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Bioinspired and Biocompatible Adhesive Coatings Using Poly(acrylic acid)-Grafted Dopamine

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ABSTRACT: Poly(acrylic acid) (PAA) was grafted with dopamine to increase its adhesion force to metal surface. Nitinol plate surfaces were then modified by coating with PAA-g-dopamine. To synthesize PAA-g-dopamine, PAA was first activated by dicyclohexylcarbodiimide and N-hydroxysuccinimide (NHS) to form PAA–NHS. Dopamine was then copolymerized with PAA–NHS in an aqueous medium at pH 8.5. We propose to increase the adhesion of adhesive PAA-g-dopamine on nitinol to improve its durability. In this article, we studied wettability, surface elemental composition, and surface morphology. Biocompatibility was also assessed by L929 fibroblast cells in vitro. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2013

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INTRODUCTION

The surfaces of most metals are positively charged. Consequently, metal stents form thrombi in biomedical applications due to interaction with negatively charged platelets.[1] The surface free energy related to high critical surface tension is another factor that affects the reactivity of metal with blood. It also indicates high thrombogenicity. To address these issues, metal surfaces can be modified with biomaterials. This process can play an important role in creating functionalized surfaces, increasing biocompatibility, and decreasing critical surface tension. Alterations in these properties can induce specific cellular responses and promote new tissue regeneration.[2–4]

The effectiveness of coating techniques on metal stents is as follows: sputtering followed by ion bombardment,[5] pulsed biased arc ion plating,[6] dip-coating,[7,8] spray-coating,[9,10] and plasma-based deposition.[11,12] The main roles of coating layers are to increase biocompatibility of the metallic surface and to inhibit intimal hyperplasia as a platform for controlled drug delivery in clinical coronary stent applications. Although the restenosis rate for patients receiving drug eluting stents is reduced compared to patients with bare metal stents, the detachment of a piece of coating layer could lead to downstream thrombosis.

Dopamine components are the most abundant component in mussel adhesive proteins. They are known to play a major role in the strong adhesive force of a mussel onto various substrates in spite of a wet environment, including inorganic and organic surfaces such as noble metals, oxides, ceramics, and plastics.[13,14] Inspired by the mussel adhesion mechanism, its main adhesive component (polydopamine) was adsorbed onto the surface of stainless steel[15–19] due to strong adhesion between the hydroxyl groups of polydopamine and the metallic surface.

Poly(acrylic acid) (PAA) is a synthetic biostable polymer with many carboxylic side chains that enable conjugation with the amine groups of dopamine through covalent linkage. Therefore, it is possible that the polymer could covalently bind onto metal surfaces treated with dopamine. We also expect an enormous increase in the durability of polymer-coated metals used in clinical applications (see Figure 1).

In this study, we explored ways to improve the adhesion of polymers on nitinol substrate through a dopamine-grafted polymer. We envisioned PAA grafted with dopamine as an adhesive component that acted for the pretreated layer on nitinol surface. The PAA grafted with dopamine would form a strong adhesive surface as the coating layer to enhance its durability. Surfacial characterizations including wettability, surface elemental analysis, and morphology were assessed by water contact angle, X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM), respectively. Adhesion strength was measured by adhesion/release testers. Finally, cell adhesion and proliferation

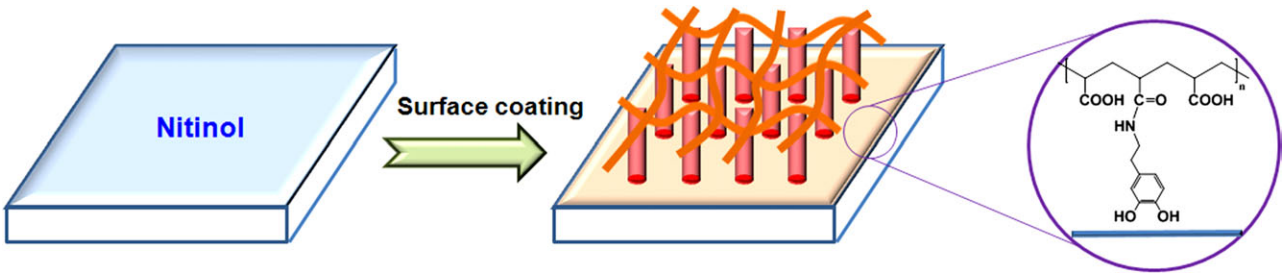

Figure 1. Schematic diagram of PAA-g-dopamine on the surface of metals. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

behaviors were also assessed on a L929 mouse fibroblast cells culture and by cytotoxicity test.

EXPERIMENTAL SECTION

Materials

Dopamine hydrochloride (98%), PAA (Mn = 450,000, 100,000), phosphate buffered saline (PBS), *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), and *N*,*N*-dimethylformamide (DMF, ≥99%) were purchased from Sigma–Aldrich Chem. Co. (St. Louis, MO, USA). Deionized water (18.2 MΩ/cm resistivity at 25°C) was used in the experiment. All other organic solvents were bought from Samchun Pure Chemicals (Ansan, Korea).

Polydopamine Formation and Surface Coating Process

In 100 mL of DMF solvent, 4.5 g of PAA (450,000) or 1 g of PAA (100,000), 1.15 g of NHS, and 2.06 g of DCC was dissolved. The reaction was carried out for 3 h at 0°C and then kept at room temperature for additional 3 h. Crude products were dialyzed by water repeatedly for 1 day. The final PAA–NHS product was lyophilized for 2 days.

Dopamine hydrochloride solution was prepared in PBS (20 mL, pH 8.5) and mixed with PAA–NHS. The final concentrations for dopamine hydrochloride and PAA–NHS were 5 mg/mL and 10 mg/mL, respectively. Nitinol plates (10 mm × 10 mm × 1 mm) were ultrasonically cleaned with acetone and ethanol for 20 min. The nitinol plates were subsequently immersed in dopamine and PAA–NHS mixture solutions at room temperature for 2 days. After 2 days, the PAA-g-dopamine coated nitinol plates were rinsed thoroughly with deionized water, dried under nitrogen atmosphere, and stored in vacuum conditions.

Characterization of Coating Surface

Characterizations of the bare nitinol surface and PAA-g-dopamine coated nitinol surface were conducted by water contact angle, XPS, and AFM.

For bare nitinol plates and PAA-g-dopamine coated nitinol plates, contact angles were measured by a direct optical determination at the water droplet interface near the contact line. A Water Contact Angle Meter (GBX DIGIDROP-Scientific Instrumentation, Romans, France) was used to investigate the wettability of coating surfaces at room temperature.

The surface elemental compositions were assessed by XPS (ESCA 2000, VG Microtech, London, UK) with an Al Kα X-ray ($h\nu$ = 1486.6 eV) source. The XPS measurement was performed with energy increments of 1 eV for broad scans and 0.1 eV for high resolution scans (narrow scan) at room temperature.

Surface morphology was observed under ambient conditions using AFM (SPA-300HV, Seiko Instruments, chiba, Japan) with a SPI 3800N controller. Etched silicon tips with a resonance frequency of approximately 70 kHz and a spring constant of about 42 N/m were used for the AFM scan. The scan rate was in the range of 1.0–2.0 Hz.

Peel Test

For peel tests, the large scaled stainless steel plates (610 mm × 260 mm × 1.5 mm) were prepared as substrates instead of the nitinol plates. Stainless steel plates were coated with 0.5 mL of aqueous solutions containing PAA (10 mg/mL, Mn = 450,000 or 100,000) grafted dopamine (PAA-g-dopamine). A bare stainless steel plate was evaluated as the control. An Adhesion/Release Tester AR-1000 (ChemInstruments, Mentor, OH, USA) was used to measure peel force. Each mean value was obtained by testing five samples.

Cell Culture

L929 mouse fibroblast cells obtained from the Korean cell line bank (Seoul, Korea) were cultivated at 37°C in a humidified atmosphere. The cells were grown in a 25 cm^2 tissue culture flask containing 10 mL Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen Corporation, Carlbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (BBP15, Timaru, New Zealand), 1% (v/v) penicillin, and streptomycin (Boehringer Mannheim, Ottweiller, Germany). The culture medium was changed every 2 days.

Metal plates were cleaned and coated by PAA-g-dopamine. The coated plates were sterilized with UV light irradiation of 30 nm and then stored at room temperature for the experiments.

Cell Adhesion and Proliferation

Fibroblastic cells (4×10^5) were cultivated in tissue culture polystyrene (TCPS) dis (24 wells) at 36.5°C in a humidified atmosphere of 5% CO_2 for 2 days. The number of mouse fibroblast cell was determined by counting the cells that adhered to the surface per unit area after 1 h, 10 h, 24 h, and 48 h. An optical microscope (CK40, Olympus, Tokyo, Japan) with a 10× objective captured images of the cells after 1 h, 10 h, 24 h, and 48 h.

Cell Viability

Cell viability was examined using an 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.[20] All

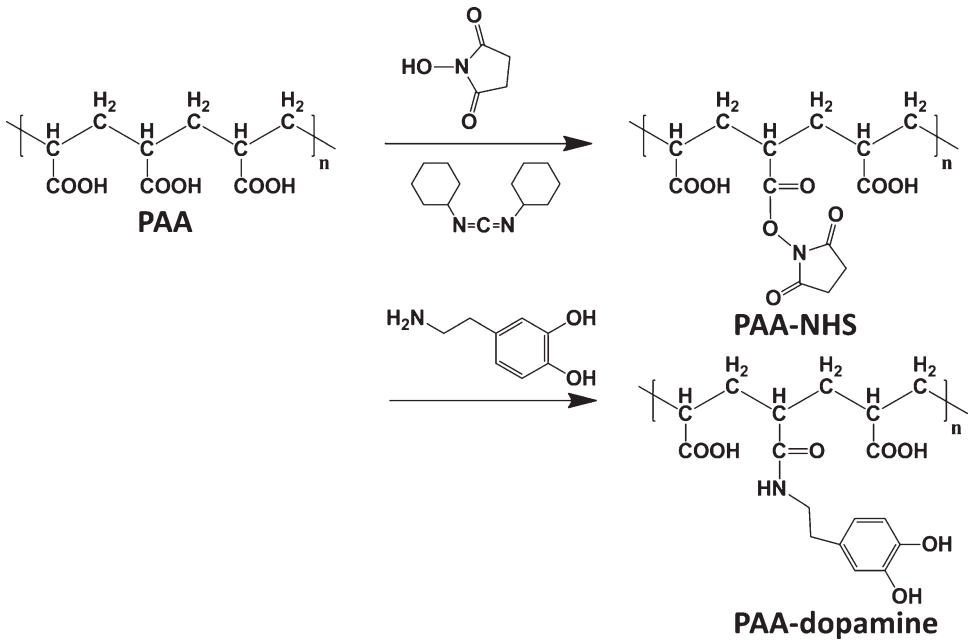

Figure 2. Synthesis scheme of PAA-g-dopamine.

the experiments were repeated at least four times to obtain average values. The 2×10^5 fibroblastic cells were seeded on coated metal surfaces in each well (24 well) at 36.5°C in a humidified atmosphere containing 5% CO_2 for 2 days. DMEM (400 μL) and MTT solution (St. Louis, MO, USA; 100 μL) was then added to each well. After an additional 4 h of incubation at 36.5°C, all solutions in the wells were removed by vacuum suction. Subsequently, dimethylsulfoxide (DMSO) (400 μL) and a glycine buffer (50 μL, pH 10.5) were added to the wells. The plate was shaken for a few minutes to thoroughly dissolve the dark blue crystals. The solution (100 μL) was then transferred to a 96 well plate, and absorbance was measured at 570 nm by an enzyme-linked immunosorbent assay (ELISA) instrument (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA). The cell viability was calculated by comparing MTT treated cell solution with control cell solution.

RESULTS AND DISCUSSION

Surface Modification

Inspired by mussel adhesive protein that contains dopamine,[14] we envisioned improved adhesion between polymer and metal surfaces if dopamine moieties could adhere firmly to metallic substrates as the first adherent layer and form covalent bonds with additional polymer coating layers. PAA containing a carboxylic group (–COOH) in its side chain was used as the model polymer in this investigation. It could react spontaneously with primary amines in dopamine through coupling reactions with NHS. DCC treatment with PAA causes a dehydration reaction between the carboxyl groups of PAA and NHS hydroxyl groups, giving rise to NHS-ester-activated PAA, as shown in Figure 2. The PAA carboxyl group was activated by DCC, and then activated PAA was reacted with NHS to give PAA–NHS. The corresponding ^{1}H-NMR chemical signals are shown in Figure 3. Activated PAA (PAA–NHS) easily reacted with a primary amine group in dopamine. The dopamine hydroxyl groups then adhered onto metallic surfaces through hydrogen bonding. PAA-g-dopamine was obtained. From ^{1}H-NMR, we have calculated the amount of dopamine in PAA-g-dopamine. The final amount of dopamine is 7 mole % of total -COOH groups in one PAA molecule with a molecular weight of 450,000.

Figure 4 shows the surface color change of nitinol metals before and after treatment with PAA-g-dopamine. Before treatment, nitinol was bright white [see Figure 4(a)]. After dip-coating for 2 days with PAA-g-dopamine, the nitinol plate surface became light yellow [see Figure 4(b)], different from its native color. This indicated the formation of a thin adherent film on the nitinol plate.

Characterization of Coating Surface

To assess the components and morphologies on the nitinol surface before and after treatment with PAA-g-dopamine, the surfaces of uncoated and coated nitinol were analyzed by XPS and AFM. As shown in Figure 5, XPS spectra changed significantly after PAA-g-dopamine coating. The main Ni (~873.807 eV) and Ti peaks (~464.035eV) were nearly suppressed after coating. Instead, carbon (~284.635eV), nitrogen (~399.612eV), and oxygen (~532.203eV) photoelectron peaks corresponding to a PAA-g-dopamine layer were observed. The theoretical nitrogen-to-carbon area ratio was 0.125 for dopamine and 0.1061 for PAA-g-dopamine if the PAA carboxylic groups all reacted with the amine groups of dopamine. However, the nitrogen-to-carbon area ratio in the XPS spectrum was 0.032 for PAA-g-dopamine coated nitinol, suggesting the existence PAA-g-dopamine on the nitinol plate surface.

AFM was used to observe the surface morphologies of untreated nitinol and PAA-g-dopamine coated nitinol. Before coating, nitinol plates were pretreated by rubbing. As seen in Figure 6(a), the surface of untreated nitinol was regular and rough. However, after PAA-g-dopamine coating, the surface showed a

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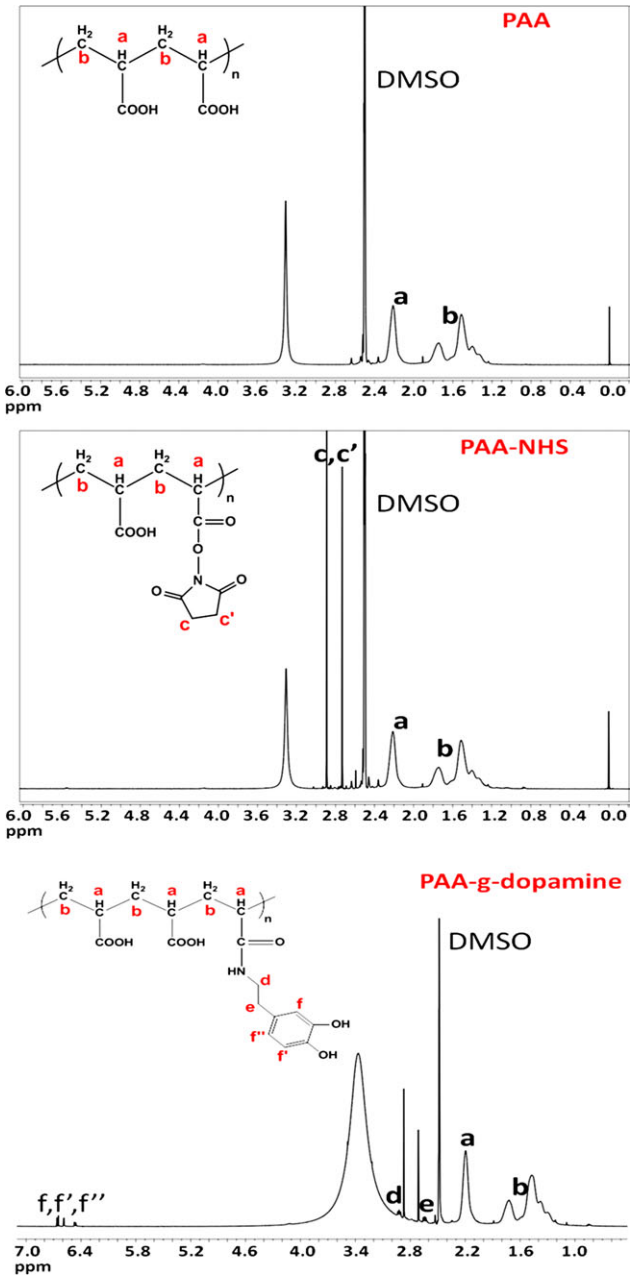

Figure 3. [1]H-NMR spectra of PAA, PAA–NHS, and PAA-g-dopamine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

more protuberant and flat morphology [Figure 6(b)]. The change in morphology implied that PAA-g-dopamine could be successfully coated on nitinol plates. Moreover, the Rt (mean value from peak to valley) of PAA-g-dopamine (root mean square of surface roughness (RMS) = 2.185 nm) was 12.86 nm, but the Rt of nitinol (RMS = 0.5031 nm) was 3.458 nm. This result suggested that PAA-g-dopamine was successfully coated on the metallic surface.

Intrinsic hydrophilicity/hydrophobicity could be significantly influenced by the chemical components of a biomaterial surface. The water contact angles were measured to evaluate the wettability of the coating layer. The water contact angle decreased significantly after treatment by PAA-g-dopamine, as shown in Table I. By calculating the water droplet interface near the contact line, the exact contact angles were found to be 75 ± 1° and 34 ± 1° for untreated nitinol and PAA-g-dopamine coated nitinol surfaces, respectively. This suggested that surface modification by PAA-g-dopamine can improve the wettability of nitinol.

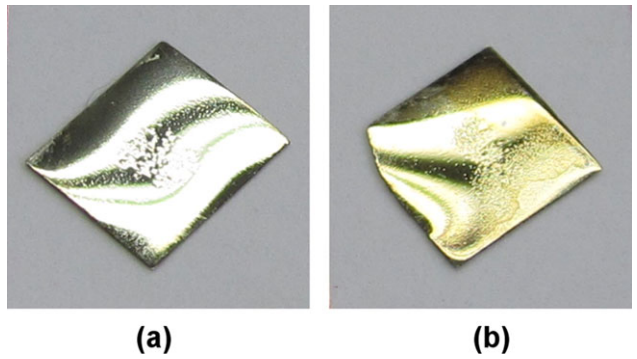

Figure 4. Photos of (a) native nitinol metal surface and (b) PAA-g-dopamine coated nitinol surface. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

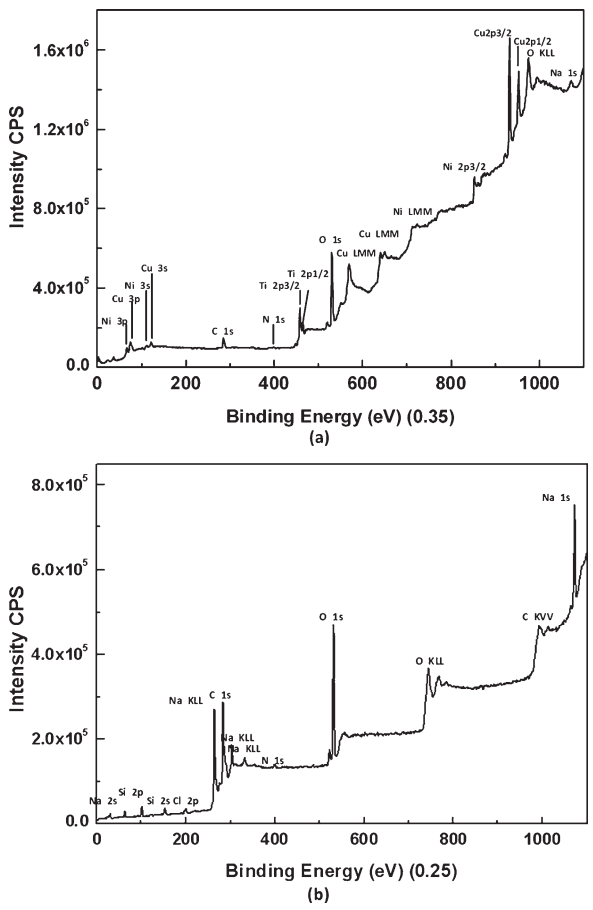

Figure 5. XPS spectra of (a) native nitinol surface and (b) PAA-g-dopamine coated nitinol surface.

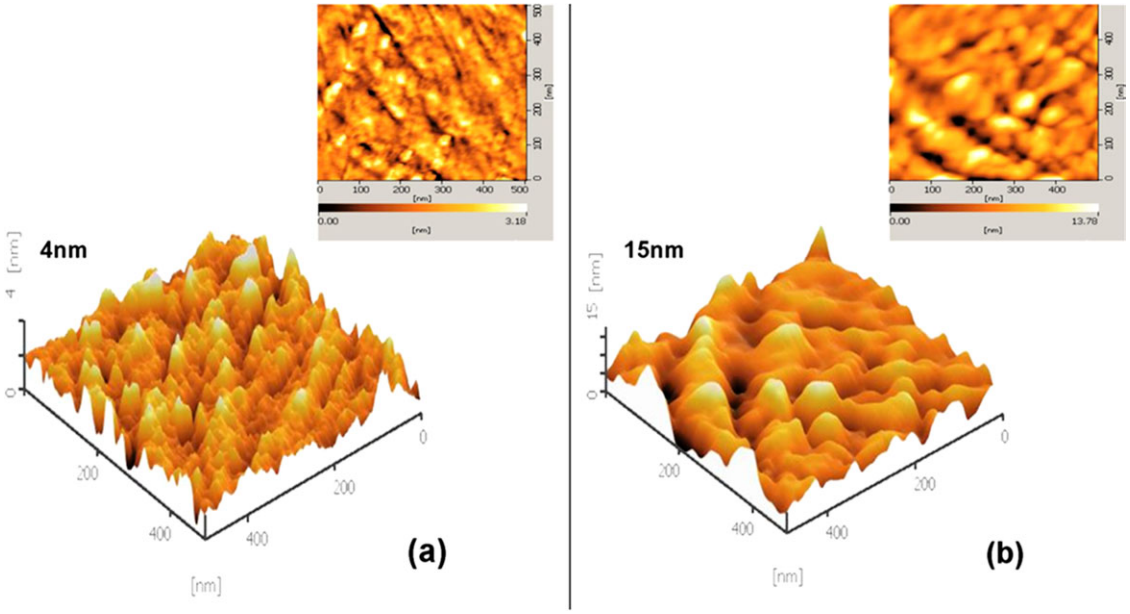

Figure 6. AFM images of (a) native nitinol surface and (b) PAA-g-dopamine coated nitinol surface. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Peel Test

Peel tests were used to measure adhesion force between a polymer layer and metal surfaces and to evaluate peeling forces. Untreated metal was used for control, while PAA-coated metal and PAA-g-dopamine coated metal were used as metal samples. The two kinds of PAA used in this research have different molecular weights (100,000 and 450,000). As shown in Figures 7 and 8, peeling force increased significantly after coating with PAA-g-dopamine for either PAA (450,000) or PAA (100,000), compared with the peeling force of untreated metal. The peeling force of PAA (450,000) was higher than that of PAA (100,000). However, after graft polymerization with dopamine, the peeling force of PAA-g-dopamine (450,000) was lower than that of PAA-g-dopamine (100,000). The enhancement ratio after polymerization with dopamine was 48.36%. This may be due to with the higher flexibility, lower molecular weight, and shorter chain length of PAA (100,000) than PAA (450,000). This suggested that chemical modification of the metal surface through covalent bonds improved the adhesion force between polymer and metal.

Table I. Water Contact Angles on Nitinol and PAA-g-dopamine Coated Nitinol Surfaces

Sample surfaces	Contact angles (degree)
Nitinol surface	75 ± 1
PAA-g-dopamine coated surface	34 ± 1

Cell Adhesion and Proliferation

Adhered cell morphology and detachment behavior were observed by optical microscopy to evaluate cell adhesion and the spreading of L929 cells on PAA-g-dopamine coating layers. The glass substrates were previously coated with PAA-g-

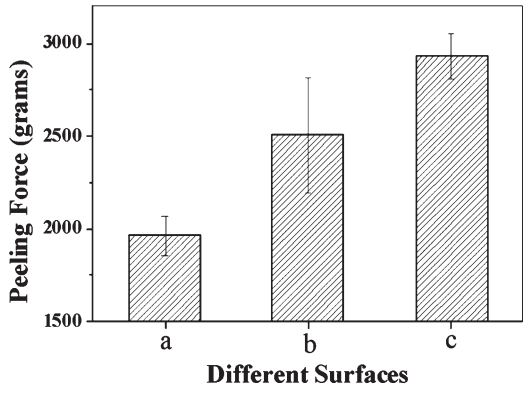

Figure 7. Peel test results of (a) metal plate, (b) PAA (450,000) coated on metal plate, and (c) PAA-g-dopamine (450,000) coated on metal plate.

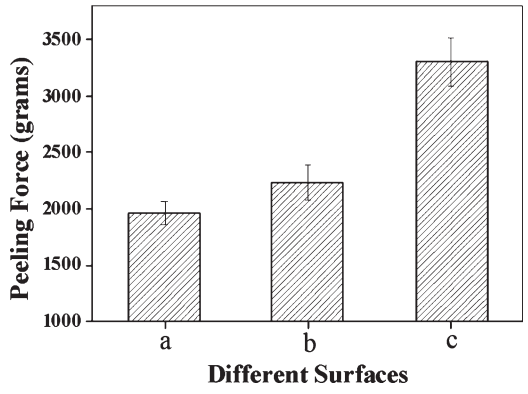

Figure 8. Peel test results of (a) metal plate, (b) PAA (100,000) coated on metal plate, and (c) PAA-g-dopamine (100,000) coated on metal plate.

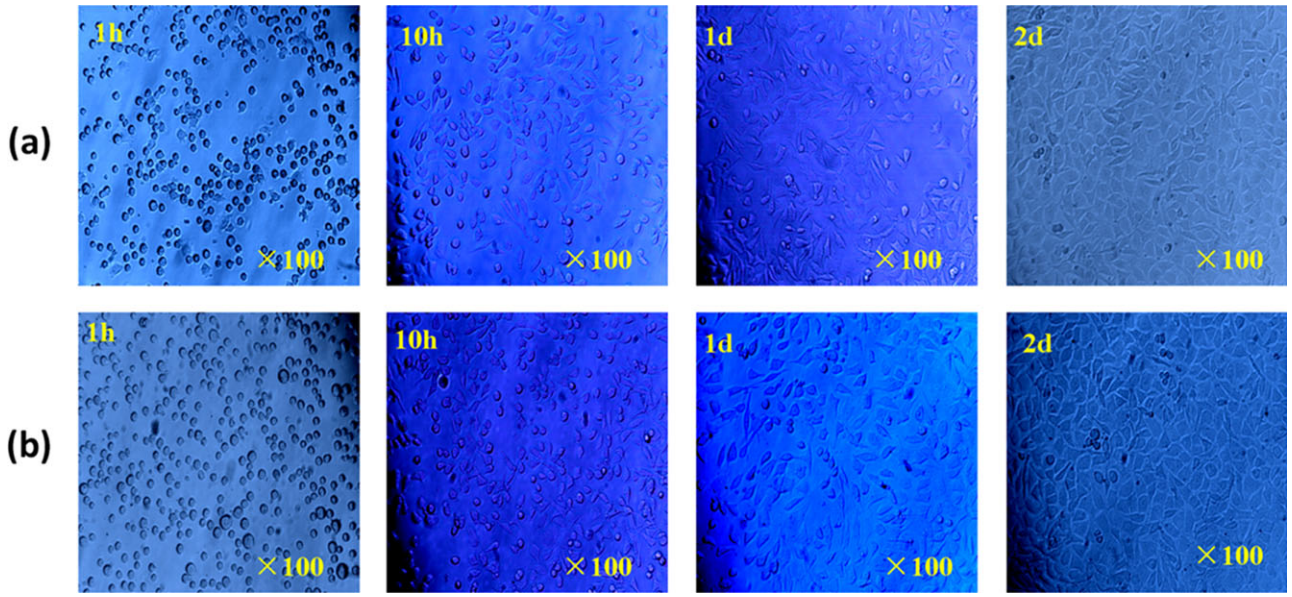

Figure 9. Optical microscopic images of L929 cell adhesion and spreading on the surface coated with (a) TCPS and (b) PAA-g-dopamine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dopamine. The control sample was polystyrene tissue culture plate (TCPS). Figure 9 shows the cell morphologies at different incubation times. From the TCPS results [Figure 9(a)], a few adhered cells began to spread. Most adhered cells show a round shape after 1 h incubation. Cells adhered on PAA-g-dopamine [Figure 9(b)] did not begin to spread in the early stages of incubation. After 10 h, some cells began to attach and spread on both TCPS and PAA-g-dopamine surfaces. One day later, many more L929 cells had spread than at 10 h, and they showed a polygonal shape. Two days later, the proliferation tendency of L929 cells was obvious. Most attached cells showed above 90% confluency on both TCPS and PAA-g-dopamine surfaces.

Correspondingly, Figure 10 shows the number of adherent L929 cells on the surfaces of TCPS and PAA-g-dopamine at different incubation times. The seeded cell number was 4×10^5/well. For all samples, the number of adherent cells increased after 10 h, 1 day, and 2 days of incubation. Results have shown that poly-dopamine components can greatly enhance cell adhesion on various coating substrates.[21] This indicates that the number of cells incubated on PAA-g-dopamine could be higher than the number incubated on TCPS surfaces.

Cytotoxicity

To test *in vitro* cytotoxicity, the viability of fibroblastic L929 cells on untreated nitinol surfaces and on PAA-g-dopamine coated nitinol was examined through MTT assay for 2 days. From Figure 11, obvious cell toxicity was not observed on the surfaces of untreated and PAA-g-dopamine coated nitinol. Cell viability on untreated nitinol was higher than that of TCPS and also did not generate any cytotoxic reaction. This result was supported by other studies that showed no cytotoxicity response to nitinol metals during short term *in vitro* testing.[22] Moreover, other researchers have shown that fibroblast cells exhibit a higher adhesion rate on smooth substrates than on rough surfaces.[23] In the present study, nitinol surface was smoother than PAA-g-dopamine surface, as shown in AFM figures.

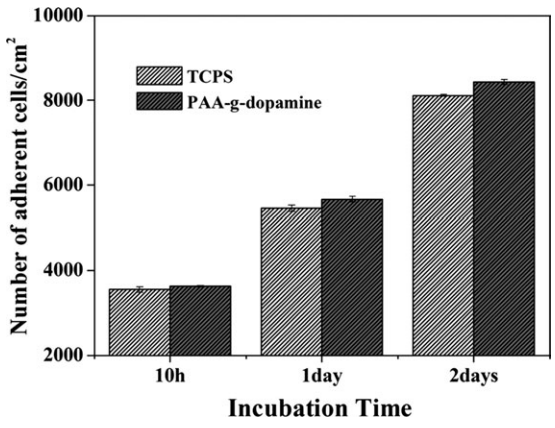

Figure 10. Number of adherent L929 cells on the surfaces of TCPS and PAA-g-dopamine.

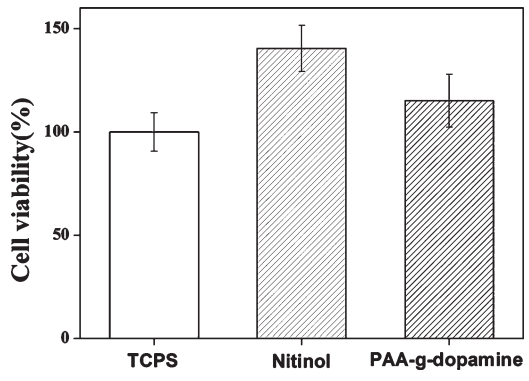

Figure 11. L929 cell viability by MTT assay on TCPS, nitinol, and PAA-g-dopamine coated nitinol surfaces.

CONCLUSIONS

In summary, we reported a facile and versatile bioinspired coating for surface modification of nitinol substrates. This method is expected to improve metal stent durability with *in vivo* environments. Inspired by the mussel adhesive mechanism, we synthesized PAA-g-dopamine through NHS reactions. Surface analysis revealed that PAA-g-dopamine adhered onto nitinol, as shown by XPS and AFM measurements. Surface wettability was assessed by water contact angles. The exact contact angles were 75 ± 1° and 34 ± 1° for untreated nitinol and PAA-g-dopamine coated nitinol surfaces, respectively. Moreover, MTT assay showed no obvious cytotoxicity on mouse fibroblastic cells. The adhesion of L929 cells on the surfaces of PAA-g-dopamine was excellent, with a spread morphology and typical polygonal shape. Dopamine is a versatile adhesive molecule that could be used to improve the adhesive strength of polymers on nitinol in future biomedical applications.

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