Albumin and Fibrinogen Adsorption, Platelet Adhesion and Activation on ePTFE and electrospun PTFE

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Statement of Purpose: Arterial bypass surgery is performed in more than 1.4 million people in the U.S. every year. Autologous grafts are used in two-third of these cases [1]. However, the use of autologous grafts is hindered by several limitations including donor site morbidity, unavailability of suitable vessels, and limited long-term success [2]. Hence, the use of synthetic materials has been explored for vascular grafts. Expanded PTFE (ePTFE) has performed well as a material for making large diameter (>6 mm) vascular grafts. However, early graft occlusion is frequently observed in small diameter (<6 mm) grafts mainly due to thrombosis [2]. Hence, there is a need to use an anti-thrombogenic material for vascular grafts. Electrospun materials have shown great potential for applications in vascular grafts. In this study, we have investigated and compared the adsorption of blood plasma proteins (albumin and fibrinogen), and the adhesion and activation of platelets on ePTFE and electrospun PTFE.

Methods: The ePTFE and electrospun PTFE films (1 cm × 1 cm) (Zeus, USA) were chemically cleaned by immersing them in ethanol for 2 min followed by 24 h drying in air. Fluorescent dye tagged albumin (Alb) and fibrinogen (Fb) were used to make protein solutions at 200 µg/mL in 0.9% NaCl. A mixture of these two protein solutions at equal volume was prepared to obtain a dual protein solution. A 100 µL of single or dual protein solution was then deposited on ePTFE and electrospun PTFE films. After incubating the samples at 37 °C for 15 min, the amount of protein adsorbed on the materials was determined using fluorescence detection microplate reader. Also, the proteins adsorbed on the sample surfaces were imaged using a fluorescence microscopy. For platelet interaction study, the blood collected from healthy donors was centrifuged at 180 g for 20 min to obtain a PRP. A 100 µL of PRP was deposited on the samples and incubated at 37 °C for 1 h. Chemically cleaned glass cover slips were used as a control in this study. The adhesion of platelets was determined using LDH assay. The activation of platelets was quantified by measuring P-selectin expression using flow cytometer. Also, SEM was used to image the adhesion and activation of platelets on the samples. A one-way ANOVA was performed to determine the statistical significance at $p < 0.05$.

Results: The amount of proteins adsorbed on ePTFE and electrospun PTFE films from a single or dual protein solution did not show any significant differences (Fig 1A,1B,2A). Also, the fluorescence microscopy images of protein adsorption were in agreement with the quantitative results (Fig 1C,2B). A significantly lesser number of platelets were adhered on electrospun PTFE than that of ePTFE (Fig 3A). Also, the activation of platelets was significantly lesser on electrospun PTFE when compared to that of glass or ePTFE (Fig 3B). SEM images were used to determine the degree of activation of platelets on glass, ePTFE, and electrospun PTFE based on their morphology. Most of the platelets adhered on glass and ePTFE were in early or intermediate pseudopodial morphology (activated) with very few cells maintaining round/discoid morphology (not activated). [3]. However, most of the platelets adhered on electrospun PTFE were not activated as evident from their round morphology.

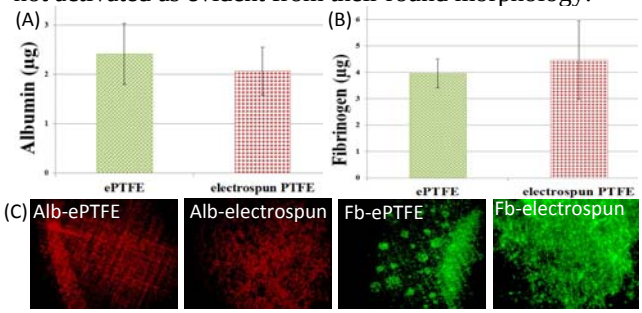

Fig 1: Amount (A and B) of Alb and Fb adsorbed; and fluorescent images (C) of Alb/Fb adsorbed on ePTFE and electrospun PTFE from single protein (Alb/Fb) solution.

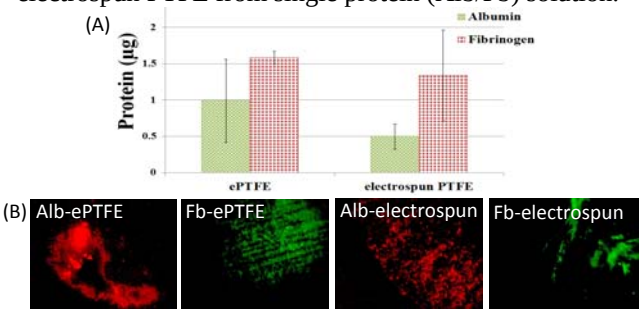

Fig 2: Amount of Alb and Fb adsorbed (A); and fluorescent images (B) of Alb/Fb adsorbed on ePTFE and electrospun PTFE from dual protein (Alb+Fb) solution.

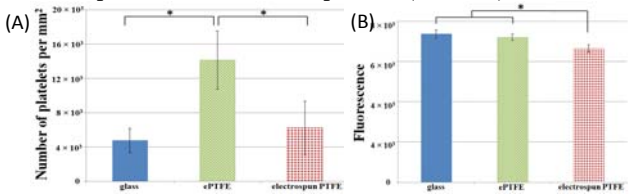

Fig 3: Platelet adhesion (A) and Platelet activation (B) on control glass, ePTFE and electrospun PTFE.

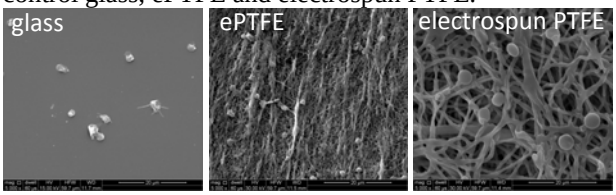

Fig 4: SEM images of platelet adhered on control glass, ePTFE, and electrospun PTFE

Conclusions: Electrospun PTFE shows better blood compatibility than ePTFE. Hence, electrospun PTFE is a promising material for making vascular grafts.

References:

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