생리식염수의 재료표면에의 분출에 의한 이중튜브의 응혈 방지

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PREVENTION OF MURAL THROMBUS IN POROUS INNER TUBE OF DOUBLE-LAYERED TUBE BY SALINE PERFUSION

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ABSTRACT

An *in vitro* experiment under laminar non-pulsatile blood flow and an acute canine *ex vivo* femoral A-V series shunt experiment were undertaken to investigate the effectiveness of saline perfusion through pores of porous tubes to prevent formation of mural thrombus. PS/SBR porous tubes were used for the *in vitro* experiment. Commercially obtained ePTFE porous tubes were etched by sodium naphthalenide, and the etched tubes were used for the *ex vivo* experiment. According to the results of the *in vitro* experiment, mural thrombus on the surface of the porous tube could be prevented by the saline perfusion. Adhered blood cells decreased semi-logarithmically with increased perfusion rate (up to 0.022 ml/min-cm^2) of isotonic saline solution. According to results of the *ex vivo* experiment, mural thrombus decreased with increased perfusion rate (upto 0.060 ml/min-cm^2).

INTRODUCTION

The prevention of thrombus formation is important in clinical situations in which blood is in contact with artificial materials because the thrombosis causes the occlusion of blood lines and implants and reduces the efficiency of membranes by decreasing its effective surface area and by altering the permeability of the membranes. The thrombosis also causes loss of blood cells and embolization from the dislodgement of a thrombus (1, 2). In extracorporeal blood circulation systems, anticoagulation is currently used to prevent the formation of clots and is achieved through the use of heparin. In general, heparin is well tolerated and devoid of serious consequences. However, it allows platelet adhesion to foreign surfaces and cause hemorrhagic complications such as subdural hematoma, retroperitoneal hematoma, hemopericardium, gastrointestinal bleeding, hemorrhage into joints, ocular and retinal bleeding, menorrhagia, and bleeding at surgical sites (3). Therefore, excessive bleeding due to heparin is a major clinical problem after perfusion for open heart surgery and perfusion for long-term extracorporeal pulmonary support (4, 5). During hemodialysis, heparin takes place in hemorrhagic complications for uremic patients who are traumatically injured and who are postoperative (6). These difficulties give rise to an interest in developing new methods of anticoagulation and developing more blood-compatible materials. Several anticoagulation methods have been developed: regional heparinization, low-dose heparinization, prostacyclin and citrate anticoagulation, saline-flushing method (2, 6, 7, 8, 9), etc. In addition, many studies for developing blood-compatible materials such as biolized surfaces, anionic surfaces, and immobilization of heparin or urokinase have been carried out (10, 11, 12, 13). The present study is to investigate the possibility to apply the saline perfusion method (14, 15, 16, 17) for anticoagulation of extracorporeal blood circulation systems without using anticoagulants.

Materials and Methods

Materials

Polystyrene/poly(styrene-co-butadiene) (PS/SBR) porous tubes made by incorporating 55 v/o powdered sugar were used for an *in vitro* blood clotting experiment. The tubes were made by a dipping method discribed in the previous paper (17). Expanded polytetrafluoroethylene tubes (ePTFE tubes, Impra, Inc.) whose inner diameter and wall thickness are 3.0 and 0.43 mm, respectively were used for *ex vivo* blood clotting experiment. Since the ePTFE tube does not permeate water under a pressure of 300 cmH_2O, which was the maximum pressure that can be applied by our experimental setup, it was chemically etched to increase permeability with Chemgrip® treating agent (Norton, Inc.), which is a PTFE etching solution made of an active form of sodium and naphthalene. The tubes used in the *in vivo* experiment were etched by the Chemgrip® treating agent diluted by a volume ratio of 1:1 with 10 w/o naphthalene

tetrahydrofuran solution to reduce the strength of the treating agent. For making control tubes, the N,N-dimethylacetamide solution of 10 w/o Pellethane® (Dow Chemicals, 2103-70A), which is a poly(etherurethane) was coated at the outer surface of the etched ePTFE tubes to prevent blood leakage. Tubes were stored in 0.9% saline solution.

***In Vitro* Experiment**

Blood circulation for blood clotting was performed under non-pulsatile flow using a flow control funnel. PS/SBR porous tubes were used for experimental tubes. Sodium citrate in citrated blood was neutralized by calcium chloride solution. Calcium chloride solution (0.5 M) was perfused in circulating blood with the rate of 2 ml/min. Sodium citrate solution (0.5 M) was perfused in the blood passed through the experimental tube (perfusion rate: 1 ml/min). The amount of blood was 200 ml, blood flow rate was 100 ml/min, and circulation time was 15 minutes.

***Ex Vivo* Experment**

An adult, conditioned male mongrel dog weighing 22.5 kg was selected for *ex vivo* experiment. Its hematocrit was 41±2%. The animal was anesthetized with intravenous Nembutal® sodium (25 mg/kg initial dose, Abbott Laboratories) and respirated. The femoral artery and vein in one leg was exposed and ligated. The artery and vein were then cannulated with feeding tube and exit sections connected to the series shunt. The shunt sections were initially filled with a degassed divalent cation-free Tyrodes solution to prevent blood-air contact. The shunt sections were composed of control tubes and perfusing tubes with different perfusing rates. Original ePTFE tube and etched ePTFE tubes were used as experimental tubes. The experiment was performed for 20 minutes without heparin injection. The blood flow rate was measured with square-wave electromagnetic flow meter (Carolina Medical Electronics, Inc.). After experiment, the experimental tubes were washed gently by injecting 50 ml of 0.9% saline solution for 3 times using a syringe.

Measurement of Blood Cell Adhesion

After blood circulation, isotonic saline solution was circulated for 2 minutes to remove non-adhered blood cells in experimental tubes. Experimental tubes were disassembled from the circulation setup and then washed in isotonic saline solution gently for 1 minute. The tubes were put in 0.9 % saline solution containing 2.5 w/o glutaraldehyde for 4 hours to fix adhered blood cells and then washed in double-distilled water thoroughly. Test samples were collected from the center of the tubes. These test samples were freeze-dried for 2 days. SEM micrographs of different regions, which were magnified to 1500 times were taken after test samples were coated by thin palladium-gold film by using a plasma sputter. Blood cells in each micrograph were counted, then the average count of blood cells in a micrograph whose actual area was 4449.8 mm^2 was calculated and converted to that in 1 mm^2 area.

RESULTS AND DISCUSSION

***In Vitro* Experiment**

Sodium citrate in citrated blood was neutralized with calcium chloride during citrated blood circulation to determine the effect of the saline perfusion on blood clotting. The hematocrit of citrated blood was 32±2%. The relative viscosity and density of citrated blood were 3.013 and 1.037 g/ml, respectively, corresponding to the Reynolds number of blood flow of 122 and wall shear rate of 79 sec^{-1}. The perfusion rates of saline solution were 0.0163 and 0.0221 ml/min-cm^2, respectively, and 55% sugar tubes were used.

The numbers of adhered blood cells are plotted against perfusion rate in Figure 1. The relationship between the number of adhered blood cells and perfusion rate is semi-logarithmic (r = 0.991). Mural thrombus and platelet aggregation and adhesion was decreased by the saline perfusion. Coagulation cascade may be prevented since blood cannot directly contact the tube surface presumably due to (1) the force of saline solution and (2) thin layer of saline solution on the tube surface. As a result of this mechanism, mural thrombus may be prevented.

***Ex Vivo* Animal Experiment**

An acute canine *ex vivo* femoral A-V series shunt experiment was performed to determine the effect of the saline perfusion in a canine. The initial mean blood flow rate was about 350-400 ml/min, the relative viscosity and density of blood were 3.911 and 1.056 g/ml, thus corresponding to Reynolds number of 668-764 and wall shear rate of 2201-2515 sec^{-1}.

SEM micrographs of the center regions of the control and perfused tubes are shown in Figure 2. An original ePTFE tube was used as a reference (control). There was large platelet adhesion and aggregation on the surface of control ePTFE tube, as shown in Figure 2(a). However, adhered RBCs were almost never found. The surface of non-perfused etched ePTFE tube (control) is shown in Figure 2(b). There was highly developed mural thrombus present. There were many fibrin fibers, and blood cells were entrapped by fibrin meshes. Therefore, etched ePTFE tube was more thrombotic than original ePTFE tube presumably due to increase of hydrophilicity and change of chemical structure. The surface of the tube with $F_{\bar{p}}$ = 0.021±0.002 ml/min-cm^2 is shown in Figure 2(c). Highly developed fibrin meshes were observed and some RBCs were entrapped in fibrin meshes. However, it seems to be

lesser developed mural thrombus than that on the control surface. This mural thrombus is similar to white thrombus (18), which is mainly an interwoven mesh of white blood cells in fibrin and is generated under high shear conditions. The surface of the tube with F_p = 0.035±0.003 ml/min-cm^2 is shown in Figure 2(d). There were fibrin fibers, some RBCs, and many platelets present. Platelet aggregation was also seen. However, the amount of fibrin fibers was relatively less, and thus mural thrombus was less developed, compared to the tube with F_p = 0.0209 ml/min-cm^2. The surface of the tube with F_p = 0.0596±0.0021 ml/min-cm^2 is shown in Figure 2(e). There were no fibrin fibers but platelets and RBCs were present. Many RBCs were entrapped in large pores, which were generated by the fracture of fibrils due to surface etching. In this case, no mural thrombus was found. Consequently, mural thrombus was decreased with increased perfusion rate of saline solution. Adhered blood cells were counted and are plotted in Figure 3. The number of adhered platelets in the ePTFE tube was 42998±3744 cells/mm^2. However, adhered blood cells in the control tube and the tubes with F_p = 0.020 and 0.035 ml/min-cm^2 cannot be counted. The number of total blood cells in the tube with F_p = 0.060 ml/min-cm^2 was 5004±1031 cells/mm^2. The blood-cell amount except blood cells entrapped in the pores was 794±563 cells/mm^2 in which platelets were 449±398 cells/mm^2. Because blood cells cannot be entrapped if there are no large pores, the number of actual adhered blood cells will be near 449 cells/mm^2. Thus, the tube with F_p = 0.060 ml/min-cm^2 is more blood-compatible than original ePTFE tube.

CONCLUSIONS

Mural thrombus at the PS/SBR porous tubes decreased with increased the saline perfusion rate. According to the results of the *ex vivo* experiment, the expanded PTFE tube became a more thrombotic material due to the surface etching with sodium naphthalenide. The saline perfusion with enough perfusion rate made the etched ePTFE tube more blood-compatible than the original ePTFE tube. Therefore, the saline perfusion is effective in the actual body to prevent blood clotting and blood cell adhesion.

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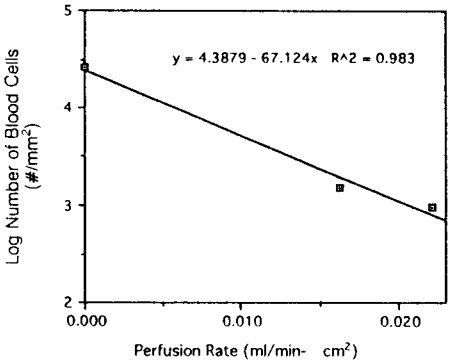


Figure 1. Plot of log number of blood cells adhered at tube wall versus perfusion rate of *in vitro* blood clotting experiment.

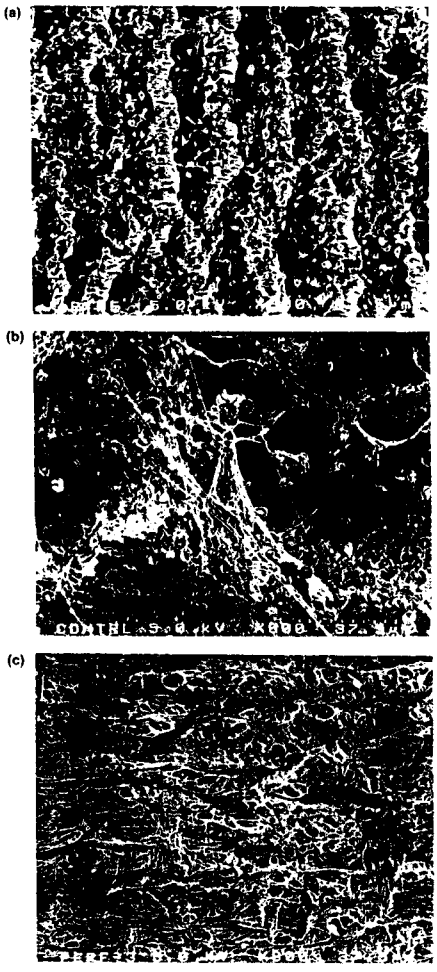
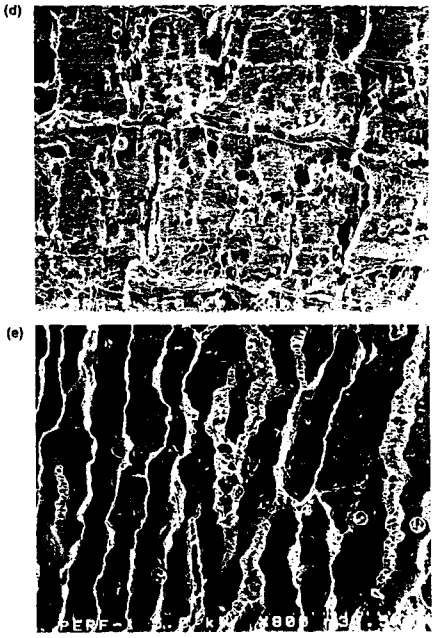


Figure 2. SEM micrographs of ePTFE tubes of *ex vivo* blood clotting experiment. (a) ePTFE tube (control); (b) Etched ePTFE tube (control); (c) F_p = 0.021 ml/min-cm^2; (d) F_p = 0.035 ml/min-cm^2; (e) F_p = 0.060 ml/min-cm^2.



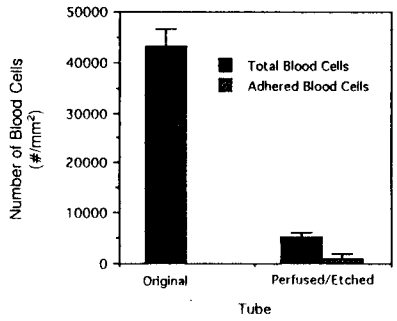


Figure 3. Numbers of adhered blood cells in original ePTFE and saline-perfused (F_p = 0.060 ml/min-cm^2) etched ePTFE tubes.