Poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) nanofibers coated with micro-patterned diamond-like carbon (DLC) for the controlled drug release

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Abstract  Attaining high hemocompatibility and promoting endothelialization are two major keys to solve the endoleak problems observed in existing stent-grafts. For the satisfactory long-term use of the stent-grafts, blocking the endoleak symptom is highly essential. This paper deals with the fabrication of electrospun nanofibers made of antithrombogenic poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) containing drug that can sustain the endothelial activity of the MPC nanofibers. Moreover, the drug release was controlled by micro-patterned diamond-like carbon (DLC) coated on the MPC nanofibers. It was found that MPC nanofibers retained excellent hemocompatibility and that the micro-patterned DLC efficiently controlled the drug-release rate of MPC fibers.

Keywords  electrospinning, nanofibers, drug-release, MPC, DLC

Introduction

The endovascular aneurysm repair (EVAR) using stent-grafts has become a standard treatment for the abdominal aortic aneurysm (AAA). The research on the introduction of hemocompatibility to the surface of the stent-grafts has been actively carried out for the lifelong use of the stent-grafts [1, 2], while endothelialization of the stent-grafts, another significant durability factor for the stent-grafts as was presented in Fig. 1, has not been much explored to prevent the fatal endoleak [3].

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Fig. 1 Stent-graft covered by antithrombogenic MPC fibers

The 3D-structural approach using fine electrospun fibers made of an antithrombogenic polymer could be highly effective for the design of a stent-graft material with sufficient cell adhesion and additional endothelialization [4–6]. Our research group has been investigating the fabrication of electrospun nanofibers of biocompatible poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) [7], one of the most frequently studied phospholipid biopolymers [8, 9]. For the promotion of endothelialization, MPC nanofibers containing the drug for the endothelialization could work as a useful drug-delivery system. We studied the controlled drug-release profile of electrospun MPC nanofibers by changing their diameters to control the surface area [7], finding that the controlled drug release from nanofibers could effectively induce cell proliferation [10].

Here, we investigated the antithrombogenicity of MPC nanofibers and attempted to control the drug-release rate from the MPC nanofibers coated with biocompatible micro-patterned diamond-like carbon (DLC) [11–13].
Experimental

Material preparations

MPC was obtained from Terumo Clinical Supply Co., Ltd. An antithrombogenic, antioxidant, and anti-inflammatory curcumin was selected as an eluting drug. MPC was mixed in ethanol to make 5 wt%, 7.5 wt%, and 10 wt% MPC solutions that were electrospun under the same experimental conditions studied before [7]. MPC nanofibers with the diameter of 164 nm, 637 nm, and 1270 nm were eventually synthesized for the platelet adhesion and activation tests. A smooth MPC film was fabricated by solvent casting as positive control and polycarbonate (PC) was prepared for negative control.

For the drug-release testing, curcumin was added to 5 wt% MPC solution, and the mass ratio of curcumin to MPC was 4 wt%. The curcumin/MPC blend solution was electrospun to fabricate MPC nanofibers containing curcumin. The curcumin/MPC nanofibers shown in Fig. 2a were then covered with a mask made of a metal grid during the DLC deposition to construct micro-patterned DLC. Fig. 2b showed the metal micro-pore grid (Screen-mesh 4S: hole dimension of 35 μm, Hitachi Maxell, Ltd.) used for the mask. DLC was deposited on MPC nanofibers by radio frequency plasma enhanced chemical vapor deposition (PECVD) (custom-build, HIRANO KOH-ON Co., Ltd), where the RF power was set at 200 W with the deposition time of 45 s. Acetylene (C₂H₂) was used as process gas at 13 Pa. The surface of MPC nanofibers coated with micro-patterned DLC was observed by laser microscopy (VK-X100 Keyence Corp.).

Platelet adhesion and activation tests

Human whole blood (45 ml) was collected from a healthy volunteer and mixed with 5 ml of acid-citrate-dextrose (ACD). The blood was then centrifuged at 1500 rpm for 10 min to separate blood corpuscles, and the resulting platelet-rich plasma (PRP) was adjusted to a concentration of $3.0 \times 10^5$ cells/μl by diluting with the platelet-poor plasma. After rinsing samples with phosphate-buffered saline (PBS), five testing substrates (polycarbonate (PC), an MPC film, and MPC fiber specimens of 164 nm, 637 nm, and 1270 nm) with a surface area of 100 mm² were incubated in a 24-well plate with 1 ml of adjusted PRP for 60 min with 5% of CO₂ gas at 37°C (n = 3 substrates for each sample). The adherent platelets were then fixed with 1 ml of freshly prepared 1.0% of glutardialdehyde for 60 min at room temperature. After the fixation, the samples were washed and freeze-dried. The dried materials were coated with osmium before the investigation by scanning electron microscopy (SEM; S-3100H, HITACHI). Adhering platelets were manually counted per unit area (37500 μm²) through the photographs captured by fluorescence microscopy (50iF-

Fig. 2 Microscopic images of: a MPC fibers by SEM, b the micro-pore grid by optical microscopy, and c the micro-patterned DLC on MPC fibers by laser microscopy.

RFL-1, Nikon).

In vitro drug-release tests from MPC nanofibers with micro-patterned DLC

Three MPC nanofiber specimens (MPC fibers, MPC fibers with micro-patterned DLC, and MPC fibers with full DLC) with the diameter of ~160 nm were soaked in 2 ml of
PBS medium (pH 7.4) at 37°C. The medium was changed every 24 h to analyze the extract from the specimens. The concentration of the extracted curcumin in the medium was measured by ultraviolet-visible spectroscopy (UV-Vis: U-2810, Hitachi, Ltd). The amount of the eluted curcumin was converted to the weight and plotted as a cumulative microgram of eluted drug against time.

Results and Discussion

Platelet adhesion and activation

Fig. 3a shows the adherent platelets counts on the five different samples. The results clearly demonstrated that the numbers of platelets per unit area for the MPC film and fibers were significantly smaller than the number of the PC substrate (P < 0.05). It was found that the number of observed platelets increased, as the diameter increased regarding the nanofiber specimens. Our previous study revealed that the MPC fibers of large diameter had higher Ra (the arithmetic average of the roughness profile) than those of small diameter [7], indicating a close relationship between the surface roughness and the antithrombogenicity of MPC fibers.

The activation levels of the attached platelet could be classified as follows [14]: (I) an early stage of adhesion of an original spherical platelet, (II) the development of pseudopodia from the attached platelet, and (III) the fully-spread platelet. The morphology of the attached platelets on PC and MPC fibers after 60 min of incubation is shown in Fig. 3b. The PC substrate exhibited a dense platelet layer with predominantly spread platelets of level (II) or (III). On the surface of the MPC fibers, in contrast, a fewer platelets were attached to the surface at the lower activation level (I). These results led to the conclusion that the antithrombogenicity of MPC was maintained even after the morphology change to nanofibers. It was also found that an enhanced antithrombogenicity was observed by the MPC fibers with smaller diameter, i.e. at lower Ra, the arithmetic average of the roughness profile.

Surface morphology of drug eluting MPC nanofibers with micro-patterned DLC

The surface of the MPC nanofibers with micro-patterned DLC deposited through a metal grid with the pore-size of microns was observed. From Fig. 2c, the pore size of the deposited micro-patterned DLC was ~35 μm square, which...
was almost the same as that of the micro-pores of the metal grid. The ratio of the DLC-deposited area to the whole surface area of the substrates was approximately 47% determined by the laser microscopy image analysis.

**Release profile of curcumin from MPC nanofibers with micro-patterned DLC**

The rheology of the 5 wt% MPC-polymer solutions in ethanol with or without curcumin (4 wt%) was studied by measuring the viscosity. It was found that the viscosity was 10.7 mPa·s for the solution with curcumin and 9.0 mPa·s for the solution without curcumin. The viscosity presented marked difference between the two samples with or without curcumin, however it was found that the morphological studies of the MPC fibers fabricated by the solutions were not much influenced by the difference in the viscosity.

The cumulative release of curcumin from MPC nanofibers to the medium as a function of time is plotted in Fig. 4. Squares represent the drug-release from the MPC fibers without coating, while triangles show the results of MPC fibers with micro-patterned DLC, and circles present the results of MPC fibers with full DLC. It was found that the released amount of curcumin was successfully controlled by changing the DLC-coated surface area.

The relationship between the cumulative released drug amount and the ratio of the covered surface area with DLC to the whole surface area (R) can be estimated using the following equation:

\[ \frac{m_{\text{MPC}}(t) - m_{\text{pDLC}}(t)}{m_{\text{MPC}}(t) - m_{\text{DLC}}(t)} \times 100 \geq R \]  

Where \( m \) is the cumulative released drug amount from each sample (MPC fibers: \( m_{\text{MPC}}(t) \); MPC fibers with micro-patterned DLC: \( m_{\text{pDLC}}(t) \); and MPC fibers with full DLC: \( m_{\text{DLC}}(t) \)). For instance, \( R \) was approximately 50% on the day 10, which matched well with the ratio of the DLC-coated surface area calculated by laser microscopy mentioned above. It was therefore found that the DLC-coated surface area determined the drug release amount of the specimens.

**Conclusions**

The antithrombogenicity of MPC nanofibers and the drug-release profile of MPC nanofibers with micro-patterned DLC were discussed. It was found that the number of platelets on the MPC specimens was suppressed and the antithrombogenicity of MPC nanofibers efficiently inhibited the platelets activation. The drug-release rate from the DLC-coated MPC specimens was well controlled by varying the deposition area of the micro-patterned DLC. These results may be highly applicable to a new stent-graft with both antithrombogenicity and well-controlled drug release that would prevent endoleak.

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**References**


10. Sahoo S, Ang LT, Goh JC-H, Toh S-L. Growth factor delivery...


