

# Quantitative SEM-Based Analysis of Blood Cell Adhesion on Hollow Fiber Membranes Using a Miniaturized Oxygenator Model and ImageJ/FIJI-Based Image Analysis

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## Research Article

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# Abstract

**Purpose** The hemocompatibility of membrane oxygenators remains inadequate in extracorporeal membrane oxygenation (ECMO), particularly regarding thrombus formation. Adhesion of blood components onto hollow fiber membrane is considered a major contributor to coagulopathy during ECMO; however, quantitative data remain limited. To enable quantitative scanning electron microscopy (SEM) evaluation of cellular adhesion without cutting the oxygenator case, we developed a miniaturized oxygenator with a removable case and evaluated the hemocompatibility of a newly modified membrane designed to inhibit platelet adhesion.

**Methods** Two types of miniaturized membrane oxygenators (membrane area:  $0.01 \text{ M}^2$ ) were constructed using OXYPHAN membranes (3M), with or without novel membrane modification ( $n = 8$  each). Two-hour in-vitro circulation tests were performed using blood from eight volunteers. Hemocompatibility was assessed by blood testing and by SEM analysis of fiber membrane using ImageJ/FIJI.

**Results** The increase in thrombin-antithrombin complex (TAT) was significantly lower in the modified membrane oxygenators than in the non-modified ones ( $\Delta \text{TAT}$ :  $9.7 \pm 4.0 \text{ ng/mL}$  vs  $27.5 \pm 19.5 \text{ ng/mL}$ ,  $p = 0.0477$ ). The modified devices reduced total adhered cell counts and platelet adhesion counts by approximately 80% ( $47.3 \pm 17.3$  vs  $228.7 \pm 116.8$ ;  $p = 0.00206$ ) and 90% ( $34.1 \pm 21.1$  vs  $358.8 \pm 298.8$ ;  $p = 0.01394$ ), respectively, compared with the non-modified devices.

**Conclusion** Our miniaturized oxygenator with a removable case enabled easy sampling of fiber membranes and quantitative SEM image analysis. This approach exhibited a marked reduction in platelet adhesion on the modified membrane and may serve as a useful method for evaluating membrane modifications.

## INTRODUCTION

In long-term use of membrane oxygenators, such as during extracorporeal membrane oxygenation (ECMO), thrombus formation inside the oxygenator has been reported [1–3]. Contact between blood and biomaterials activates host defense mechanisms and induces inflammatory reactions and thrombogenesis [4, 5]. To suppress these adverse reactions, several strategies have been adopted, including heparin/polymer coating of membranes [6, 7], improvement of intradevice hemodynamics [8], and optimization of anticoagulation dosing and monitoring [9]. However, these measures have not yet provided a complete solution. To evaluate the extent to which such countermeasures improve oxygenator biocompatibility (hemocompatibility), numerous studies have employed in-vitro simulated cardiopulmonary bypass modes, clinical studies, and large-animal cardiopulmonary bypass (CPB) experiments assessing systemic inflammatory response syndrome (SIRS) and organ injury, as well as analyses of inflammatory and coagulation markers in blood [6, 7, 10, 11]. In contrast, although adsorption of blood components onto hollow fiber membranes is considered a key factor in ECMO-associated coagulopathy, available knowledge remains limited [12]. The importance of scanning electron microscopy (SEM) analysis for evaluating blood cell adhesion on hollow fiber membranes has been emphasized [13], but prior SEM studies have mainly provided qualitative assessments of thrombus and protein/cell deposition [10, 13, 14]. Quantitative evaluation of the number of cells adhered to membranes has not been reported.

At the final stage of oxygenator development, regulatory approval requires testing in accordance with ISO 7199 and national regulatory authority requirements [15]. Prior to that stage, large-animal CPB experiments and ex-vivo simulation of CPB using human blood and clinical oxygenators are commonly used [7, 16]. However, in early development stage without industrial collaboration, manufacturing techniques that are typically proprietary- such as centrifugal polyurethane potting and precise cutting of potting end faces- are difficult for non-specialist researchers to implement. This situation represents a barrier to applying novel academic findings in this field.

In clinical ECMO research, internal observation of exchanged oxygenators often requires cutting open the case with a power saw, which is labor intensive and produces substantial debris, making SEM analysis technically difficult [13, 17]. Although the need for research-scale miniaturized oxygenators has recently been recognized and new models have been proposed, these still rely on industrial cooperation for centrifugal potting and end-face cutting [18].

We therefore developed a miniaturized oxygenator that can be constructed without industrial technical support, using a simple adhesive-based hollow fiber end fixation method and a case structure that allows partial removal for easy sampling of internal hollow fibers.

The purpose of this study was to determine whether this new miniaturized oxygenator enables quantitative SEM-based evaluation of a newly modified membrane designed to suppress blood cell adhesion. Using this model, we constructed oxygenators with modified and non-modified hollow fiber membranes, performed two-hour in-vitro circulation tests with identical human blood, systematically sampled hollow fibers after partial case removal, and quantitatively evaluated adhered cell counts by SEM image analysis.

## **MATERIALS AND METHODS**

### **Concept of the Design of a Small Research Oxygenator Model**

The design concept of the new oxygenator had two main features:

(1) Construction of an ultra-small oxygenator with one-quarter the membrane area ( $0.01\text{m}^2$ ) of a rat oxygenator ( $0.04\text{m}^2$ ) without industrial technical support [19]; and (2) use of a highly transparent PMMA case that allows visual inspection and partial disassembly to enable sampling of hollow fibers from arbitrary internal locations.

To achieve (1), we selected an adhesive injection method in which a dispenser nozzle is inserted between hollow fiber bundles to apply adhesive. Because fiber mats used in current clinical oxygenators have very narrow fiber spacing that prevents nozzle insertion, we instead used spool-type hollow fiber membranes, cut to a specified length and aligned in parallel.

To facilitate fiber alignment and prevent downward adhesive flow, we designed and developed a holding disc with 169 small holes (perforated disc) (Supplementary Fig. 1). These discs were placed at both ends of the

oxygenator, and 169 hollow fibers were passed through the holes and fixed by adhesive application on the disc surface.

To achieve (2), the oxygenator case was divided into four parts assembled with screws. Wide joining surfaces and external adhesive sealing were used to prevent blood leakage while still allowing later partial disassembly (Fig. 1A).

## **Application and Evaluation of the Newly Developed Membrane Surface Modification**

A newly developed surface modification designed to suppress platelet adhesion was applied to polypropylene hollow fiber membranes (OXYPHAN capillary membrane, 3M Membrana, Germany). The modification concept is based on controlling the adsorbed water of the membrane surface to reduce nonspecific interactions with blood components. Detailed chemical composition and fabrication processes are outside the scope of the present study.

The antithrombogenicity of the modified membrane surface was preliminarily evaluated using a human platelet-rich plasma (PRP) immersion test. Membrane samples were immersed in 1 ml of PRP for 15 minutes under platelet activation conditions, adding adenosine diphosphate (ADP) stimulation and centrifugation at 250 g, to assess the robustness of platelet adhesion suppression. Adhered platelets on the membrane surface were observed by scanning electron microscopy (SEM) and compared with those on non-modified membranes.

## **Construction of Membrane Oxygenators**

Polypropylene hollow fiber membrane (OD 380  $\mu\text{m}$ , ID 280  $\mu\text{m}$ , OXYPHAN capillary membrane, 3M Membrana, Germany) was cut into 65-mm lengths. Half of the fibers were modified by the aforementioned method, while the remaining fibers were left unmodified. A total of 169 fibers (modified and non-modified) were inserted through the perforations of two discs positioned at the upper and lower ends of the blood chamber under a surgical magnifying glass.

A dispenser nozzle was inserted aligned fiber rows to apply a light-curing acrylic adhesive (LOCTITE AA3924, Henkel, Germany; qualified under ISO 10993 protocol), which was cured by LED light irradiation. O-ring and disc were then placed at both ends of the oxygenator. All parts were fabricated from mirror-polished PMMA and secured with screws and sealed with adhesives (LOCTITE AA3951, AA3972).

The blood chamber dimensions were 18 mm in diameter and 50 mm in length, with a priming volume of 6.6 ml. Sixteen oxygenators were fabricated. Gas ports were sealed using the same light-curing adhesive. After washing the blood pathway with 1L of ultrapure water and drying, all oxygenators, circuit components, and thermocouple sensors were sterilized using ethylene oxide gas. Eight modified and eight non-modified oxygenators were prepared for comparative experiments.

# Comparative Study of Modified and Non-Modified Membrane Oxygenators

This study was reviewed and approved by the Medical Ethics Committee of the Shimane University Faculty of Medicine (approval No. 20200825-1). Written informed consent was obtained from eight healthy volunteers. Approximately 20 ml of blood was collected from each volunteer using a syringe containing 100 units of heparin. About 6 mL was separated for baseline laboratory testing, and the remaining blood was used to prime the oxygenator and the simulated circulation circuit. For each volunteer, blood collection was performed twice on separate days for testing with the non-modified and modified membrane oxygenators.

The oxygenators and PVC tubing were primed with saline solutions. A pair of bead-type K thermocouple sensors were inserted at the blood inlet and outlet and connected to a data logger. Immediately after blood priming, circulation was initiated at 10 mL/min using a roller pump (Reglo Digital Pump, Antilia Scientific, USA) in a closed-loop circuit. Room temperature was maintained at 26°C. Blood temperature at the oxygenator inlet was controlled at 36°C using a thermostatic bath. Oxygen gas flow was maintained at 0.1 L/min (Fig. 1B).

**B** Closed-loop *in vitro* circulation system used for the blood perfusion experiment. T/S denotes insertion site of a pair of thermocouple sensors and blood sampling. Eight modified and eight non-modified membrane oxygenators were comparatively evaluated using the same blood obtained from eight volunteers for both types of oxygenators.

In addition to activated coagulation time (ACT) and blood gas analysis, the following laboratory tests were performed at baseline and after 2 hours of circulation (SRL, Japan): blood cell count, TAT (thrombin-antithrombin complex), F1 + 2 (prothrombin fragment 1 + 2), IL-8,  $\beta$ -TG (beta-thromboglobulin), and PF-4 (platelet factor 4).

After 2 hours of perfusion, each oxygenator was rinsed with 1 L of gravity-fed normal saline until the effluent became clear. A fixative solution (2.5% glutaraldehyde) was then infused via the blood port and stored at 4°C for 3 hours. After replacement with washing buffer (0.1 mol/L phosphate buffer), the adhesive sealing the joint of one case component was carefully removed using a No. 10 scalpel. With the oxygenator immersed in phosphate buffer, the screws were removed and one case component was detached to expose the hollow fibers. Hollow fiber samples (15 mm length) were then collected (Fig. 2A, B).

Sampling sites comprised four regions: proximal to the blood inlet, distal from the blood inlet, proximal to the blood outlet, and distal from the blood outlet. From each region, two adjacent fibers were collected from defined layer depths (second-third layers and eleventh-twelfth layers, Fig. 2A).

Samples were dehydrated through graded ethanol (50%, 70%, 90%, 99.5%) followed by tert-butyl alcohol, then substituted, frozen, and vacuum-dried. Two hollow fiber samples were mounted per specimen stub and sputter-coated with a thin layer of gold (Eiko VX-10A Multicoater, Japan).

# Quantitative Analysis of Adhered Cells on Membranes from SEM Images

SEM observation was performed by using a JSM-6510 microscope (JEOL, Japan). Each sample was first scanned at x50 magnification over its full length to identify the region with the highest cell adhesion, followed by imaging at x4000, x2000(x1000), x400, and x150 magnifications. Because x400 was the lowest magnification at which platelet morphology could be reliably identified, subsequent quantitative analyses were performed using x400 images.

SEM analysis was conducted on samples obtained from eight non-modified and eight modified oxygenators, with two fibers sampled from each four locations per device, yielding a total of 128 hollow fiber samples. Counts from adjacent paired samples were averaged for analysis.

Cell counts from SEM images were quantified using ImageJ/FIJI [20, 21]. Images were converted 8-bit, thresholded using defined algorithms, and segmented. Particle quantification was performed using the “Analyze Particles” command according to ImageJ particle analysis workflows [21–24].

For platelet counting, the particle size threshold was set to  $\geq 20$  pixels to exclude smaller particles. The “Exclude on edge” option was enabled. To avoid analytical bias between comparison groups, the same threshold algorithm (Otsu or MaxEntropy) was applied consistently to both modified and non-modified membrane images. When clearly incorrect particle selection occurred, manual correction was performed.

Because “Analyze Particles” provides total particle counts but does not distinguish leukocytes, erythrocytes, and platelets, manual classification was performed using the ImageJ/FIJI plugin “Cell Counter” [21–23].

## Statistical Analysis

Variables with apparent skewness were summarized as median (interquartile range [IQR]), whereas non-skewed variables were expressed as mean  $\pm$  standard deviation (SD). For paired comparisons between two devices, paired t-tests were applied to non-skewed data, and Wilcoxon signed-rank exact tests were used for skewed data.

The validity of counts generated using the ImageJ/FIJI “Analyze Particles” function was evaluated by assessing agreement with manual counts obtained using the “Cell Counter” plugin [21–23]. After averaging values at each sample location, concordance was assessed on a logarithm scale using Spearman’s correlation, Kendall’s tau, and Lin’s concordance correlation coefficient (CCC) [25, 26]. The association between SEM-derived cell counts and laboratory parameters were evaluated using both Pearson and Spearman correlation coefficients, with Pearson correlation performed on log-transformed data. All statistical analyses were performed using R version 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided p value  $< 0.05$  was considered statistically significant.

## RESULTS

# Comparison of Platelet Adhesion Between Unmodified and Modified Membranes in Immersion Tests

In the PRP immersion test, platelet adhesion on the modified membranes was markedly reduced compared with that on the non-modified membranes (Fig. 3). Representative SEM images demonstrated rarely adhered platelets and aggregation on the modified membrane under ADP- and centrifugation-stimulated conditions.

These results indicate that the surface modification effectively suppressed platelet adhesion in a simplified *in vitro* setting.

## Comparison of Hematologic, Coagulation, and Inflammatory Markers

Blood samples were obtained twice from each of eight volunteers (3 men, 5 women, mean age 41.4 years) and used for both two-hours perfusion experiments with non-modified and modified membrane oxygenators.

At the end of perfusion, oxygenator inlet blood temperature was  $36.4 \pm 0.2^{\circ}\text{C}$  in the non-modified group and  $36.6 \pm 0.2^{\circ}\text{C}$  in the modified group, while outlet temperature was  $35.5 \pm 0.4^{\circ}\text{C}$  and  $35.6 \pm 0.5^{\circ}\text{C}$ , respectively, with no significant differences between groups ( $p = 0.1725$  and  $p = 0.3429$ ). Baseline ACT values were 414 (401, 633) s in the non-modified group and 426 (311, 718) s in the modified group ( $p = 1.0$ ). After perfusion, ACT values were 766 (495, 966) s and 977 (743, 1000) s, respectively, without significant difference ( $p = 0.158$ ). PaO<sub>2</sub> at the end of perfusion was  $562.8 \pm 56.6$  mmHg in the non-modified group and  $569.8 \pm 66.8$  mmHg in the modified group, without significant difference between groups ( $p = 0.8162$ ).

Hematologic, coagulation, platelet activation, inflammatory, and complement activation markers before and after perfusion, together with their changes ( $\Delta$  values) and  $p$  values, are summarized in Table 1. The decrease in hemoglobin after circulation was small but significantly greater in the modified membrane group ( $p = 0.04967$ ). Among coagulation markers, the increase in TAT was significantly smaller in the modified group ( $p = 0.0477$ ). F1 + 2 also showed a trend toward a smaller increase in the modified group ( $p = 0.1059$ ). No significant differences were observed for other markers.

| Table 1 Comparison of blood compatibility data between non-modified and modified membrane small oxygenators                                     |                    |                  |                    |                    |                    |                    |   |         |
|-------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|------------------|--------------------|--------------------|--------------------|--------------------|---|---------|
| Timing of sampling                                                                                                                              | Before circulation |                  | After circulation  |                    | Delta              |                    |   |         |
| Membrane type                                                                                                                                   | non-modified       | modified         | non-modified       | modified           | non-modified       | modified           | n | P-value |
| RBC (x104/ $\mu$ L)                                                                                                                             | 458.5 $\pm$ 29.7   | 460.9 $\pm$ 19.5 | 430.6 $\pm$ 31.5   | 411.7 $\pm$ 22.1   | 32 (25, 37.8)      | 40 (25, 63)        | 8 | 0.07969 |
| Hematocrit (%)                                                                                                                                  | 42.4 $\pm$ 4.1     | 42.3 $\pm$ 3.3   | 39.0 $\pm$ 3.9     | 37.6 $\pm$ 3.2     | 3.8 (2.9, 4.8)     | 3.9 (2.9, 5.8)     | 8 | 0.1953  |
| Hemoglobin (g/dL)                                                                                                                               | 13.5 $\pm$ 1.5     | 13.6 $\pm$ 1.3   | 12.7 $\pm$ 1.5     | 12.1 $\pm$ 1.2     | 0.9 (0.6, 1.2)     | 1.1 (0.8, 1.9)     | 8 | 0.04967 |
| Platelet (x104/ $\mu$ L)                                                                                                                        | 27.9 $\pm$ 8.4     | 29.0 $\pm$ 9.0   | 22.6 $\pm$ 7.1     | 24.7 $\pm$ 9.1     | 5.3 $\pm$ 2.6      | 4.3 $\pm$ 0.9      | 8 | 0.2134  |
| WBC (/ $\mu$ L)                                                                                                                                 | 5400 $\pm$ 676     | 5800 $\pm$ 460   | 2725 $\pm$ 792     | 3100 $\pm$ 644     | 2675 $\pm$ 962     | 2738 $\pm$ 619     | 8 | 0.7256  |
| TAT ( $\eta$ g/mL)                                                                                                                              | 2.2 (1.9, 3.3)     | 1.5 (1.2, 2.0)   | 32.0 $\pm$ 20.2    | 10.2 $\pm$ 4.3     | 27.5 $\pm$ 19.5    | 9.7 $\pm$ 4.0      | 8 | 0.04777 |
| F1 + 2 (pmol/L)                                                                                                                                 | 246 (114, 489)     | 146 (104, 235)   | 481 (286, 586)     | 182 (13, 236)      | 157 $\pm$ 146      | 33 $\pm$ 49        | 8 | 0.1059  |
| $\beta$ -TG ( $\eta$ g/mL)                                                                                                                      | 98.5 (62.3, 366.5) | 94 (75.5, 141)   | 1308.8 $\pm$ 632.3 | 1167.5 $\pm$ 287.4 | 1007.8 $\pm$ 459.2 | 954.2 $\pm$ 257.1  | 4 | 0.7128  |
| PF-4 ( $\eta$ g/mL)                                                                                                                             | 35.5(21.8, 171.5)  | 33.5 (22.3, 44)  | 632.8 $\pm$ 243.6  | 467.3 $\pm$ 65.1   | 490.0 $\pm$ 178.7  | 383.2 $\pm$ 54.9   | 4 | 0.2532  |
| Interleukin-8 (pg/mL)                                                                                                                           | 1.8 (0, 8.6)       | 0 (0, 10.9)      | 64.6 (17.2, 194.5) | 41.7 (5.4, 130.5)  | 59.7 (17.2, 168.0) | 41.7 (4.2, 120.8)  | 8 | 0.5469  |
| SC5b-9 ( $\eta$ g/mL)                                                                                                                           | 116.5 $\pm$ 28.6   | 117.4 $\pm$ 34.2 | 1195.2 $\pm$ 206.3 | 1173.9 $\pm$ 389.8 | 1078.7 $\pm$ 188.7 | 1092.0 $\pm$ 376.8 | 6 | 0.9373  |
| Data are presented as mean $\pm$ SD or median (IQR). Statistical analyses were performed by paired t-tests or Wilcoxon signed-rank exact tests. |                    |                  |                    |                    |                    |                    |   |         |
| TAT: thrombin-antithrombin complex; F1 + 2: prothrombin fragment 1 + 2; $\beta$ -TG: beta-thromboglobulin; PF-4: platelet factor 4.             |                    |                  |                    |                    |                    |                    |   |         |

## Comparison of Adhered Cell Counts by SEM Image Analysis

There was no macroscopic thrombus inside in all sixteen oxygenators. However, there were four samples with small thrombus (100–500  $\mu$ m) out of eight samples in one non-modified oxygenator. Therefore, these samples were excluded from SEM analysis.

In the modified oxygenator group, there was no thrombus inside oxygenator or on the membrane samples. A representative SEM images of hollow fiber membrane from a non-modified oxygenator after two hours of in-vitro perfusion are shown in Fig. 4A. Numerous activated platelets with pseudopod were scattered across the membrane surface, with a smaller number of erythrocytes and leukocytes also present.

Using the semi-automatic “Analyze Particles” command in ImageJ/FIJI, the adhered cell count in this field was 555 (Fig. 4B).

**B** Semi-automated particle detection of the same image using Image J/FIJI (“Analyze Particles” function); number of adhered cells = 555. The inset highlights platelets pairs incorrectly identified as a single particle.

However, when platelets were densely distributed, multiple platelets were sometimes counted as a single particle. Manual counting using the “Cell Counter” plugin yielded a count of 640 in the same image (Fig. 4C).

**C** Manual counting of the same image using the “Cell Counter” plugin; number of adhered cells = 640. Red arrows indicate platelet pairs misclassified by the semi-automated method.

Higher magnification images (x 2000) demonstrated activated platelet morphology with pseudopod formation and spreading, as well as clearly visible membrane pores (Fig. 4A).

In non-modified membranes, pronounced platelet adhesion occasionally resulted in the formation of platelet aggregates attached to the hollow fiber surface (Fig. 4D).

**D** Representative SEM image (x400) showing platelet aggregations on a non-modified membrane. Red arrows are the largest aggregation.

In such cases, semi-automatic analysis identified large aggregates as single particles, leading to underestimation (n = 360) (Fig. 4E).

**E** Semi-automated analysis of the same image using Image J/FIJI “Analyze particles” function; number of adhered cells = 360. The largest platelet aggregate indicated by the red arrow was identified as a single particle.

In contrast, Manual counting of the same image yielded a total of 1411 particles (Fig. 4F).

**F** Manual counting of the same image using the “Cell Counter” plugin; number of adhered cells = 1,411. Numerous platelets with the aggregate (red arrow) were individually counted.

In contrast, modified membranes showed markedly weaker cellular adhesion, with few platelets and occasional leukocytes (Fig. 5). At high magnification (x4000), membrane pores remained clearly visible (Fig. 5).

Systemic comparison using the “Analyze Particles” command demonstrated significantly fewer adhered cells on modified membranes at three of four sampling locations (Table 2). The modified devices reduced total adhered cell counts by approximately 80% ( $47.3 \pm 17.3$  vs  $228.7 \pm 116.8$ ;  $p = 0.00206$ ) compared with the non-modified devices. Manual counting using the “Cell Counter” plugin showed similar results, with

significantly fewer adhered cells on the modified membranes at three of four sites compared with the non-modified ones ( $64.2 \pm 29.8$  vs  $410.1 \pm 280.9$ ;  $p = 0.01942$ ).

Table 2

Comparison of total adhered cell counts\* on either modified membranes or non-modified ones; analyses by both "Analyze particles" and "Cell Counter" in Image J/FIJI

| Count method                                                                                                                                                                                                                                                                                                            | Cell counting by "Analyze particles" |                   |         | Cell counting by "Cell Counter" |                    |         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-------------------|---------|---------------------------------|--------------------|---------|
| Site of sample excision                                                                                                                                                                                                                                                                                                 | membrane type                        |                   | p-value | Membrane type                   |                    | p-value |
|                                                                                                                                                                                                                                                                                                                         | non-modified                         | modified          |         | non-modified                    | modified           |         |
| 1                                                                                                                                                                                                                                                                                                                       | 101.8 (82, 270.9)                    | 35.3 (15.5, 66.3) | 0.01563 | 127.5 (76.9, 512)               | 69.8 (21.5, 79.3)  | 0.02344 |
| 2                                                                                                                                                                                                                                                                                                                       | 176.3 (73.1, 347.3)                  | 34.8 (12.8, 59.1) | 0.07813 | 263.8 (81.6, 546.6)             | 38.5 (15.4, 91.3)  | 0.05469 |
| 3                                                                                                                                                                                                                                                                                                                       | $296.2 \pm 178.5$                    | $47.6 \pm 28.4$   | 0.0044  | $659.1 \pm 642.6$               | $58.4 \pm 30.3$    | 0.02966 |
| 4                                                                                                                                                                                                                                                                                                                       | $232 \pm 172.7$                      | $46.1 \pm 29.1$   | 0.0132  | 144 (452.2, 732.5)              | 47.5 (21.1, 109.6) | 0.04688 |
| mean of 1–4                                                                                                                                                                                                                                                                                                             | $228.7 \pm 116.8$                    | $47.3 \pm 17.3$   | 0.00206 | $410.1 \pm 280.9$               | $64.2 \pm 29.8$    | 0.01942 |
| Data are presented as mean $\pm$ SD or median (IQR). Statistical analyses were performed by paired t-tests or Wilcoxon signed-rank exact tests. "Analyze particles" function is a semi-automated segmentation and analysis tool and "Cell Counter" is a plugin tool which is useful for manual counting in ImageJ/FIJI. |                                      |                   |         |                                 |                    |         |

Manual platelet counting demonstrated significantly fewer adhered platelets on modified membranes at three of four sampling sites (Table 3). The modified devices reduced platelet adhesion counts by approximately 90% ( $34.1 \pm 21.1$  vs  $358.8 \pm 298.8$ ;  $p = 0.01394$ ) compared with the non-modified devices.

Table 3  
Comparison of adhered platelet counts on either modified membranes or non-modified ones.

| Site of sample excision                                                                                                                                                                                                                                                          | membrane type         |                   | p-value  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------------|----------|
|                                                                                                                                                                                                                                                                                  | non-modified          | modified          |          |
| 1                                                                                                                                                                                                                                                                                | 64.8 (28.1, 438.9)    | 15.3 (10.9, 31.9) | 0.007813 |
| 2                                                                                                                                                                                                                                                                                | 257.4 ± 245.6         | 32.3 ± 29.1       | 0.02995  |
| 3                                                                                                                                                                                                                                                                                | 274.8 (662.4, 1419.9) | 40.3 (14.1, 56.9) | 0.02344  |
| 4                                                                                                                                                                                                                                                                                | 27 (18.5, 718.5)      | 28 (9, 62.3)      | 0.07161  |
| mean of 1–4                                                                                                                                                                                                                                                                      | 358.8 ± 298.8         | 34.1 ± 21.1       | 0.01394  |
| Data are presented as mean ± SD or median (IQR). Statistical analyses were performed by paired t-tests or Wilcoxon signed-rank exact tests. Counting of adhered platelets on either non-modified membranes or modified ones was performed by "Cell Counter", ImageJ/FIJI plugin. |                       |                   |          |

## Agreement Between Semi-Automatic and Manual SEM Cell Count

To evaluate the validity of cell counts obtained using the semi-automated "Analyze Particles" function in the ImageJ/FIJI for quantifying cells adhered to membranes in SEM images, we compared these measurements with counts derived from the manual reference method using the ImageJ/FIJI "Cell Counter" plugin. Although four samples were excluded due to small thrombus, the counts of adhered cells were averaged over two fiber samples per each location in device, and thereby only one data point was excluded, leaving 63 data points in the subsequent analyses. After logarithmic transformation, Lin's concordance correlation coefficient (CCC) was 0.924 (95% CI: 0.885 to 0.95) (Fig. 6). In addition, Spearman's rank correlation coefficient was  $\rho = 0.916$  ( $p < 2.2 \times 10^{-16}$ ), and Kendall's rank correlation coefficient was  $\tau = 0.813$ , further demonstrating a strong concordance between the two counting methods.

## Correlation between SEM-derived platelet counts and laboratory markers

To investigate the association between morphological changes observed on SEM and alterations in hematological parameters, linear regression models were fitted with  $\Delta\beta$ -TG values and  $\Delta$ PF-4 as dependent variables and the number of platelets adhered to the membranes as the independent variable, all on a logarithmic scale. A total of ten observations were analyzed, comprising four paired and two unpaired data. Assuming that  $\Delta\beta$ -TG and  $\Delta$ PF-4 follow a log-normal distribution, conditional means (with arithmetic means adjusted by adding half of the residual variance) and 95% confidence intervals (CIs) were estimated and are presented in Fig. 7. These results demonstrate a clear non-linear relationship that adequately characterizes the associations.

A correlation between  $\Delta\beta$ -TG and platelet counts (SEM) was 0.870 (95%CI: 0.531 to 0.969) for Pearson and 0.867 ( $p = 0.00268$ ) for Spearman.  $\Delta$ PF-4 showed a comparably strong correlation: 0.899 (95%CI: 0.621 to

0.976) for Pearson and 0.830 ( $p = 0.00556$ ) for Spearman.

## DISCUSSION

We first developed a miniaturized oxygenator with a partially removable case. Using this model and a novel hollow fiber membrane modification technique, we evaluated a modified hollow fiber membrane oxygenator designed to reduce platelet adhesion and improve hemocompatibility. Using a controlled in-vitro circulation model with human blood, we demonstrated that the modified membrane significantly reduced platelet and total cell adhesion compared with the non-modified membrane.

To the best of our knowledge, this study is the first to successfully quantify the number of blood cells—particularly platelets—adhering to hollow fiber membranes in oxygenators. A previous animal study by Kundu and colleagues established a quantitative SEM-based method that measures “percent area densities” on the membranes in evaluating thrombosis in ECMO without anticoagulation [27]. Zang and coworker reported a protocol of quantitative analysis of clot deposition on oxygenator membranes using SEM images [17]. Both approaches quantified deposition area rather than absolute cell counts. Measurement of coverage area may be more informative than cell counting when platelet aggregation or spreading is extensive (see Fig. 4D, Fig. 4E, and Fig. 4F).

These prior studies evaluated deposits or clots across the entire membrane samples and were more comprehensive than our approach. Nevertheless, the method used in this study—selecting and analyzing only the most reactive region, not all length of sample—provides high sensitivity for detecting adhesion reactions while remaining relatively practical in terms of workload.

SEM observations, particularly at high magnification, revealed that platelets on non-modified membranes frequently exhibited activated morphology, including pseudopod formation, spreading, and aggregation. In contrast, modified membranes showed minimal platelet adhesion, and the membrane pore structure remained clearly visible even after perfusion. These morphological findings support the quantitative results and indicate improved surface biocompatibility.

Laboratory data further supported reduced thrombogenic activation with the modified membrane. In particular, the increase in TAT3 was significantly smaller in the modified group, and F1 + 2 showed a similar trend. Platelet activation markers ( $\beta$ -TG, PF-4) were strongly correlated with the number of platelets adhering to the hollow fiber membranes (Fig. 7). Both are chemokine proteins abundantly stored in platelet alpha granules and released upon platelet activation, thereby further promoting coagulation [28–30]. In other ex-vivo simulation models using human blood,  $\beta$ -TG elevation was similarly suppressed in PMEA-coated oxygenators and heparin-coated oxygenators but markedly increased in non-coated oxygenators [7, 16]. In the present study, no significant differences between groups were observed for  $\beta$ -TG, PF-4, likely due to the small sample size ( $N = 4$ ).

To evaluate the validity of cell counts obtained using the semi-automated approach (the “Analyze Particles” function) in ImageJ/FIJI, manual counting was defined as the reference standard [21–23], and agreement between the two methods was assessed using Lin’s concordance correlation coefficient (CCC), a statistical metric specifically developed to quantify concordance between a novel test method and a reference method.

Lin's CCC was 0.924 (95% CI: 0.885–0.95), indicating a high level of agreement [31–33]. Spearman's and Kendall's correlation analyses likewise demonstrated strong association with manual counting.

Therefore, although semi-automated analysis is useful for standardized comparative studies, complementary manual validation remains important, particularly in high-density regions. Manual counting using the Cell Counter plugin also enables discrimination among cell types, such as leukocytes and erythrocytes, but is labor intensive for large datasets. Watershed or adjustable watershed-based segmentation methods may facilitate separation of fused cells (platelets) [34]. However, in the present study, these approaches did not reliably separate tightly aggregated platelet clusters and occasionally introduced additional segmentation errors.

ImageJ/FIJI provides a machine learning plugin, Trainable Weka Segmentation, which enables ROI-based classification of erythrocytes, leukocytes, and background. With sufficient training data, a robust machine learning model can be developed [35]. We plan to investigate the applicability of this approach for classifying cell populations adhering to hollow fiber membranes.

The findings of this study may contribute to the development of a novel evaluation method for hollow fiber membrane modification in membrane oxygenators and suggest that direct analysis of the membrane surface may provide a more sensitive assessment than measurement of blood components alone. Because blood components can also be damaged by roller pumps [36], this effect may have obscured the benefits of membrane modification shown in blood-based assays.

Quantification of cells—particularly platelets—adhering to membranes following *in-vitro* circulation with human blood enables objective evaluation of modified membranes and, when combined with coagulation and inflammatory biomarkers, may support the establishment of a more comprehensive evaluation framework for membrane oxygenators. Upon contact with artificial surfaces, the contact activation system is initially triggered, generating factor  $\text{Xa}$ , active kallikrein, and bradykinin, thereby initiating coagulation and inflammation pathways [28, 37]. Prior to cellular adhesion to hollow fiber membranes, plasma proteins such as fibrinogen and von Willebrand factor are known to absorb onto the membrane surface [5, 12, 28, 37–39]. Further investigation of these processes on hollow fiber membranes will be necessary to advance hemocompatibility.

This study has several limitations. First, the circulation model was *in vitro* and employed low flow conditions compared with clinical extracorporeal circulation. Second, the sample size was limited. Further *in-vivo* and high-flow investigations will be necessary to confirm clinical relevance.

Despite these limitations, the combined approach of controlled circulation testing, SEM surface analysis, and quantitative image processing provides a practical and reproducible framework for evaluating membrane surface modifications of oxygenators.

The use of a miniaturized oxygenator with a removable case enabled direct sampling of hollow fiber membranes and quantitative SEM image analysis. This approach clearly demonstrated a substantial reduction in platelet adhesion on the newly modified membrane and may serve as a useful method for

evaluating membrane surface modifications. Reduced surface adhesion was associated with lower coagulation activation markers, supporting improved hemocompatibility of the modified membrane.

## Declarations

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**Ethics Statement** This study was reviewed and approved by the Medical Ethics Committee of the Shimane University Faculty of Medicine (approval No. 20200825-1). Written informed consent was obtained from eight healthy volunteers.

**Data Availability** Data supporting the findings of this study are available from the corresponding author on reasonable request.

**Competing interests** The authors have no conflicts of interest to report.

## References

1. Brodie D, Bacchetta M (2011) Extracorporeal membrane oxygenation for ARDS in adults. *N Engl J Med* 365(20):1905–1914
2. Villalba CAF, McMullan DM, Reed RC, Chandler WL (2022) Thrombosis in extracorporeal membrane oxygenation (ECMO) circuits. *ASAIO J* 68(8):1083–1092. <https://doi.org/10.1097/mat.0000000000001605>
3. Staessens S, Moussa MD, Pierache A, Rauch A, Rousse N, Boulleaux E, Ung A, Desender L, Pradines B, Vincentelli A, Mercier O, Labreuche J, Duhamel A, Van Belle E, Vincent F, Dupont A, Vanhoorelbeke K, Corseaux D, De Meyer SF, Sus S (2022) Thrombus formation during ECMO: Insights from a detailed histological analysis of thrombus composition. *J Thromb Haemost* 20(9):2058–2069. <https://doi.org/10.1111/jth.15784>
4. Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD (2007) The role of complement in biomaterial-induced inflammation. *Mol Immunol* 44(1–3):82–94. <https://doi.org/10.1016/j.molimm.2006.06.020>
5. Millar JE, Fanning JP, McDonald CI, McAuley DF, Fraser JF (2016) The inflammatory response to extracorporeal membrane oxygenation (ECMO): a review of the pathophysiology. *Crit Care* 20:387
6. De Somer F, Van Belleghem Y, Caes F, Francois K, Arnout J, Bossuyt X, Taeymans Y, Van Nooten G (2002) Phosphorylcholine coating offers natural platelet preservation during cardiopulmonary bypass.

- Perfusion 17(1):39–44. <https://doi.org/10.1191/0267659102pf526oa>
7. Zimmermann AK, Aebert H, Reiz A, Freitag M, Hussein M, Ziemer G, Wendel HP (2004) Hemocompatibility of PMEA coated oxygenators used for extracorporeal circulation procedures. *ASAIO J* 50(3):193–199
  8. Gravlee GP, Davis RF, Stammers AH, Ungerleider RM (eds) (2008) *Cardiopulmonary bypass, principles and practice*. Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo: Wolters Kluwer, Lippincott Williams & Wilkins, Philadelphia
  9. McMichael ABV, Ryerson LM, Ratano D, Fan E, Faraoni D, Annich GM (2022) 2021 ELSO adult and pediatric anticoagulation guidelines. *ASAIO Journal* 68(3):303–310
  10. Suhara H, Sawa Y, Nishimura M, Oshiyama H, Eng B, Yokoyama K, Saito N, Matsuda H (2001) Efficacy of a new coating material, PEMA, for cardiopulmonary bypass circuits in a porcine model. *Ann Thorac Surg* 71:1603–1608
  11. Suehiro S, Shimizu K, Imai K, Nii A, Akeho K, Nataka H, Yamaguchi A, Matsumoto K, Oda T (2017) Polymer-coated cardiopulmonary bypass circuit attenuates upregulation of both proteases/protease inhibitors and platelet degranulation in pigs. *Perfusion* 32(8):645–655. <https://doi.org/10.1177/0267659117715506>
  12. Callaghan S, Cai T, McCafferty C, Van Den Helm S, Horton S, MacLaren G, Monagle P, Ignjatovic V (2020) Adsorption of blood components to extracorporeal membrane oxygenation (ECMO) surfaces in humans: a systemic review. *J Clin Med* 9(10):3272
  13. Beely BM, Campbell JE, Meyer A, Langer T, Neggard K, Chung KK, Cap AP, Cancio LC, Batchinsky AI (2016) Electron microscopy as a tool for assessment of anticoagulation strategies during extracorporeal life support: the proof is on the membrane. *ASAIO Journal* 62(5):525–532
  14. Roberts TR, Harea GT, Singha P, Sieck KN, Beely BM, Wendorff DS, Choi JH, Ande S, Handa H, Batchinsky AI (2020) Heparin-free extracorporeal life support using tethered liquid perfluorocarbon: A feasibility and efficacy study. *ASAIO J* 66(7):809–817
  15. ISO ISO 7199:2024(en) Cardiovascular implants and artificial organs— Blood-gas exchangers (oxygenators). <https://www.iso.org/standard/83184.html>. Accessed at 2026/5/1
  16. Teligui L, Dalmayrac E, Corbeau JJ, Bouquet E, Godon A, Denomme AS, Binuani P, Verron L, Boer C, Baufreton C (2016) Ex vivo simulation of cardiopulmonary bypass with human blood for hemocompatibility testing. *Perfusion* 31(5):376–383. <https://doi.org/10.1177/0267659115599454>
  17. Zang Y, Roberts TR, Harea GT, Beely BM, Perez L, Ande S, Batchinsky M, Lee JH, Thraikill M, Reynolds MM, Batchinsky AI (2023) Quantitative analysis of clot deposition on extracorporeal life support membrane oxygenators using digital and scanning electron microscopy imaging techniques. *Bio-protocol* 13(18): e4814. 10.21769/Bioprotoc,4814, <https://creativecommons.org/licenses/by-nc/4.0/>
  18. Arens J, Schraven L, Kaesler A, Flege C, Schmitz-Rode T, Rossaint R, Steinseifer U, Kopp R (2023) Development and evaluation of a variable, miniaturized oxygenator for various test methods. *Artif Organs* 47(4):695–704. <https://doi.org/10.1111/aor.14465>

19. Cresce GD, Walpoth BH, Mugnai D, Innocente F, Rungatscher A, Luciani GB, Zaniboni A, Battistuzzi G, Tessari M, Kalangos A, Mazzucco A, Faggian G (2008) Validation of a rat model of cardiopulmonary bypass with a new miniaturized hollow fiber oxygenator. *ASAIO J* 54(5):514–518
20. Schindelin J, Arganda-Carreras I, Frise E, Kaying V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji - an open-source platform for biological image analysis. *Nat Methods* 9(7):676–682
21. Roels J, Vernailen F, Kremer A, Gonçalves A, Aelterman J, Luong HQ, Goossens B, Philips W, Lippens S, Saeys Y An interactive ImageJ plugin for semi-automated image denoising in electron microscopy. *Nat Commun* 11:7712020. <https://doi.org/10.1038/s41467-020-14529-0>
22. Siritantikorn S, Jintaworn S, Noisakran S, Suputtamongkol Y, Paris D, Blacksell SD (2012) Application of ImageJ program to the enumeration of *Orientia tsutsugamushi* organisms cultured in vitro. *Trans R Soc Trop Med Hyg* 106:632–635
23. Grishagin IV (2015) Automatic cell counting with ImageJ. *Anal Biochem* 473:63–65. <https://doi.org/10.1016/j.ab.2014.12.007>
24. Image J, Docs P, Analysis Automatic Particle counting. [https://imagej.net/imaging/particle-analysis?utm\\_source=chatgpt.com](https://imagej.net/imaging/particle-analysis?utm_source=chatgpt.com), accessed at 2026/5/1
25. Lin LI (1989) A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45(1):255–268. <https://doi.org/10.2307/2532051>
26. Ma Y (2012) On inference for Kendall's  $\tau$  within a longitudinal data setting. *J Applied Statistics* 39(11):2441–2452. <https://doi.org/10.1080/02664763.2012.712954>
27. Kundu SK, Salley SO, Whittlesey GC, Klein MD (1989) Extracorporeal membrane oxygenation without anticoagulation: a study using quantitative scanning electron microscopy. *J Lab Clin Med* 114:58–62
28. Weber M, Steinle H, Golombek S, Hann L, Schlensak C, Wendel HP, Avci-Adali M (2018) Blood-contacting biomaterials: In vitro evaluation of the hemocompatibility. *Front Bioeng Biotech* 6. :Article 99, <https://doi.org/10.3389/fbioe.2018.00099>
29. Reed G (2004) Platelet secretory mechanisms. *Semin Thromb Hemost* 30(4):441–450
30. Egan K, van Geffen JP, Ma H, Kevane B, Lennon A, Allen S, Neary E, Parsons M, Maguire P, Wynne K, O'Kennedy R, Heemskerk JWM, Ainle FN (2017) Effect of platelet-derived  $\beta$ -thromboglobulins on coagulation. *Thromb Res* 154:7–15. <https://doi.org/10.1016/j.thromres.2017.03.023>
31. McBride GB A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. *NIWA Client Rep HAM* 2005–2062
32. Maier A, Koc Y, Steinfurth L, Kettemann D, Norden J, Riitano A, Schmitt P, Kolzarek F, Subramanian S, Munch C, Spittel S, Meyer T (2026) Agreement between the harmonized and the self-explanatory versions of the revised ALS functional rating scale in a clinical setting. *Muscle Nerve* 73(2):250–259. <https://doi.org/10.1002/mus.70092>
33. Ohara K, Nakamura H, Kouda K, Fujita Y, Mase T, Momoi K, Nishiyama T (2023) Similarities and discrepancies between commercially available bioelectrical impedance analysis system and dual-energy X-ray absorptiometry for body composition assessment in 10-14-year-old children. *Sci Rep* 13:17420

34. ImageJ user guide Process, Watershed. ImageJ User Guide - IJ 1.46r | Process Menu, accessed at 2026/3/15
35. Arganda-Carreras I, Kaynig V, Rueden C, Eliceiri KW, Schindelin J, Cardona A, Seung HS (2017) Trainable Weka segmentation: a machine learning tool for microscopy pixel classification. *Bioinformatics* 33(15):2424–2426. <https://doi.org/10.1093/bioinformatics/btx180>
36. Van Oeveren W, Tiellu IF, de Hart J (2012) Comparison of modified Chandler, roller pump, and ball valve circulation models for in vitro testing in high blood flow conditions: application in thrombogenicity testing of different materials for vascular applications. *Int J Biomater* 2012 Article ID 673163:7
37. De Mel A, Cousins BG, Seifalian AM (2012) Surface modification of biomaterials: a quest for blood compatibility. *Int J Biomater* 2012 Article ID 707863:8
38. Steiger T, Foltan M, Phillipp A, Mueller T, Gruber M, Bredthauer A, Krenkel L, Birkenmaier C, Lehle K (2019) Accumulations of von Willebrand factor within ECMO oxygenators: potential indicator of coagulation abnormalities in critically ill patients? *Artif Organs* 43(11):1065–1076
39. Saito N, Motoyama S, Sawamoto J (2000) Effects of new polymer-coated extracorporeal circuits on biocompatibility during cardiopulmonary bypass. *Artif Organs* 24(7):547–554. <https://doi.org/10.1046/j.1525-1594.2000.06520.x>

## Figures

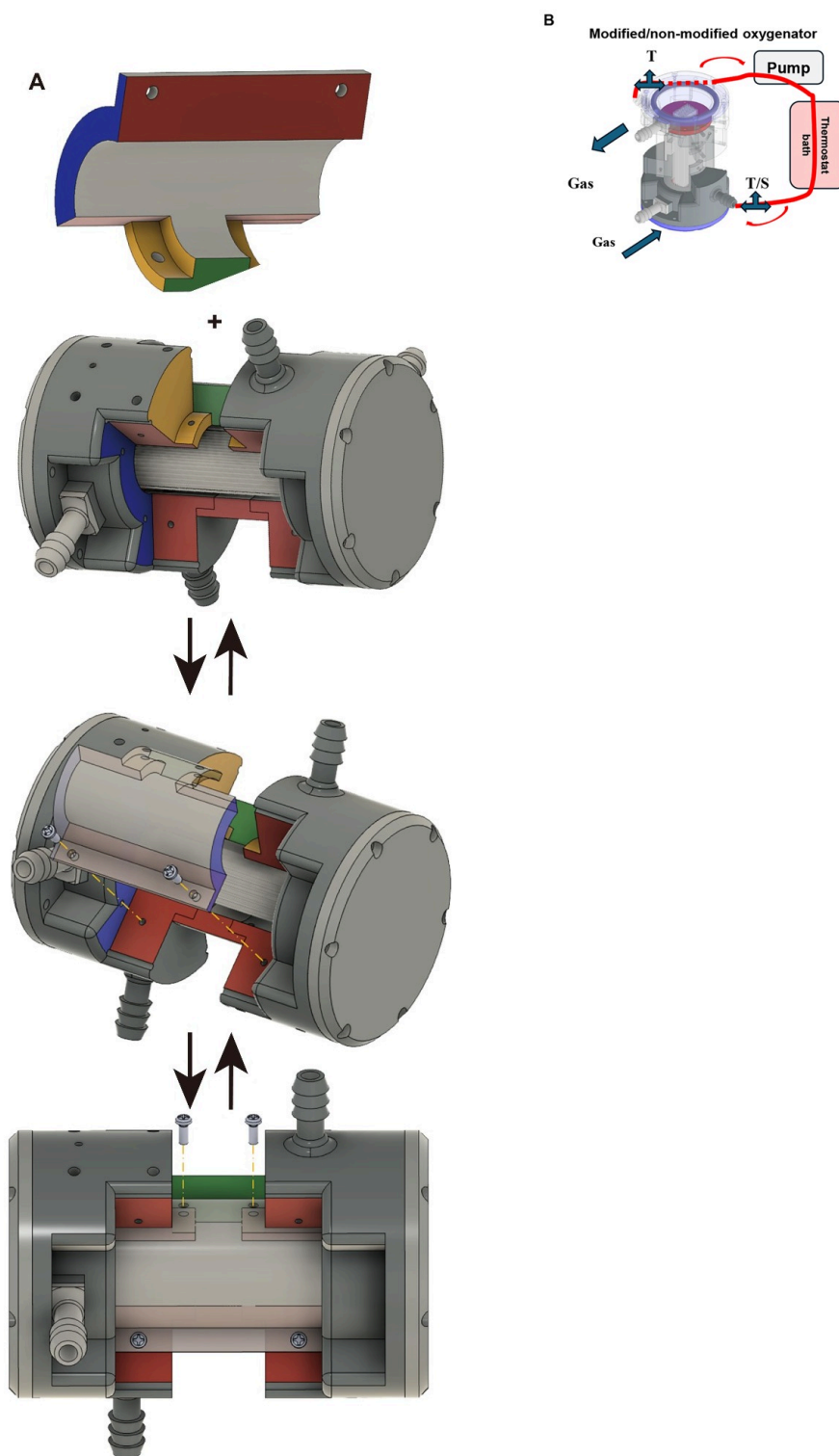


**Figure 1**

Three-dimensional design of the newly developed miniaturized oxygenator and experimental setup. **A** Three-dimensional schematic of the membrane oxygenator with a partially removal case. The anterior half of the case can be detached or reattached by removing the screws and adhesive. Color coding indicates the mating surfaces between components.

**B** Closed-loop *in vitro* circulation system used for the blood perfusion experiment. T/S denotes insertion site of a pair of thermocouple sensors and blood sampling. Eight modified and eight non-modified membrane oxygenators were comparatively evaluated using the same blood obtained from eight volunteers for both types of oxygenators.

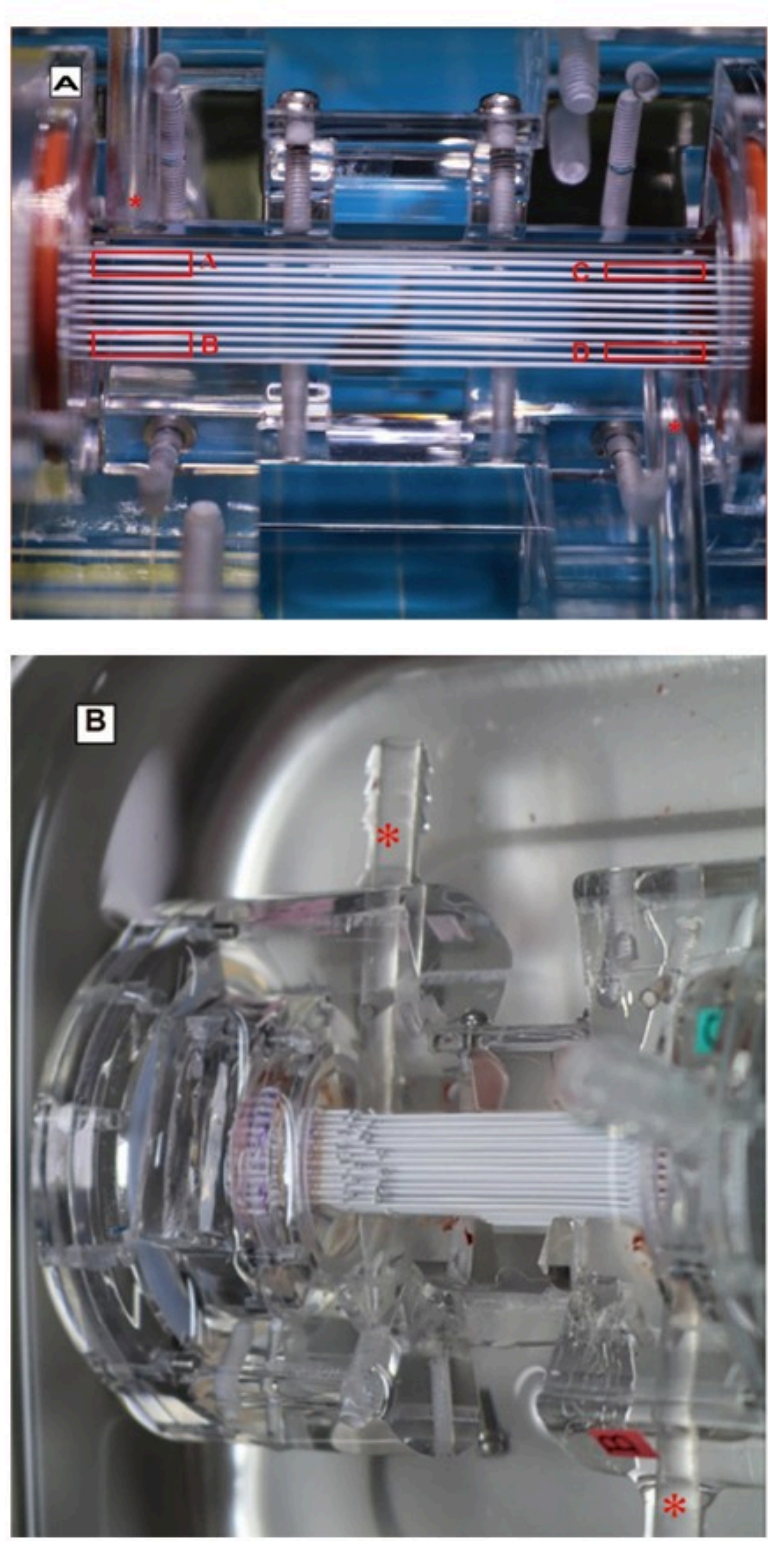


**Figure 2**

Sampling method for SEM analysis. **A** Definition of sampling locations and layers depths within the fiber bundle. Asterisks indicate the blood inflow and outflow regions; A–D denote sampling sites. **B** Representative photo of hollow fibers sampling. The front case was already detached. No thrombus formation was observed within the oxygenator after 2 h of closed-loop *in vitro* circulation.

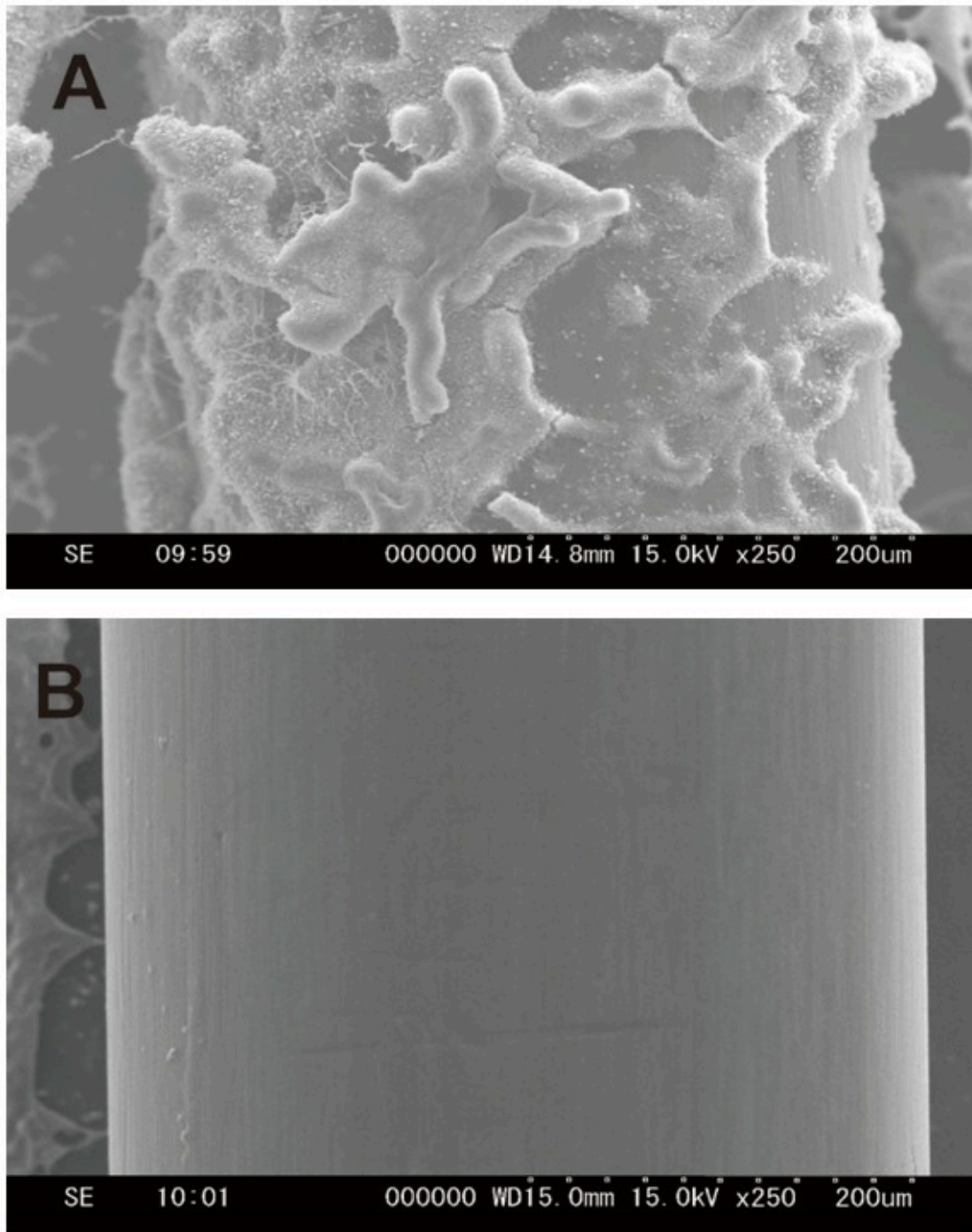


**Figure 3**

Evaluation of the newly developed membrane surface modification in immersion tests. **A** SEM image of non-modified membrane (OXYPHAN) demonstrating pronounced platelet adhesion. **B** SEM image of a modified membrane showing minimal platelet adhesion.

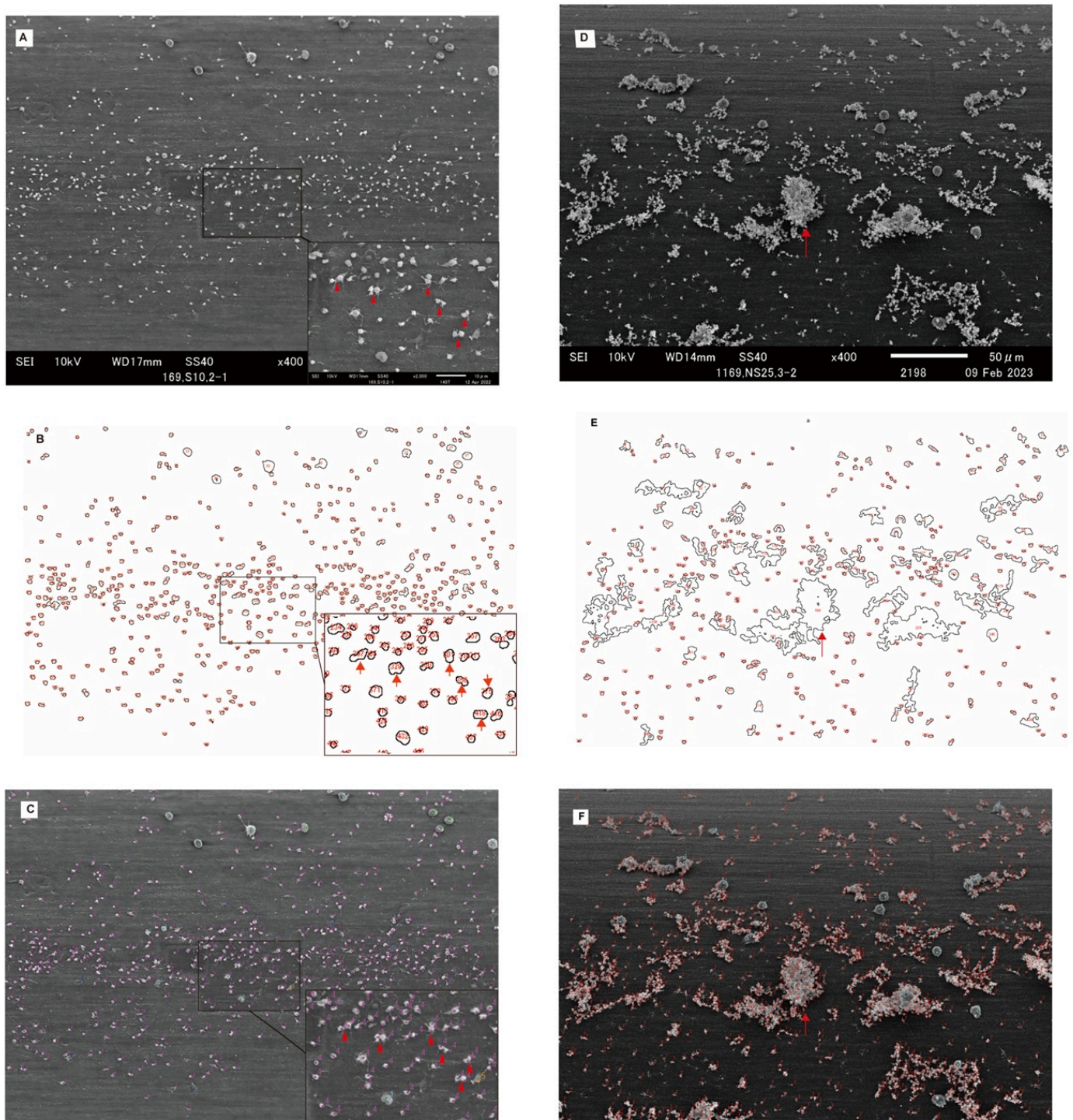


**Figure 4**

Representative SEM images on a non-modified membrane and ImageJ analysis workflow. **A** SEM image (x400) of blood cells adhered to a non-modified membrane following perfusion. The higher magnification inset (x2000) shows numerous activated platelets with pseudopods distributed across the membrane surface. Red arrows indicate platelets pairs incorrectly identified as a single particle by the "Analyze particle" function (see panels **B** and **C**)

- B** Semi-automated particle detection of the same image using Image J/FIJI (“Analyze Particles” function); number of adhered cells = 555. The inset highlights platelets pairs incorrectly identified as a single particle.
- C** Manual counting of the same image using the “Cell Counter” plugin; number of adhered cells = 640. Red arrows indicate platelet pairs misclassified by the semi-automated method.
- D** Representative SEM image (x400) showing platelet aggregations on a non-modified membrane. Red arrows are the largest aggregation.
- E** Semi-automated analysis of the same image using Image J/FIJI “Analyze particles” function; number of adhered cells = 360. The largest platelet aggregate indicated by the red arrow was identified as a single particle.
- F** Manual counting of the same image using the “Cell Counter” plugin; number of adhered cells = 1,411. Numerous platelets with the aggregate (red arrow) were individually counted.

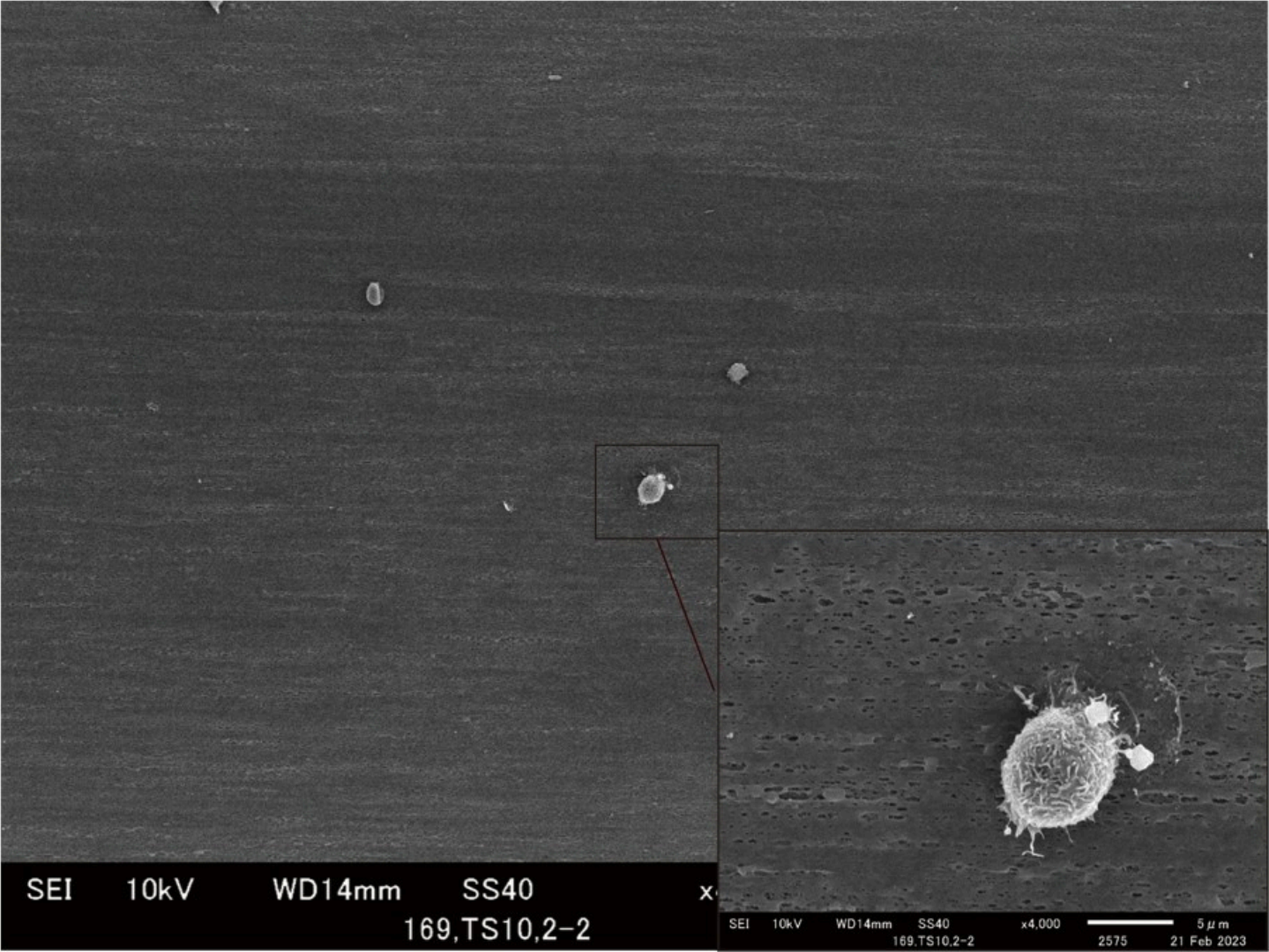


**Figure 5**

Representative SEM images (x400, x4000) on a modified membrane after perfusion and ImageJ analysis workflow. Adhered cells were quantified using the semi-automated “Analyze particles” function in Image J/FIJI; number of adhered cells = 10. The higher magnification inset (x4000) demonstrates that membrane pores remain patent after 2 h of *in-vitro* circulation.

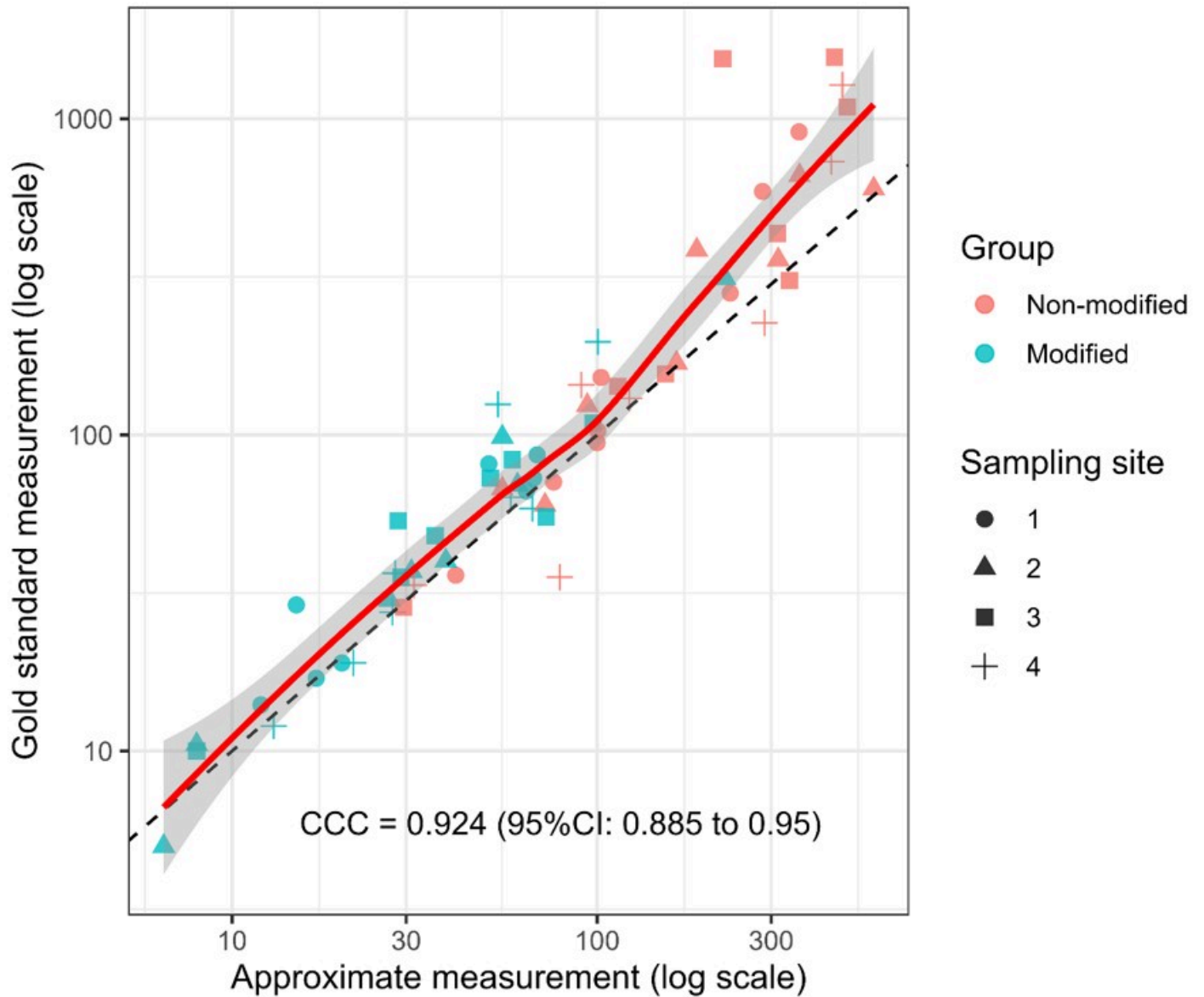


Figure 6

Lin’s concordance correlation coefficient (CCC) comparing cell counts obtained by “Analyze particles” function (approximate measurement) and the “Cell Counter” plugin (manual counting: reference standard).

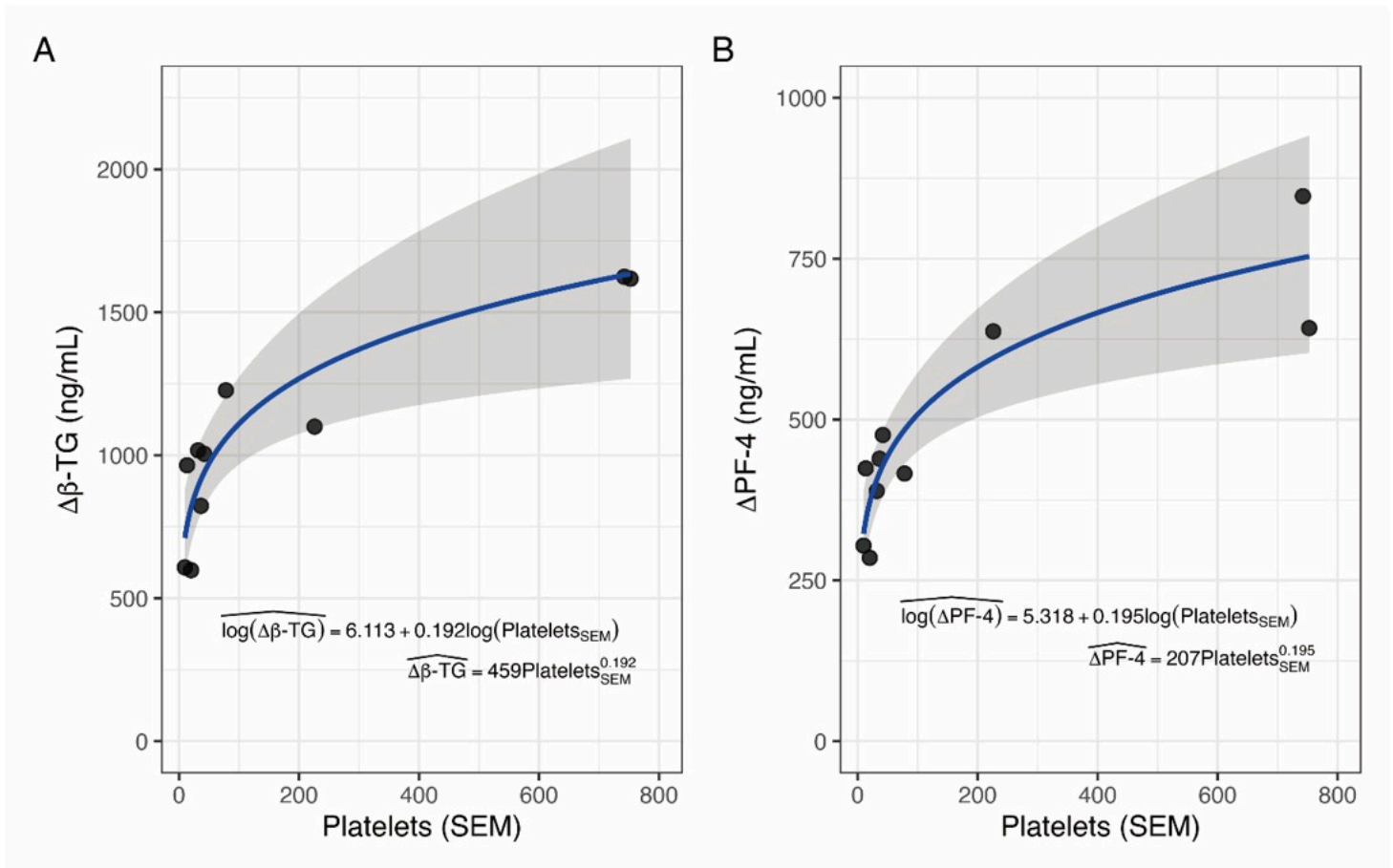


**Figure 7**

Correlation between SEM-derived platelet counts and laboratory markers. Conditional means and 95% CIs are shown. **A** Correlation between adhered platelet counts on membranes (SEM) and changes ( $\Delta$ ) in blood  $\beta$ -TG levels. **B** Correlation between adhered platelet counts on membranes (SEM) and changes ( $\Delta$ ) in blood PF-4 levels.

## Supplementary Files

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- [SupplementalFig.1.tif](#)