Electrocoating of stainless steel coronary stents for extended release of paclitaxel

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Abstract: Nonbiodegradable polymer coating based on *N*-(2-carboxyethyl)pyrrole (PPA) and butyl ester of PPA (BuOPy) were successfully electrodeposited on a stainless steel stent surface using cyclic voltammetry. Chemical composition of the coating was examined by X-ray photoelectron spectroscopy. Polymer stability was examined by immersing the coated stent into 1:1 solution of fetal calf serum:seline solution up to 1 year and implantation subcutaneously in mouse for 1 week. Morphology changes were then recorded by scanning electron microscopy. Paclitaxel loading was carried out by immersion into drug solution and its release was detected by HPLC. The results show that thin (single micrometers), uniform coating with various morphology and hydrophobicity can be created by electrochemical deposition. The polymer did not show significant histopathological or morphological changes *in vitro* and *in vivo*. The surface properties allow loading appropriate amounts of paclitaxel and release it slowly up to a month. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 00A: 000–000, 2008

Key words: pyrrole; electrocoating; stainless steel; stent; local drug delivery

INTRODUCTION

The implantation of coronary stents is a widely accepted therapy of obstructive coronary artery disease. The application of stents in coronary lesions showed promising results in overcoming the main limitation of angioplasty alone, by providing mechanical scaffold to the vessel wall. Still, this method is not free of complications. Stent implantation causes wall injury, moreover, the metal surface of the stent itself, constitutes a thrombogenic foreign body. A number of studies have focused on development of biocompatible coating, such as heparin,[1] phospholylcholine,[2] and different methacrylate derivatives.[3] According to long-term biocompatibility investigation in humans, these coatings are blood tolerable. These biocompatible candidates can be used as a carrier for local antirestenotic drug administration. Different strategies have been developed to deliver and release the therapeutic agent. Two most successful drug eluting systems, currently approved by FDA are methacrylate and polyethylene based, nonbiodegradable polymer coating loaded with sirolimus (Cypher by Cordis and Johnson and Johnson)[4] and Translute [poly(styrene-*b*-isobutylene-*b*-styrene)] (SIBS) polymer coating with paclitaxel (Taxus by Boston scientific).[5] A drug can be incorporated during stent coating, either by attaching it to the polymer functional groups or loading it into the polymeric coating after its creation. Nonbiodegradable polymers need to provide blood tolerance after the specified therapeutic period.

A number of approaches for stent coating have been developed. One is dipping the stent into the polymer-drug solution and drying the deposited film on the stent. This coating has some significant disadvantages, such as "bridging," pooling and lack of uniformity, which leads to difficulties in scaling up the process. Uniform, continuous coating can be achieved by spraying the stent with the polymer solution. Nevertheless, the thickness of the resulting coating is greater than desired (~8–15 μm). Another method applied is chemical vapor deposition (CVD), which allows obtaining uniform coatings of controlled thickness and morphology. CVD methods require high temperature, pressure environments, and complex equipment.[6]

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Considering all these disadvantages there is a need for a simple, reproducible, coating process to create uniform, thin composites on the stent surface. Since stents are made of stainless steel it is possible to use its conducting properties to deposit the coating electrochemically.

About three decades ago, the discovery of high conductivity in organic conjugated materials, such as polyacetylene, stimulated studies and synthesis of new polymers.[7] These materials have received special attention while developing electrocatalysts, biosensors, optoelectronic devices, and anticorrosive coatings. Among conducting polymers, polypyrrole, polyaniline, and polythiophene[8,9] are the most often used.

Recent studies have shown that polypyrrole is biocompatible and hemocompatible.[10,11] *N*-pyrrole derivatives are attractive candidates for electropolymerization of reactive substrates such as stainless steel due to their relatively low oxidation potential. We have reported in detail the synthesis and characterization of pyrrole derivatives and the coating of stainless steel with these polymers.[12] In this contribution we examine the application of electropolymerization of *N*-pyrrole derivatives for coating stents. Specifically, we study the physical and chemical properties of the coatings and the drug release from the polymer coated stents. We found that the *N*-(2-carboxyethyl)pyrrole (PPA) and butyl ester of PPA (BuOPy) are nontoxic and biocompatible. Moreover, pBuOPy coated stent elutes paclitaxel over an extended period of 3 weeks.

EXPERIMENTAL

Materials

316L stainless steel plates (10×20 mm^2) were used for characterizing the electrodeposited polymers, while 316L stainless steel stents were applied for stability, flexibility, *in vitro* drug release, and animal biocompatibility studies. The stents had a length of 12 mm, a surface area of $\sim$18 mm^2, and a closed diameter of 1.5 mm (both plates and cardiovascular stents were produced by STI Laser Industries, Israel). Paclitaxel was purchased from Sigma-Aldrich.

Pyrrole (Fluka) was purified from short oligomers and oxidized byproducts by distillation before use. 1-(2-Cyanoethyl)pyrrole, tetrabutyl ammonium tetrafluoroborate (TBATFB), and other reagents were obtained from Sigma-Aldrich and used without further purification. Silica gel for column chromatography was purchased from Merck, Germany. Sodium dodecyl sulfate (SDS), disodium hydrogen phosphate, and sodium dihydrogen phosphate were purchased from Reidel-de Haën. All aqueous solutions were prepared from deionized water (Barnstead Easy pure UV system).

Procedures and characterization

Monomer analysis

^{1}H NMR spectra of synthesized monomers were recorded on a Varian 300-MHz instrument with $CDCl_3$, DMSO-d_6, and D_2O as solvents. Values were recorded as parts per million relative to the internal standard TMS (tetramethylsilane).

Infrared (IR) spectroscopy was performed with a PerkinElmer System 2000 Fourier transform infrared spectrometer (FTIR) on samples cast on NaCl plates from chloroform or dichloromethane solution. EIMS and CIMS spectra were performed with VG Autospec M250Q.

Electrochemical system

Electrochemical measurements were conducted with 630B electrochemical analyzer (CH Instruments, Austin, TX) using a single compartment three-electrode glass cell. The reference electrode was an Ag|AgBr wire that was used in organic media. The latter has a potential of 0.448 V versus ferrocene-ferrocenium (Fc/Fc^+). A 0.5 mm diameter platinum wire or graphite rod was used as an auxiliary electrode. 316L stainless steel plates or stents were used as working electrodes.

Surface analysis

X-ray photoelectron spectroscopy (XPS) was used for chemical analysis of the polymer surface with Axis Ultra spectrometer (Kratos Analytical, Manchester, UK), and Mg Kα radiation of 1486.71 eV. Data were collected and analyzed by a vision processing program. Surface morphology of the coatings before and after drug release was determined by scanning electron microscopy (SEM). Contact-angle measurements were performed with a Rame-Hart (model 100) (Mountain Lakes, NJ) telescopic goniometer. Thickness of the polymer coatings were determined by profilometery (P-15, KLA-Tencor, San Jose, CA). The amount of paclitaxel in buffer solutions was determined by high-performance liquid chromatography (HPLC, Hewlett Packard, Waldbronn, Germany) system composed of an HP 1100 pump, HP 1050 UV detector, and HP ChemStation data analysis software.

Methods

Monomer preparation

N-methyl pyrrole was distilled from a commercially available product: ^{1}H NMR ($CDCl_3$): 6.77 (t, 2H, CH-β), 6.33 (t, 2H, CH-α), 3.80 (s, 3H, N—CH3). The synthesis of PEG2000 diester of PPA (PEG2000 dipy) was previously reported.[12]

Other *N*-substituted monomers were synthesized as follows:

1. *N*-(2-carboxyethyl)-pyrrole (pyrrole propionic acid, PPA) was synthesized according to the literature,[13]

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with slight modifications: 1-(2-Cyanoethyl)pyrrole (1 equiv.) was added to a KOH (5 equiv.) aqueous solution and refluxed until no more ammonia gas was released. The mixture was cooled and acidified with concentrated hydrochloric acid to pH > 4, until the product precipitated in the reaction medium, and then extracted by diethyl ether, while maintaining the low pH by adding HCl during extraction. The organic phase was dried over magnesium sulfate and evaporated to dryness, yielding an off-white product that was recrystallized from boiling *n*-heptane. The yield was about 90%. ^{1}H NMR ($CDCl_3$) δ: 6.68 (t, 2H, CH-β); 6.15 (t, 2H, CH-α); 4.21 (t, 2H, N—CH_2); 2.82 (t, 2H, CH_2COO). FTIR (NaCl): 3108, 2949, 1705, 1502, 1208 cm^{-1}. EIMS ($C_7H_9NO_2$, MW = 139.1) m/z (%): 139.1 (100), $[M]^+$; 94.1 (44), $[M—COOH]^+$; 80.1 (97), $[M—CH_2COOH]^+$ Elemental analysis: C, 60.33 wt % (calcd. 60.42); H, 6.70 wt % (calcd. 6.52); N, 9.99 wt % (calcd. 10.07).

2. Butyl ester of PPA (BuOPy) was synthesized by esterification of PPA in excess of butyl alcohol over night (70°C) in the presence of catalytic amount of *p*-toluene sulfonic acid and magnesium sulfate, as a drying agent. The solvent was evaporated to dryness and the ester residue was washed with saturated sodium bicarbonate solution and ethyl acetate. The product was purified on a silica gel column using dichloromethane as eluent. The yield was 52%. ^{1}H NMR ($CDCl_3$) δ: 6.66 (t, 2H, CH-β), 6.13 (t, 2H, CH-α), 4.21 (t, 2H, N—CH_2), 4.09(t, 2H, $COOCH_2$), 2.76 (t, 2H, CH_2COO), 1.59(m, 2H, COO—CH_2—CH_2), 1.35 (m, 2H, COO—CH_2—CH_2—CH_2), 0.92 (t, 3H, CH_3). FTIR (NaCl):3095, 2959, 1734, 1500, 1167, 1090, 724 cm^{-1}. EIMS ($C_{11}H_{17}NO_2$, MW = 195.26) m/z(%): 195.2 (100), $[M]^+$; 94.1 (95), $[M—(C=O)OBu)]^+$; 80.1 (93%), $[M—CH_2(C=O)OBu)]^+$. Elemental analysis: C, 67.50 wt % (calcd. 68.02); H, 8.83 wt % (calcd. 8.78); N, 7.07 wt % (calcd. 7.16).
3. PEG400 diester of PPA (PEG400 dipy) was synthesized by esterification with two equivalents of PPA per PEG dialcohol group under azeotropic reflux overnight in toluene with catalytic amount of *p*-toluene sulfonic acid. The PEG derivative was purified by chromatography with silica gel column using 10% methanol in dichloromethane as eluent.

PEG400 dipy:1H NMR (DMSO-*d*6) δ: 6.66(d, 4H, CH-β), 5.11 (d, 4H, CH-α), 4.20 (t, 8H,N—CH_2) 4.23 (t, 8H,$COOCH_2$), 3.66 (t, 4H, $COOCH_2CH_2$), 3.63 (m 36H, PEG400), 2.79 (t, 4H, CH_2COO). FTIR (NaCl): 2863, 1736, 1461, 1355, 1188, 1138 cm^{-1}. CIMS (isobutane) m/z (%): 701 (2) $[MH]^+$ for $C_{34}H_{56}N_2O_{13}$ MW = 700; 657 (5) $[MH]^+$ for $C_{32}H_{52}N_2O_{12}$ MW = 656; 613 (20) $[MH]^+$ for $C_{30}H_{48}N_2O_{11}$ MW = 612; 569 (65) $[MH]^+$ for $C_{28}H_{44}N_2O_{10}$ MW = 568; 525 (100) $[MH]^+$ for $C_{26}H_{40}N_2O_9$ MW = 524; 481 (46) $[MH]^+$ for $C_{24}H_{36}N_2O_8$ MW = 480.

Surface pretreatment

316L stainless steel plates were treated with 2000 grit emery paper following by gentle polishing with 1 and 0.05 μm alumina paste. Cleaned plates were washed in an ultrasound bath with DDW and acetone (15 min for every solvent) and dried with a gentle stream of nitrogen. 316L stainless steel stents were treated with dichloromethane solution, followed by dipping the stents in 40% solution of HNO_3 for 5 min at room temperature (23°C), then rinsed with DDW three times, and finally cleaned in an ultrasound bath with DDW and acetone and dried with a gentle stream of nitrogen.

Electropolymerization of pyrrole derivatives

The clean electrodes were immersed into a solution of 0.1*M* pyrrole derivative and 0.1*M* TBATFB in acetonitrile (ACN) at room temperature. A potential sweep between −0.4 and 1.4 V versus Ag|AgBr (5 cycles) was applied (unless otherwise mentioned). The coated working electrode surface was rinsed with pure ACN and dried with a gentle stream of nitrogen.

Coating thickness measurement

All surface measurements were carried out on 316L stainless steel plates. Poly(propanoic acid)pyrrole (pPPA), poly(butyl ester)pyrrole (pBuOPy), poly(propanoic acid: butyl ester)pyrrole (9:1) (see Scheme 1) were prepared electrochemically as described above and in our previous report.[12] Their thickness was measured on plates with polymer coatings deposited by applying 5 potential cycles each, using profilometery. S1

Contact angle measurements

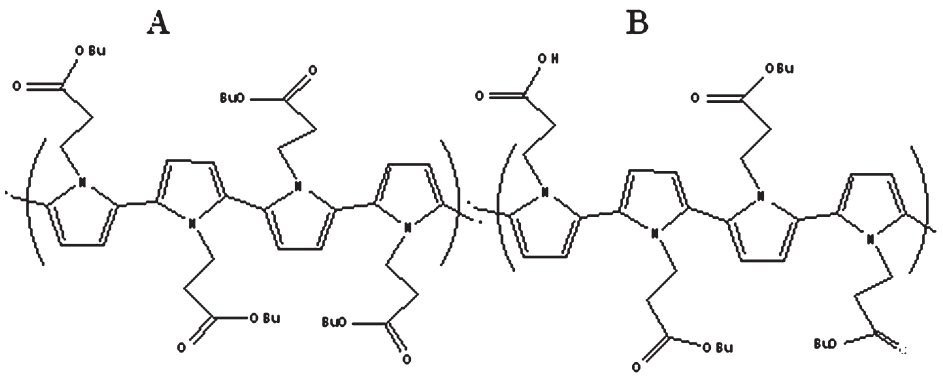
Contact angle of homo and copolymers were measured using the sessile drop method: a small droplet of water was placed on the coated surface, and the tangent angle between the drop and the surface was measured. This measurement was repeated three times for each sample, and the average values are reported.

Polymer stability

Coated stent samples were placed in 1:1 solution of fetal calf serum:saline with 0.1% of PEN-STREP-NEOMYCIN solution (10,000 U/mL, 10 mg/mL, 10 mg/mL, respectively) (Biological industries, Kibbutz Beit Haemek, Israel) up to 1 year. Before examination, coated samples were rinsed three times with DDW and dried.

In vivo biocompatibility

Two female C3H mice 10- to 11-weeks-old with average weight of 20 g (Harlan Laboratories, Israel) were anesthetized by Intraperitoneal (IP) injection with 5% chloral hydrate (BioLab Laboratories, Israel) in saline solution. Injection dosage was 3.2 mg per 10 g mouse weight with actual injection volume of 130–150 μL. Stents were washed



Scheme 1. Chemical structure of pBuOPy (A) and p(BuOPy:PPA) (9:10) (B).

in ethanol (70% v/v) and air-dried before implantation. Two stents, bare metal and polypyrrole coated were implanted subcutaneously in each mouse. At 7 days post-implantation, the mice were sacrificed, the implants were taken for chemical analysis and the surrounding tissue was fixed in 4% neutrally buffered formaldehyde and subjected to histopathological examination. Tissues were trimmed, embedded in paraffin, and routinely processed for light microscopy. Sections were stained with hematoxylin and eosin. A board-certified toxicological pathologist (A.N.) performed the histopathological evaluation. Each sample was evaluated and graded for histopathological changes. The reactive and inflammatory changes were assigned severity grades of 0–4 representing unremarkable, minimal, mild, moderate, and marked changes, respectively. Evaluated parameters included the presence of the capsule and histological components of the capsule, that is, inflammatory cells including giant cells, fibroblasts, and mature collagen. Polymer morphology changes after implantation were examined by electron microscopy after all implanted stents were washed with pure water and dried.

Ethics committee at the Hebrew University in Jerusalem (NIH approval number: OPRR-A01-5011) has reviewed our application for animals' study and found it compatible with the standards for care and use of laboratory animals (ethics committee-research number: MD-80.04-3, date: 05/01/2003).

Drug absorption and release conditions

Stents were used for the pyrrole-derivative coating. Drug-polymer interaction was examined using paclitaxel as antiproliferation agent and polymer coatings of different pyrrole derivatives. Drug was absorbed by swelling in paclitaxel ACN solution and evaporation. The drug is entrapped within the hydrophobic pyrrole matrix coating. The amount of drug loaded into the polymer coated stent was examined by varying the concentration of the drug loading solution in ACN. The polymer coated plates were immersed in solutions of 10, 15, 20 mg/mL paclitaxel in ACN.

Total paclitaxel amount embedded in the polymer matrix was determined after the excess surface drug layer was washed out and the inner content was leached out to pure ACN. Finally, drug loading was carried out by immersion of the polymer coated plate or stent into 20 mg/mL paclitaxel ethanolic solution for 0.5 h and dried in air. The absorbed drug was passively defused out of the polymer to the buffer phosphate, pH 7.4 with 0.3% SDS at 37°C. Paclitaxel amount in buffer solutions was determined by HPLC using C18 reverse-phase column (LichroCart® 250-4, Lichrospher® 100, 5 μm). A mixture of 55% ACN: 45% water at a flow rate of 1 mL/min was used as eluent and UV detection at 230 nm.

RESULTS AND DISCUSSION

Pretreatment

Dipping the stent in nitric acid forms a homogeneous chromium oxide layer. During this treatment the outer oxide layer is removed and a new protective continuous oxide layer, richer in Cr compared to the bulk, is built.[14,15] Surface changes caused by acid pretreatment were revealed by XPS. Cr/Fe ratios before and after the treatment were 1.25 and 1.65, respectively. Similarly, the Fe_2O_3/Fe^0 ratios were 2 and 1.3, respectively. These results can be explained by dissolution of the Fe_2O_3 and the formation of a Cr richer layer. AQ3

Electropolymerization and polymer coating characteristics

Electropolymerization of pyrrole monomers on the stent surface was effective in forming a polypyrrole layer with no bridges effect (Fig. 1). Final properties of the resulted coating depended on various parameters. The thickness of the coating can be controlled very easily by the duration of the electropolymerization process. Figure 2 shows repetitive cyclic voltammetry (CV) of BuOPy electropolymerization. It is clearly seen that the currents of the doping/undoping process inside the growing film increase with each cycle. This is correlated with the thickness growth of the BuOPy film.[16] We have shown[16] that F1 F2

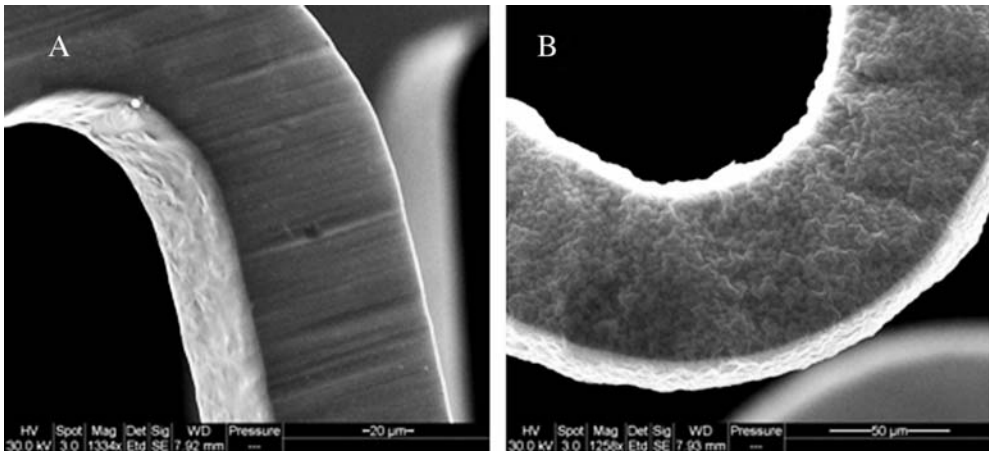


Figure 1. Scanning electron microscopy images of 316L stainless steel stent surface before and after electrodeposition of pBuOPy using CV.

the increase of the current associated with the doping process varies linearly with the thickness of the polymer between approximately 0–2 μm. The thicknesses of pBuOPy, pPPA, p(Buopy:PPA) (9:1) deposited by applying 5 CV are 1.8,* 0.4, 1.2 μm, respectively.

The substituent size on the oxidizable pyrrole influences the electrochemical behavior of the monomer.[17–19] The effect of N-substituent is remarkable; the oxidation potential wave, $E_{pk,a}$, of PPA is shifted to positive values by about 0.65 V as compared with unsubstituted pyrrole. The electropolymerization rate slows down, affecting the polymer yield. Table I T1 summarizes the relationship between the size of the N-substituent, its oxidation potential, and thickness of the film obtained as a result of five CV cycles. It can be seen that the oxidation potential of PEG400 dipy is shifted dramatically (~1 V) and its thickness is less than 0.4 μm. PEG2000 dipy does not polymerize indicating that the length of the PEG chain creates a significant steric barrier toward electropolymerization.

Chemical analyses of the resulted polymer coatings were conducted by XPS. Figure 3 F3 shows the XPS survey spectra of bare and pPPA coated stents. The metal signals such as Cr (576.17 ($2p_{3/2}$) and 587.1 ($2p_{1/2}$) eV) and Fe (707.3 ($2p_{3/2}$) and 720.5 ($2p_{1/2}$) eV) disappeared and instead 1s oxygen, carbon, and nitrogen signals (533, 285–290, 400, respectively) attributed to an organic coating appear. This alludes to the total coverage of the metal surface by an organic film. Oxygen originating from the metal oxide (531 eV) is exchanged with oxygen from the carboxylic group of PPA (533 eV), while the nitrogen signal stems from the pyrrole unit. Figure 4(A) F4 shows the C s1 high resolution spectrum curve-fitting analysis, which indicates the presence of different types of carbons that can be assigned to C—C (283.94 eV), C=C, C—H (shared peak) (285.07 eV), C—N (286.30 eV), O—C=O (289.23 eV). It can be noted, that the major peak (285.07 eV) is related to the π-conjugated backbone of the polymer. High resolution spectra of O 1s of bare and pPPA coated stainless steel plate are shown in Figure 4(B,C), respectively. Two different oxygen species were found in each spectrum. For a bare plate a signal at 531.57 eV corresponding to O—H, while a minor signal at 530.25 eV related to M=O species, can be assigned. pPPA coating has a carboxyl group, which contains carbonyl and hydroxyl in equal amounts. Indeed, the ratio between atomic percent of carbonyl

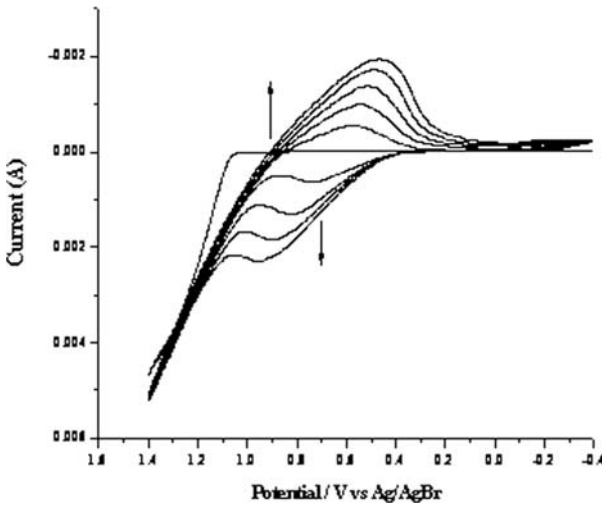


Figure 2. CV of 0.1*M* of pBuOPy in 0.1*M* acetonitrile solution of TBATFB at scan rate of 0.1 V/s (E vs. Ag/AgBr) on stainless steel plate.

*The kinetics of pBuoPy electrodeposition is faster than pPPA, according to our findings.[15] Therefore the thickness of pBuoPy is greater than that of pPPA.

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TABLE I
Relationship Between Substituent Length of the Monomer, Oxidation Potential, and Thickness of the Resulted Film

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Monomer	Number of Methylene Groups	$E_{pk,a}$ (V vs. Ag/AgBr)	Thickness of the Polymer (μm)
Pyrrole	0	0.71	1.9
N-Methyl pyrrole	1	0.84	1.5
PPA	2	1.36	0.4
PEG400dipy	24	~1.6	<0.4
PEG2000dipy	104	No polymerization	–

The films were deposited using CV (the deposition solution consisted of 0.1*M* of the monomer and 0.1*M* TBATFB in acetonitrile).

(332.25 eV) and hydroxyl (333.8 eV) oxygen species in pPPA film is 1.04.

The contact angles of homo and copolymers of BuOPy and PPA were examined. The contact angles of pBuOPy, p(BuOPy:PPA) (9:1), p(BuOPy:PPA) (1:1), and pPPA are 90°, 87.5°, 53°, 35°, respectively. It is evident that increasing the fraction of the hydrophilic component, that is PPA, in the course of electropolymerization decreases the contact angle of the resulted polymer.

Polymer coating *in vitro* and *in vivo* stability was investigated to ensure the compatibility of polymer coating for medical applications. As nonbiodegradable polymer coating, polypyrrole film has to adhere very strongly to the metallic surface and retain on the surface through time. In addition, the coating must be noninflammatory and nontrombogenic.

For studying the durability and stability of the coatings, the stents were incubated in 1:1 solution of fetal calf serum and saline consisting of 0.1% of PEN-STREP-NEOMYCIN up to 1 year. Figure 5(A,B) represents light microscopy and SEM images of a coated stent before and after 8 month of incubation. Neither delamination from the stent surface nor morphological changes could be detected during and after this period of incubation. *In vivo* stability tests (coated stent were implanted subcutaneously in mouse for 1 week) also did not reveal any morphological changes of the polymer coating [Fig. 5(C)]. F5

Histopathological evaluation indicated that no histopathological changes were noted in samples of the skin overlying the site used in the other groups as site of implantation (samples from a blank mouse—region without stent) [Fig. 6(A)]. In the tissue samples, taken from two mice and used for testing the biocompatibility at sites of bare as well as coated stent implantation region, presence of minimal capsule composed of mature connective tissue, and very minimal (grade 0–1 of 4) sparse mononuclear cells, was noted [Fig. 6(B,C)]. No evidence for an active irritation (inflammation) was noted as well. It can be concluded that the tested implants, under the present experimental conditions, showed very good biocompatibility without any evidence of local adverse effect. F6

Drug loading and release

The effect of paclitaxel concentration on the amount of drug loaded into polymer coated on stent was determined (Table II). Maximal paclitaxel loading was detected after immersing the coated stent into drug loading solution of 20 mg/mL. It can be seen that the hydrophobic paclitaxel shows superior interaction with the hydrophobic polypyrrole coating of pBuOPy. Nevertheless, we found that pBuOPy T2

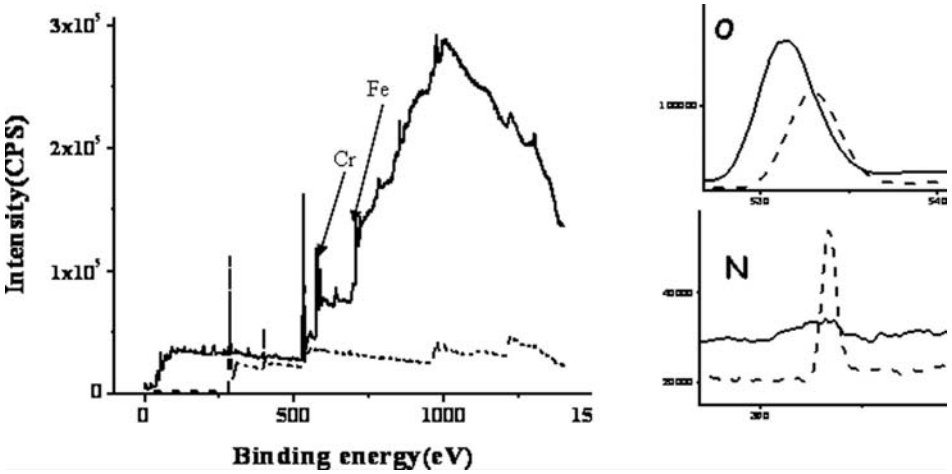

Figure 3. A wide survey scan, low resolution XPS (0–1400 eV) of bare (——) and pPPA coated (—) (~0.4 μm thick) stainless steel plate. Close up (on the right) to oxygen (O) and nitrogen (N) signals detected on bare (——) and pPPA coated (—) stainless steel surfaces by high resolution XPS.

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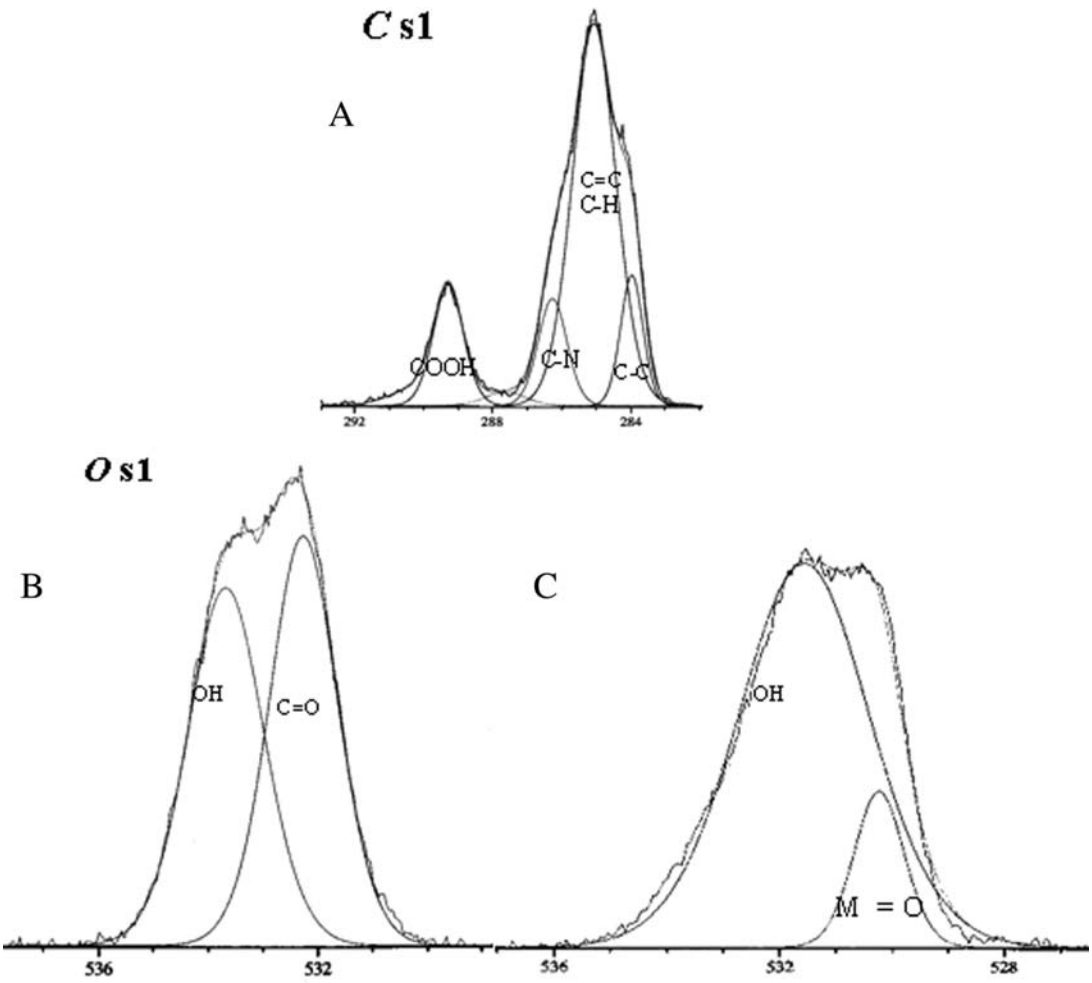

Figure 4. High resolution XPS spectra of C1s (A), O1s (B) core level region of pPPA coated stainless steel plate, and O1s (C) of bare stainless steel plate.

has poor adhesion to the stent. Hence, we carried out adhesion tests (according to standard test ASTM D 7027) to the different polypyrrole derivatives. These showed a significant stronger adhesion of pPPA to the substrate than pBuOPy.[20] Therefore, we have decided to examine a copolymer of BuOPy and PPA that possesses better hydrophobicity and therefore also better adhesion as a means of obtaining a better coating. P(BuOPy:PPA) (bulk ratio 9:1) was chosen, in favor of its relatively high contact angle (87.5°) and significant stronger adhesion to the stent surface. Drug loading and release experiment were carried out with both homo-pBuOPy and p(BuOPy:PPA) (bulk ratio 9:1) to verify that the drug loading behavior does not change dramatically by minor addition of a hydrophilic monomer (PPA). Paclitaxel was entrapped into the polymer coating by diffusion and maintained inside the coating due to the rough morphology of the surface. As a result of swelling the polymer from fairly high paclitaxel containing solution, drug precipitation and recrystallization on the surface is expected.[21] Despite surface washing before the release experiment, significant amount (~25%) of drug retained on the surface. Cumulative paclitaxel release from pBuOPy, p(BuOPy:PPA) (bulk ratio 9:1), and bare stent loaded with equal amounts of drug was investigated in phosphate buffer solution with 0.3% SDS (Fig. 7). The solubility of the paclitaxel increased from less than 1 μm/mL in DDW[22,23] to about 17 μm/mL in buffer phosphate solution with 0.3% SDS. F7

Two phases of drug release were found for polymer coated stents: a rapid release of the drug from the coating within the first 24 h, followed by slow release of the drug up to 3 weeks in comparison with only one phase release (rapid release within first hours) from paclitaxel loaded bare stent. This behavior is typical for systems with high drug loads.[24] In these cases the slow drug release is related with the time required for drug to pass through the polymer bulk.[5] In the case of polypyrrole coating the polymer structure is neither affected

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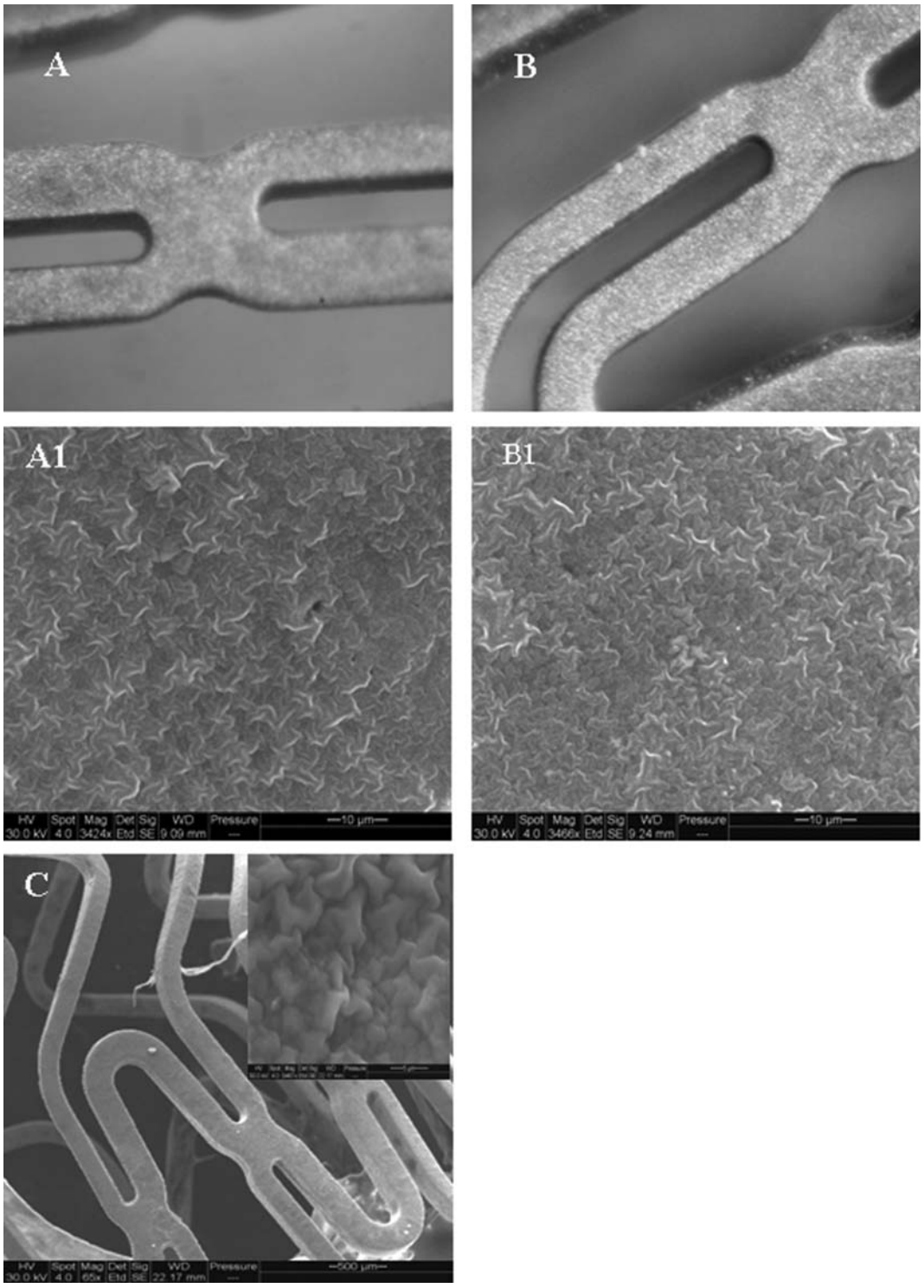

Figure 5. Light microscopy and SEM images of stent before (A,A1) and after (B,B1) incubation in 1:1 solution of fetal calf serum and saline with 0.1% of PEN-STREP-NEOMYCIN solution at 37°C shaking for 8 months. SEM images of the polypyrrole coated stent struts (C) and close up to the morphology on the polymer film (C capture) after subcutaneous implantation in mouse. (Light microscopy magnification 100×, SEM image scale bar represents: A1,B1-10 μm, C-500 μm, C capture-1 μm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.] AQ5

by drug loading nor by drug release. Total drug release from coated stents occurred within 3 weeks. This rather fast release can be moderated by applying the top coating that would function as a diffusion barrier for the drug.[4] Figure 8 shows SEM images of the p(BuOPy:PPA) coating before drug F8

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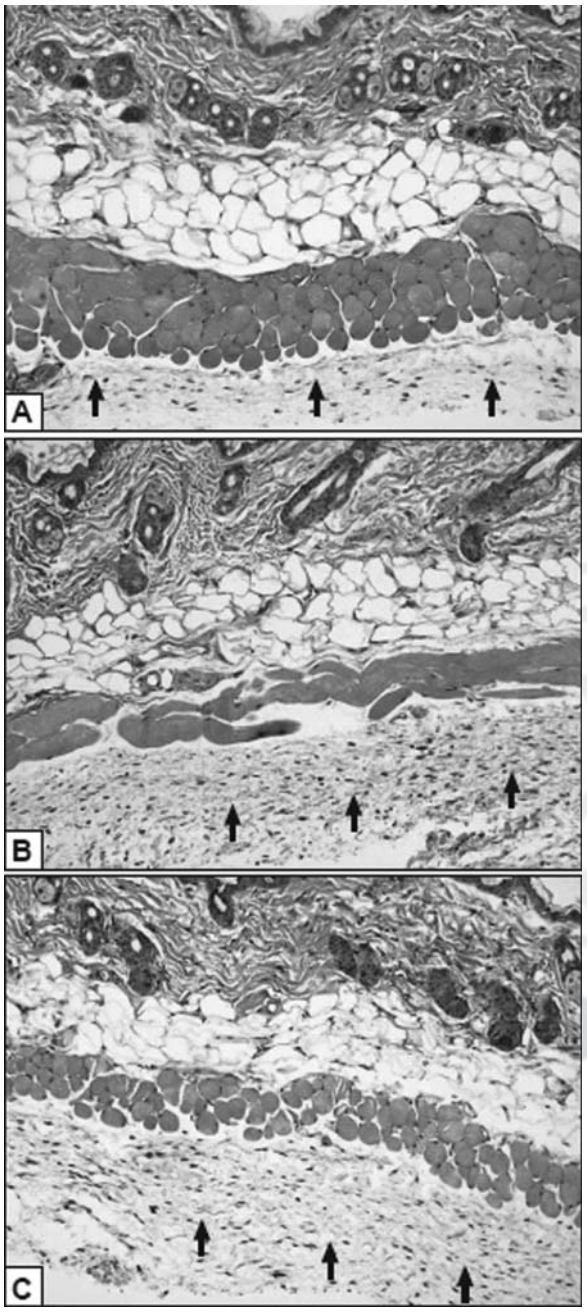

Figure 6. Histopathology of implantation sites. (A) Skin sample from a blank mouse (without stent implantation)—no abnormality detected. (B) Skin sample from bare stent implantation region. Note the presence of minimal capsule (arrows) composed of mature connective tissue, and very minimal (grade 0–1 of 4) sparse mononuclear cells. Compare to figure A, same region (arrows). (C) Skin from coated stent implantation region. Note the presence of minimal capsule (arrows) composed of mature connective tissue, and very minimal (grade 0–1 of 4) sparse mononuclear cells. Compare to figure A, same region (arrows). All pictures with original magnification, 10×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Polymer Composition and Paclitaxel Loading Solution Concentration Influence on Final Drug Amount on Stent

Polymer Coating	Paclitaxel Concentration in Loading Solution (mg/mL)	Paclitaxel Amount on Coated Stent ($\mu g/mm^2$)
BuOPy	20	0.82
	15	0.53
	10	0.32
PPA	20	0.16

loading (A) and after (B) release to phosphate buffer solution with 0.3% SDS. As can be seen, the surface morphology does not change. A reasonable explanation can be that paclitaxel is released by the same way as it is loaded without affecting the polymer structure. The data presented confirm the suitability of the polypyrrole coatings for drug eluting system. With regard to the physical and chemical properties of these films, BuOpy:PPA (9:1) showed superior adhesion properties to the stainless steel stent surface due to the hydrophilic component of PPA, with no significant morphological and drug release changes.

CONCLUSIONS

The development of simple and effective coating processes in treating medical devices used in cardiology and orthopedics is becoming a crucial demand in applying these devices. The efforts until now have shown promising device coating systems particularly for stents.[4,5] This work demonstrates an electrocoating method for stents, based on conducting coating of polypyrrole derivatives. Polypyrrole derivatives are biocompatible and retained on the stent surface after drug release. Chemical and physical properties of the prepared coatings were studied. The electrocoating process allows controlled thickness that can

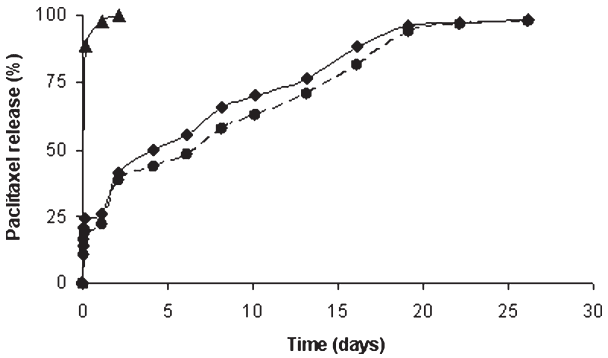

Figure 7. *In vitro* release profile of paclitaxel from bare metal stent (—▲—), PBuOPy (—◆—), and P(BuOPy:PPA) (9:1) (– –■– –), coated stents into 0.1*M* buffer phosphate pH = 7.4 with 0.3% SDS at 37°C. Each symbol represents the cumulative amount of drug released, detected by HPLC.

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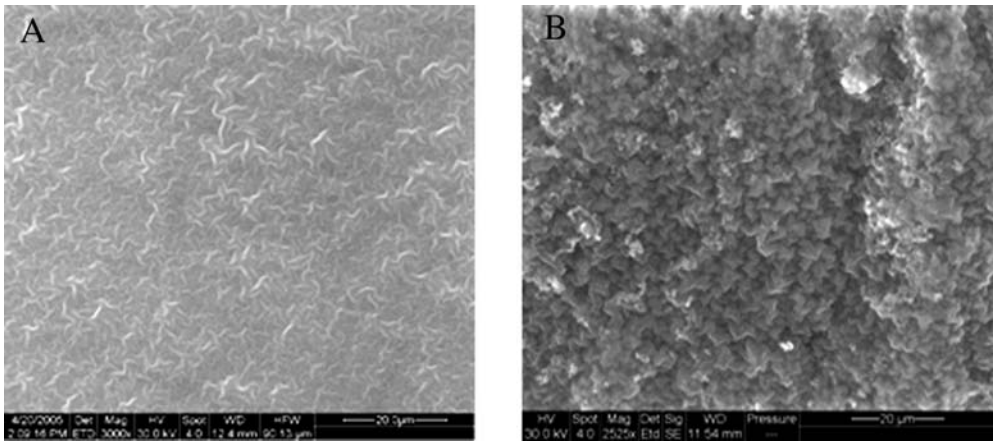

Figure 8. SEM images of the p(BuOPy:PPA) coating before drug loading (A) and after (B) drug release to phosphate buffer solution with 0.3% SDS up to 3 weeks. Scale bar represents 20 μm.

vary between 0.1 and 2 μm uniformity, defined morphology, and *in vitro* and *in vivo* stability. Copolymerization allows achieving a desired morphology, better adhesion properties to the stent and appropriate drug absorbing characteristics. Paclitaxel loaded in the poly(pyrrole) coating was constantly released over 3 weeks.

References

1. Van der Giessen WJ, Van Beusekom HMM, Larsson R, Serruys PV. Heparin-coated coronary stents. Curr Interv Cardiol Rep 1999;1:234–240.
2. Whelan DM, van der Giessen WJ, Krabbendam SC, van Vliet VA, Verdouw PD, Serruys PW, van Beusekom HMM. Biocompatibility of phosphorylcholine coated stents in normal porcine coronary arteries. Heart 2000;83:338–345.
3. Bar FW, van der Veen FH, Benzina A, Habets J, Koole LH. New biocompatible polymer surface coating for stents results in low neointimal response. J Biomed Mater Res 2000;52:193–198.
4. Morice MC, Serruys PW, Sousa JE. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med 2002;346:1773–1780.
5. Ranade SV, Miller KM, Richard RE, Chan AK, Allen MJ, Helmus MN. Physical characterization of controlled release of paclitaxel from the TAXUS™ Express2™ drug-eluting stent. J Biomed Mater Res A 2004;71:625–634.
6. Lahann J, Klee D, Thelen H, Bienert H, Vorwerk D, Hoecker H. Improvement of haemocompatibility of metallic stents by polymer coating. J Mater Sci Mater Med 1999;10:443–448.
7. Vernitskaya TV, Efimov ON. Polypyrrole: A conducting polymer; its synthesis, properties and applications. Russ Chem Rev 1997;66:443–457.
8. Kanazawa KK, Diaz AF, Geiss HR, Gill DW, Kwak JF, Logan JA, Rabolt JF, Street JB. Organic metals: Polypyrrole, a stable synthetic metallic polymer. J Chem Soc Chem Commun 1979;19:854–855.
9. Tanaka K, Shichiri T, Yamabe T. Influence of polymerization temperature on the characteristics of polythiophene films. Synth Met 1986;16:207–214.
10. Zang Z, Roy R, Dugre JF, Tessier D, Dao HL. In vitro biocompatibility study of electrically conductive polypyrrole-coated polyester fabrics. J Biomed Mater Res 2001;57:63–71.
11. Wang X, Gu X, Yuan C, Chen S, Zhang P, Zhang T, Yao J, Chen G, Chen F. Evaluation of biocompatibility of polypyrrole in vitro and in vivo. J Biomed Mater Res A 2004;68:411–422.
12. Weiss Z, Mandler D, Shustak G, Domb JA. Pyrrole derivatives for electrochemical coating of metallic medical devices. J Polym Sci Part A: Polym Chem 2004;42:1658–1667.
13. Maeda S, Corradi R, Armes SP. Synthesis and characterization of carboxylic acid-functionalized polypyrrole-silica microparticles. Macromolecules 1995;28:2905–2911.
14. Thamaraiselvi TV, Kannan S, Balalmurugan A, Rajeswari S. Predicting the susceptibility of HNO_3 treated 316 LMV alloy to localized attack—An electrochemical approach. Trends Biomater Artif Organs 2003;17:19–23.
15. Drogowska M, Menard H, Brossard L. Electrooxidation of stainless steel AISI 304 in carbonate aqueous solution at pH 8. J Appl Electrochem 1996;26:217–225.
16. Okner R, Domb JA, Mandler D. Electrochemical formation and characterization of copolymers based on *N*-pyrrole derivatives. Biomacromolecules 2007;8:2928–2935.
17. Diaz AF, Castillo J, Kanazawa KK, Logan JA, Salamon M, Fajardo O. Conducting poly-*N*-alkylpyrrole polymer films. J Electroanal Chem 1982;133:233–239.
18. Cross MG, Walton D, Morse NJ, Mortimer RJ, Rosseinsky DR, Simmonds DJ. A voltammeric survey of steric and β-linkage effects in the electropolymerization of some substituted pyrroles. J Electroanal Chem 1985;189:389–396.
19. Haase V, Beck F. Electrodeposition of N-substituted polypyrroles on iron and the CIPL strategy. Electrochim Acta 1994;39:1195–1205.
20. Okner R, Shaulov Y, Tal N, Domb AJ, Mandler D. Under preparation.
21. Dhanikula AB, Panchagnula R. Development and characterization of biodegradable chitosan films for local drug delivery. AAPS J 2004;6:e27.
22. Konno T, Watanabe J, Ishihara K. Enhanced solubility of paclitaxel using water-soluble and biocompatible 2-methacryloyloxyethyl phosphorylcholine polymers. J Biomed Mater Res A 2003;65:210–215.
23. Liggins RT, Hunter WL, Burt HM. Solid state characterization of paclitaxel. J Pharm Sci 1997;86:1458–1463.
24. Acharya G, Park K. Mechanisms of controlled drug release from drug eluting stents. Adv Drug Deliv Rev 2006;58:387–401.
25. Westedt U, Wittmar M, Hellwig M, Hanefeld P, Greiner A, Schaper AK, Kissel T. Paclitaxel releasing films consisting of poly(vinyl alcohol)-*graft*-poly(lactide-*co*-glycolide) and their potential as biodegradable stent coatings. J Controlled Release 2006;111:235–246.

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