Hollow Fiber Membrane Oxygenator Reduces Platelet Loss During Simulated Extracorporeal Circulation

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ABSTRACT

Contact between blood and the extracorporeal circuit results in adverse alterations in platelet number and function. To determine the effects of surface composition and flow on these changes, 450 ml of heparinized (5 U/ml) blood from random, aspirin-free volunteers was recirculated at a flow rate of twice the circulating volume for 2 hrs at 37°C through circuits with an identical surface area (1.0m²). These circuits contained either a spiral coil, silicon rubber, membrane oxygenator (SC) or a hollow fiber, polypropylene membrane oxygenator (HF). In SC circuits (n=5), platelet counts fell to 10±3% (±SEM p <.05) at 5 min. rising to 60 ±9% (p < .05) at 2 hrs. of extracorporeal circulation (ECC). In contrast, in HF circuits (n=5), the platelet count fell to only 53±7% (p < .05) at 5 min of ECC and rose to 82 + 14% (p > .05) at 2 hrs. Plasma levels of the platelet-specific protein platelet factor (PF) 4 rose to 2780 ±222 ng/ml (p <.05) by 5 minutes, reaching 6338 ±767 ng/ml (p < .05) at 2 hours in SC circuits. In contrast, PF4 in HF circuits rose to 836±73 ng/ml and 2640 ± 410 ng/ml (p < .05), respectively. Although platelets in the SC circuits became insensitive to ADP (≥ 50 uM) within 5 min, platelets from HF circuits aggregated to ADP (17 uM) despite 2 hrs of ECC. We conclude that the hollow fiber membrane oxygenator not only reduces adhesion, maintaining the circulating platelet count, but also reduces platelet protein release and preserves platelet function as well.

INTRODUCTION

Interaction between blood and synthetic surfaces results in platelet adhesion, aggregation and degranulation. [1-3] Consistently, extracorporeal circulation (ECC), which involves extensive contact between blood, air, and the synthetic surfaces of the circuit, results in thrombocytopenia and significant functional impairment of platelets. [4,5] Although membrane oxygenators have eliminated the blood-to-air interface, their large surface area and flow characteristics continue to inpact on the hemostatic system.[6,7,8] The purpose of this study was to determine the extent to which oxygenator design contributes to adverse platelet changes during extracorporeal circulation.

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MATERIALS AND METHODS

Extracorporeal circuits with a surface area of 1.0 m² were assembled from 100 cm of medical grade 1/4 inch polyvinylchloride tubing[a], a 400 ml polyvinylchloride venous reservoir bag[b] and either a spiral coil silicon rubber membrane oxygenator[c] or a hollow fiber polypropylene membrane oxygenator[d] with 0.8 m² surface interface. Circuits were flushed in 100% CO_2 for a minimum of fifteen minutes prior to priming. The tubing and oxygenator were then primed by gravity flow through the circuit. Blood was recirculated by a shimmed, barely occlusive, calibrated double roller pump[e] for a period of two hours at a rate of twice the circulating volume in milliliters per minute. Blood temperature was maintained at 37°C using either the integral heat exchanger in the hollow fiber oxygenator or a warming pad[f] wrapped around the venous reservoir bag for the spiral coil oxygenator that was connected to a heat pump.[g] Ventilation was maintained at 1.0 L/min with a 95%/5% oxygen-carbon dioxide mixture.

SAMPLE ACQUISITION

These studies were approved by the University of Pennsylvania Committee on Human Investigations and the National Institutes of Health. Written and informed verbal consent was obtained from each donor prior to venipuncture.Volunteers abstained from all medications for at least two weeks prior to donation. 450 ml of human blood was drawn directly into the venous reservoir containing 2250 units of beef lung heparin and 1.65 gm of glucose. All blood samples were drawn into syringes containing 3.8% trisodium citrate (9:1

a. Medtronic, Anaheim, CA
b. and d. Terumo, Piscataway, NJ
c. Sci-Med Life Systems Inc., Minneapolis, MN
e. Sarns, Inc., Ann Arbor, MI
f. Cobe Laboratories, Lakewood, CO
g. Radiometer, Copenhagen, Denmark

FIGURE 1

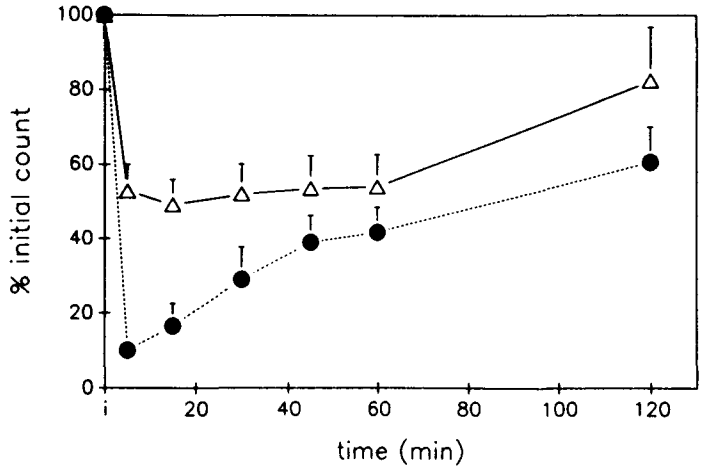


FIGURE 2

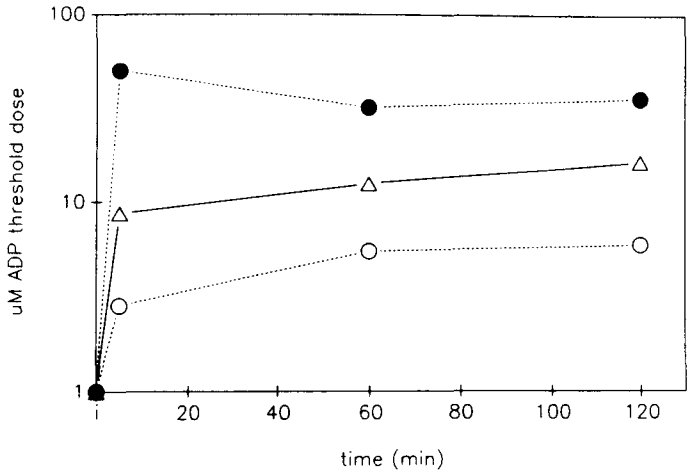


FIGURE 3

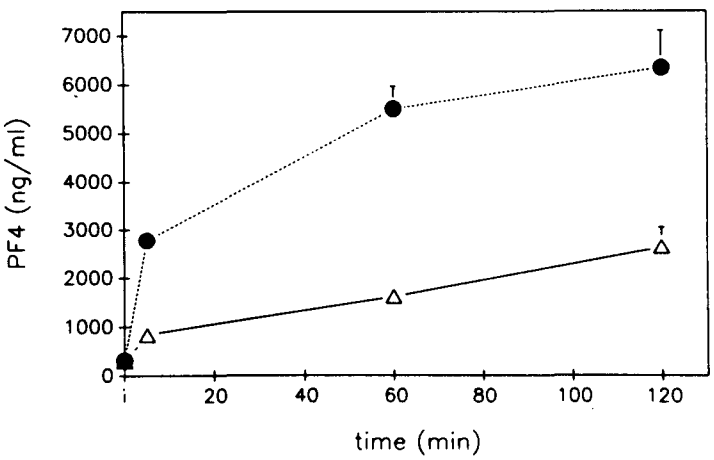
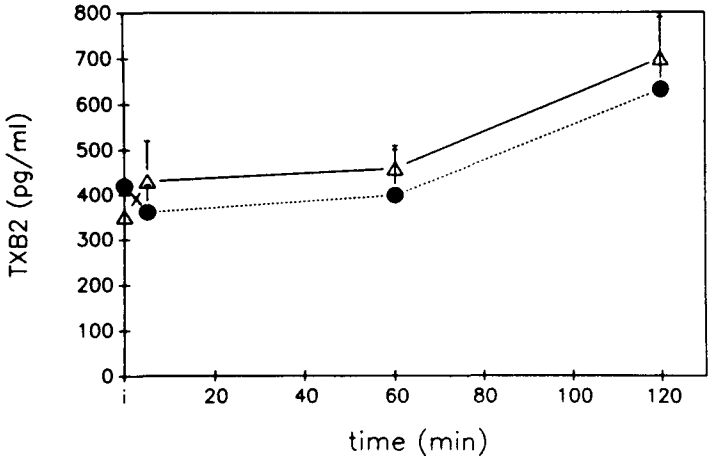


FIGURE 4



V/V). 10 ml aliquots of blood for control samples were obtained from the reservoir bag after priming but prior to the start of recirculation, incubated at 37°C, and assessed at five, 60, and 120 minutes for platelet function. Twenty milliliter aliquots were drawn from the circuit after five, 60, and 120 minutes of recirculation for platelet function and release studies. One milliliter samples were drawn at 15, 30, and 45 minutes for platelet counts. Samples for assay of platelet release products were processed and frozen at -70°C for later analysis.

PLATELET STUDIES

PLATELET COUNT

Whole blood platelet counts were obtained with a Coulter Z-F cell counter and checked when necessary by phase microscopy.[9]

PLATELET AGGREGATION

Platelet-rich plasma (PRP) was prepared from a 10 ml sample of citrated whole blood as previously described.[10] Samples were centrifuged at 150g for 10 min at 25°C. Following gentle aspiration of the PRP (350,000±50,000 platelets/ul), the remaining blood was centrifuged at 12,000 g for five minutes at 25°C in a microcentrifuge to obtain platelet-poor plasma (PPP) with a platelet count of less than 1000/ul. Platelet aggregation studies previously described determined that in aspirin-abstaining donors the platelet release reaction is complete at the 95% confidence level when adenosine diphosphate (ADP)-induced aggregation exceeds 62%.[4] Thus, complete second wave aggregation is defined as more than 62% light transmission through PRP at five minutes. The threshold dose of aggregating agent is defined as the lowest logarithmic concentration of aggregating agent necessary to produce second wave aggregation. Normal reactivity in control samples was demonstrated using 1-5 uM ADP. The maximum concentration of ADP tested was 50 uM.[12]

PLATELET FACTOR 4

A 4.5 ml sample of citrated whole blood was transferred to plastic tubes containing 10% disodium ethylene-diaminetetraacetic acid (EDTA), 5.4 mg/ml theophyllin, and 3 x 10^{-3} M PGE_1 that was immediately centrifuged at 2000 g for 20 minutes at 4°C to obtain platelet-poor plasma. The resultant PPP was centrifuged at 12,000 g for two minutes at room temperature in a microcentrifuge.[13]

The appearance of the platelet-specific protein platelet factor (PF) 4 in plasma was used to indicate the occurrence of the platelet release reaction. The plasma levels of PF4 were quantitated by radioimmunoassay with a specific antibody[h] as previously described.[14] The sensitivity of this assay is 1 ng/ml.

THROMBOXANE B2

A 4.5 ml sample of citrated whole blood was transferred to a plastic tube containing EDTA for a final concentration of 10mM and spun for PRP. When preparing PPP from the PRP, indomethacin was added to give a final concentration of 10 uM. The aliquot was then centrifuged at 2000 g for 10 minutes at room temperature.[13]

Thromboxane B2, the stable end-product of thromboxane A2, used as an indicator of platelet activation, was measured in plasma by radioimmunoassay with a specific antibody[i] as previously described.[15] The sensitivity for this assay is 25 pg/ml.

h. Abbott Laboratories, North Chicago, IL

i. Dupont-New England Nuclear, Boston, MA

STATISTICAL ANALYSIS

Mean, standard deviation and standard error of the mean were calculated for all groups. Differences within each group were compared utilizing a one-way analysis of variance. Duncan's Multiple Range t-test was used to make comparisons among sampling periods within each oxygenator group. A two-way analysis of variance was used to compare differences between the spiral coil and hollow fiber groups. A p value $< .05$ was considered statistically significant.

RESULTS

PLATELET COUNTS

In spiral coil oxygenator circuits (n=5), mean platelet counts, expressed as a percentage of the initial platelet count, decreased to 10±3% (mean + standard error of the mean) ($p < .05$) of initial levels within five minutes of recirculation (**Figure 1**). After 120 minutes of recirculation, platelet counts reached 60 ±9% ($p < .05$). In contrast, platelet counts in the hollow fiber oxygenator circuits (n=5) fell to 53±7% ($p < .05$) of initial levels during the first five minutes of ECC and rose to 82 ±14% at two hours ($p > .05$) (**Figure 1**). The circulating platelet count was significantly preserved in blood recirculated through the hollow fiber oxygenator as compared to that in the spiral coil oxygenator.

PLATELET AGGREGATION

Aggregation of platelets obtained from control samples (n=10) that were merely incubated at 37°C for two hours demonstrated essentially normal reactivity when challenged with ADP (2-5 uM) (**Figure 2**). After five minutes of recirculation, the platelets from the spiral coil oxygenator circuits were unresponsive even when challenged with maximum concentration of ADP (50 uM). This loss of reactivity persisted throughout recirculation. In contrast, platelets from hollow fiber oxygenator circuits were less responsive to the threshold dose of ADP after five minutes of recirculation (9 uM), but remained reactive even after two hours of recirculation (16 uM) (**Figure 2**).

PLATELET FACTOR 4

Initial plasma levels of platelet factor 4 in both membrane oxygenator groups were similar prior to recirculation (310 ng/ml) (**Figure 3**). In the spiral coil oxygenator circuits, plasma levels of PF4 rose to 2780±222 ng/ml within five minutes of recirculation ($p < .05$), reaching 6338± 767 ng/ml after two hours ($p < .05$), indicating extensive granule release (**Figure 3**). In contrast, in the hollow fiber oxygenator circuits plasma levels of PF4 increased to 836±73 ng/ml after 5 minutes ($p > .05$) and rose to only 2640±410ng/ml during the two hour recirculation

time ($p < .05$) (**Figure 3**). Recirculation of blood through the hollow fiber oxygenator released significantly less PF4 than blood recirculated through the spiral coil oxygenator.

THROMBOXANE B2

Initial plasma levels of thromboxane B2 were similar for both circuits (420 ± 81 pg/ml spiral coil vs. 353 ± 64 pg/ml hollow fiber), with no significant differences (**Figure 4**). Spiral coil oxygenator circuits demonstrated plasma levels of 361 ± 61 pg/ml after five minutes rising to 632 ± 157 pg/ml after two hours of recirculation (**Figure 4**). In hollow fiber oxygenator circuits, plasma levels rose from 431 ± 89 pg/ml after five minutes or recirculation to 700 ± 125 pg/ml over two hours ($p > .05$) (**Figure 4**). No statistical difference in generation of thromboxane levels was noted for blood recirculated through either membrane oxygenator.

DISCUSSION

Following exposure to synthetic surfaces, platelets will adhere, aggregate, and release granule contents.[1-3] Thus, it is not surprising that cardiopulmonary bypass which requires extensive blood-surface contact is associated with thrombocytopenia, prolonged bleeding times and excessive blood loss.[4,5] During extracorporeal circulation, a primary site of activation of platelets is, predictably, the oxygenator.[10] Although membrane oxygenators minimize a blood-gas interface, the surface area remains extensive and reactive towards platelets.[16]

Currently, two designs for clinically used membrane oxygenators are the hollow fiber and the spiral coil. We have demonstrated that compared to the spiral coil design, the hollow fiber design preserves the circulating platelet count, reduces platelet granule release, and preserves platelet reactivity even after two hours of simulated extracorporeal circulation. Although welcomed, this preservation of platelet number and function in the hollow fiber design would not have been predicted and remains unexplained.

Platelets do not interact directly with synthetic surfaces.[3] Actually, within seconds of surface contact with blood, a protein layer is absorbed which can extend to 200 A^{O}.[17,18] This protein layer is comprised largely of fibrinogen and is highly reactive.[19,20] Furthermore, since all synthetic materials develop this protein layer, their behavior in contact with blood tends to be stereotyped.[6,21] Thus, we would predict that increased platelet damage in spiral coil circuits is not due to the fact that they are composed of silicon rubber rather than polyvinylchloride as in the hollow fiber circuits.

It was previously believed that the blood volume-surface area ratio was critical in determining thrombogenicity of a device. In the 1970s, much effort was expended to increase this ratio as much as possible.[8] This concept, however, has never been adequately tested and, in any case, our circuits had equivalent surface areas and blood-surface area ratios. More likely, it is the device design which is critical in determining the thrombogenicity of a complex device like an oxygenator. Indeed, it is known that adverse hemodynamics participate most directly in activation of hemostatic elements and can even obscure the contribution of the synthetic surface.[22,23] To permit adequate gas exchange, spiral coil oxygenators actually encourage secondary currents which increase the residence time of formed blood elements and increase the likelihood of thrombosis.[24] Our prediction would be that the hollow fiber design encourages laminar flow and reduces secondary currents. Thus, residence time is reduced and activation of platelets on synthetic surfaces less likely. However, two oxygenators of identical design but composed of different materials would be required to fully test this hypothesis. This work underscores the need for device testing in biomaterials research. Often synthetic materials are placed in highly stylized systems which are not predictive of performance in a clinical setting. For example, our results would not likely have been duplicated had we used polyvinylchloride or silicon rubber in a simple flow chamber. Furthermore, it underscores the need for use of human blood as four species are adequately predictive of the human behavior.[25]

In summary, simulated extracorporeal circulation with both spiral coil and hollow fiber oxygenators is associated with extensive alterations in platelet numbers and function. Hollow fiber design, however, appears to be substantially less active in this regard. Furthermore, it is increased laminar flow within the device which is likely to be responsible for these salutary effects. Finally, through device design optimalization, excessive bleeding following acute cardiopulmonary bypass will be reduced and horizons extended for all applications of acute and long-term extracorporeal circulation.

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