Biocompatible PEG Grafting on DLC-coated Nitinol Alloy for Vascular Stents

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ABSTRACT: The surfaces of Nitinol (TiNi), a popular metal alloy for arterial stents were thin-coated with diamond-like carbon (DLC) and then grafted with poly(ethylene glycol) (PEG) to increase biocompatibility. The TiNi control, DLC-coated TiNi (TiNi–DLC), and the PEG-grafted TiNi–DLC (TiNi–DLC–PEG) surface characteristics and biocompatibility were evaluated. The hydrophilicity of the TiNi–DLC–PEG significantly increased and the amount of both oxygen and nitrogen on the TiNi–DLC–PEG also increased compared to the TiNi control and TiNi–DLC due to the grafted PEG. The ratio between albumin and fibrinogen was higher on the PEG-grafted surface than the other surfaces when tested with human blood components; the platelet adhesion decreased the most on the TiNi–DLC–PEG surface, indicating improved blood compatibility. For *in vivo* tests using a rat model, the samples that were implanted for 6 weeks formed fibrous tissue; the tissue layer was much thinner on the PEG-grafted sample than the other two groups. The present results indicate that PEG-grafted TiNi–DLC surface may be effective in enhancing biocompatibility of blood-contacting biomaterials including vascular stents.

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KEY WORDS: stent, nitinol (TiNi alloy), surface modification, diamond-like carbon (DLC), poly(ethylene glycol) (PEG), biocompatibility.

INTRODUCTION

Stent placement is an effective clinical treatment for the life-threatening peripheral vascular occlusive diseases. However, the long-term success of this procedure is hampered by the development of chronic in-stent restenosis, due to the neointimal hyperplasia, that frequently occurs to the patients who received stent placement in the peripheral arteries [1–3]. Restenosis is a re-narrowing of a blood vessel, caused by the growth of fibrous tissue at the site of angioplasty or stent implementation. The fact that nonspecific adsorption of blood components significantly compromises the function of devices in contact with blood shows that the surface of implanted stents must have durable biocompatibility. Due to its super elasticity and shape-memory properties, titanium–nickel (TiNi) alloy (Nitinol) has been widely used for self-expanding vascular stents, vena cava filters, and wires for orthodontic applications [4,5]. Although nickel is known to induce hypersensitive reactions and tissue necrosis, TiNi has proven to be safe and biocompatible in many *in vitro* and *in vivo* studies based on the presence of a surface oxide film that prevents nickel from corrosion and leaching. This oxide layer is at the interface and can react with circulating blood and with living tissue as follows: (1) by electron exchange (redox reaction), (2) by proton exchange (hydrolysis), and (3) by complex formation (metal ion-organic molecule binding). The poly(crystalline oxide) on metals has been reported to have low corrosion resistance in physiological fluid and may release positively charged ions upon interacting with circulating blood. After implantation, the release of metallic ions from stents can act as a trigger to active fibrinopeptide that can form severe thrombosis, stimulate fibroblast growth, and promote protein adsorption and platelet adhesion [6].

Several studies have been directed toward specific chemical modifications of blood-contacting surfaces to develop better biocompatible biomaterials. Poly(ethylene glycol) (PEG) is known as a highly biocompatible, hydrophilic polymer [7]. Surface modification using hydrophilic polymers decreases protein adsorption and reduces platelet and cell adhesion [8–11]. The role of PEG is explained by its unique properties, such as excluded volume on the surface and flexible hydrophilic chain motion against proteins and cells, along with its nontoxicity and nonimmunogenicity [12,13].

Methods for surface modification using PEG include a simple physical adsorption, self-assembled monolayer (SAM) [14,15], chemical coupling [16,17], and graft polymerization [18]. For instance, Whitesides and co-workers [14,15] demonstrated that SAM composed of oligo-PEG decreased protein adsorption. The surface grafting of PEG has been applied not only to polymer but also to metals and glasses surfaces [19,20].

In this study, biocompatible PEG was grafted on TiNi alloy through a two-step procedure (Figure 1). Bare TiNi surface was doped with diamond-like carbon (DLC) and then grafted with PEG. Carbon is a blood-compatible, inert ceramic and thus widely used in blood-facing device. Thin DLC film was produced using a variety of techniques, such as ion beam deposition, radio frequency plasma-enhanced chemical vapor deposition (r.f.-PECVD), and pulsed laser deposition [21–23]. Biocompatibility of DLC was confirmed using human fibroblast and osteoblast-like cells *in vitro* or through animal implantations *in vivo* [24,25]. DLC was also tested for hemocompatibility and found very promising [26,27]. However, there are some controversies over DLC: clinical tests of DLC-coated vascular stents exhibited that DLC coating might not provide significant improvement in restenosis rate as compared to the uncoated stents [28]. Nonetheless it is notable that beneficial effect of DLC would come only when the coated layer is stable during the expansion of stents.

We hypothesize that utilization of PEG on the DLC-coated TiNi would have an additive benefit in enhancing biocompatibility over bare TiNi stent. To demonstrate the hypothesis, three test groups were selected: TiNi control, TiNi–DLC, and TiNi–DLC–PEG. After the surface characterization of them, both protein adsorption and platelet adhesion *in vitro* were examined. In addition, through *in vivo* implantation of each sample, the formation of fibrous tissue was evaluated in terms of tissue thickness around the implants.

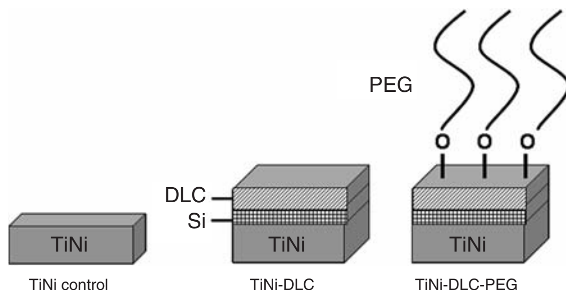

Figure 1. A schematic illustration of the process of surface modification of TiNi. After the DLC coating, biocompatible PEG is grafted on the DLC-coated layer.

MATERIALS AND METHODS

Materials

Nitinol (TiNi alloy) was purchased from Biosmart Co. Korea. Poly(ethylene glycol) (PEG; MW 2000), hexamethylene diisocyanate (HDI), dibutyltin dilaurate (DBTDL), ammonium hydroxide, hydrogen peroxide, stannous octoate, bovine serum albumin, and human fibrinogen were purchased from Aldrich Chemical Co. USA.

Synthesis of Isocyanated PEG

To isocyanate PEG, a tin catalyst (0.2% DBTDL), PEG (6.25×10^{-3} mol) were reacted with HDI (13.1×10^{-3} mol) dissolved in 10 mL of toluene under nitrogen at 40°C for 45 min. One side of the isocyanated PEG (OCN-PEG-NCO) was then reacted with methanol to produce a methylated PEG-NCO (MPEG-NCO). The final product was stored in the moisture-free vacuum chamber for further use.

DLC Coating on TiNi Alloy

The DLC layer on the TiNi alloy ($10 \times 10 \times 0.5\ mm^3$) was prepared using 13.56 MHz RF plasma-assisted chemical vapor deposition (PACVD) technique. The samples were placed on water-cooled cathode, where the RF power was delivered through impedance matching network. The precursor gases used in the deposition process were benzene and diluted silane ($SiH_4/H_2 = 10:90$). Prior to the deposition, after the samples were sputter cleaned with Ar plasma for 15 min, the DLC layers were then deposited under a bias voltage of -400 V and deposition pressure of 1.33 Pa. The film thickness was 0.55 ± 0.01 μm as measured by an alpha step profilometer.

PEG Grafting on TiNi–DLC

Before PEG grafting on the surface of TiNi–DLC, steps were taken to remove the impurities and oxide on the surface of TiNi–DLC. After the samples were dipped in distilled water and acetone for 5 min, respectively, they were immersed for 5 min in a mixture (1 : 1 : 5, v/v/v) of 25% ammonium hydroxide, 30% hydrogen peroxide, and distilled water at 80°C. The samples were then sonicated for 30 min in a solution of butanol and water (9 : 1, v/v) at room temperature. After rinsing in ethanol and triple distilled water three times, the samples were

vacuum-dried for 24 h to obtain an oxidized TiNi–DLC. These samples were reacted with 0.2 g isocyanated PEG (MPEG-NCO) in 2 mL of toluene and 0.004 mL of stannous octoate at 40°C for 24 h, to produce PEG-grafted TiNi–DLC.

Surface Characterization

Chemical structures of TiNi control, TiNi–DLC, and TiNi–DLC–PEG were analyzed using attenuated total reflection-Fourier transform infrared (ATR-FTIR, IFS 66 spectrometer, Bruker, Germany). Post-treatment changes on the chemical compositions were also investigated using electron spectroscopy for chemical analysis (ESCA; S-Probe Surface Science, Mountain View, CA, USA). The relative atomic percent was calculated from the peak areas reflecting atomic sensitivity factors. Water contact angle, an indicator of surface wettability, was determined using an optical bench-type contact angle goniometer (Digidrop, GBX Scientific Instrument, France). A drop of water was deposited onto the sample surface and direct microscopic measurement of contact angle was then carried out. Surface morphology of the modified samples was observed using scanning electron microscope (SEM, Hitachi 2500C, Japan).

Protein Adsorption and Platelet Adhesion

Both control and surface-modified samples were hydrated for 30 min in phosphate-buffered saline (PBS), then immersed for 1 h in human fibrinogen (0.3 mg/mL) and in bovine serum albumin (3 mg/mL), respectively, and rocked in a water bath at 37°C. After rinsing three times in PBS, the samples were treated with 5% sodium dodecyl sulfate (SDS) for 24 h to detach any proteins physically adsorbed on the surface. The amount of total protein was measured using BCA protein assay kit. Human blood was mixed with 3.8% sodium citrate solution in the ratio of 1 : 9 (v/v). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were separately obtained by centrifugation at 250 g for 10 min and at 1500*g* for 20 min, respectively. The number of platelets in PRP was adjusted to 1×10^5 platelets/mL by adding PPP. After the samples were incubated for 1 h in the platelet suspension (2 mL) at 37°C and washed three times with PBS, the adhered platelets on samples were fixed by putting them in 2% glutaraldehyde for 1 h. The samples were dehydrated sequentially with the aqueous 50, 70, 90, and 100% ethanol solution, and then, vacuum-dried overnight. The images of platelet-adhered surfaces for each sample were taken using SEM.

In Vivo Rat Implantation

Samples ($n = 6$, each group) of TiNi control, TiNi–DLC, and TiNi–DLC–PEG were individually implanted in both sides of the thigh muscles of six rats. Anesthesia was carried out with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (15 mg/kg). Once the thigh muscles were incised, each sample was inserted in the muscle and then the skin was sutured to close the wound. After 6 weeks, the rats were sacrificed and the implanted samples were retrieved for histological analysis. The formation of fibrous tissue around the samples was visualized using hematoxylin & eosin (H&E) staining. Through a microscopic examination, the thickness of fibrous tissue was measured three times for each sample and represented on average.

Statistical Analysis

All the data were presented as mean ± standard deviation (SD). As compared to the TiNi control, a statistically significant difference was sought using the Student's t-test. The difference was considered significant when the p-value was less than 0.05.

RESULTS AND DISCUSSION

In this study, the biocompatible compounds, DLC and PEG, were coated and grafted onto TiNi alloy surfaces. It was anticipated that through two-step surface treatment, a synergistic effect may arise for TiNi–DLC–PEG in improving its biocompatibility. The surface modification of TiNi alloy sample was performed and evaluated by surface analyses.

The chemical structure of the PEG-grafted TiNi–DLC–PEG surfaces displayed additional ART-FTIR spectrum peaks for CH stretching at $2800\,cm^{-1}$ and an ether peak at $1100\,cm^{-1}$ [17], when compared to both the TiNi control and TiNi–DLC (Figure 2). For further analysis of surface chemistry, when atomic percents of carbon, oxygen, and nitrogen on the surface were determined using ESCA, a significant increase of oxygen level on the TiNi–DLC–PEG indicated the presence of grafted PEG (Table 1) [11]. The detection of nitrogen was indicative of the isocyanated PEG presence. The TiNi–DLC exhibited the highest percent carbon. The high level of oxygen in the TiNi control occurred during the oxidation process that sharply decreased after DLC was applied in the thin layer. The PEG grafting dramatically changed the water contact angle from 71° to 18° (Table 1). While the DLC coating

was barely effective in improving surface hydrophilicity of the TiNi control, the PEG-grafted sample exhibited the lower contact angle. Thus, the surface of TiNi–DLC–PEG was significantly hydrophilized due to PEG [7,12,19]. Taken at 1000× magnifications by SEM, the differences in the surface morphologies appeared substantial before or after surface modification. The TiNi control was rather smooth while the DLC-coated surface displayed a wavy topography (Figure 3). The surface morphology of PEG-grafted TiNi–DLC was even more topographically pronounced compared to the other two samples. This indicated that the PEG grafting had a dominant effect on the surface as also indicated by the data in Figure 2.

Since the coated PEG can be stripped from the surface when in contact with physiological solutions, improvements in coating stability are essential and several approaches have been proposed [12,19].

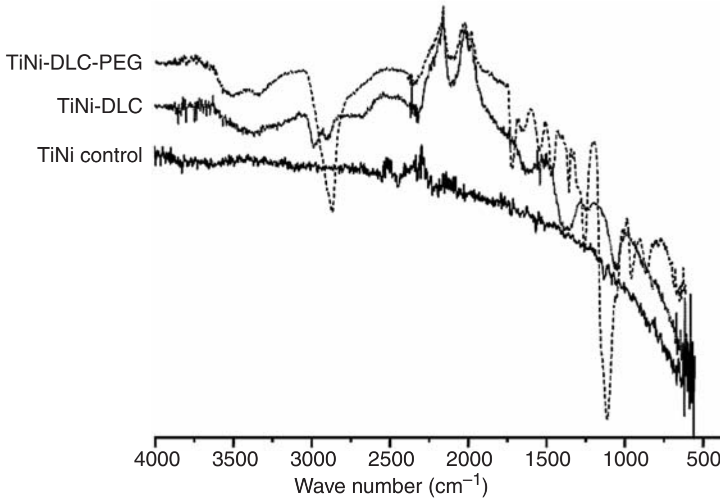

Figure 2. ATR-FTIR spectra of TiNi control, TiNi–DLC, and TiNi–DLC–PEG.

Table 1. Surface characteristics of TiNi control and surface-modified TiNi.

Sample	ESCA atomic percent			Contact Angle (degree)
	C	O	N	
TiNi Control	39.22	59.94	0.84	71 ± 2
TiNi–DLC	85.32	13.93	0.75	70 ± 1
TiNi–DLC–PEG	66.90	29.90	3.20	18 ± 1

In this work, TiNi–DLC layer was oxidized and then coupled with a PEG molecule, where one hydroxyl group was replaced with more reactive isocyanate group. This established a covalent link between oxidized TiNi–DLC and isocyanated PEG. The binding seems to be stable but still need to be worked on to ensure a long-term stability of current PEG layer.

The biocompatibility of the surface-treated TiNi was examined using human blood components. The amounts of albumin and fibrinogen adsorbed onto the TiNi samples were different and appeared to be influenced by surface treatment of TiNi (Figure 4). The average concentrations of albumin and fibrinogen adsorbed on the TiNi control

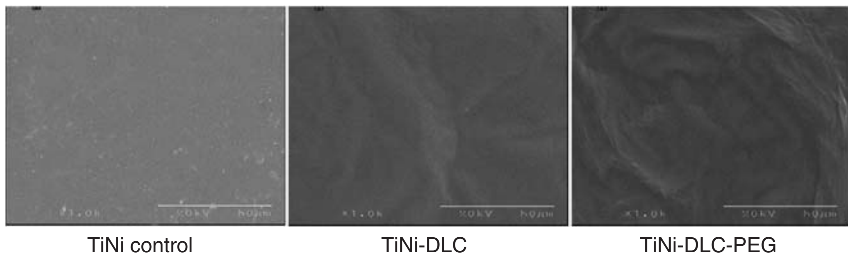

Figure 3. Comparison of the surface morphology (1000×) using SEM before and after surface modification.

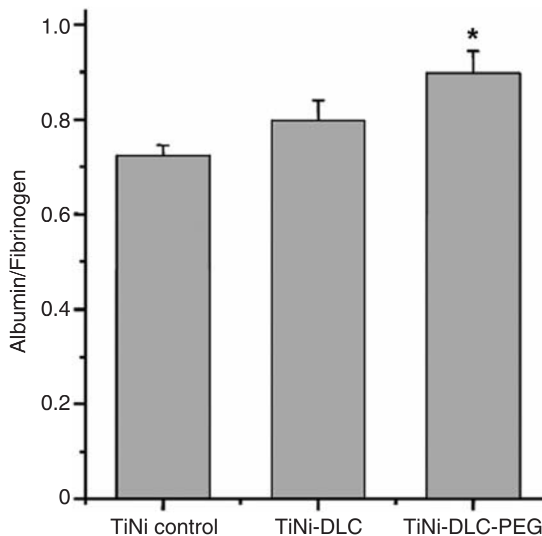

Figure 4. Measurement of albumin to fibrinogen ratio adsorbed on the surfaces of TiNi control, TiNi–DLC, and TiNi–DLC–PEG.

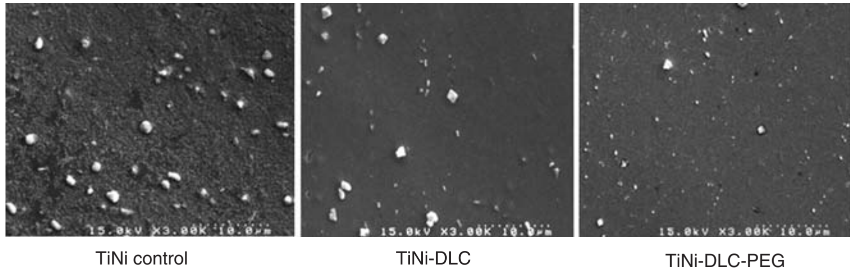

Figure 5. SEM images (3000×) of platelet adhesion on the surface of TiNi control, TiNi–DLC, and TiNi–DLC–PEG, respectively.

were 0.09 and 0.125 μg/cm^2, respectively, while those on the PEG-grafted group were 0.125 and 0.14 μg/cm^2, respectively. In general, more albumin and less fibrinogen adsorbed on the surface are positive signs of blood compatibility of biomaterials [8–10]. The hydrophilic properties of PEG, as identified from the contact angle, may have kept the fibrinogen clusters from adhering to the surface. When the platelet adhesion was examined using SEM, the dispersion of adhered platelets was rather widespread on the TiNi while the DLC-coated TiNi was only marginally effective in reducing the attachment of platelet (Figure 5). Interestingly, the PEG-grafted TiNi–DLC had the most pronounced decrease in platelet adhesion [9,12,19]. These results indicate that blood protein adsorption and platelet adhesion may be substantially suppressed on the PEG-grafted surface, implying enhanced blood compatibility. This information is in good agreement with the previous reports [19,29]. Many theories have been proposed to explain such antifouling behavior by PEG, including a high molecular mobility, lack of protein binding sites, large excluded volume, osmotic repulsion, and hydrophilicity [7,12,19]. Protein-resistant PEGylated surfaces are frequently described as a liquid assembly of highly mobile molecules and oligomeric segments that offer few binding sites to most proteins, as well as very short interaction time between PEG and proteins.

For the evaluation of *in vivo* performance of PEG-grafted TiNi–DLC, samples were implanted for 6 weeks and retrieved from sacrificed rats (Figure 6). All of the recovered implants had fibrous tissue around them regardless of the surface treatments. The result of H&E tissue staining, however, showed that notable differences in the thickness of the deposited tissue as seen listed in Table 2 (Figure 7). It deceased from TiNi control (85.4 μm) and TiNi–DLC (76.0 μm) to TiNi–DLC–PEG (61.6 μm), indicating that the PEG-graft tissue compatibility played a role in retarding fibrous tissue growth *in vivo* [30,31].

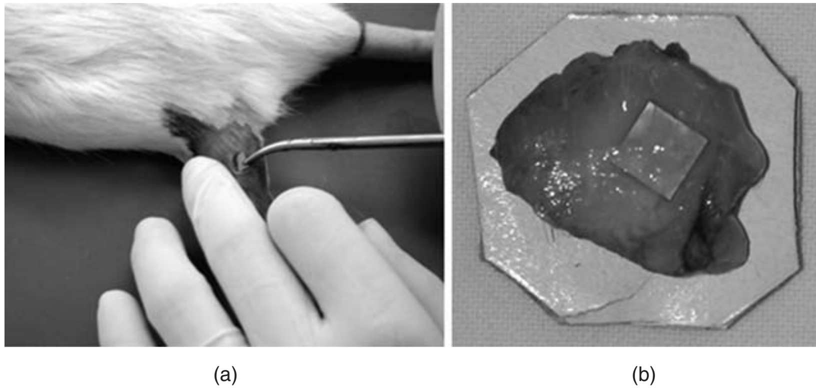

Figure 6. A view of implanted sample *in vivo* (a) and the retrieved one after 6 weeks implantation (b).

Table 2. Thickness of fibrous tissues deposited on the implanted samples.

Sample	Thickness (μm)
TiNi Control	85.4 ± 0.71
TiNi–DLC	76.0 ± 1.51
TiNi–DLC–PEG	61.6 ± 0.06

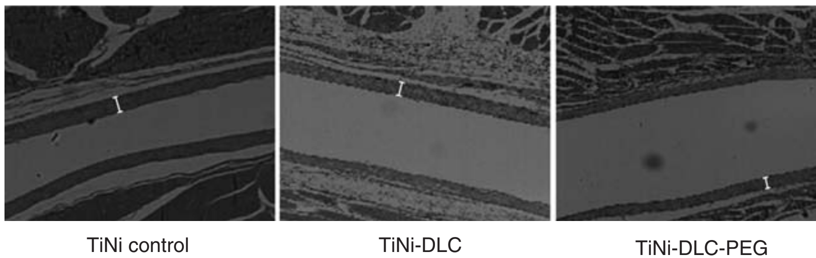

Figure 7. H&E staining of the fibrous tissues formed on the implanted samples. The thickness of fibrous tissue is marked in the white bar.

CONCLUSIONS

To improve the biocompatibility of TiNi alloy surfaces, they were coated with diamond-like carbon (DLC) and then grafted with a layer of PEG, which was confirmed by surface analyses. The PEG-grafted TiNi–DLC exhibited considerably reduced protein adsorption and platelet adhesion compared to TiNi control and TiNi–DLC. After being implanted *in vivo* for 6 weeks, the TiNi–DLC–PEG sample had substantially delayed

fibrous tissue growth around the implant compared to the controls. The present work indicates that the PEG-grafted TiNi–DLC significantly improved the biocompatibility and has potential application for vascular stent modification.

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REFERENCES

1. Windecker, S., Mayer, I., Pasquale, G.D., Maier, W., Dirsch, O., Groot, P.D. et al. (2001). Stent Coating with Titanium-Nitride-Oxide for Reduction of Neointimal Hyperplasia, *Circulation*, **104**: 928–933.
2. Lewis, A.L., Tolhurst, L.A. and Stratford, P.W. (2002). Analysis of a Phosphorylcholine-based Polymer Coating on a Coronary Stent Pre- and Post-Implantation, *Biomaterials*, **23**: 1697–1706.
3. Thierry, B., Merhi, Y., Bilodeau, L., Trepanier, C. and Tabrizian, M. (2002). Nitinol versus Stainless Steel Stents: Acute Thrombogenicity Study in an *Ex Vivo* Porcine Model, *Biomaterials*, **23**: 2997–3005.
4. Duerig, T., Pelton, A. and Stockel, D. (1999). An Overview of Nitinol Medical Applications, *Mater. Sci. Eng., A Struct. Matter.: Prop. Microstruct. Process.*, **273/275**: 149–160.
5. El Medawar, L., Rocher, P., Hornez, J.-C., Traisnel, M., Breme, J. and Hildebrand, H.F. (2002). Electrochemical and Cytocompatibility Assessment of Nitinol Memory Shape Alloy for Orthodontic Use, *Biomol. Eng.*, **19**: 153–160.
6. Shin, C.C. and Shin, C.M. (2003). Characterization of the Thrombogenic Potential of Surface Oxides on Stainless Steel for Implant Purposes, *Appl. Surface Sci.*, **219**: 347–362.
7. Lee, J.H., Lee, H.B. and Andrade, J.D. (1995). Blood Compatibility of Polyethylene Oxide Surfaces, *Prog. Polym. Sci.*, **20**: 1043–1079.
8. Han, D.K., Park, K.D., Ryu, G.H., Kim, U.Y., Min, B.G. and Kim, Y.H. (1996). Plasma Protein Adsorption to Sulfonated Poly(Ethylene Oxide)-Grafted Polyurethane Surface, *J. Biomed. Mater. Res.*, **30**: 23–30.
9. Han, D.K., Jeong, S.Y., Kim, Y.H., Min, B.G. and Cho, H.I. (1991). Negative Cilia Concept for Thromboresistance: Synergistic Effect of PEO and Sulfonate Groups Grafted onto Polyurethanes, *J. Biomed. Mater. Res.*, **25**: 561–575.

10. Zhang, F., Kang, E.T., Neoh, K.G. and Huang, W. (2001). Modification of Gold Surface by Grafting of Poly(Ethylene Glycol) for Reduction in Protein Adsorption and Platelet Adhesion, *J. Biomater. Sci. Polym. Edn.*, **12**: 515–531.
11. Han, D.K. and Hubbell, J.A. (1997). Synthesis of Polymer Network Scaffolds from L-Lactide and Poly(Ethylene Glycol) and their interaction with Cells, *Macromolecules*, **30**: 6077–6083.
12. Amiji, M. and Park, K. (1993). Surface Modification of Polymeric Biomaterials with Poly(Ethyleneoxide), Albumin, and Heparin for reduced Thrombogenicity, *J. Biomater. Sci. Polym. Edn.*, **4**: 217–234.
13. Fuerteges, F. and Abuchowski, A. (1990). The Clinical Efficacy of Poly(Ethylene Glycol)-Modified Proteins, *J. Control. Rel.*, **11**: 139–148.
14. Pale-Grosdemage, C., Simon, E.S., Prime, K.L. and Whitesides, G.M. (1991). Formation of Self-assembled Monolayers by Chemisorption of Derivatives of Oligo(Ethylene Glycol) of Structure $HS(CH_2)_{11}(OCH_2CH_2)_mOH$ on Gold, *J. Am. Chem. Soc.*, **113**: 12–20.
15. Harder, P., Grunze, M., Dahint, R., Whitesides, G.M. and Laibinis, P.E. (1998). Molecular Conformation in Oligo(Ethylene Glycol)-Terminated Self-assembled Monolayers on Gold and Silver Surfaces Determines their Ability to resist Protein Adsorption, *J. Phys. Chem. B*, **102**: 426–436.
16. McPherson, T.B., Shim, H.S. and Park, K. (1997). Grafting of PEO to Glass, Nitinol, and Pyrolytic Carbon Surfaces by Irradiation, *J. Biomed. Mater. Res. Appl. Biomater.*, **38**: 289–302.
17. Han, D.K., Jeong, S.Y., Ahn, K.-D., Kim, Y.H. and Min, B.G. (1993). Preparation and Surface Properties of PEO-sulfonate Grafted Polyurethanes for Enhanced Blood Compatibility, *J. Biomater. Sci. Polym. Edn.*, **4**: 579–589.
18. Tseng, Y.C. and Park, K. (1992). Synthesis of Photoreactive Poly(Ethylene Glycol) and its Application to the Prevention of Surface-Induced Platelet Activation, *J. Biomed. Mater. Res.*, **26**: 373–391.
19. Kim, Y.H., Han, D.K., Park, K.D. and Kim, S.H. (2003). Enhanced Blood Compatibility of Polymer Grafted by Sulfonated PEO via a Negative Cilia Concept, *Biomaterials*, **24**: 2213–2223.
20. Chognot, D., Six, J.-L., Léonard, M., Dellacherie, E., Faivre, B., Bonneaux, F. et al. (2008). Synthesis and *In Vivo* Studies of Protein C-loaded Nanoparticles with PEO Modified Surfaces, *J. Bioact. Compat. Polym.*, **23**: 49–68.
21. Li, D.J., Cui, F.Z. and Gu, H.Q. (1999). Studies of Diamond-like Carbon Films Coated on PMMA by Ion Beam Assisted Deposition, *Appl. Surf. Sci.*, **137**: 30–37.
22. Sanchez-Lopez, J.C., Donne, C., Fontaine, J., Belin, M., Grill, A., Patel, V. et al. (2000). Diamond-like Carbon Prepared by High Density Plasma, *Diamond Relat. Mater.*, **9**: 638–642.

23. Shim, K.S., Kim, S.M., Bae, S.H., Lee, S.Y., Jung, H.S. and Park, H.H. (2000). Fabrication and Characterization of Diamond-like Carbon Thin Films by Pulsed Laser Deposition, *Appl. Surf. Sci.*, **154/155**: 482–484.
24. Butter, R., Allen, M., Chandra, L., Lettington, A.H. and Rushton, N. (1995). *In Vitro* Studies of DLC Coatings with Silicon Intermediate Layer, *Diamond Relat. Mater.*, **4**: 857–861.
25. Du, C., Su, X.W., Cui, F.Z. and Zhu, X.D. (1998). Morphological Behavior of Osteoblasts on Diamond-like Carbon Coating and Amorphous C-N Film in Organ Culture, *Biomaterials*, **19**: 651–658.
26. Nurdin, N., Francois, P., Moret, M., Unal, K., Krumeich, J., Aronson, B.O. et al. (2003). Hemocompatible Diamond-like Carbon (DLC) Surfaces, *Eur. Cells Mater.*, **5**: 44–45.
27. Roy, R.K. and Lee, K.-R. (2007). Biomedical Applications of Diamond-like Carbon Coatings: A Review, *J. Biomed. Mater. Res. Appl. Biomater.*, **83**: 72–84.
28. Airoldi, F., Colombo, A., Tavano, D., Stankovic, G., Klugmann, S., Paolillo, V. et al. (2004). Comparison of Diamond-like Carbon-Coated Stents versus Uncoated Stainless Steel Stents in Coronary Artery Disease, *Am. J. Cardiol.*, **93**: 474–477.
29. Han, D.K., Park, K.D. and Kim, Y.H. (1998). Sulfonated Poly(Ethylene Oxide)-Grafted Polyurethane Copolymer for Biomedical Applications, *J. Biomater. Sci. Polym. Edn.*, **9**: 163–174.
30. Olayo, R., Ríos, C., Salgado-Ceballos, H., Cruz, G.J., Morales, J., Olayo, M.G. et al. (2008). Tissue Spinal Cord Response in Rats after Implants of Polypyrrole and Polyethylene Glycol obtained by Plasma, *J. Mater. Sci. Mater. Med.*, **19**: 817–826.
31. Han, D.K., Park, K.D., Jeong, S.Y., Kim, Y.H., Kim, U.Y. and Min, B.G. (1993). *In Vivo* Biostability and Calcification-Resistance of Surface-Modified PU-PEO-SO_3, *J. Biomed. Mater. Res.*, **27**: 1063–1073.