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Short term evaluation of material blood compatibility using a microchannel array

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Abstract New short-term evaluation of material blood compatibility was attempted using a microchannel array with human blood under a flow condition. The microchannel array chips were made of silicon, having 8,736 microchannels of 10 μm-wide, 30 μm-long, and 4.5 μm-deep on the average, as the models of capillary blood vessels. Titanium, chromium, albumin and collagen were coated onto the chips to examine the difference of material blood compatibility and the effect of protein adsorption on it. The time for the first 100 μl portion of whole blood to pass through the channels (blood pass-through time, BPT) was measured under a pressure difference of 20 cmH_2O. Simultaneously, the flow behavior of blood cells was observed by an optical microscope. The BPT tends to correlate well with the level of platelet adhesion. The highest BPT as well as platelet adhesion was observed on collagen, followed by titanium, chromium, silicon, and albumin. These results indicate that the BPT can detect the different levels of platelet adhesion and thrombus formation on microchannel surface and that the protein adsorption onto chip surface can influence BPT. We concluded that this method could be applied to evaluate initial blood compatibility of materials within several minutes in vitro.

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Introduction

On the development of new materials for biomedical uses, animal experiments have often been employed to evaluate the materials blood compatibility as screening tests before clinical studies. However, enormous expenses, long periods, and animal sacrifices are necessary for these experiments. Animal models are often pointed out their differences from human being, and the results of these experiments do not always correspond with those of clinical studies. To overcome this difference in biological species, some trials to establish a new in vitro method for blood compatibility evaluation were done using human blood such as platelet adhesion and complement protein adsorption test in static [1–6] or flow conditions [6–10]. Platelet adhesion test is normally performed in the absence of flow, but this is not appropriate because platelet adhesion physiologically occurs under flow conditions. No standard system of a flow chamber has been established for the platelet adhesion test, so these studies must be performed using hand-made systems, which generally require a large volume of blood and make it difficult to perform with human blood. Consequently, there is no appropriate method to evaluate materials blood compatibility in vitro, and a new in vitro method is anticipated to be developed.

Recently, a microchannel array, which is a model of a capillary blood vessel prepared on silicon by a

photolithographic technique, has attracted interest in the field of microrheology of red blood cells [11]. An advantage of the microchannel array is that it enables us to monitor the interaction between blood cells and material surface under a flow condition in real time with a very small amount of blood.

The first event in blood/biomaterial interaction is the adsorption of proteins onto material surface [12]. Then, platelets adhere to the material surface over the adsorbed protein layer, reacting with them [12]. Some of the adsorbed proteins such as fibrinogen (Fb) and von Willebrand factor can activate platelets to release blood coagulation factor, resulting in thrombus formation. Therefore, the nature of the adsorbed protein layer on material surface deeply influences the following thrombus formation. For example, Fb-coated surfaces are thrombogenic, whereas albumin-coated surfaces are less reactive to platelets [13]. The same process of thrombus formation will occur on the surface of the microchannel array. If the thrombus is formed in the microchannels, it is expected to decrease the blood flow rate, increasing the blood pass-through time (BPT) of a certain quantity of blood. Therefore, the initial process of thrombus formation on material surface can be detected using the microchannel array when BPT is measured and the blood cell behavior is observed.

In this paper, we applied the microchannel array technique to short-term evaluation of material blood compatibility as a new in vitro evaluation method using human blood under a flow condition. We investigated the platelet adhesion on the surface of silicon and two kinds of thin metal films, titanium (Ti) and chromium (Cr), to demonstrate that this technique can evaluate the different level of platelet adhesion caused by different surface. We also investigated platelet adhesion onto the chips coated by albumin (Alb) and collagen (Col) to show that protein adsorption onto microchannel surface influences platelet adhesion into the microchannels.

Materials and methods

Preparation of metal- or protein-coated microchannel array chips

A microchannel array chip made by silicon (Bloody6–7, Hitachi Haramachi Electronics Co. Ltd, Hitachi, Japan; abbreviated as Si) was used. This chip was covered by silicon oxide layer of a thickness of 800 nm which was prepared by acid etching. Details of the microchannel array chip are shown in Fig. 1. The dimension of a microchannel is 10 μm-wide, 30 μm-long, and 4.5 μm-deep on the average. A total number of channels on each Si was 8,736. Four types of surface were prepared on Si; Ti, Cr, Alb and Col.

About 50 nm-thickness of Ti and Cr films were deposited onto Si chips by a sputter deposition apparatus. The area of the cross section of metal-coated microchannel is estimated to be 97.5% of that of the non-treated Si, which can be ignored. Contact angles of water to Si, Ti and Cr chips were measured by surface tester (FACE CA-W, Kyowa Interface Science Co. Ltd, JAPAN).

Alb, which does not take part in blood coagulation reaction, was coated onto Si chips just before the measurement as follows; one of the Si chips was soaked into 1% (w/v) bovine serum albumin (A-2153, Sigma-Aldrich co. Ltd, USA) solution in phosphate buffer saline [PBS (–)] for 2 h at room temperature. Surplus Alb was removed from the chip by washing it three times with PBS (–).

Col, which encourages thrombus formation, was coated onto Si as follows; Si chips were dipped into collagen solution [0.03% (w/v) in HCl aq. (pH 3.0)] for one night in a refrigerator and surplus collagen was removed from the chip by rinsing it three times with PBS (–), followed by distilled water as well. These chips were dipped into 1% (w/v) bovine serum albumin solution for 2 h and rinsed three times with PBS (–) to avoid non-specific adsorption to the uncoated parts of the Si surface.

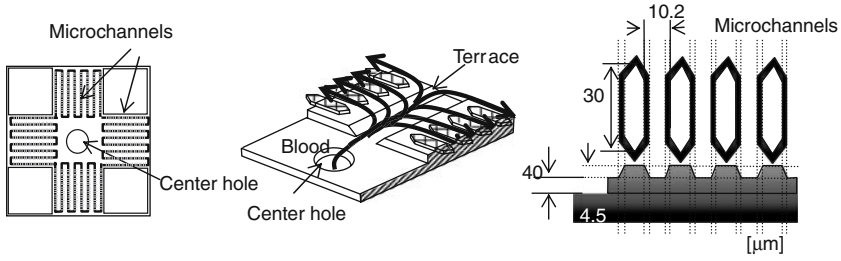

Fig. 1 Diagram of a microchannel array chip. The size of the chip is 1.5×1.5 cm^2. Blood sample is supplied into a center hole from the back of the chip (**a**). Blood flows into each microchannel from the center hole (the arrow in **b**). The average size of the microchannel is 10 μm-wide, 30 μm-long, and 4.5 μm-deep (**c**). 8736 channels are prepared in parallel on the chip

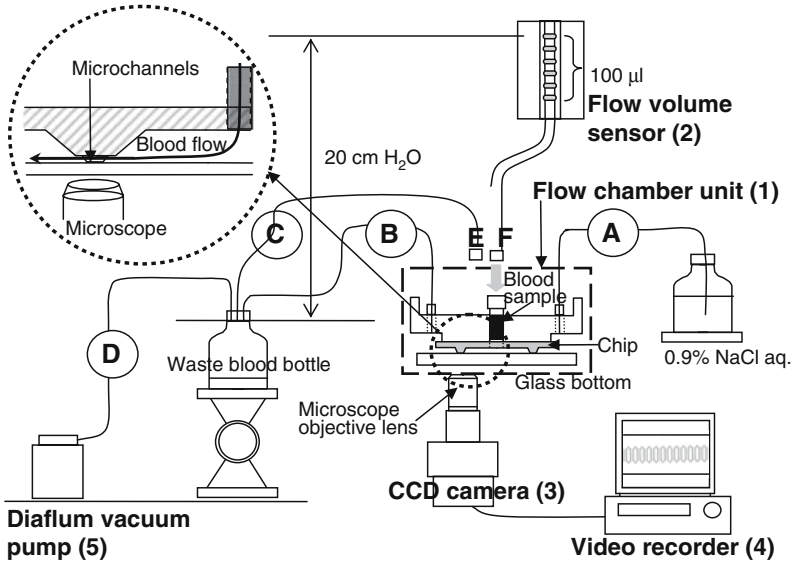

Fig. 2 Diagram of the equipment for the measurement of blood flow rate through the microchannels. Overview of the instrument shows flow chamber unit (1), flow volume sensor (2), CCD camera (3) with video recorder (4) and diaflum vacuum pomp (5). A, B, C, and D indicate solenoid operated valves whereas E and F indicate silicon tubes. Before the measurement, tube E is connected to the center hole of the chamber unit to wash the chip surface with 0.9% NaCl by opening valve A, C, and D while turning on the pump. At the measurement, blood sample is poured into the center hole of the chamber unit. Tube F is connected to the center hole and filled with 0.9% NaCl. Then, valve B and D are opened to start the measurement. Blood flows from the center hole into the microchannels. Blood coagulation on the microchannel is observed by a microscope with a CCD camera and blood pass-through time is measured by flow volume sensor and a stop watch. After the measurement, tube F is disconnected from the center hole of the chamber unit and the blood sample is washed into the waste bottle by turning on the pump

Blood compatibility evaluation

The principle of the blood compatibility evaluation using a microchannel array is schematically shown in Fig. 2. First, a chip was set upside down into 0.9% NaCl solution in a flow chamber unit to form microchannel array at a gap between the glass bottom of the unit. After the process of informed consent, blood was collected via venepuncture of an antecubital vein from a healthy, drug free young person with a sterile disposable syringe and immediately mixed with heparin solution (1,000 IU/ml) which will be 5% of the whole volume in a polypropylene tube [10]. After ca. 200 µl of 0.9% NaCl solution was removed from the center hole of the microchannel array chip, the same amount of whole blood was poured into there. A tube was connected from the center hole to the flow volume sensor and filled with 0.9% NaCl solution. Then, the valve connected to a vacuum pump was opened into the air, which siphons the blood through the microchannel array under a pressure difference of 20 cmH_2O (1961Pa). The BPT, which is the pass-through time of the first 100 µl portion of blood through the microchannel array, was measured by a microchannel array flow analyzer (MC-FAN KH-3; Hitachi Haramachi Electronics Co. Ltd. Ibaraki, Japan). Simultaneously, the flow behavior of blood cells in the channels was observed by an optical microscope and recorded on a video tape. After the measurement, the adhered cells were roughly removed by the strong suction of 400 mmHg (53329Pa) pressure difference and collected into a waste bottle. This study was approved by the Ethics Committee of NIMS.

Results

Effect of the time after blood collection

To examine whether or not the BPT is influenced by the time after blood collection, the BPT was measured every 15 min after blood collection using 8 Si chips with the blood of the donor A as shown in Fig. 3a. The BPTs were 35–40 s till 75 min after blood collection. After 90 min, BPT kept increasing. Over 105 min, the closure of the microchannel arrays was remarkably observed and the BPT could not be obtained. Fig. 3b shows the microscopic video images of the microchannel array surface of these measurements when the 100 µl of blood passed through. At 45 and 60 min,

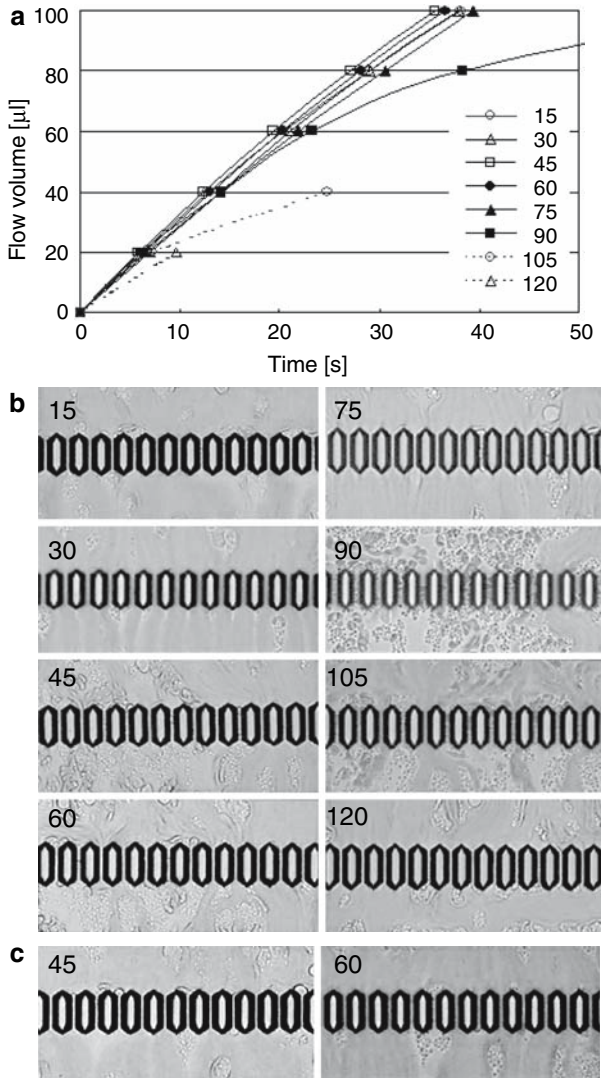

Fig. 3 The BPTs of 8 Si chips measured every 15 min after blood collection using the blood of donor A (**a**), the microscopic images of the microchannels when 100 μl of blood passed through (**b**), and the images of other parts of the microchannels at 45 and 60 min when 100 μl of blood passed though (**c**). Figures indicate the time (min) after blood collection. The blood flows from top to bottom in (**b**) and (**c**)

relatively high number of platelet adhered on the chip surface in spite of the short BPT. Fig. 3c shows the images of other parts of the microchannels at 45 and 60 min after 100 μl of blood passed through. Less platelet adhered on these areas, suggesting that the images for 45 and 60 min (Fig. 3b) were very specific portion of 8,736 channels. Extreme closure of the microchannel arrays by platelet coagulation was observed at 105 and 120 min (Fig. 3b). In general, the level of platelet adhesion observed on the microchannel array surface correlates with the BPT.

Metal- and protein-coated chips

To investigate the platelet behavior on different surface, the BPTs of the Ti, Cr, Alb and Col chips were measured. Based on the results of the Si chip measurements, the level of the blood coagulation correlates with the time after blood collection. Therefore, the BPT of the Si chip was alternately measured with a sample chip to be a control.

In this measurement, we use the blood collected from five healthy young people. We performed blood cell count with a portion of the collected blood to examine the correlation of blood cell counts and the thrombogenicity. Table 1 showed the average counts of the white blood cell (WBC), red blood cell (RBC), Hemoglobin (Hgb), Hematocrit (HCT), and platelet (PLT). The blood cell count is measured for the blood sample collected at the same time for blood compatibility evaluation, and they are quite stable except WBC. As a feature, donor A was low in HCT, and donor C and D were almost the same in number. Donor A and D tend to have shorter BPTs of Si chips than other donors.

Titanium-coated chips

Figure 4 shows the BPTs of Si and Ti chips using the blood collected from donor A on three different days, donor B, and donor C. The BPTs of Ti chips were longer than those of corresponding Si chips even when the donor and experimental days changed. Figure 4f shows the typical video images of the Si and Ti chips when the 100 μl of blood passed through. Thrombus formation was observed on Ti chip as well as Si chip. During the measurement, the detachment of the thrombus from the surface was observed both on Si and on Ti chips. After the measurement, platelets adhered to the Ti was removed by cleaning procedure using negative 400 mmHg pressure, however platelets on the Si was not removed, suggesting the weaker platelet adhesion on Ti than that on Si. The same result was obtained in the case of donor B and C on subsequent days (not shown).

Chromium-coated chips

Figure 5 shows the BPTs of the Si and Cr chips using the blood collected from donor A, C, and D with typical images of platelet adhesion onto these chips when 100 μl of blood passed through. Platelet adhesion onto Cr surface was frequently observed just after the blood flow started. The BPTs of Cr chip was longer than those of Si chips in all cases. No detachment of

Table 1 Average blood cell counts of 5 donors

Donor	WBC ($\times 10^3$/ml)	RBC ($\times 10^6$/ml)	Hgb (g/dl)	HCT (%)	PLT ($\times 10^4$/ml)
A	4.31 ± 0.68	4.02 ± 0.17	11.94 ± 0.68	34.05 ± 2.40	23.3 ± 5.31
B[a]	5.00	4.82	16.00	46.30	22.20
C	6.42 ± 0.73	4.66 ± 0.15	14.47 ± 0.72	42.51 ± 2.98	20.99 ± 3.47
D	6.31 ± 0.79	4.63 ± 0.35	15.43 ± 0.92	42.89 ± 4.07	23.84 ± 6.51
E	7.00 ± 0.68	3.92 ± 0.16	13.87 ± 0.21	36.90 ± 2.84	16.03 ± 2.06

Average ± standard deviation is shown on the table

[a] Only one measurement is performed because of the temporal unavailability of the blood cell analyzer

adhering platelets was observed on the Cr chip during the measurement. After the measurement, no platelets adhered to the Cr were removed form the surface by cleaning process using negative pressure of 400 mmHg, suggesting the higher adhesion strength of platelet to Cr than Si chip. The same results were obtained in the cases of donor B and C on subsequent days (not shown).

Albumin-coated chip

The typical BPTs of Si and Alb-coated chips using the blood collected from donor A, C, and D with the typical video microscopic images of these chip surfaces are shown in Fig. 6. The BPT of the Alb-coated chip was shorter than that of the Si chip. Platelet adhesion was seldom observed on Alb-coated chip.

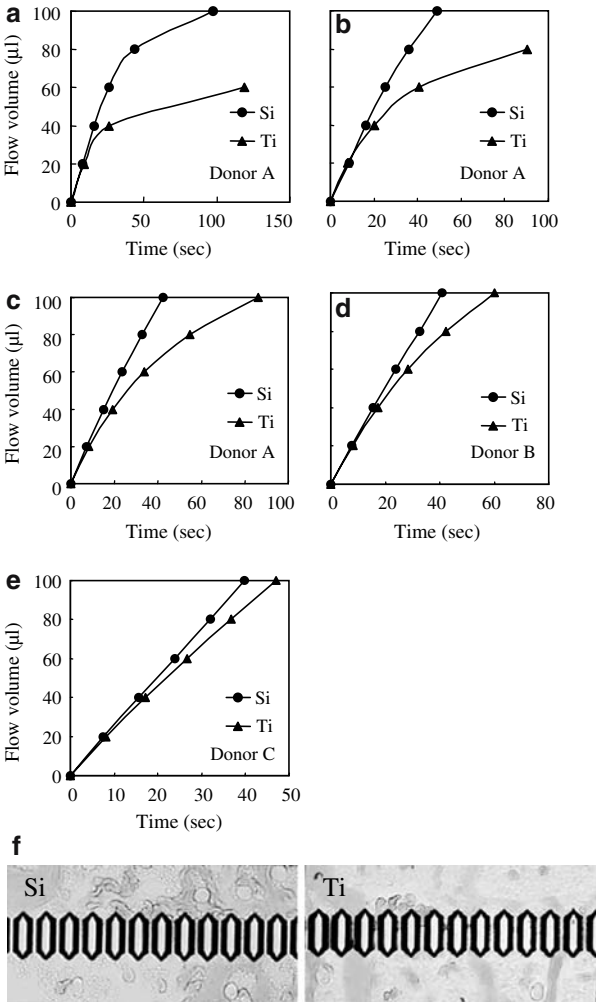

Fig. 4 The BPTs of Si and Ti-coated chips using the blood of donor A on other days (**a–c**), donor B (**d**), and Donor D (**e**), and the typical microscopic images of the microchannels when 100 µl of blood passed through (**f**). The blood flows from top to bottom in (**f**)

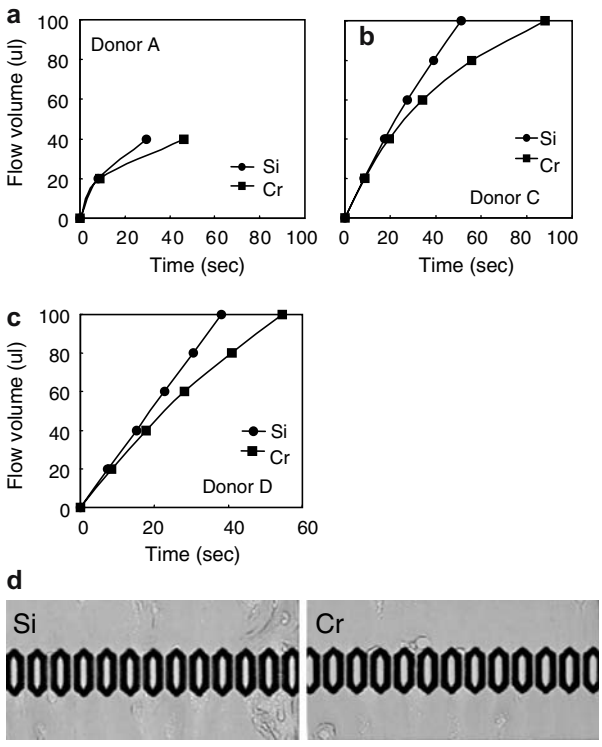

Fig. 5 The BPTs of Si and Cr-coated chips using the blood of donor A (**a**), donor C (**b**) and donor D (**c**), and the typical microscopic images of the microchannels when 100 µl of blood passed through (**d**). The blood flows from top to bottom in (**d**)

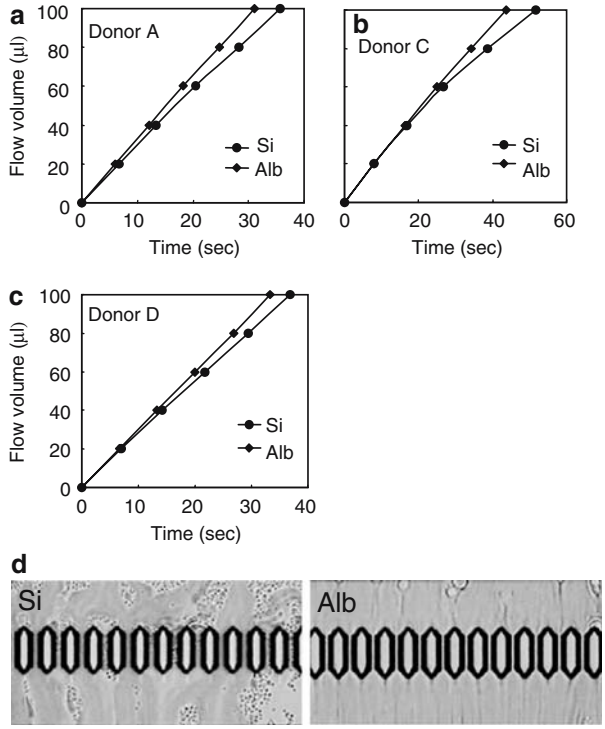

Fig. 6 The BPTs of the Si and Alb-coated chips using the blood of donor A (**a**), donor C (**b**) and donor D (**c**), and the typical microscopic images of the microchannels when 100 µl of blood passed through (**d**). The blood flows from top to bottom in (**d**)

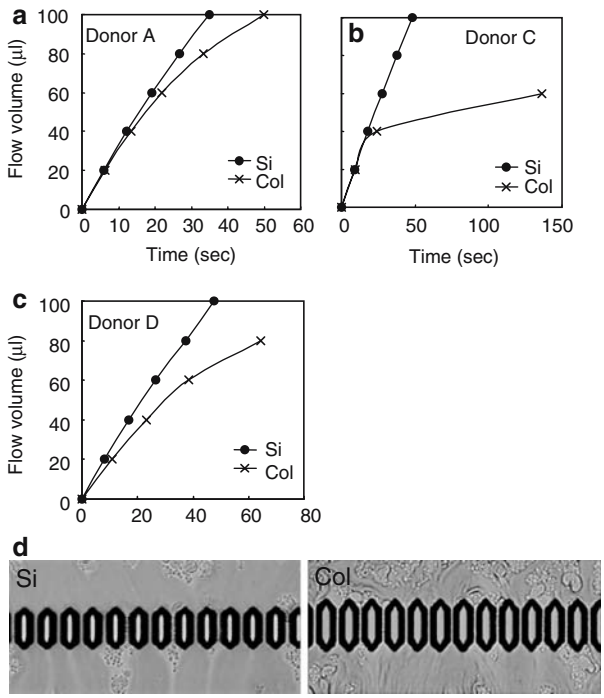

Fig. 7 The BPTs of the Si and Col-coated chips using the blood of donor A (**a**), donor C (**b**), and D (**c**), and the typical microscopic images of the microchannels when 100 µl blood passed through (**d**). The blood flows from top to bottom in (d)

Collagen-coated chip

Figure 7 shows the BPTs of the Si and Col-coated chips using the blood collected from donor A, C, and D with the typical images of the Col-coated and Si chip surface when the 100 µl blood passed through. Almost all the BPTs of the Col were longer than those of the Si. Many platelets were adhered on the Col surface. After the measurements, these chips were cleaned using negative 400 mmHg pressure and platelets on the Col-coated chip was not removed, suggesting the higher adhesion strength of platelet to Col than Si chip.

Comparison of the data for metal- and protein-coated chips

Since blood compatibility evaluation of these four types of chip surfaces are repetitively performed on individual days, obtained BPTs were standardized with the BPTs of Si and plotted as shown in Fig. 8 to compare to the BPTs of each surface. The X-axis represents the BPT of the Si when 100 µl of blood passed through, and the Y-axis represents the standardized BPT of each surface to that of the control, Si. A–E in the Fig. 8 indicates the donors. Mostly, the BPTs of Si were shorter than 100 s. The standardized BPTs of Ti and Col were higher than 1, suggesting that Ti and Col are relatively thrombogenic in comparison with Si though the standardized BPT scattered in a large range. The standardized BPTs of Cr were slightly larger than 1, suggesting that Cr has similar level of the thrombogenicity to Si. The standardized BPTs of the Alb were below 1, suggesting Alb-coating reduces thrombogenicity of Si chip. The tendency of the thrombogenicity of these surfaces increased in the order of Alb, Si, Cr, Ti and Col.

Discussion

Evaluation of material blood compatibility has mainly been performed in static condition such as platelet adhesion test using platelet rich plasma [1–4] and blood clotting (coagulation) time measurement [14]. Recently, several flow chamber systems are developed to evaluate blood compatibility in dynamic condition [6–10, 15–17]. Some of them designed to reduce the necessary amount of blood for application of human blood [7, 8, 15]. In this study, a microchannel array is introduced, which enables us to evaluate material blood compatibility using human blood under flow condition.

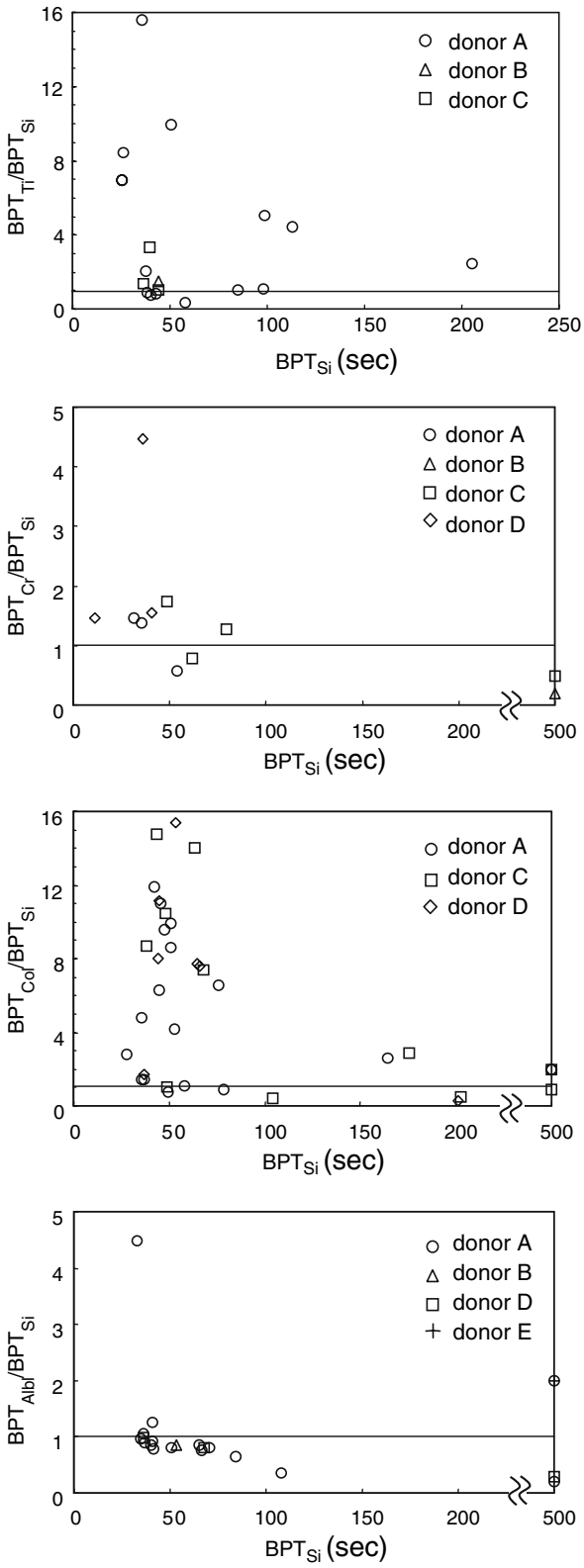

Fig. 8 The BPTs of the Ti-, Cr-, Alb- and Col-coated chips standardized by the BPT of the Si (control). X-axis indicate the BPT of the Si when 100 μl of blood passed through and Y-axis indicate the standardized BPT of the each sample to that of Si. The letters A–E in the graphs indicate the donors

When platelet adhesion and thrombus formation on the microchannel array surface increased, closure of the channels also increased, resulting in the increase of the BPT. The BPT is the time that the sum of the blood amount passing through 8,736 microchannels achieves 100 μl, that is, it reflects the condition of every channel such as occlusion or reopening. As shown in Fig. 3, the amount of adhering platelets correlates well with the BPTs. Because of the limited view of the microscope objective as 12 of 8,736 channels, the BPT is a practically better index to explain the thrombus formation on the microchannel array.

For the experiment using fresh human blood, anticoagulation of blood is required. In this study, heparin was used as an anticoagulant, which inhibits the serine proteases (thrombin and factor X) in association with its cofactor antithrombin III [18, 19]. Therefore, platelet adhesion and activation can occur even in the presence of the heparin though the formation of fibrin network is inhibited. Actually, blood anticoagulated with heparin is employed for the research on platelet function. P. G. Groot et al. recommended using 20 IU/ml heparin for the research on the pathophysiological mechanism of platelet adhesion without the influence of the activation of the coagulation cascade [9]. Since the half life of heparin is 60–90 min [19], we examined the effect of the time after blood collection on BPT (see Fig. 3). The BPT increased with the time after blood collection drastically after 90 min, indicating that the measurement of the BPT should be performed within 90 min after blood collection to keep the effect of anticoagulant constant.

Adsorbing protein to material surface greatly influences the material blood compatibility. Coating material surface with protein such as Fb, which are involved in coagulation cascade, increased thrombus formation [13], whereas coating the surface with inert proteins such as Alb reduced thrombus formation [20], because the pre-coated protein layer inhibits the adsorption of platelet-adhesive proteins. To examine the effect of adsorbing protein to the channel surface on the BPT, Col and Alb were pre-coated on the Si chips. Col has the binding domain to platelet-adhesive protein such as von Willebrand factor [21], so platelet activation and thrombus formation will occur, when blood contacts to it. As shown in Figs. 6 and 7, Col-coated chip has longer BPT than that of uncoated Si chip, whereas Alb-coated chip has shorter BPT. It confirmed that the protein adsorption influenced the platelet adhesion and thrombus formation at microchannel surface.

Adsorbed protein layer differs with materials because adsorption behavior is influenced by physicochemical

properties of material surface such as point of zero charge, dielectric constant, hydorophilicity/hydrophobicity, wettability, etc [22, 23]. In this study, Ti and Cr were coated to Si chips to demonstrate the difference of materials in blood compatibility. The surfaces of these metals as well as Si are covered by thin oxide layer such as several nanometers in thickness [24]. Some of the physicochemical properties of Cr_2O_3, SiO_2, and TiO_2, the major oxide composition of Cr, Si, and Ti, are shown in Table 2 [25]. Since these surfaces form hydroxyl groups in aqueous solution by hydration, their surface charge depends on the pH of the solution. Cr_2O_3 has similar point of zero charge to the pH of blood (7.15–7.35), suggesting that the surface of Cr_2O_3 is not charged in blood. SiO_2 and TiO_2, however, have lower points of zero charge than the pH of blood, suggesting that these surfaces are negatively charged in blood. Alb, which is negatively charged in neutral solution with the isoelectric point of 4.7, is confirmed to increase its adsorption to surface hydroxyl group on TiO_2 surface by the existence divalent cations such as Ca^{2+} and Mg^{2+} [26]. Fb is also negatively charted in neutral solution with the isoelectric point of 5.8. Therefore, adsorption of Alb and Fb is expected to be less encouraged on Cr_2O_3 than TiO_2 and SiO_2. Dielectric constant relates with the polarization in inter- or intra-molecule. TiO_2 has the highest value among these surfaces, suggesting the easier adsorption of charged or polarized protein molecules. The smaller contact angle indicates the higher hydrophilicity. Alb and Fb is known to have higher affinity to hydrophobic surface than hydrophilic surface [27]. Alb adsorbs more rapidly to hydrophobic surface than hydrophilic surface whereas the adsorption rate of Fb to hydrophobic surface is slightly larger than that to hydrophilic surface [27]. For the hydrophobic surface, the Alb adsorption is much quicker than that of Fb since the size of Alb is much smaller than that of Fb. From these consequences, the adsorption of albumin is lower on TiO_2 surface than Cr_2O_3 and SiO_2 surface suggesting higher Fb adsorption in the short-term adsorption such as 1 min or shorter. As is described before, the adsorption of Fb induces thrombus formation on the channel surface, resulting in the higher BPT. In the present study, platelet adhesion level and BPTs differed with materials and increased in the order of $Si \leq Cr < Ti$ (see Fig. 8). This result corresponds well to the above estimation.

Since most of metallic biomaterials have been used as a replacement of hard tissue, blood compatibility of metals is not widely and systematically investigated; studies on limited metals such as Ti, Ti-6Al-4 V, Co-Cr alloy and 316L stainless steel are available [7, 10, 28–30]. Based on these studies, it is suggested that material blood compatibility is influenced by the time and condition contacting to blood as well as the thickness and structure of the surface oxide film. The clotting time of rabbit blood increased in the order of glass, Ti, LTIC, and TiO_2 of 400 nm in thickness [31], suggesting the thicker TiO_2 layer gives higher blood compatibility. The thickness of TiO_2 oxide layer also influences the protein adsorption such as albumin and Fb [32, 33]. Concerning the blood contacting time, the amount of adsorbed thrombin on Ti surface is higher than that on glass at earlier stage such as 1–16 min incubation with whole blood [5]. In our system, platelet adhesion and thrombus formation on microchannel surface is evaluated within a few minutes of contact with blood, and our result that the Ti-coated chip has higher BPT than Si corresponds well to this result on thrombin adsorption. Therefore, our system has the possibility to be applied as a short-term evaluation of material blood compatibility in vitro using human blood, however further investigation is required to confirm the correlation with the results in vivo. In this study, sputter deposition of metals was applied to the microchannel chips because it can easily cover the whole surface (even the side walls) of the microchannels with a very thin and homogenous layer, avoiding the drastic change of channel dimension. As described before, the data of in vivo blood compatibility evaluation of metals is limited, resulting in the difficulty of direct comparison of our results to in vivo results. Generally, in vivo blood compatibility evaluation is more

Table 2 Physicochemical properties of Cr_2O_3, SiO_2, and TiO_2 [25] and contact angles of water to Cr, Ti, and Si chips

Element	Oxide	Point of zero charge (pH°)	Dielectric constant	Contact angle(°)
Cr	Cr_2O_3	6.5–7.4	12.0 (r.t., 2×10^6 Hz)	86.2
Si	SiO_2(quartz)	1.8–2.8	4.5–4.6 (r.t., 10^5 Hz)	70.4
	(zol)	1–1.5		
Tiα	TiO_2	4.8		60.5
	(rutilu)	5.5–6.7	85.8–170 (25°C, 10^6 Hz)	
	(anatase)	6.0		

r.t.: room temperature

Contact angles of water to these chips were measured by surface tester (FACE CA-W, Kyowa Interface Science Co. Ltd, JAPAN)

performed with polymeric materials rather than metals since polymeric materials have been applied to the medical devices and containers in contact with blood. In future, the blood compatibility evaluation of polymer-coated chips will be studied to confirm the correlation of the results between our system and in vivo experiments.

Application of human blood to our system arise the question whether or not the BPT reflects the individual difference in blood property such as thrombogenic activity. Since the BPT increases along the closure of the microchannels by thrombus formation during the measurements, it may be influenced by the number of the platelet in a unit volume of the blood. We performed blood cell count and found that the blood property was almost the same between donor C and D (see Table 1). However, the BPTs of the donor D were shorter than those of the donor C (See Fig. 6, for example). Furthermore, as shown in Fig. 6, the BPTs of donor A are similar to those of donor D for Si and Alb, whereas their blood properties are quite different. Therefore, we concluded that the BPTs were not influenced by blood cell count. Nevertheless, the tendency of the blood compatibility of these four materials was the same even when the donor changed. In this study, the blood of only five donors was used to confirm that this system could detect platelet adhesion and activation at material surface by measuring the BPT. To specify the effect of the donor or blood cell count, further investigation should be performed with a larger number of the donors.

As described in the Introduction, animal study and in vitro platelet adhesion test in static or flow condition have been performed to evaluate materials blood compatibility. However, many problems remain in these evaluation methods such as species miss-matching and the inappropriate situation far from the vascular environment. In this study, a new method is developed to evaluate materials' initial blood compatibility in vitro. In this system, we could evaluate initial level of blood coagulation on microchannel array surface by measuring BPT and observing platelet adhesion in situ. The advantage of this system is that; it requires only a small amount of blood (100 μl) and one measurement can be performed within several minutes. Therefore, we could apply this system for a patient to select optimal implanting materials for blood contacting devices with his blood easily and quickly, such as a bed-side test. We also found that the initial blood coagulation on the microchannel array surface depends on the initial protein adsorption to it. Therefore, this system can be modified to study protein adsorption onto the material surface under a flow condition at high sensitivity.

In this study, we applied the pressure difference of 20 cmH_2O to siphon blood into the microchannel array to achieve quick measurement within several minutes, which only allows us to observe initial platelet activation. However, the pressure difference, the shape, and the number of microchannels can be modified to perform measurements in various conditions. These features will contribute to elucidate the mechanism of initial blood coagulation induced by contacting to artificial material or applying shear stress to platelets.

Conclusion

In this study, we applied a microchannel array technique to in vitro blood compatibility evaluation of different surface. The BPT and the behavior of platelets differ with the kind of materials; Si, Ti, Cr, Alb- and Col-coated surface. The BPT correlates with the platelet adhesion levels on the material surface, which is expected to reflect the initial blood coagulation on the surface in vivo. In this research, it was confirmed that protein adsorption on the material surface influenced platelet adhesion and activation. Platelet adhesion can be detected sensitively using this microchannel array system. The blood compatibility evaluation using the microchannel system can be performed within a few minutes with a small amount of human whole blood, suggesting that this system can be a quick evaluation method for initial blood compatibility of materials in vitro as well as the evaluation of the mechanism of blood coagulation on material surface under shear flow.

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