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Analysis of the Adsorbed Plasma Proteins in the Moving Actuator type Total Artificial Heart

=Abstract=

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Plasma protein adsorption is the first event in the blood-material interaction and influences subsequent platelet adhesion towards thrombus formation. Thromboembolic events are strongly influenced by surface characteristics of materials and fluid dynamics inside the blood pump. *In vitro* flow visualizaion and an amimal experiment with the moving actuator type TAH were performed in order to investigate fluid dynamic effects on the protein adsorption. The different levels of shear rate inside the ventricle were determined by considering the direction of the major opening of four heart valves in the implanted TAH and the visualized flow patterns as well. Each ventricle of the explanted TAH was sectionalized into 12 segments according to the shear rate level. The adsorbed protein on each segment was quantified using the ELISA method after soaking in 2%(w/v)SDS/PBS for two days. Adsorbed protein layer thicknesses were measured by the immunogold method under TEM. The SEM observation show that right ventricle (RV), immobilized with albumin, displayed different degrees of platelet adhesion on each segment, whereas the left ventricle (LV), grafted by PEO-sulfonate, indicated nearly same platelet adhesion behavior, regardless of shear rates. The surface concentrations of adsorbed proteins in the low shear rate region are higher than those in the high region, which was confirmed statistically. A modified adsorption model of plasma protein onto polyurethane surface was suggested by considering the effect of the fluid dynamic characteristics.

INTRODUCTION

Thromboembolic complication in artificial hearts is one of the major factors which disturb their long term implantation. These thromboembolic events are strongly influenced by surface characteristics of materials and fluid dynamics inside the blood pump. It has been generally accepted that plasma protein adsorption would influence subsequent platelet adhesion and activation, and also plays a major role *in vivo* towards initiation of thrombus formation at the blood-material interface. Boretos et al. [1] and Matsuda et al. [2] worked on protein adsorption on the artificial ventricular surfaces of the

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implanted devices. Nojiri et al. [3] also observed the thickness and distribution of adsorbed protein on the artificial ventricular surface by scanning and transmission electron microscope (SEM and TEM). Recently, Mabuchi et al. [4] also evaluated the adsorbed plasma proteins on the surfaces of implanted artificial hearts quantitatively. However, fluid dynamic effects on these phenomena have not been well-defined yet. From this point of view, we studied the effect of shear rates on the blood-material interactions of protein adsorption and their relationship with platelet adhesion on the blood-contacted surface of the implanted TAH.

MATERIALS AND METHODS

The moving actuator type TAH has two (right and left) solution-cast double sacs, four quick connectors, four Bjrok-Shiley heart valve prosthesis (Shiley, Inc., Irvine, CA), two woven Dacron outflow grafts, and two inflow cuffs [5, 6]. Two surface modification techniques were applied to the blood-contacting surfaces of the implanted TAH: albumin is immobilized on the right ventricular surface and PEO-sulfonated on the left one[7, 8]. The moving actuator type TAH was implanted into a female calf of 95Kg weight for 7 hours. The shear rate distribution inside of ventricle was determined by considering the directions of the major opening of the four heart valves in the implanted TAH and the flow pattern was visualized as well. We sectionalized both of the blood-contacted ventricles to 12 Parts according to the level of the shear rate after the death of the animal. Because the analysis of adsorbed protein might be influenced by the size of the ventricle segment, the number of segments was limited to 12 for each ventricle. Platelet adhesion and its morphology were observed by SEM. Adsorbed protein layer thickness and distribution were measured using the immunogold method under TEM [9, 10]. The adsorbed plasma protein of each segment was quantified by the ELISA method[11].

FLOW VISUALIZATION OF TAH

In order to obtain flow patterns in the artific-, ial ventricle, we developed a transparent pump system(TPS) with one ventricle and a simple circulatory system. Polystyrene particles (IRA 904, Amberite ion exchange resin, Rohm & Hass Co., Philadelphis, PA), less Than 100um in diameter, were suspended in the testing fluid as scatterers and planar He-Ne laser light source illuminated the artificial ventricle located in the center of TPS through a cylindrical lens. Pictures of flow patterns during various parts of cardiac cycles were captured using a still photo camera (Sam Sung Minolta, alpha 9000) at shutter speed of 1/30, 1/60, and 1/125 seconds and Kodak 35mm ASA 400 Tri-X films. The testing fluid was a blood analogue fluid consisting of 36.7 vol. % glycerine and distilled water. In ord-er to select photographing time, a digital time displayer which indicates systolic and diastolic phase at the interval of 1/30 and 1 /60 sec was made. The displayed time was photographed together with the flow pattern.

SEM OBSERVATION

Samples 1mm×1mm were cut from sectionized segments and soaked in the Karnovsky's fixative for 24 hours at 40℃. The morphology of adhered platelet was examined with a Hitachi S-510 SEM at 15kV. The degree of platelet adhesion was evaluated by counting the numbers of adhered platelets of the overall surface.

TEM OBSERVATION

Samples from each specimen were sliced into 0.5mm-thick strips under a stereo microscope. The strips were stained with 1% (w/v) O_5O_4 so-

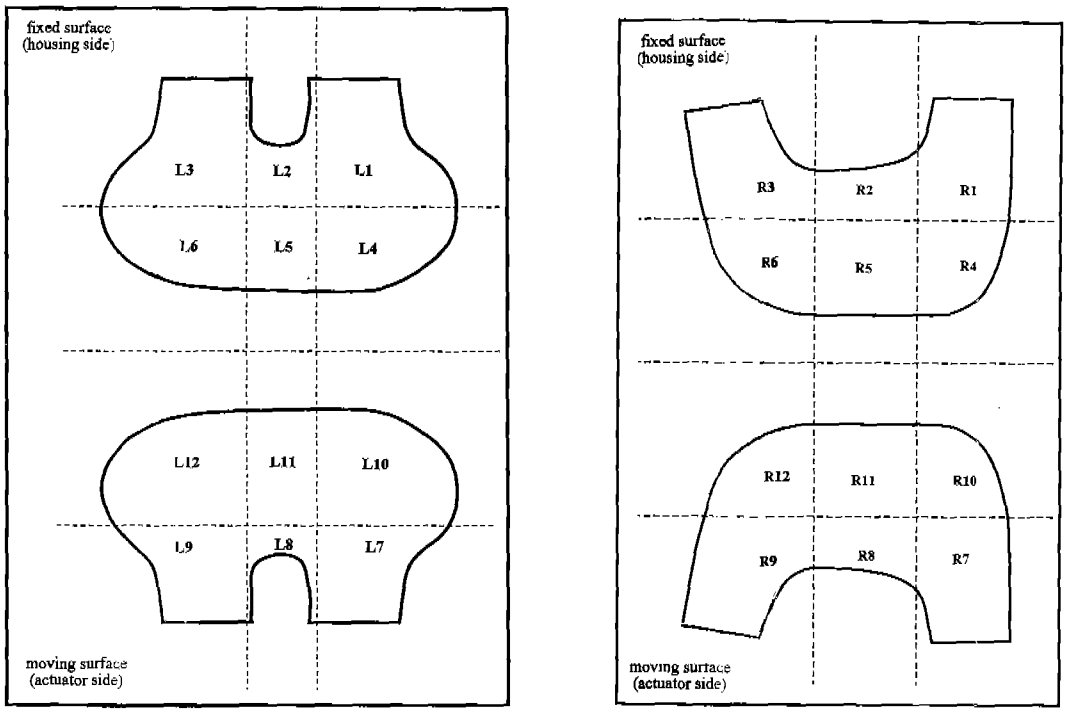

Fig. 1 Schematic diagram of the sectionized ventricle of the moving actuator type TAH; (a)left ventricle (b)right ventricle

lution for 30 minutes to measure the total thickness of the adsorbed protein layers. Untreated polyurethane(PU) and albumin-immobilized samples were also stained as controls.

Monospecific rabbit antisera (primary antisera, IgG fraction) against bovine albumin(BSA) and IgG were used(Sigma Chemical Co., St. Louis, MO). IgG fraction of rabbit antisera against bovine fibrinogen was obtained from immunized rabbits. The IgG fraction was isolated using protein A sepharose CL-4B(Pharmacia, Uppsala, Sweden). The samples were incubated in rabbit primary antisera against each protein for 24 hours at 40℃, and then rinsed with PBS -BSA containing 0.05% Tween-20(PBS-Tw). In the reaction of samples with primary antiserum against BSA, PBS-Gelatin was used for its specificity instead of PBS-BSA. The samples were incubated in gold conjugated second antibody(mean particle size=10nm, Sigma Chemical Co., St Louis, MO) for 1 hours at room temperature. After rinsing with PBS-Tw, the samples were immersed in a 1%(w/v) O_5O_4 solution for 10minutes followed by fixing in the Karnovsky's solution for 5 minutes, and then rinsed with PBS buffer (pH 7.4). The samples were freeze-dried overnight, and a cross-sectional view was observed with a Hitachi TEM (H-600EM, Japan).

QUANTIFICATION OF ADSORBED PROTEINS

The plasma proteins on each specimens of the TAH were eluted with 2%(w/v) SDS/PBS (pH 7.4) for 2 days and dialyzed against PBS (pH 7.4). The protein samples (0.1ml each) were added into each well of the microelisa plate (Dynathech, Chantilly, VA) and incubated overnight at 40℃. After washing with PBS-Tw, the samples were incubated in 0.1ml of rabbit primary antisera (same as in TEM observation) at an appropriate dilution at room temper-

ature. Then the samples were washed with PBS -Tw and incubated in 0.1ml of peroxidase conjugated second antibody(Sigma Chemical Co., St. Louis, MO) at a dilution of 1:1500 for 2 hours at room temperature. After rinsing with PBS-Tw, 0.2ml of ELISA substrate solution (0.1% ABTS, 0.005% H_2O_2,0.09M $N_aH_2PO_4$,0.05M citric acid, pH 4.6) was added to each well, mixed, and then the absorbance of each well was read at 405nm in the microelisa reader (Dynatech, Chantilly,VA).

THEORETICAL CONSIDERATION

Adsorption kinetics are often analyzed in the Langmuir approximation which consists of assuming the rate of adsorption is proportional to the fraction of uncovered surface as follows;

$$\frac{dB}{dt} = k\left(1 - \frac{B}{B_{max}}\right) \qquad (1)$$

where B is the surface concentration of adsorbed protein, k is the rate constant for adsorption, and B_{max} is the surface coverage limit.

In order to consider the effect of the fluid dynamic characteristics on the protein adsorption, a modified Langmuir adsorption model was proposed. In this model, a monolayer protein adsorption and no conformational change of the adsorbed protein were assumed. Under flow condition, adsorbed proteins are desorbed by fluid shear stress. The average normal stress on a protrusion by fluid shear stress is proportional to shear rate under linear approximation of the tubular flow[12]. Therefore, the protein desorption rate can be assumed to be proportional to wall shear rate. The adsorption rate might be also dependent on the shear rate, but it was assumed to be constant. A modified Langmuir approximation of protein adsorption under flow condition is proposed as follows;

$$\frac{dB}{dt} = k\left(1 - \frac{B}{B_{max}}\right) - k'(r)B \qquad (2)$$

where k′is the desorption rate of adsorbed protein which is dependent on the wall shear rate (r). The adsorbed protein concentration was calculated by solving equation(2) with different desorption rates.

RESULTS

Complex flow patterns inside the artificial ventricle can be observed and can be easily changeable by changing control parameters such as heart rate and stroke length. In diastolic phase, two large vortex are formed and several flow separation areas can be observed. In order to categorize specimens of each ventricle according to the level of the shear rate, as well as the flow patterns, the following three criteria were also considered;

-The shear rate in the direction of the major opening area of the heart valve prosthesis is higher than that in the minor one;

-The shear rate near the flow separation area is lower than that of other area;

-The shear rate on the moving surface of the ventricle is lower than that on the fixed one.

After the death of animal, the ventricles of the TAH were washed mildly with normal saline and sectionalized into 12 segments as shown in Fig. 1-(a)and (b). These samples were categorized according to level of shear rate as shown in Table 1. The amount of adsorbed proteins on each segments are also shown in Table 1. The surface concentrations of three adsorbed plasma proteins in low shear rate regions were higher than those in high shear rate region, which can be also confirmed statistically by T-test as shown in Table 2. No significant difference was obtained in thse amounts of adsorbed concentrations between the right and left ventricles. For the case of the rig-

Table 1 Amounts of adsorbed proteins on each segments and shear rate levels

Segment	Amounts of adsorbed proteins (ng/cm²)			Shear rate level
	Albumin	fibrinogen	IgG	
R1	10.32	5.02	9.27	6
R2	20.72	105.68	16.82	3
R3	27.73	78.66	15.02	1
R4	15.39	48.97	8.57	6
R5	22.88	76.42	12.42	3
R6	25.52	66.67	11.50	2
R7	20.33	45.17	8.79	1
R8	18.34	14.05	7.63	2
R9	20.04	53.26	9.84	5
R10	15.62	31.05	6.29	4
R11	16.65	37.45	7.87	4
R12	14.37	22.47	6.34	5
L1	15.81	54.80	9.90	6
L2	38.64	136.09	17.08	3
L3	21.34	64.03	13.78	1
L4	12.71	43.65	6.36	6
L5	19.78	83.19	12.47	3
L6	8.69	34.05	4.55	4
L7	27.72	44.01	9.57	1
L8	37.10	93.40	18.37	2
L9	10.67	64.03	12.34	5
L10	16.70	42.32	7.68	4
L11	15.02	53.60	9.21	2
L12	10.75	22.24	8.44	5

Table 2 T-test results :p value[1]

Compared Groups	Albumin	fibrinogen	IgG
High SR[2] Low SR	0.0001	0.004	0.008
Right V. Left V.	0.876	0.319	0.615
Hight SR Low SR in Right V.	0.004	0.049	0.028
Hight SR Low SR in Left V.	0.007	0.038	0.021

[1] If p value is less tha 0.05, null hypothesis must be rejected.
[2] high SR:shear rate leved (4~6)
Low SR: Shear rate level (1~3)

Table 3 Values of the parameters used in the modified adsorption model.

Parameters	Values
Bmax	1.0 ug/cm²
k	0.1 cm/sec
k[1]	0
	0.01
	0,001cm/sec

ht ventricular surface with albumin immobilized PU, we could see different degrees of platelet adhesion on each segment with different shear rate on SEM observation. Meanwhile, there was little difference in the case of sulfonated PU-PEO surface of the left ventricle. One of typical SEM pictures is shown in Fig. 2. From TEM observation, the average

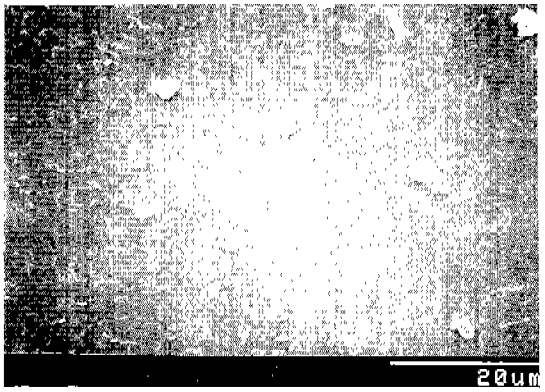

Fig. 2 SEM picture of adhered platelets on albumin immobilized surface of R3 segment

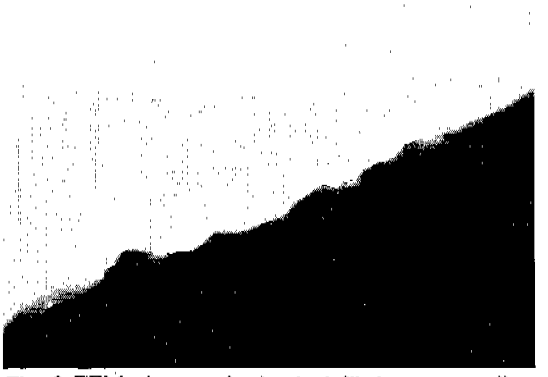

Fig. 3 TEM picture of adsorbed fibrinogen on albumin immobilized surface of R3 segment

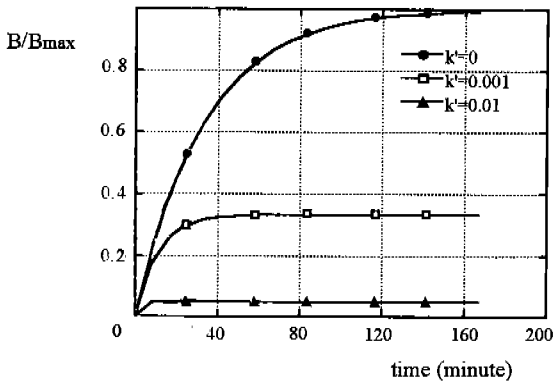

Fig. 4 Kinetics of the protein adsorption with different desorption rate constants calculated from the equation(2) and values of the parameters in Table 3

thickness of adsorbed protein layers was about 1500 Å and a typical TEM picture of adsorbed fibrinogen is shown is Fig. 3. The solution of the equation(2) was shown in the Fig. 4 using the parameters listed in Table 3. Less protein was adsorbed in the case of the higher desorption rate as shown in Fig. 4.

DISCUSSION

Three plasma proteins were adsorbed in a multilayer during seven hours, implantation, which was confirmed by TEM pictures and consideration of the protein sizes. Mabuchi et al. [4] observed that adsorbed proteins were unevenly distributed on the artificial ventricles. In our experiment, we found that amount of adsorbed proteins was correlated with levels of shear rates. Different shear rates might influence on the adsorption and/or desorption rates. Pitt and Cooper [13] also suggested that the albumin adsorption on the polymer surface could be influenced by the shear rate through *in vitro* experiments. Correlative relationship between the amount of adhered platelets and adsorbed protein could not be found in this experiment. This might be caused from short implantation time and qualitative nature of SEM observation. The present observation relating the protein adsorption with the shear rate may be significant for the future design and control of the artificial heart, because the protein adsorption pattern can lead to the specific thrombotic phenomenon in long term implantation experiments.

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