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Estrogen release from metallic stent surface for the prevention of restenosis

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Abstract

For the prevention of coronary restenosis, estrogen was coupled onto a metallic stent and in vitro release of estrogen was investigated. Estrogen was introduced to the metal surface using a hydrolysable covalent bond for local sustained delivery of drug as follows: (i) the stainless steel (SS) surface was activated with silane by plasma polymerization, (ii) the activated surface (SS–Si surface) was treated with acrylic acid by plasma polymerization (SS–Si–AAc surface), and (iii) 17β-estradiol (E_2) was covalently linked to the carboxyl group on that surface (SS–Si–AAc–E_2 surface). The modified surfaces were characterized by X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FT-IR) spectroscopy, and water contact angle measurement. The amount of E_2 was measured by UV–visible spectrophotometry and high performance liquid chromatography (HPLC). The in vitro release profile of E_2 demonstrated sustained release of E_2 in aqueous buffer. In summary, a novel method of immobilizing estrogen onto a metallic stent surface using plasma polymerization has been developed. The obtained results attest to the usefulness of the estrogen-releasing stent for preventing restenosis.

Keywords: Restenosis; Estrogen-releasing stent; Estrogen immobilization; In vitro release; Metallic stent

1. Introduction

Coronary artery diseases (CAD) are still a leading cause of death in industrialized countries. Percutaneous transluminal coronary angioplasty (PTCA) and bypass surgery are well-established methods of treating these patients [1,2]. However, revascularization induces thrombosis and neointimal hyperplasia, which in turn cause restenosis in 30–40% of coronary arteries within 6 months after successful balloon angioplasty and in over 60% of aorta-coronary saphenous vein bypass grafts within 5 years following surgical intervention [3]. Despite extensive research on the incidence, timing, mechanisms, and pharmacological interventions in humans and animal models, no therapy consistently prevents this difficult problem [4,5].

Local administration of pharmacologic agents

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directly to the site of coronary intervention has been advocated as a means of concentrating drug in the injured arterial tissue to inhibit restenosis, and several designs of local delivery catheters and drug-eluting stents have been evaluated in patients and animal models [6]. The main benefits of local drug delivery are attenuation of toxic side-effects of potent drugs by decreasing the general drug burden and the potential for prolongation of local pharmacodynamic effects by physical or chemical binding of the drug to the delivery vehicle. Recently, development of techniques to reliably provide sustained local drug delivery has focused on the use of synthetic or biologic polymers as matrices for drug incorporation and elution [7–9]. However, a consistent limitation of these local delivery techniques appears to be the rapid washout of agents from the arterial wall within hours or even minutes of administration [10]. The immobilization of pharmacological agents on the stent surface and their sustained release from the coating is a very promising approach to prevent post angioplasty restenosis [11]. The coating materials as well as the coating process and the choice of drug to be immobilized are critical to therapeutic effectiveness [12]. Therefore, it was considered that effective drug should be successfully immobilized on the stent surface by a proper process in this study.

The protective effects of estrogen for restenosis have been investigated by several researchers. The incidence of coronary artery disease in premenopausal women is lower than in men and increases after the menopause [13–15]. Estrogen replacement therapy in postmenopausal women markedly reduces cardiovascular events [13,16,17]. Hence, female sex hormones and 17β-estradiol in particular may protect women against vascular disease [13]. A major effect of estrogen is that it inhibits vascular smooth muscle cell proliferation and migration [18]. In recent studies, prolonged systemic administration of estrogen has been shown to inhibit intimal hyperplasia in animal studies [19,20].

Plasma polymerization of organic compounds to produce thin coatings has been an area of research since the early 1970s [21,22]. The films are highly cross-linked, very strongly bound to almost every substrate and are relatively chemically and thermally stable [23,24]. The cross-linking of plasma polymers makes a strong matrix on substrates, and the formation of thin films on substrates has the effect of inhibiting cracks arising from the increased internal stresses as coating film thickness increases [25,26]. Therefore, we have chosen a plasma treatment technique for immobilization of drug onto stent surface.

Local drug release from a drug-bound stent might minimize the toxic side-effects of estrogen by decreasing the general drug dose which will lower the drug's exposure to circulation. Besides, the estrogen-releasing stent in this study has advantages such as the avoidance of additional therapeutic procedures and site-specific delivery in balloon-injured coronary artery. Immobilization of the drug on the stent surface using biodegradable ester bond allows sustained release of the drug, thus improving the efficacy of the restenosis treatment.

In this study, estrogen was coupled onto metal surface using plasma treatment and the physicochemical properties of estrogen bond surfaces were investigated, including in vitro drug-releasing behavior.

2. Materials and methods

2.1. Materials

Stainless steel (SS) plates of 5 mm in thickness were initially rinsed by sonicator with 99.5% ethanol. The plates were then dipped into chromic acid solution for 30 min. Finally they were cleaned with distilled deionized water. Cleaned plates were stored under vacuum. 17β-Estradiol (E_2), *N*-(dimethylamino) pyridine (DMAP) and dicyclohexylcarbodiimide (DCC) were purchased from Sigma (St. Louis, MO, USA). Tetrahydrofuran (THF) was purchased from Mallinckrodt Baker (Philipsburg, NJ, USA). Benzalkonium chloride (BKC) was purchased from Aldrich (Milwaukee, WI, USA). All other chemicals were used as received without further purification.

2.2. Estrogen immobilization onto SS surface

Estrogen was immobilized onto a stainless steel stent surface which was pretreated using plasma.

Initially the plasma-polymerized silane coated SS surface (SS–Si) was prepared using hexamethyldisilane (HMDS). This was prepared in a chamber with a linear microwave source at a power of 300 W and frequency of 2.46 GHz. The SS–Si surface was further plasma polymerized using acrylic acid monomer to introduce reactive carboxylic group on the SS surface (SS–Si–AAc).

The plasma deposition system comprised a radio frequency (RF) power source (13.56 kHz). The RF supply was coupled via copper bands to a deposition chamber that was evacuated to a base pressure of ~1 Pa. The deposition chamber consisted of cylindrical Pyrex® glass. The flow of acrylic acid vapor into the deposition chamber was regulated through the use of manually adjusted metering valves. Acrylic acid flow rate was monitored throughout all procedures and a constant flow rate of 0.8 sccm was maintained. The pressure in the chamber was kept constant (30 Torr) for all depositions using a valve on the vacuum pumping line. The deposition power of 10 W was supplied for 30 min. Acrylic acid was deposited on the SS–Si surface positioned down-stream of the region where gas plasma was formed. After one side of the plate sample was treated, the other side was treated under the same conditions (SS–Si–AAc).

Finally, E_2 was covalently coupled to carboxyl group on the SS–Si–AAc surface by DCC method. E_2 (1.0 mmol) was dissolved in 10 ml THF, followed by the addition of DCC (1 mmol) and DMAP (0.1 mmol). SS–Si–AAc surface was immersed under shaking in glass tube containing the reaction mixture at 50 °C for 24 h. The plate was taken out from the reaction mixture and was rinsed with pure THF repeatedly. The cleaned plate was dried thoroughly under vacuum overnight.

2.3. Surface characterization

2.3.1. XPS measurements

Modified SS surfaces were characterized by X-ray photoelectron spectroscopy (XPS). The XPS spectra were acquired using an ESCALAB MK-II spectrometer (VG Scientific, UK) with a Mg Kα X-ray source (1486.6-eV photons) at power of 400 W. The core-level signals were measured at a photoelectron take-off angle of 90°. Binding energies of elemental source were referenced to the C1s carbon peak at 284.6 eV. In XPS survey spectra, Si, O and C peaks were measured and C1s peak was analyzed by narrow scan spectra. In a particular spectrum, the distinct elemental peaks were fitted by Gaussian curves and the full-width at half-maximum (FWHM) for the Gaussian peaks was maintained constant for all components.

2.3.2. Static contact angle

The contact angle of modified surfaces was measured by the Sessile drop method. The contact angles were obtained using a DGD Fast/60 contact angle goniometer (GBX, France) at constant room temperature under ambient laboratory conditions. Five plates of each surface were measured and the measurements of at least five spots on each plate sample were recorded.

2.3.3. FT-IR measurements

The FT-IR spectra were acquired using infrared reflection absorption spectroscopy (IRRAS) at a grazing angle of 80° on a Magna-IR™ 550 spectrometer (Nicolet, USA). An HgCdTe detector was used and a GRASEBY SPACE grazing angle reflectance accessory was attached to the spectrometer.

2.4. In vitro release

E_2 release from surface was analyzed by high-performance liquid chromatography (HPLC) in vitro. E_2 immobilized plates were placed in stopped 15-ml conical tubes containing 5 ml of 1.0% BKC solution. The tubes were placed in an shaking orbital incubator operating at 100 rpm, 32-mm orbit diameter, and maintained at a temperature of 37 °C. Samples were taken daily for HPLC analysis. Chromatographic methods were employed with a C_{18} bonded reversed phase column, an acetonitrile–water (50:50, v/v) mobile phase and a UV detection wavelength of 281 nm. A series of solutions ranging from 0.266 to 272.4 μg/ml were prepared for the HPLC calibration curve [27].

3. Results and discussion

The process of SS surface modification by silanization, introduction of carboxyl group and immobili-

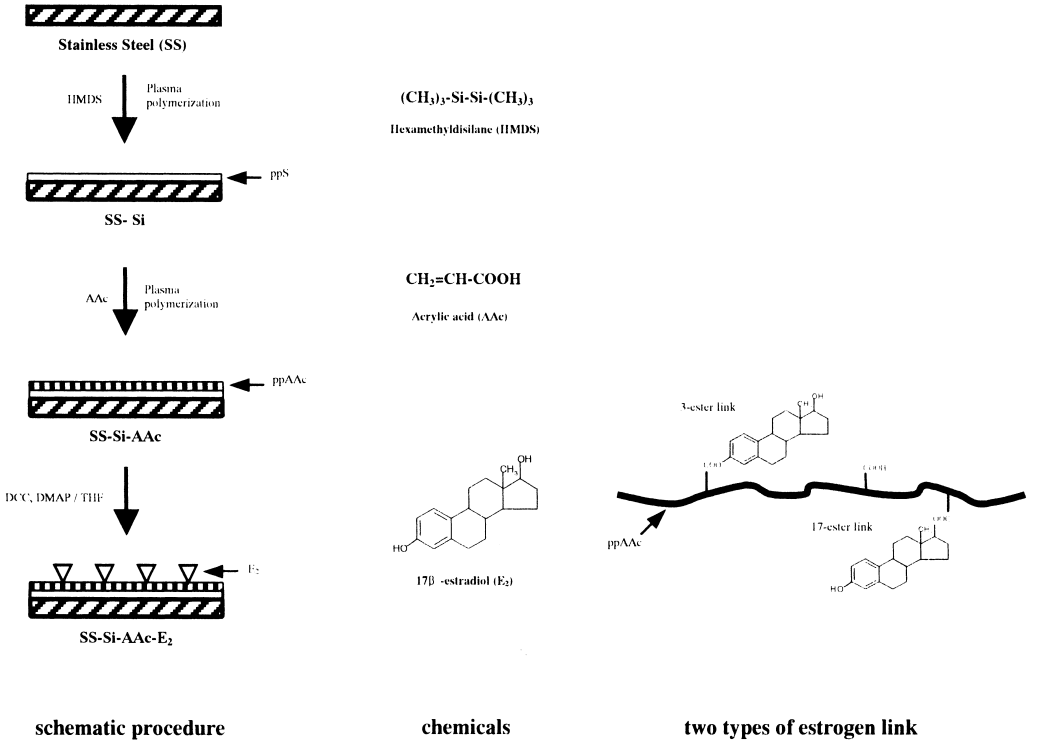

Fig. 1. Schematic procedure of E_2immobilization onto SS surface.

zation of 17β-estradiol is shown in Fig. 1. Silanization on SS surface was carried out by plasma polymerization using HMDS (SS–Si). Acrylic acid was then deposited by plasma polymerization on the silanized surface (SS–Si–AAc). Finally, $E_2$was successfully immobilized by chemical bonding between hydroxyl group of E_2 and carboxyl group on the SS–Si–AAc using DCC (SS–Si–AAc–E_2).

3.1. Surface characterization

3.1.1. Silanization of SS surface: SS–Si surface

Components on SS surface were derived from the intrinsic carbon component of stainless steel and trace amounts of hydrocarbon or hydrated oxide contaminants on the SS surface (Table 1). In the

Table 1
Atomic composition of surfaces measured by XPS

Surface	Treated with	Atomic composition (%)				
		C			O	Si
		C–C/C–H	C(=O)O–	C–O–		
SS	–	21.5	8.7	–	69.8	–
SS–Si	Hexamethyldisilane	31.0	–	8.6	17.9	23.5
SS–Si–AAc	Acrylic acid	42.9	16.3	12.0	27.9	–
SS–Si–AAc–E_2	17β-Estradiol	51.6	20.4	6.8	5.2	–

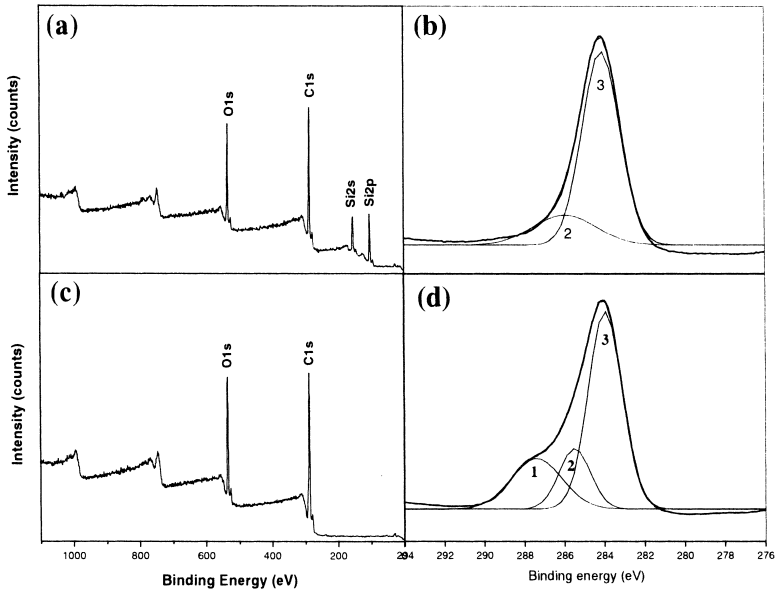

Fig. 2. XPS survey and C1s core level spectra from silanized surface (SS–Si) (a,b), and carboxylated surface (SS–Si–AAc) (c,d). 1: C(=O)–O (BE = 287.4 eV); 2: C–O (BE = 286.5 eV); 3: C–C/C–H (BE = 284.2 eV). 2 in (b): C–Si/C–H. BE = Binding Energy.

silanized surface (SS–Si), silicon composition was observed and oxygen composition was drastically decreased. In Fig. 2(b), the C1s core level spectrum of the silanized surface was fitted with two peak components with binding energies at 284.2 eV for C–Si/C–H species and 286.5 eV for C–O species. These results indicate that HMDS was plasma-polymerized to form thin film on SS surface. C–Si/C–H peak arose from natural structure of HMDS, and C–O peak was induced by incorporation of air in plasma and exposure in air after plasma process. For SS–Si surface, ([C(=O)–O] + [C–O])/[C] ratio is lower than for any other surfaces, and [C(=O)–O]/[C] is approximately zero (Table 2). These results are correlated with the theoretical structure of plasma polymerized HMDS which contains not carbonyl group (C=O) but ether group (C–O).

In IR-RA spectra (Fig. 3), the Si–O–Si peak observed from 1070 to 1120 cm^{-1} is of particular interest. This peak suggests that formation of Si–O–Si bridges is affected by the presence of oxygen in the plasma. $Si–CH_x–$ peaks also appeared at 1260 and 840 cm^{-1}; this result arises from the chemical structure of ppHMDS network on SS surface.

Silanization provided hydrophobic surface with contact angles of 80.9° (Table 3). This could be

Table 2
Summary of atomic composition ratios

Surface	Treated with	Ratios of compositions		
		[O]/[C]	([C(=O)–O] + [C–O])/[C]	[C(=O)–O]/[C]
SS	–	2.31	0.29	0.29
SS–Si	Hexamethyldisilane	0.45	0.22	–
SS–Si–AAc	Acrylic acid	0.39	0.40	0.23
SS–Si–AAc–E_2	17β-Estradiol	0.07	0.35	0.26

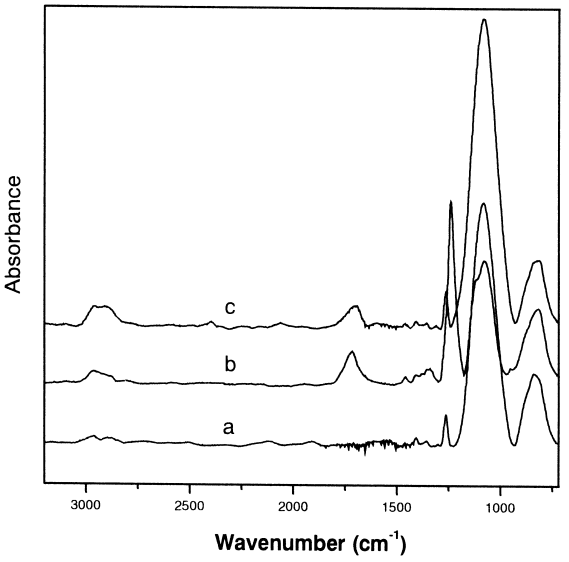

Fig. 3. IR-RAS spectra of (a) SS–Si, (b) SS–Si–AAc, and (c) SS–Si–AAc–E_2 surfaces.

attributed to the hydrophobic character of HMDS containing silicon and methyl group.

3.1.2. Deposition of carboxyl group: SS–Si–AAc surface

In the wide scan spectrum of XPS, Si signals disappeared and intensity of oxygen signal increased (Table 1, Fig. 2). In Fig. 2(d), C1s core level spectrum of SS–Si–AAc surface was fitted with three peak components with binding energies at 284.2 eV for C–C/C–H, 286.5 eV for C–O and 287.4 for C(=O)–O. C(=O)–O peak indicates existence of carbonyl group derived from carboxyl group of plasma-polymerized acrylic acid (ppAAc) film. In Table 2, ([C(=O)–O]+[C–O])/[C] ratio is the highest, and this correlates well with other results. In contrast, [O]/[C] ratio decreased after plasma polymerization of acrylic acid. For the formation of carboxyl group, although oxygen content derived from acrylic acid was added to the surface, carbon content of acrylic acid and loss of oxygen through plasma process resulted in comparatively increased carbon content, and therefore, decreased [O]/[C] ratios are shown in XPS spectrum.

In Fig. 3, C=O bridges from 1690 to 1720 cm^{-1} in spectra b and c are a distinctive structure of carboxyl group, and it is confirmed that carboxyl group was introduced onto SS–Si surface.

The ppAAc deposited surface (SS–Si–AAc) had a contact angle of less than 60°. This surface is less hydrophobic than the SS–Si surface due to the carboxyl group moiety in ppAAc on SS–Si surface.

3.1.3. Immobilization of E_2: SS–Si–AAc–E_2 surface

XPS data show that carbon related compositions increased due to $E_2$with a large amount of carbon (Tables 1 and 2). E_2 immobilized surface shows the contact angle of ~77.4° which is higher than that of SS–Si–AAc (57.5°). This might be due to the bound hydrophobic E_2, resulting in higher contact angle. These results suggest that E_2 was covalently bonded on plasma-polymerized surface.

3.2. Chemical stability of estrogen

Chemical stability of E_2 through modification processes and release test was confirmed by comparing HPLC and UV–visible spectra obtained from E_2 samples released from SS surfaces with those derived from freshly prepared solutions of E_2. Fig. 4

Table 3
Static contact angle of modified surfaces

Surface	Treated with	Contact angle (deg.)[a]
SS	–	69.0±7.2
SS–Si	Hexamethyldisilane (HMDS)	80.9±2.7
SS–Si–AAc	Acrylic acid	57.5±0.6
SS–Si–AAc–E_2	17β-Estradiol	77.4±1.4
E_2[b]	17β-Estradiol	95.8±1.4

[a] Mean values±S.D. ($n=5$).
[b] SS surface was coated with estradiol solution.

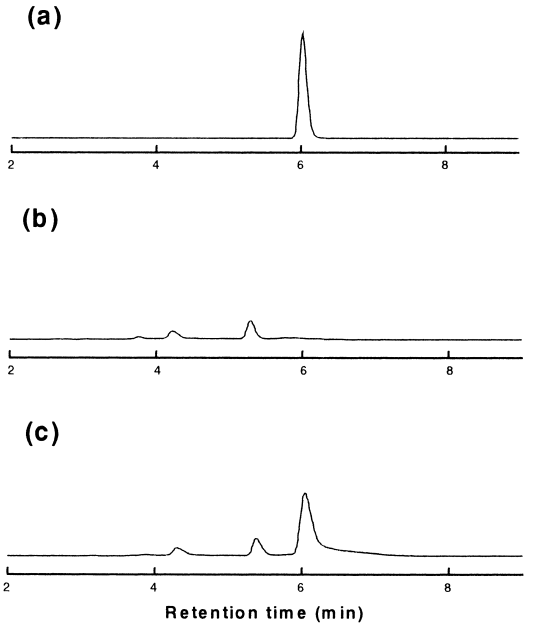

Fig. 4. Retention time of (a) estrogen standard solution, (b) 1% BKC solution, and (c) estrogen released in 1% BKC solution, measured by HPLC.

shows that E_2 released in media has the same chemical structure as fresh E_2 dissolved in aqueous solution.

3.3. In vitro release of estrogen

In vitro E_2 release behavior from plasma-modified SS surfaces was investigated.

Hydrolysable ester linkage of estrogen (17β-estradiol) to carboxyl group allows estrogen to be released from the stainless steel surface under aqueous conditions. Release profile of estrogen from the modified SS surface is shown in Fig. 5. Surface-bound E_2 was determined by HPLC. The content of estrogen on the surface was measured to be $\sim 12 \pm 4.2\ \mu g/cm^2$. Released estrogen was also observed by HPLC for ~2 weeks. The curve is extrapolated as linear on all the recorded values for 14 days.

This release profile result is significant. An initial release burst did not occur. Generally, drug release from polymer-based matrices and polymer-coated

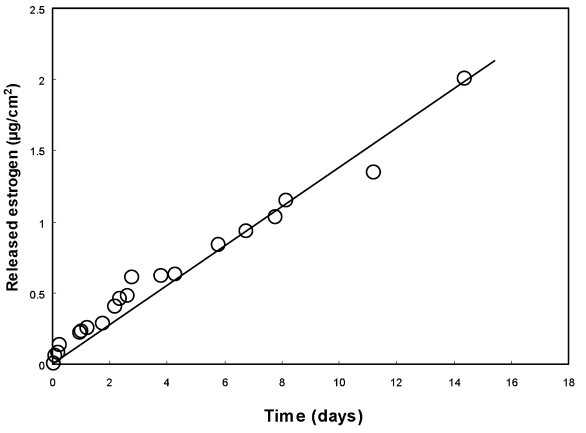

Fig. 5. Cumulative release profile of estrogen from modified stainless steel surface ($y = 0.005x$, $r^2 = 0.964$).

stent is followed by initial bursting. But, in this study, the covalent bond of drug on surface prevents drug loss by initial bursting. As a result, toxic side-effects caused by the high dose release and the loss of potent hormone, estrogen, seemed to be effectively inhibited.

Immobilization of estrogen onto stent allows sustained release of the drug, thus prolonging the period of treatment. For drug delivery systems for the treatment of restenosis, the cellular mechanisms in restenotic neointimal formation should be understood. Studies on the porcine coronary injury model using immunocytochemical methods show that after injured to artery, smooth muscle cells (SMCs) begin to proliferate ~24 h later and migrate from adventitia through media to neointima; 2 weeks the cellular proliferation is largely completed [28,29]. Based on this mechanism, the 2 weeks until proliferation is completed is very important to prevent restenosis. For this reason, our release system is beneficial to prevent restenosis.

E_2-releasing stent for in vivo studies has been prepared using the same reaction condition as SS surface. In vivo efficacy of E_2-releasing stent is under investigation in a porcine coronary injury model. The preliminary results demonstrated significantly lower restenosis incidence of E_2-releasing stent (8.58%) than non-treated stent (11.62%). Long-term in vivo performance is the subject to further study.

4. Conclusions

A novel drug-releasing stent was developed through chemical coupling of estrogen onto plasma treated stainless steel stent surface. Surface characterizations demonstrated unique properties of estrogen bound surface and plasma treated surface. The amounts of estrogen bound to the surface can be regulated by changing immobilization conditions. The estrogen-releasing stent is expected to be effective for sustained and site specific drug delivery to the coronary artery, resulting in minimized restenosis.

Acknowledgements

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