Fluorinated diamond-like carbon as a potential coating for re-endothelialization of intravascular stent platform

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Statement of Purpose: Stenting is currently the most frequently performed percutaneous coronary intervention for the treatment of coronary artery disease. However, stent thrombosis remains a severe complication after stent implantation owing to its high morbidity and mortality. Since the introduction of drug-eluting stents (DES), most interventional centers have noted stent thrombosis up to 3 years after implantation, which is the complication rarely seen in bare-metal stents. The endothelium is a single layer of endothelial cells lining the vascular wall and plays an integral part in maintaining vascular homeostasis. DES impairs endothelial function of the coronary artery, which potentially increases the risk of late in-stent thrombosis. To solve this problem, surface coating by thin films promoting endothelialization and having non-thrombogenicity is considered to be an effective technique. Diamond-like carbon (DLC) has been actively studied, and a number of promising results have indicated its good biocompatibility, hemocompatibility, and non-thrombogenicity. Surface fluorination of materials has generally been found to create surfaces with improved blood compatibility, hydrophobicity, and chemical stability. It has been reported that incorporation of fluorine into DLC film greatly reduces film hardness but largely preserves other DLC properties. In this study, we fabricated fluorinated DLC (F-DLC) by combining the advantages of fluorination and DLC characteristics and evaluated both the non-thrombogenicity of F-DLC and the stability of an endothelial cell layer on F-DLC. The goal of the study is to attain superior non-thrombogenicity by promoting surface endothelialization.

Methods: DLC and F-DLC films were prepared on silicon substrates using the radio frequency (RF) plasma enhanced chemical vapor deposition (CVD) method. RF (13.56 MHz) power and total pressure were fixed at 200 W and 13.3 Pa, respectively. DLC film samples were deposited from acetylene (C_2H_2) F-DLC film samples from a mixture of C_2H_2 and hexafluoroethane (C_2F_6). We prepared polycarbonate (PC) and gelatin as controls for non-thrombogenicity test and endothelialization test, respectively. For non-thrombogenicity test, each sample was incubated with platelet-rich plasma ($3.0 \times 10^5/\mu L$) isolated from human whole blood in 24-well plates at 37ºC for 30 mins in an atmosphere containing 5% CO_2 gas. After incubation, each sample was examined using optical microscope and scanning electron microscope (SEM). For endothelialization test, human umbilical vein endothelial cells (HUVECs) (1×10^4 cells/mL) were seeded on the samples and incubated in 24-well plates at 37ºC for 24 hrs in an atmosphere containing 5% CO_2 gas. After incubation, each sample was examined and the amount of the cells on each sample was calculated using SEM and adsorption spectrometer.

Results: Figure 1 shows the representative optical microscopic images of adherent platelets on each surface. As described below, the platelet adhesion on F-DLC was significantly inhibited compared with that on the other samples. Figure 2 shows the amounts of adhered HUVECs on each sample. This result suggested that larger amount of HUVECs adhered on gelatin (as a positive control) compared with on the other samples; however, HUVECS grew well on both DLC and F-DLC. These results demonstrate that F-DLC has great potential for both non-thrombogenicity and endothelialization.

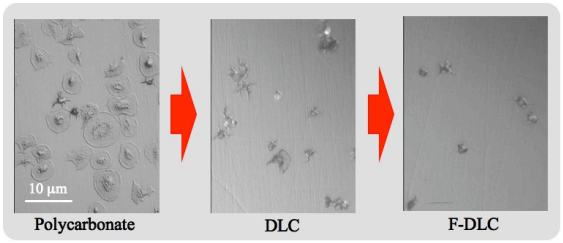

Figure 1. Adherent platelets on each sample

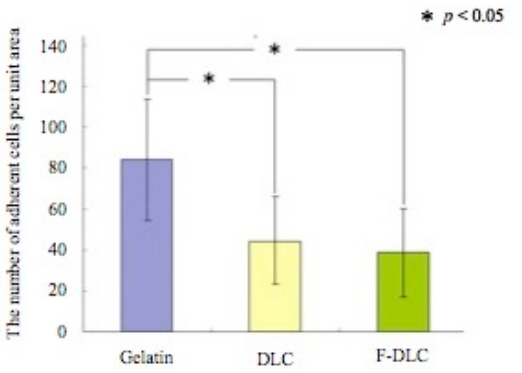

Figure 2. The number of adherent HUVECs on each sample

Conclusions: F-DLC coating can promote endothelialization as well as enhance non-thrombogenic properties. F-DLC thus appears to be a promising material for intravascular stent platform. Further *in vivo* investigations are needed to confirm the prevention of delayed stent endothelialization.

References:

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