NJC

PAPER

Cite this: *New J. Chem.*, 2018, **42**, 3722

Chemical coatings relying on the self-polymerization of catechol for retrievable vena cava filters

Songhao Bao,†[a] Jialin Kang,†[a] Chengzhao Tu,[a] Chengfeng Xu,[a] Lin Ye,*[ab] Huan Zhang,*[c] Hui Zhao,[d] Aiying Zhang,[ab] Zengguo Feng[ab] and Fuxian Zhang[c]

Received 25th October 2017,
Accepted 26th January 2018

DOI: 10.1039/c7nj04138a

rsc.li/njc

The heparin–dopamine conjugate was synthesized by an amidation reaction between heparin's carboxyl and dopamine's amine, whereas the paclitaxel (PTX)-catechol conjugate was prepared *via* an esterification reaction between the carboxyl in 3-catechol-1-acetic acid and PTX's hydroxyl. The former can be chemically coated on a Ti–Ni alloy substrate *via* pH sensitive self-polymerization of the catechol moieties in aqueous solution, and the latter can also be chemically attached to a Ti–Ni alloy substrate in acetone by the thermo-sensitive self-polymerization of catechol. Successful chemical coating of heparin and PTX can be proven by static contact angle and XPS measurements, respectively. *In vitro* evaluation showed that the heparin coating exhibited anti-thrombosis properties, whereas the PTX coating also illustrated anti-intimal hyperplasia properties. Consequently, the promising potential to develop chemical coatings relying on the self-polymerization of catechol moieties to effectively extend the *in vivo* retention time of retrievable vena cava filters in the clinic was shown.

Introduction

Blood clotting formed in deep vein vessels can migrate with circulation within the blood vessel. When thrombosis migrates from the vena cava to pulmonary circulation, severe problems are caused for patients and sometimes these can even be fatal. This is known as Pulmonary Embolism (PE).[1–3] One of the therapies for PE is the implantation of Vena Cava Filters (VCFs).[4–7] The purpose of VCFs is to capture emboli and prevent them from migrating to the pulmonary circulation. However, the use of both traditional VCFs, which remain inside the vessel permanently once being implanted, and retrievable VCFs (RVCFs), which are retrieved if the mortal PE risk is gone, will encounter severe side effects such as vena cava/in-filter thrombosis and intimal hyperplasia.[4–8] These may cause vena cava occlusion and increase the risk of vessel damage for RVCFs during retrieval.

Drug eluting stents (DESs) have shown significant anti-thrombosis and anti-intimal hyperplasia success due to the coating of certain drugs on the surface of bare stents.[9–12] DESs containing both heparin as an anti-thrombosis drug and PTX as an anti-intimal hyperplasia drug have been prepared and showed that the two drugs can work synergistically to exhibit favourable effects.[9] This progress inferred that anti-thrombosis and anti-intimal hyperplasia properties may be endowed to bare RVCFs by coating the corresponding drugs on the surface. Previously, we have coated polycaprolactone (PCL) coating containing rapamycin and heparin onto an Aegisy RVCF (Lifetech Scientific Corporation in Shenzhen, China) and *in vivo* experiments showed that the coating can significantly decrease the risk of intimal hyperplasia within 20 days.[13] However, due to the poor compatibility with PCL, the loaded heparin exhibited burst release and its anti-thrombosis activity was not satisfactory. Furthermore, because there were only intermolecular interactions present between the PCL coating and the RVCF, the coating was a typical physical coating and its stability was not satisfactory either. Finally, the PCL physical coating was invalid after 20 days *in vivo*, while the treatment for mortal PE risk may need a period longer than 20 days. Thus, it remains a challenge to develop a long-term effective and stable coating with anti-thrombosis and anti-intimal hyperplasia properties for RVCFs.

In 2007, Messersmith and colleagues[14–16] reported a bio-inspired method for multifunctional surface coatings, which was inspired by mussels' natural adhesive protein Mefp5. It was found that Mefp5's well-performing adhesive properties on various surfaces, including metal and alloy surfaces, may be associated with a dopamine/catechol-rich structure which is

[a] *School of Materials Science and Engineering, Beijing Institute of Technology, Beijing 100081, China. E-mail: yelin@bit.edu.cn*
[b] *Beijing Key Laboratory of Construction Tailorable Advanced Functional Materials and Green Applications, Beijing 100081, China*
[c] *Shijitan Hospital, Capital Medical University, Beijing 100038, China. E-mail: ahuan.1984@163.com*
[d] *Luhe Hospital, Capital Medical University, Beijing 101149, China*
† Contributed to this paper equally and in alphabetical order by surname.

electron-rich and can form ligand bonds or strong electrostatic forces on the surfaces *via* its self-polymerization[17–25] so as to form a stable chemical coating. Ma and colleagues[26,27] firstly deposited a polydopamine coating on the surface by the self-polymerization of dopamine and then grafted anti-fouling polymers to the polydopamine coated surface by UV irradiation. The anti-fouling polymers can inhibit protein and platelet adhesion so as to exhibit anti-coagulation properties.

To mimic the adhesive nature of Mefp-5, one can covalently connect dopamine/catechol into the target compound, and then coat the target compound on the substrate *via* the polymerization of dopamine/catechol. Based on this idea, we prepared two functional chemical coatings, a heparin coating and a paclitaxel (PTX) coating, and coated them on a titanium–nickel (Ti–Ni) alloy substrate which is used for making RVCFs. As mentioned before, heparin coatings can inhibit in-filter thrombosis. Compared with anti-coagulation polymers such as PEG,[26] polysarcosine,[27] *etc.*, heparin can activate antithrombin III to inactivate serials of blood coagulation factors so as to stop the coagulation process at a much earlier stage. More importantly, heparin is now in clinical use. Thus, heparin was chosen as the anti-thrombosis coating in this paper. Similarly, PTX as a major drug in DESs was selected to be the anti-intimal hyperplasia coating herein as it has a similar function to rapamycin for the inhibition of intimal proliferation in RVCFs.[13] Firstly, we covalently grafted dopamine and catechol moieties to heparin and PTX to prepare heparin–dopamine and PTX–catechol conjugates. Then, two conjugates were separately coated on the Ti–Ni alloy substrate by virtue of dopamine/catechol's adhesive properties. Since the drugs were covalently bound to the surface instead of being encapsulated in the PCL coating, they were assumed to be retained on the RVCF surface for a long time so that the heparin and PTX chemical coating may have a much longer duration of anti-thrombosis and anti-intimal hyperplasia properties than the previous PCL physical coating. Additionally, the chemical coatings should also be much more stable. Thus, this chemical coating strategy relying on the self-polymerization of catechol was expected to show promising potential to prolong the *in vivo* retention time of RVCFs enough to meet clinical requirements.

On the other hand, instead of coating with heparin or PTX individually, clinical practice requires the coating of heparin and PTX coatings together on RVCFs in order to endow anti-thrombosis and anti-intimal hyperplasia properties simultaneously. The successful preparation of heparin–dopamine and PTX–catechol compounds and their coating procedures on alloy surfaces relying on the self-polymerization of catechol in this manuscript may provide a theoretical basis to successfully prepare long-term effective RVCF coatings with simultaneous anti-thrombosis and anti-intimal hyperplasia properties in the near future.

Experimental

Materials and measurements

Heparin sodium salt was purchased from Solarbio Life Science Co., Ltd, China and 2-morpholino-ethane sulfonic acid (MES) was purchased from Alfa Aesar, USA. Phosphate-buffered saline (PBS, pH = 7.4), toluidine blue O (TBO), 3-hydroxytyramine hydrochloride (dopamine hydrochloride), and 2-(3,4-dihydroxyphenyl)acetic acid were all purchased from J&K Scientific LTD, China. PTX was obtained from Beijing Hapten and the Protein Biomedical Institute. Ti–Ni alloy (titanium–nickel) substrates (10 mm × 10 mm, 2 mm thick) were purchased from Beijing Baikai Technology Co., Ltd, China. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were all purchased from Beijing Zhongshenghuateng Technology Co., Ltd, China. Dialysis membranes (MWCO = 3500 Da) were purchased from the Beijing branch of Hongda Biotechnology Co., Ltd, China. The other common chemical reagents were of analytic grade and purchased from Beijing Chemical Manufacture, China.

^{1}H-NMR data were obtained using a BRUKER spectrometer (400 MHz); D_2O was used as the solvent. IR spectra were acquired using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR, Shimadzu IRTracer-100). X-ray photoelectron spectroscopy (XPS) measurements were performed using a PHI Quantera II SXM, Pekin Elmer. Scanning electron microscopy (SEM) observations were conducted on a COXEM EM30-plus. UV-vis spectra were obtained using Shimadzu UV-1800. The contact angle measurements were performed on a Phoenix 300 goniometer. Static water contact angles were measured at three different locations on each sample, and the average values are reported.

Preparation and characterization of chemical heparin coating

Briefly, heparin (200 mg) and dopamine (50 mg) were dissolved in 0.05 M MES buffer (pH 5.3, 30 mL, 0.1 M NaCl). After complete dissolution, NHS (0.0575 g, 0.5 mmol) and EDC (0.1915 g, 1 mmol) were subsequently added to the heparin solution to activate the carboxylic acid groups of the heparin molecules. The solution was vigorously stirred for 24 h under N_2 protection at 25 °C (the temperature should not be lower than 20 °C, otherwise gel would be formed), and the pH was maintained at 5.3. After the reaction was stopped, 3 mL saturated NaCl solution and 60 mL cold ethanol were added sequentially. After centrifugation, the precipitated crude product was re-dissolved in 10 mL NaCl solution (1 M), and then 100 mL ethanol was added again. This purification step was repeated thrice to minimize the electrostatic interactions between heparin and dopamine. Then the product was transferred to a dialysis membrane (MWCO = 3500 Da) and dialyzed for 24 h to remove unreacted coupling reagents and dopamine. Acidified water (1 mL HCl (5 M) in 1 L deionized distilled water) was used for the dialysis procedure. The heparin–dopamine conjugate was finally obtained by lyophilization. The substitution ratio (the number of conjugated dopamine molecules per molecule of heparin) was calculated by a UV spectrum based on the intensity of benzene's absorption peak (280 nm).

The heparin–dopamine conjugate was dissolved in 10 mM Tris buffer solution (5 mg mL^{-1}, pH 8.5), and the Ti–Ni substrate was polished to eliminate the oxidation layer on the surface and cleaned using ultrasonication in ethanol solvent for 10 min before use. The alloy substrate was immersed into

the buffer solution. The solution was firstly under ultrasonication for 2 min and then stirred at 70 °C for 24 h for coating. Then, the coated substrate was removed from the solution and washed in aqueous solution with stirring for 24 h to completely remove the uncoated conjugate. Finally, the coated substrate was dried using a vacuum.

The surface heparin content of the coated substrate was measured using the TBO method.[28–31] The sulfonic groups on the surface can form complexes with the TBO dye. To determine the surface heparin content, the method is described as followed. Briefly, six standard heparin sodium solutions (0 μg mL^{-1}, 6 μg mL^{-1}, 10 μg mL^{-1}, 15 μg mL^{-1}, 20 μg mL^{-1} and 25 μg mL^{-1}) were prepared using PBS as a solvent. Then, 2 mL toluidine blue (0.01 M HCl, 0.2 wt% NaCl and 0.04 wt% TBO zinc chloride double salt) was added into the standard solution and gently shaken for 30 min at 37 °C, resulting in complexation of TBO with heparin. Afterwards, 2 mL *n*-hexane was added and gently shaken for 30 min. The complex was extracted into the organic layer and the unreacted TBO remained in the aqueous phase. The calibration curve was made according to the absorbance of the aqueous phase at 590 nm using UV spectroscopy. Subsequently, the coated substrate was immersed in 2 mL PBS solution, and then the solution was manipulated according to the steps mentioned above. The surface heparin content of the coated substrate was calculated from a calibration curve.

The bare alloy and heparin coated substrate were cut into 1 × 1 cm rectangular shapes and placed into the incubation tube. Citrated standard plasma (100 μL) and Pathromtin SL (100 μL) were then pipetted into a test tube and pre-warmed to 37 °C, and incubated for 1 or 10 min at 37 °C. Then, the incubated solution was transferred to a test tube and calcium chloride solution (100 μL, 0.025 M) was added at 37 °C. Finally, the timer on a fibrintimer (TEChrom IV plus, China) started to measure the clotting time.

A platelet adhesion experiment was carried out to examine the interaction between blood and the materials *in vitro*.[32] Blood collection was approved by the animal ethics committee of Beijing Shijitan Hospital, Capital Medical University and in compliance with the National Guideline on Administration of Lab Animal in China and Regulation on Administration of Lab Animal in Beijing. Platelet rich plasma (PRP) was prepared by centrifuging 45 mL of fresh whole blood from a dog donor with 5 mL of a 3.8 wt% sodium citrate solution at 1500 rpm for 15 min and 5000 rpm for 20 min. The bare Ti–Ni alloy was employed as a negative control. After having contact with the two substrates for 180 min, the PRP was removed and the substrates were gently rinsed thrice with PBS to remove non-specifically adhered platelets. The substrates' surfaces were then observed by SEM.

Preparation and characterization of chemical PTX coating

0.1 g paclitaxel, 0.02 g 2-(3,4-dihydroxyphenyl)acetic acid, 0.048 g DCC and 0.014 g DMAP were dissolved in 10 mL dichloromethane in a flask bottle in an ice-bath. The solution was stirred vigorously for about 1 h to activate the DMAP and DCC. Then the ice-bath was removed and the mixture was stirred and reacted in room temperature for about one day under N_2 protection. After the reaction, the product was purified by column chromatography. A mixture of methanol and chloroform (1 : 20) was chosen as the mobile phase and silica gel was chosen as the stationary phase. Thin layer chromatography (SLC) was applied to determine whether the product was separated. After separation, the product was concentrated by a rotary evaporator and dried in a vacuum oven overnight.

The PTX conjugate was self-polymerized and adhered on the surface of the Ti–Ni alloy substrate in acetone. Briefly, the procedure was as follows: the conjugate was dissolved in the acetone solvent in a flask (10 mg/50 mL) and the processed substrate (polished to eliminate the oxidation layer on the surface and cleaned by ultrasonication in ethanol for 10 min before use) was placed at the bottom of the flask. The solution was refluxed in an oil-bath for one day. After coating, the coated substrate was taken out from the solution and washed in acetone with stirring for 24 h at room temperature to completely remove the uncoated conjugate. Finally, the coated substrate was dried in a vacuum oven for about 1 day.

The substrates with a dimension of 1 cm^2 in triplicate were incubated in 1 mL of media for a period of 24 hours. Endothelial cells (ECs) and smooth muscle cells (SMCs) were obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products and cultured in 96-well flat-bottomed plates. After 24 h of culturing at 37 °C in a humidified atmosphere with 5% CO_2, a cell monolayer was obtained. Various substrate samples were placed into a 24-well culture plate and cell suspension (100 μL) was seeded onto the substrates. The cell-seeded substrates were maintained at 37 °C for 3 h under 5% CO_2, and then 1.5 mL of culture medium (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin) was added to each well. At predetermined intervals (3d for ECs and 5d for SMCs), 60 μL of MTT solution (5 mg mL^{-1} in PBS) was added to each well, followed by incubation at 37 °C for 4 h. Then the upper medium was carefully removed and the intracellular formazan was dissolved by adding 500 μL of DMSO to each well. The absorbance of the produced formazan was measured at 570 nm with a microplate reader (ZS-2, Beijing). The cell viability (CV) was calculated according to the following equation.

$$\mathrm{CV}\% = \frac{E_a}{C_a} \times 100\% \quad (1)$$

where E_a and C_a are averaged absorptions of the experimental group and the control group, respectively.

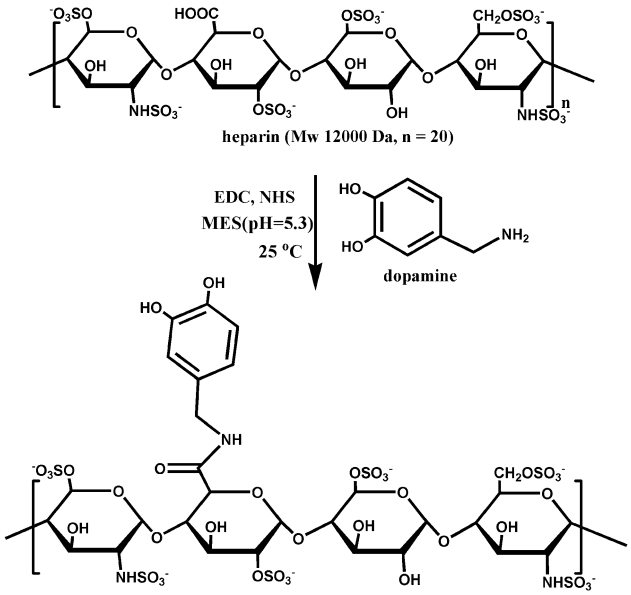
Results and discussion

The synthesis of the heparin–dopamine and PTX–catechol conjugates

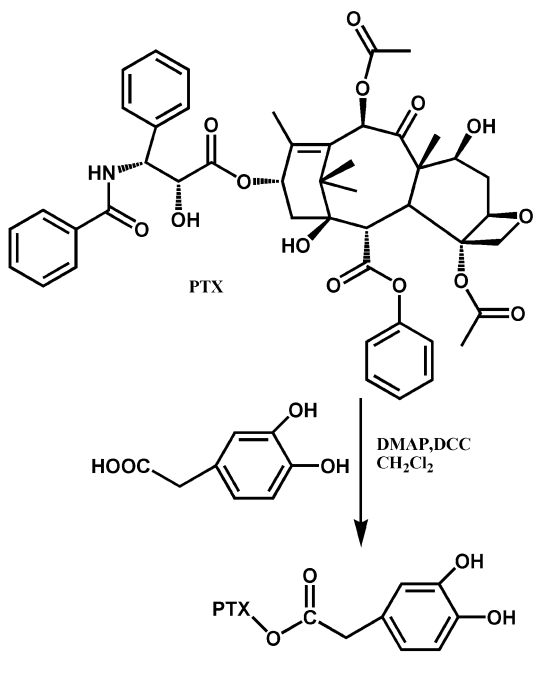
As mentioned above, the dopamine and catechol groups should be covalently introduced into the coating molecules, heparin and PTX, in order to obtain stable chemical coatings. Heparin contains abundant functional groups including carboxyl so it can react with dopamine's amine to prepare heparin–dopamine

conjugates as shown in Scheme 1a. PTX possesses several hydroxyls in its moiety thus 2-(3,4-dihydroxyphenyl)acetic acid was grafted into PTX *via* the esterification between its carboxyl and PTX's hydroxyl as shown in Scheme 1b.

The IR spectra of heparin and heparin–dopamine conjugates are exhibited in Fig. 1a. The broad absorption peaks corresponding to hydroxyl at around 3400 cm^{-1} and C–O vibration at 1057 and 1235 cm^{-1} were found in heparin and the conjugate's



a. The preparation protocol of heparin-dopamine conjugate



b. The preparation protocol of PTX-catechol conjugate

Scheme 1 The synthetic route for heparin–dopamine (a) and PTX–catechol (b) conjugates.

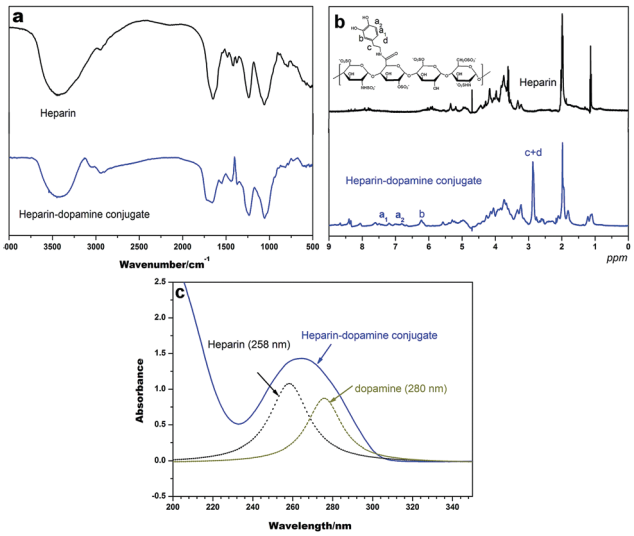


Fig. 1 IR (a), NMR (b) and UV (c) characterization of the heparin–dopamine conjugate.

spectra, while the peak of the amide II band at 1552 cm^{-1} and aromatic C–H vibration at 3064 cm^{-1} appeared in the conjugate, inferring the successful introduction of dopamine into heparin. Fig. 1b depicts the ^1H NMR spectra of heparin and the heparin–dopamine conjugate. Due to the presence of variable sequences in heparin's moiety, it's very difficult to resolve heparin's NMR spectrum. But the occurrence of the aromatic proton's resonance peaks at δ = 6.50–7.50 ppm as well as the dopamine's methylene peak at δ = 2.8 ppm strongly hinted at the successful preparation of the heparin–dopamine conjugate in addition to the FTIR result. The UV results presented in Fig. 1c also showed the successful conjugation of dopamine because of the presence of benzene's absorption at 280 nm in the conjugate. Furthermore, the intensity at 280 nm was used to quantify the substitution ratio of heparin's carboxyl groups, and this was calculated as 34%. Since there are dozens of carboxyl groups in one heparin molecule, this substitution ratio should be high enough to confirm that the heparin–dopamine conjugate can form a chemical heparin coating on metal surfaces. Furthermore, surface heparin content on coated substrates was determined as 20 μg cm^{-2} using the TBO method.

The FTIR spectra of PTX and PTX–catechol conjugates are shown in Fig. 2a. The absorption peaks of aliphatic hydroxyl at 3250 cm^{-1}, alkane at around 2930 and 2850 cm^{-1}, carbonyl at 1725 cm^{-1}, and aromatic backbone at 1620 cm^{-1} were found in both spectra as these functional groups are present in both PTX and the PTX–catechol conjugate. However, the phenolic hydroxyl group's peak at 3320 cm^{-1} occurred in the conjugate's spectrum indicating the successful introduction of the catechol moiety. Furthermore, the peak (m/z = 1005.8) corresponding to the mono-substituted PTX–catechol conjugate was found in its mass spectroscopy spectrum in Fig. 2b, whereas the peaks of the di- and tri-substituted conjugate were not present. This implied that the as-prepared PTX–catechol conjugate was mono-substituted. Since there are three hydroxyls which can be substituted in the PTX, the as-prepared conjugate may be the

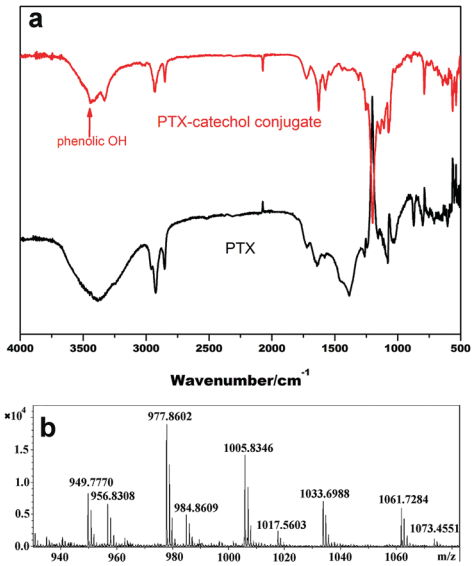


Fig. 2 The IR (a) and mass (b) characterization of the PTX–catechol conjugate.

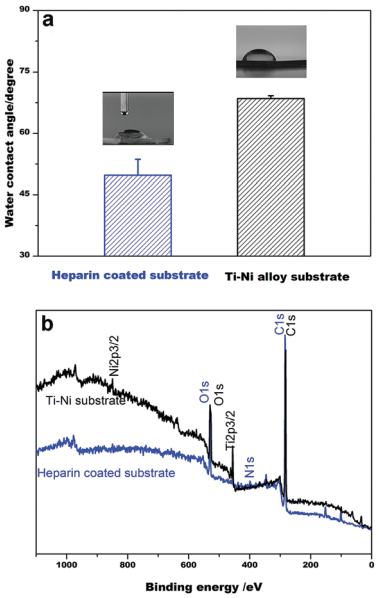


Fig. 3 The static contact angle (a) and XPS (b) characterization of the chemical heparin coating on the Ti–Ni alloy substrate.

mixture of three mono-substituted products whose polarities were too approximate to be separated by column chromatography. On the other hand, it was thought that the catechol's substituted position may not influence its self-polymerization and adhesion properties so that the mixture can be used to form chemical coating without further separation.

The preparation of the heparin and PTX chemical coating on the Ti–Ni alloy surface

The chemical coating process was conducted in aqueous solution by pH sensitive self-polymerization of the heparin–dopamine conjugate and the surface hydrophilic–hydrophobic properties would be changed by the formation of the chemical coating on the Ti–Ni alloy surface. Water contact angle measurements are a convenient way to assess the surface properties, and the results before and after heparin's coating are shown in Fig. 3a. The bare Ti–Ni alloy surface possessed a higher contact angle of about 68.5° and the water drop's shape was approximated to a circle in the image, indicating the hydrophobicity of the surface. The contact angle of the Ti–Ni alloy surface with the chemical heparin coating decreased to 50.1° and the water drop's shape was close to an oval, implying that the surface became more hydrophilic after the heparin coating. The more hydrophilic surface was attributed to the hydrophilic nature of the heparin coating, and also clearly indicated successful chemical coating by the heparin–dopamine conjugate. Furthermore, XPS was conducted to analyze the surface element composition before and after the chemical heparin coating, as shown in Fig. 3b. Before coating, the elements Ti (459 eV for Ti2p) and Ni (853 eV for Ni2p) could be clearly found in the bare alloy substrate, whereas the C1s at 281 eV and O1s at 531 eV may correspond to the carbon contaminate and oxidate layer on the surface, respectively. However, the signals of the Ti and Ni elements almost disappeared after coating, while both C1s and O1s were still present, as the organic conjugate contained a lot of carbon and oxygen atoms. Additionally, a new binding energy peak at 346 eV corresponding to N1s occurred. Since both the dopamine and heparin contained N atoms in their moieties, the occurrence of the N1s peak implied the presence of the heparin–dopamine conjugate on the alloy surface. Furthermore, the very weak Ti and Ni signals may imply that the majority of the alloy surface was covered by the heparin coating and that the thickness of the heparin coating was larger than the penetration limitation of the XPS technique (approximately 10 nm). Consequently, both contact angle and XPS results demonstrated the successful chemical coating by the self-polymerization of the heparin–dopamine conjugate.

Similarly, the chemical PTX coating could also be achieved by the self-polymerization of the PTX–catechol conjugate. However, the PTX–catechol conjugate wasn't dissolved in water so the chemical PTX coating was conducted in acetone under refluxing. The water contact angle results before and after the PTX coating are shown in Fig. 4a. It can be seen that the contact angle increased from 68.5° to 94.1° after PTX coating, indicating that the alloy surface became more hydrophobic after coating. Since PTX is a hydrophobic molecule, the increased hydrophobicity implied successful chemical PTX coating on the alloy surface. The XPS results shown in Fig. 4b further illustrate the preparation of the PTX coating on the alloy surface as the binding energy peaks of Ti2p and Ni2P can hardly be found after coating.

The *in vitro* evaluation of the heparin and PTX coatings

The *in vitro* anti-thrombosis properties of the heparin coating were firstly checked by an activated partial thromboplastin time (APTT) test. Fig. 5a shows the APTT results of the alloy and heparin coated alloy substrates. It shows that the plasma immersed by the heparin coated alloy substrate exhibited a

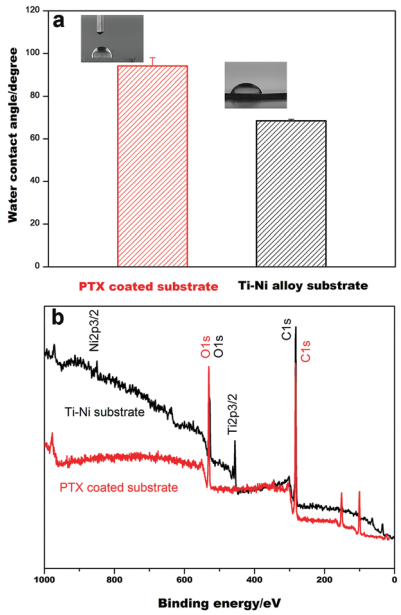


Fig. 4 The static contact angle (a) and XPS (b) characterization of the chemical PTX coating.

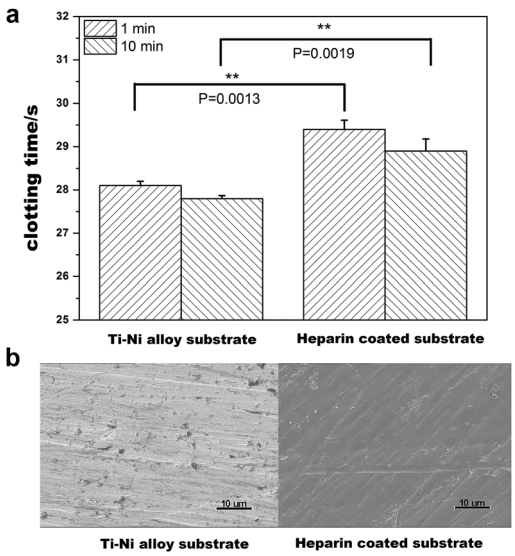


Fig. 5 The *in vitro* anti-thrombosis properties of the heparin coating on the Ti–Ni alloy substrate ((a) APTT and (b) platelet adhesion).

longer coagulation time than that of plasma immersed by the alloy substrate, whether the immersion time was 1 or 10 min. Furthermore, significant differences (P = 0.0013 for 1 min and P = 0.0019 for 10 min) are exhibited for the two samples, which shows that the as-prepared heparin coating could effectively improve the substrate's anti-thrombosis properties. Heparin is able to bind antithrombin III (AT III) to enhance its ability to inactivate the coagulation factors IIa and Xa so that the endogenous anti-thrombosis process is blocked. Thus, heparin is widely used as an anti-coagulation drug in clinics. In the heparin coating proposed herein, heparin was bound to the alloy substrate by dopamine moieties so that it may lose molecular mobility to some extent. However, the extension of the coagulation time implies that the heparin coating still possesses anti-coagulation properties.

The SEM images of the alloy substrate and the heparin coated alloy substrate after platelet adhesion are shown in Fig. 5b. It shows that rare platelets with a round shape can be found in the heparin coated alloy substrate, whereas there are many platelets deposited in the alloy substrate. This result inferred that the heparin coated alloy substrate possessed an increased anti-platelet adhesion ability compared to the bare alloy substrate. Platelet and endogenous anti-thrombosis activation both play major roles in blood coagulation. Since the above results show that heparin coating can effectively suppress these two factors, it is worth expecting that the as-prepared chemical heparin coating can play a certain role in anti-thrombosis *in vivo* after it is coated on RVCFs. Additionally, since the heparin coating was covalently bound to the alloy surface, its anti-thrombosis properties are thought to be effective long-term.

The coating's *in vitro* inhibition in ECs and SMCs is depicted in Fig. 6. The bare alloy substrate didn't show any inhibition in ECs and SMCs as its cell viability was quite close to 100%. The cell viability on the heparin coating was slightly lower than that on the bare alloy substrate, and there was a significant difference ($P < 0.0001$) for ECs and no significance ($P = 0.7976$) for SMCs. At least, it can be said that the heparin coating will not lead to intimal hyperplasia. On the other hand, the PTX coating possessed significant inhibition ($P < 0.0001$) in SMCs as its cell viability obviously decreased to 65.5%, while it also exhibited significant inhibition ($P < 0.0001$) in ECs. Although both ECs and SMCs are involved in intimal hyperplasia, their functions are different. Usually, the defective endothelium caused by RVCF implantation may give off the signals to induce the SMC phenotype to change from a contractile to a synthetic type. The synthetic SMC would proliferate excessively so as to form the intimal hyperplasia. Thus, the SMC inhibition ability of the PTX coating confirms its anti-intimal hyperplasia properties. Similar to the heparin coating, the covalently bound PTX is also thought to be effective long-term.

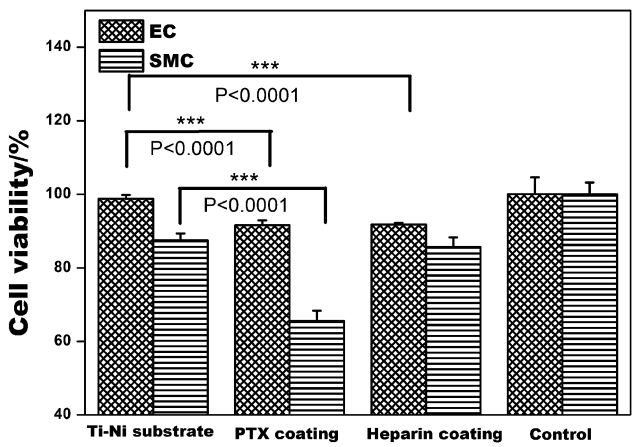


Fig. 6 The *in vitro* anti-intimal hyperplasia properties of the PTX and heparin coatings on the Ti–Ni alloy substrate.

However, although the individual heparin or PTX coatings showed promising *in vitro* anti-thrombosis or anti-intimal hyperplasia properties when investigated separately in this paper, use in clinical practice requires the coating of heparin and PTX together on RVCFs in order to endow anti-thrombosis and anti-intimal hyperplasia properties simultaneously. Thus, it is desirable to prepare a functional coating containing both heparin and PTX for RVCFs. Because there is no common solvent for the two as-prepared conjugates, this target wasn't visualized in this paper. However, further work will be done in our lab in the near future in order to coat PTX and heparin together onto the surface of RVCFs. We will try to prepare the hydrophilic PTX conjugate by firstly covalently grafting catechol to β-cyclodextrin (β-CD) and then including PTX by catechol substituted β-CD.

Conclusions

Two catechol-containing conjugates, heparin–dopamine and PTX–catechol, were successfully synthesized. The as-prepared conjugates can be covalently coated on to Ti–Ni alloy substrates *via* self-polymerization of their catechol moieties, separately. The heparin coating exhibited anti-thrombosis properties *in vitro*, whereas the PTX coating illustrated anti-intimal hyperplasia properties. Consequently, this study showed promising potential for the development of clinical anti-thrombosis and anti-intimal hyperplasia coatings for RVCFs by virtue of a chemical coating strategy relying on the self-polymerization of catechol moieties.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (81570431) and the National Undergraduate Training Program for Innovation and Entrepreneurship in the Beijing Institute of Technology (2017-B68).

References

1 F. Zhang, O. Luo and H. Zhang, *Chin. J. Pract. Surg.*, 2010, **30**(12), 1023.
2 G. Meyer, E. Vicaut and T. Danays, *et al.*, *N. Engl. J. Med.*, 2014, **370**, 1402.
3 C. Becattini, G. Agnelli, F. Germini and M. C. Vedovati, *European Respiratory Society*, 2014, **43**, 1678.
4 P. Mismetti, S. Laporte and O. Pellerin, *et al.*, *J. Am. Med. Assoc.*, 2015, **313**(16), 1627.
5 S. Sarosiek, M. Crowther and M. J. Sloan, *JAMA Intern. Med.*, 2013, **173**(7), 513.
6 A. Murie, D. Jiménez and D. Aujesky, *et al.*, *J. Am. Coll. Cardiol.*, 2014, **63**(16), 1675.
7 P. D. Stein and F. Matta, *Am. J. Med.*, 2014, **127**(3), 222.
8 J. M. Andreoli, R. J. Lewandowski, R. L. Vogelzang and R. K. Ryu, *J. Vasc. Interv. Radiol.*, 2014, **25**(8), 1181.
9 T. G. Kim, H. Lee, Y. Jang and G. T. Park, *Biomacromolecules*, 2009, **10**, 1532.
10 G. G. Stefanini and G. R. Holmes, *N. Engl. J. Med.*, 2013, **368**, 254.
11 Z. Yang, S. Zhong and Y. Yang, *et al.*, *J. Mater. Chem. B*, 2014, **2**, 6767.
12 T. Hu, J. Yang and K. Cui, *et al.*, *ACS Appl. Mater. Interfaces*, 2015, **7**(22), 11695.
13 H. Zhao, F. Zhang, F. Liang, L. Ye, H. Zhang and L. Niu, *J. Thromb. Thrombolysis*, 2016, **41**, 422.
14 Y. Cong, T. Xia and M. Zou, *et al.*, *J. Mater. Chem. B*, 2014, **2**, 3450.
15 H. J. Cho, S. K. Perikamana and J. H. Lee, *et al.*, *ACS Appl. Mater. Interfaces*, 2014, **6**(14), 11225.
16 H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science*, 2007, **318**, 426.
17 I. You, S. M. Kang, Y. Byun and H. Lee, *Bioconjugate Chem.*, 2011, **22**, 1264.
18 F. Ponzio, J. Barthès and J. Bour, *et al.*, *ACS Chem. Mater.*, 2016, **28**(13), 4697.
19 Y. Liu, K. Ai and L. Lu, *ACS Chem. Rev.*, 2014, **114**(9), 5057.
20 L. Zhang, S. Roy and Y. Chen, *et al.*, *ACS Appl. Mater. Interfaces*, 2014, **6**(21), 18644–18652.
21 Q. Liu, N. Wang, J. Caro and A. Huang, *J. Am. Chem. Soc.*, 2013, **135**(47), 17679.
22 J. Park, T. F. Brust and H. J. Lee, *et al.*, *ACS Nano*, 2014, **8**(4), 3347.
23 N. F. D. Vecchia, A. Luchini and A. Napolitano, *et al.*, *Langmuir*, 2014, **30**, 9811.
24 Y. Song, G. Ye and F. Wu, *et al.*, *Chem. Mater.*, 2016, **28**, 5013.
25 D. Chai, Z. Xie and Y. Wang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 17974.
26 Q. Gao, M. Yu and Y. J. Su, *et al.*, *Acta Biomater.*, 2017, **51**, 112.
27 Q. Gao, P. Li and H. Y. Zhao, *et al.*, *Polym. Chem.*, 2017, **8**, 6386.
28 L. Ye, X. Wu and H. Y. Duan, *et al.*, *J. Biomed. Mater. Res., Part A*, 2012, **100**, 3251.
29 L. Ye, X. Wu and Q. Mu, *et al.*, *J. Biomater. Sci., Polym. Ed.*, 2011, **22**, 389.
30 N. N. Duan, X. Geng and L. Ye, *et al.*, *Biomed. Mater.*, 2016, **11**, 035007.
31 J. Cao, X. Geng and J. Wen, *et al.*, *J. Biomed. Mater. Res., Part A*, 2017, **105**, 2806.
32 I. H. Bae, I. K. Park and D. S. Park, *et al.*, *J. Mater. Sci.: Mater. Med.*, 2012, **23**, 1259.

