, J. Y. Lee, W. J. Kim, S. J. Kang, S. W. Lee, C. W. Lee, S. W. Park, S. C. Yun, H. C. Gwon, M. H. Jeong, Y. S. Jang, H. S. Kim, P. J. Kim, I. W. Seong, H. S. Park, T. Ahn, I. H. Chae, S. J. Tahk, W. S. Chung and S. J. Park, *J Am Coll Cardiol*, 2010, **56**, 117.

3 C. Bauters, J. M. Lablanche, F. Leroy and M. E. Bertrand, *Arch Mal Coeur Vaiss*, 1992, **85**, 1515.

4 M. S. R. Frombach, W. Seger, *Gesundheitswesen*, 1997, **59**, 447.

5 J. R. Sindermann, V. Verin, J. W. Hopewell, H. P. Rodemann and J. H. Hendry, *Cardiovasc Res*, 2004, **63**, 22.

6 S. M. S. McGinty, C. McCormick, M. Wheel, *Math Med Biol*, 2014.

7 T. F. Luscher, J. Steffel, F. R. Eberli, M. Joner, G. Nakazawa, F. C. Tanner and R. Virmani, *Circulation*, 2007, **115**, 1051.

8 G. Sydow-Plum and M. Tabrizian, *Mater Sci Tech-Lond*, 2008, **24**, 1127.

9 J. N. Zhao, R. Falotico, T. Nguyen, Y. Cheng, T. Parker, V. Dave, C. Rogers and J. Riesenfeld, *J Biomed Mater Res B*, 2012, **100B**, 1274.

10 E. C. Perin, *Rev Cardiovasc Med*, 2005, **6 suppl 1**, S13.

11 A. S. Puranik, E. R. Dawson and N. A. Peppas, *Int J Pharmaceut*, 2013, **441**, 665.

12 (a) S. N. Sehgal, *Clin Biochem*, 1998, **31**, 335; (b) W. Khan, S. Farah, A. Nyska and A. M. S. Lee, S. A. Yoo, C. S. Cho, P. G. Suh, W. U. Kim and S. H. Ryu, *J Immunol*, 2006, **177**, 5585.

13 M. N. Babapulle and M. J. Eisenberg, *Circulation*, 2002, **106**, 2734.

14 A. Sedaghat, J. M. Sinning, K. Paul, G. Kirfel, G. Nickenig and N. Werner, *Clin Res*

Cardiol, 2013, **102**, 413.

15 J. Domb, *J Control Release*, 2013, **168**, 70.

16 (a) A. T. O. S. Vaina, P.W. Serruys, *Minerva Cardioangiol*, 2005, **53**, 341; (b) J. Zhang, *Int Heart J*, 2014, **55**, 213.

17 (a) K. H. Min, K. Park, Y. S. Kim, S. M. Bae, S. Lee, H. G. Jo, R. W. Park, I. S. Kim, S. Y. Jeong, K. Kim and I. C. Kwon, *J Control Release*, 2008, **127**, 208; (b) C. J. Creel, M. A. Lovich and E. R. Edelman, *Circ Res*, 2000, **86**, 879; (c) C. W. Hwang, D. Wu and E. R. Edelman, *Circulation*, 2001, **104**, 600.

18 (a) I. H. Bae, I. K. Park, D. S. Park, H. S. Lee, M. H. Jeong, *J. Mater Sci Med*, 2012, **23**, 1259; (b) Y. Z. Wu, L. Shen, q. B. Wang, X. Hu, J. Xi, J. Y. Qian, and J. B. Ge, *Chinese Med J*, 2012, **125**, 983

Figure Legends

Figure 1. Schematic design of abluminal and luminal coated stents: The abluminal surface of the stent elutes the antiproliferative agent and the luminal surface of the stent elutes the re-endothelialization agent.

Figure 2. Optical microscopy image of a-1& 4.a-2 : Luminal side coated stent (a-1. luminal side focused and a-2. abluminal side focused respectively), b-1 & b-2 : Abluminal side coated stent (b-1 luminal side focused and b-2 abluminal side focused respectively), c-1& c-2 : Abluminal and Luminal side coated stent (c-1 of luminal side focused and c-2 of abluminal side focused respectively). The microscopy has a magnification of 5X.

Figure 3. Scanning electron microscopy analyses of luminal side coated stent (a-1 & a-2 respectively), abluminal side coated stent (b-1 & b-2 respectively), abluminal and luminal side coated stent (c-1 & c-2 respectively). The microscopy has a magnification of x50 and x100.

Figure 4. Fluorescence microscope image of luminal side coated stent (a-1 & a-2 respectively), abluminal side coated stent (b-1 & b-2 respectively), abluminal and luminal side coating stent (c-1 & c-2 respectively). The microscopy has a magnification of x10 and x20.

Figure 5. Coating thickness analyses (a) and AFM tapping mode images (b) of DALC

(abluminal coating layer (●) luminal coating layer (○)).

Figure 6. Cumulative amount of released sirolimus (○) and WPP (●) from DLC(a), DAC(b), and DALC(c).

Figure 7. Cell viability against HUVEC with (a) A10 cell (b) abluminal side coated stent with sirolimus (DAC) and luminal side coated stent with WPP (DLC), and abluminal and luminal coating stent with sirolimus and WPP (DALC) after 3 days. Five experiments (N=5) were performed for each concentration per drug.

Figure 8. Representative SEM images of harvested arteries on stents for 14 days of post-implantation (a, a-1: DALC, b: DAC, and c: DLC).

Figure 9. 3D schematic representation of novel dual ultrasonic spray coating system.

1. Moving cylinder for ultrasonic nozzle position movement
2. Adjustment device for position of ultrasonic nozzle that is able to change the position of the nozzle:
3. Ultrasonic nozzle: The device for spraying a coating solution for coating the surface of the Stent:
4. Syringe pump: A pump for supplying a coating solution to ultrasonic nozzle:
5. Moving Motor of reflecting plate for inside coating: to move the reflecting plate for coating the inside of Stent,

6. Cylinder for Jig fixed: On / off control of Jig fixed device

7. Rotation Motor & Gear: Motor & Gear that brings a rotary motion to Jig as coating that can be uniformly on stent surface:

8. Jig fixed device for inside coating: Locking device of the jig for the internal coating.

Figure 10. The working procedure for inside stent coating.

1. Move to fixed coating jig after pin provided with stent

2. After inserted into the fixture, the supply pin is removed.

3. Ultrasonic nozzle which is a type of needle that is moved to the inside of stent, after coating the inside of the stent.

4. Final coated stent product

Fig. 1.

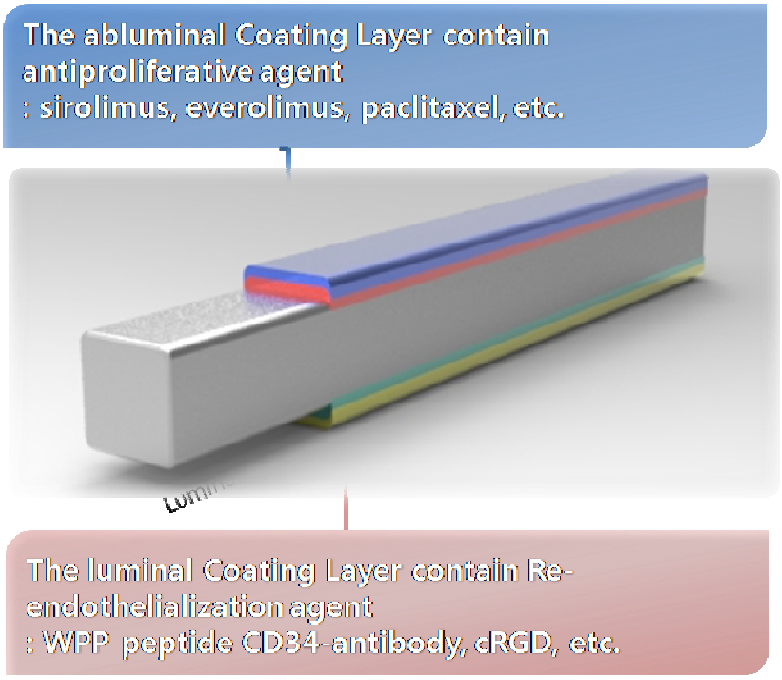


Fig. 2.

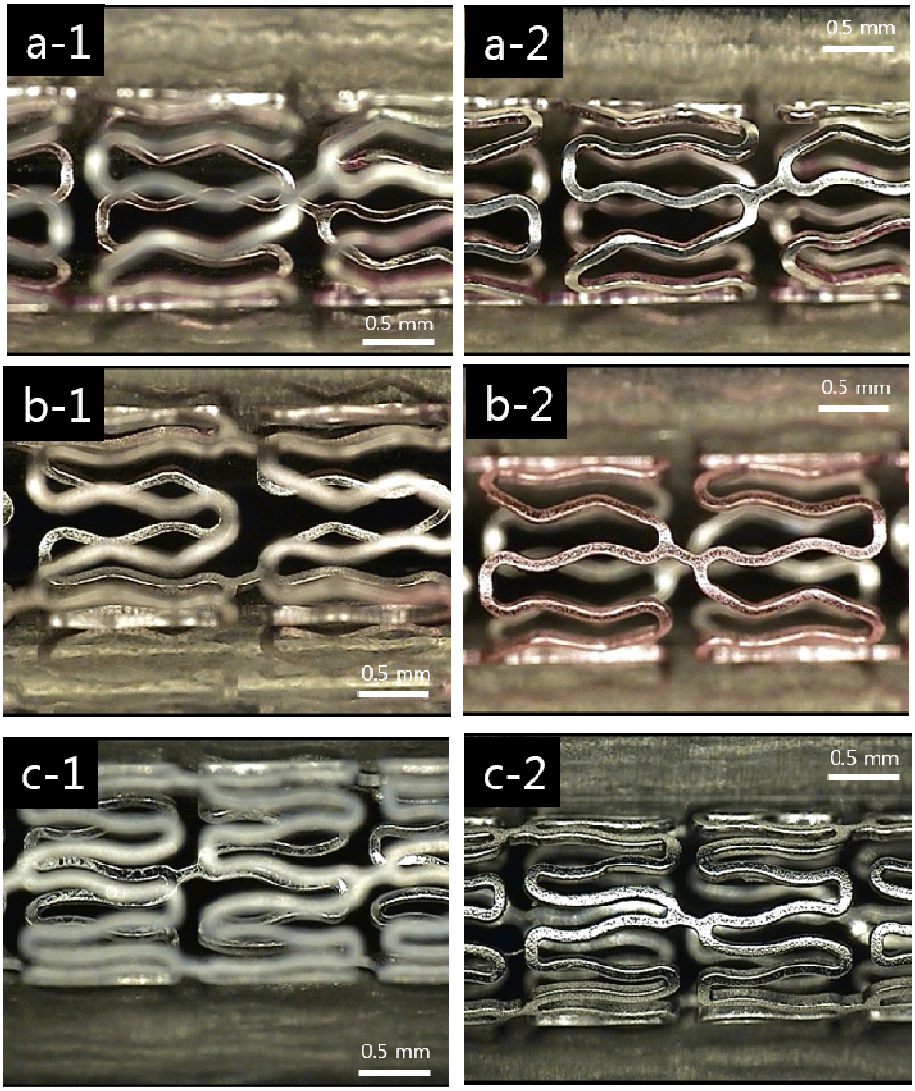


Fig. 3.

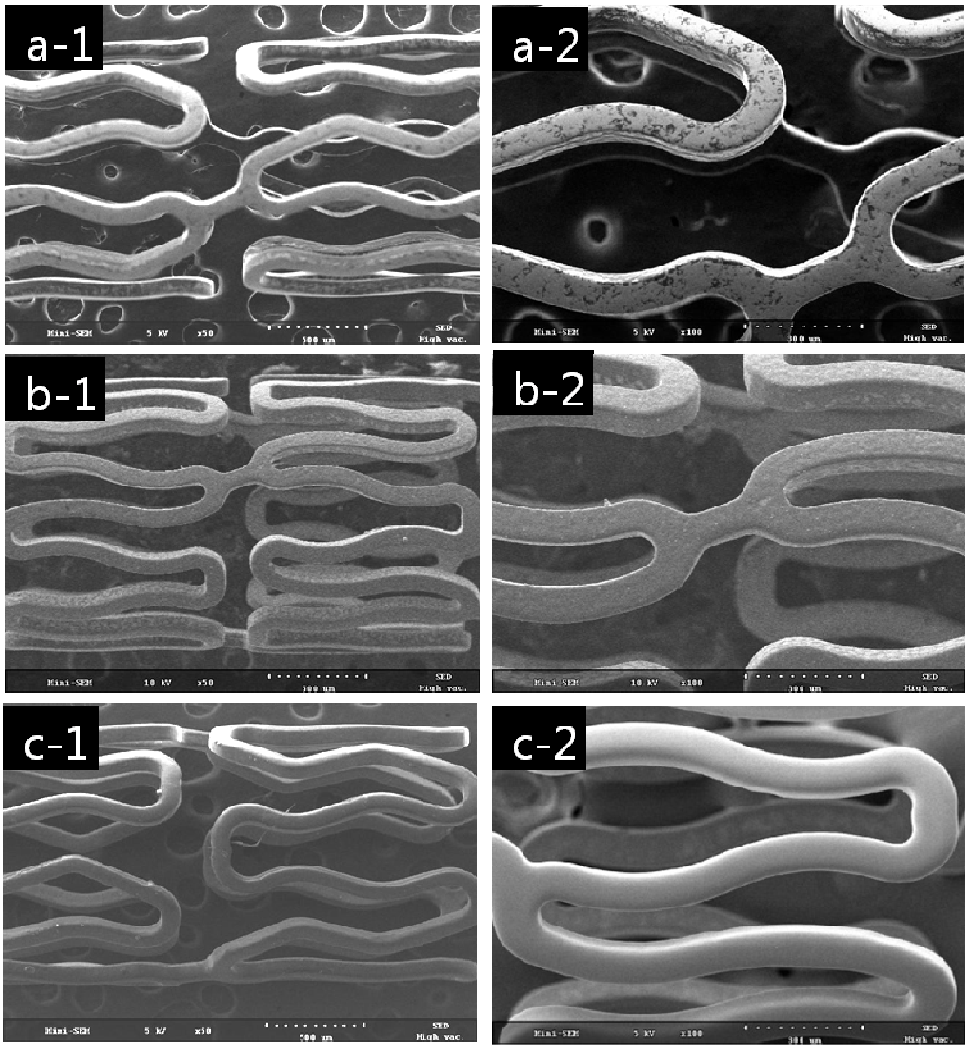


Fig. 4.

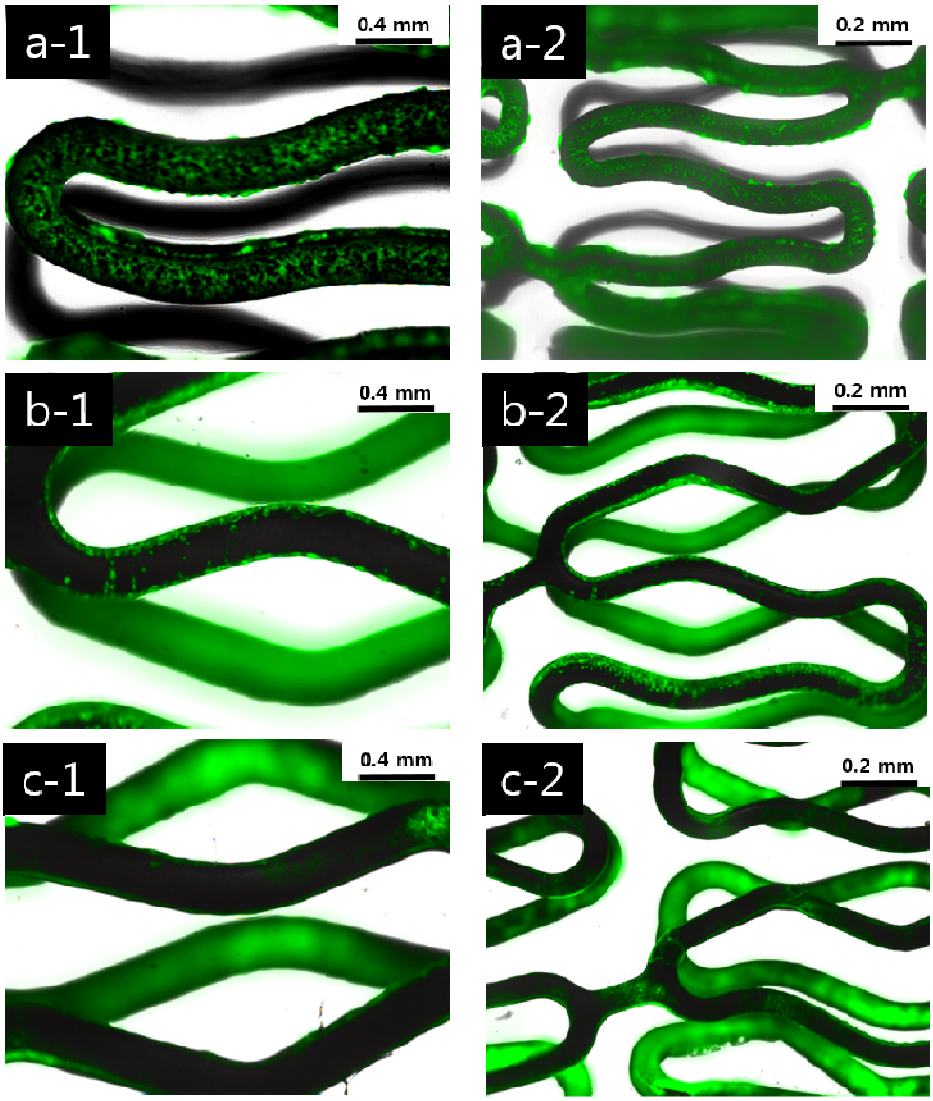


Fig. 5.

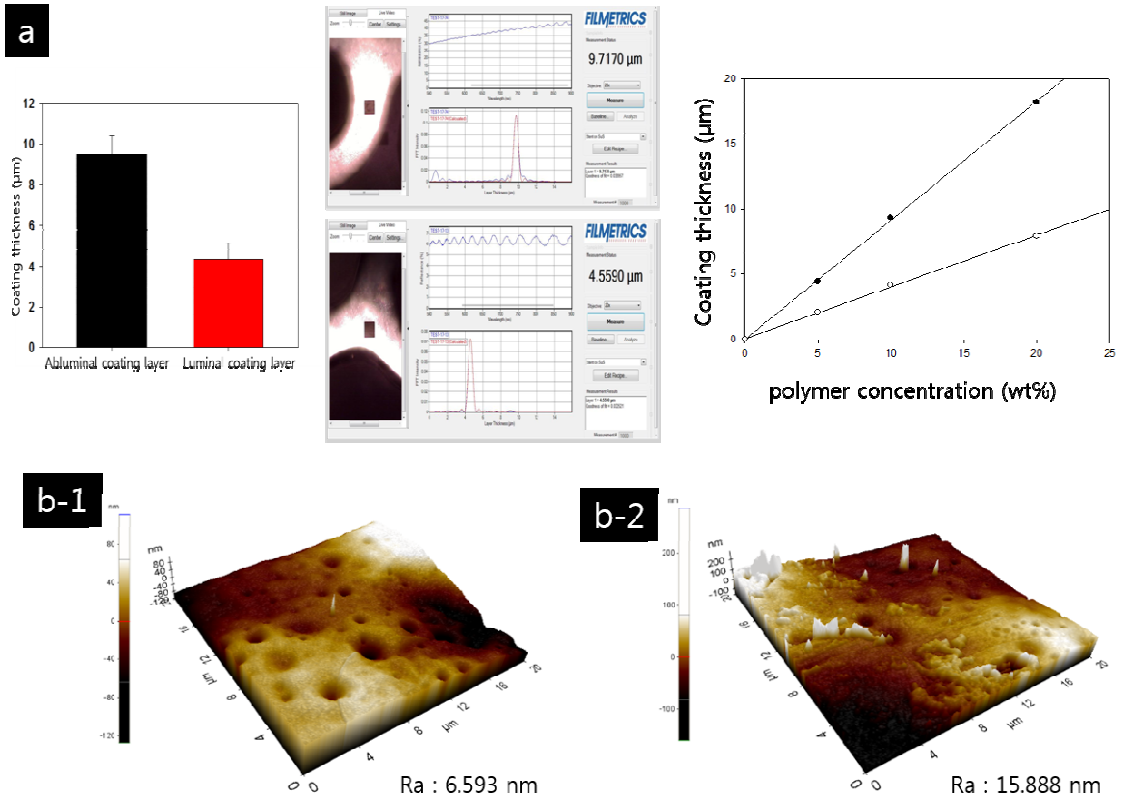


Fig. 6.

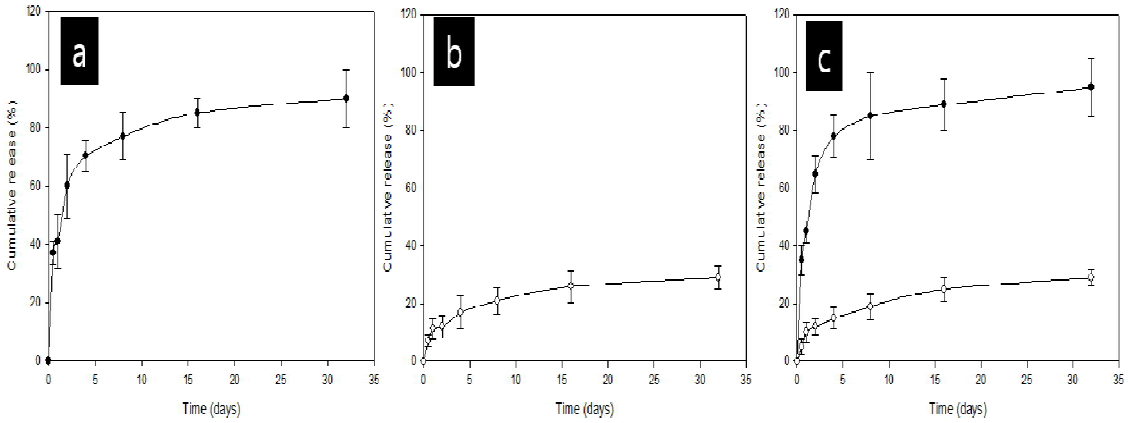


Fig. 7.

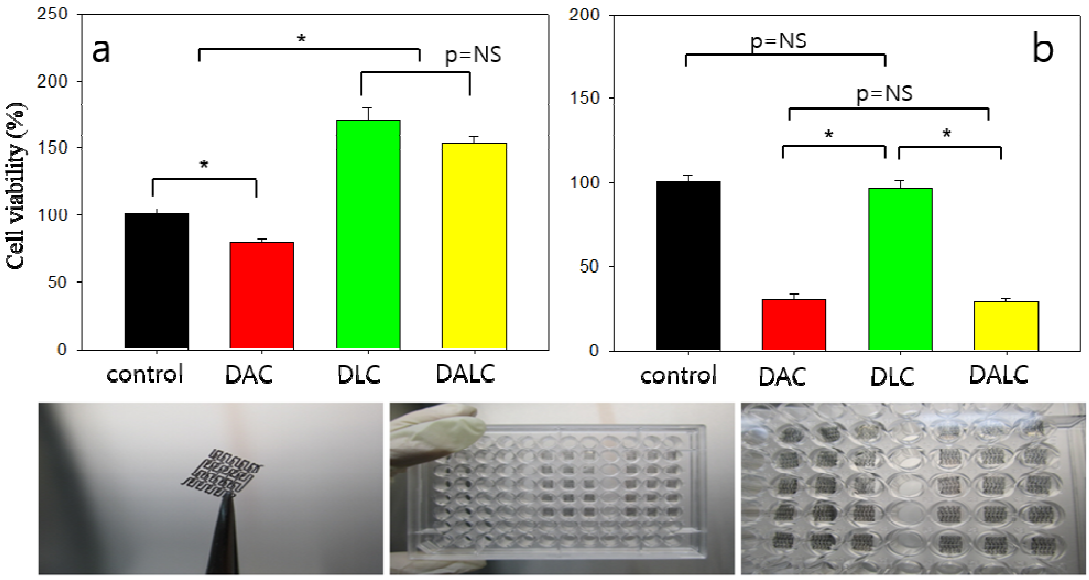


Fig. 8.

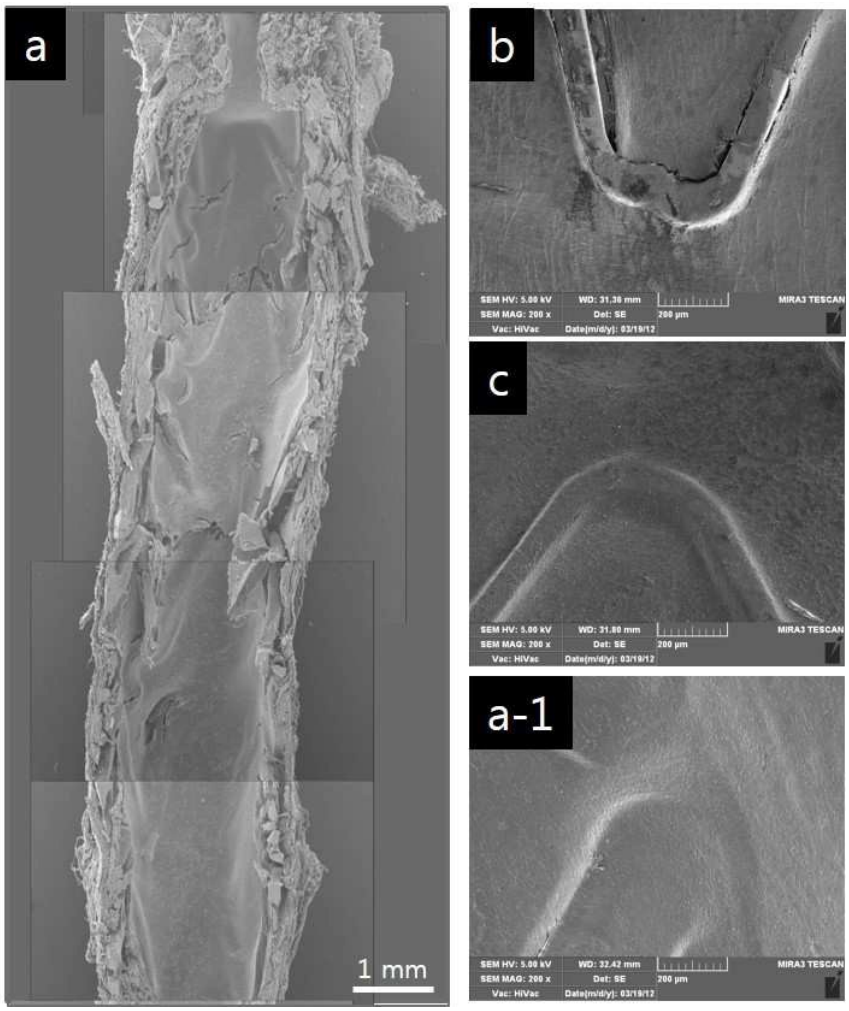


Fig. 9.

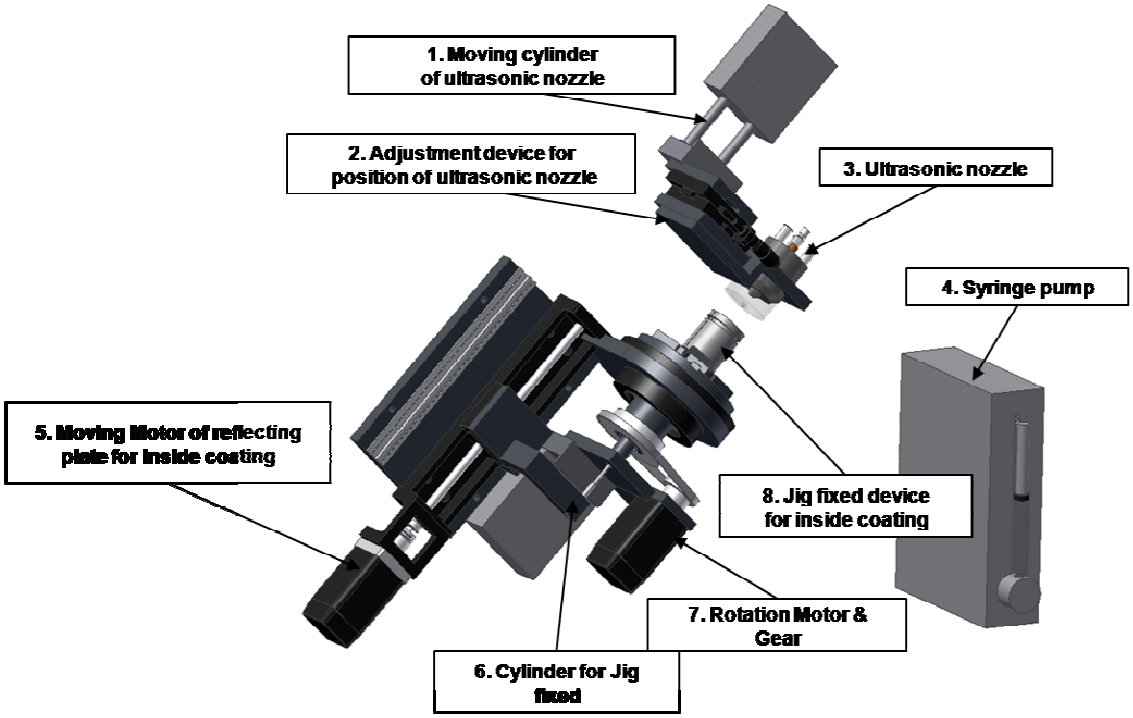


Fig. 10.