Alkalemic conditions result in blood clotting in the circuit soon after initiating cardiopulmonary bypass

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Abstract

Purpose: This study investigated the relationship between blood clotting in the circuit soon after initiating cardiopulmonary bypass (CPB) and echinocytes that appear with alkalemia, using a recirculation circuit filled with heparinized bovine blood.

Methods: Alkalemic conditions in the recirculation circuit were prepared by adding various concentrations of $NaHCO_3$ to the priming fluid. Albumin was also added to confirm its inhibitory effect on blood clotting. Blood pH, hold-up, the pressure gradient, and red blood cell (RBC) reduction rate were monitored. Blood clots were examined microscopically.

Results: Although blood pH was elevated under all experimental conditions, clotting in the circuit increased with increased concentrations of HCO_3^-. Albumin inhibited the clotting under the same alkalemic conditions. Microscopic findings revealed echinocytes in the blood clots.

Conclusions: The shape of echinocytes was transformed by a reduction in the Donnan

equilibrium ratio because of changes in pH inside and outside the RBC membrane. Blood clotting in the circuit soon after initiating CPB may be caused by echinocytes that appear under alkalemic conditions. This was inhibited by albumin, suggesting that the addition of albumin to the priming fluid may prevent such clotting in the circuit after initiating CPB.

Keywords: alkalemic conditions, blood clotting, echinocyte, Donnan equilibrium ratio, albumin

INTRODUCTION

Blood clotting has occasionally been reported in the cardiopulmonary bypass (CPB) circuit soon after initiating CPB despite adequate anticoagulation management with heparin.[1-7] This can cause an obstruction in the venous reservoir filter and membrane oxygenator, and replacement of these parts may become necessary during surgery. Two theories have been proposed to explain this clotting in the circuit: platelet aggregation/thrombus formation[2-5] and agglutination of the echinocytes that appear with alkalemia.[6,7] However, the underlying mechanisms have yet to be elucidated.

Prior to the initiation of CPB, priming fluid is re-circulated to evacuate any air from the recirculation circuit and to prevent a reduction in temperature. However, oxygen insufflation and the addition of sodium bicarbonate to the priming fluid may result in extreme alkalization.

It has been postulated that blood clotting in the circuit soon after initiating CPB is caused by echinocytes that form as a result of contact between the alkalized priming fluid and blood. We have previously investigated *in vitro* the frequency of echinocytes, platelet aggregation, and blood coagulation abilities under alkalemic conditions,[8,9] demonstrating that the frequency of echinocytes increased under such conditions.[8] In contrast, platelet aggregation and blood coagulation were not enhanced under these conditions.[9] We also confirmed that the presence of albumin under alkalemic conditions inhibited the appearance of echinocytes. This suggested that blood clotting in the circuit soon after initiating CPB could be prevented with albumin.[8]

The aim of the present study was to confirm these previous findings by investigating the relationship between various alkalemic conditions and the

appearance of clotting in the circuit during CPB, using a recirculation circuit filled with heparinized bovine blood.

MATERIALS AND METHODS

An experimental recirculation circuit was prepared with a venous reservoir (OXIA RE40; JMS, Hiroshima, Japan), a roller pump (Caps Roller Pump; Stockert GmbH, Freiburg, Germany), and a membrane oxygenator (OXIA LP; JMS, Hiroshima, Japan) (Fig.1).

Fig.1

The priming fluid was prepared with a 7% $NaHCO_3$ solution (MEYLON Injection 7%, Otsuka Pharmaceuticals, Tokyo, Japan), 25% albumin solution (Alb; Cohn Fraction V, pH 7.0; Wako, Osaka, Japan; dissolved in saline and the final concentration adjusted to 25%), and lactated Ringer's solution (Lactec Injection, Otsuka Pharmaceutical, Tokyo, Japan). The experiment was performed under six different alkaline conditions involving different amounts of the priming liquid in a total volume of 1,000 mL were examined (Table 1).

Table.1

To confirm whether any increase of blood clotting in the circuit was because of the appearance of echinocytes under alkalemic conditions, and to reduce the clotting, albumin was added under alkalemic conditions. Three sets involved different volumes of $NaHCO_3$: no addition (control), 100 mL ($NaHCO_3$ 100), and 200 mL ($NaHCO_3$ 200). Three further sets included different volumes of albumin (50 mL (Alb 50), 100 mL (Alb 100), and 200 mL (Alb 200)) after the addition of 100 mL $NaHCO_3$, resulting in final albumin concentrations of 1.6, 2.6, and 4.1 g/dL, respectively. These were combined with lactated Ringer's solution to adjust the priming fluid volume to a total of 1,000 mL.

For each of the six conditions, the priming liquid was added into the circuit,

which was then stabilized at a blood flow of 2 L/min at a 37° C solution temperature for 5 min. Subsequently, 400 mL of heparinized bovine blood (with the heparin isolated from the intestinal mucosa of pig at a concentration of 15,000 IU/L), stored overnight at 4° C after blood collection, was circulated under each condition. At the same time, oxygen insufflation of the membrane oxygenator was started at 1 L/min.

After initiating CPB, the following items were measured for a 120-min recirculation period (or for 60 min in the experiment with $NaHCO_3$ 200).

1. CPB Parameters

1) Blood pH

Because blood pH changes with the addition of $NaHCO_3$ to the priming liquid and oxygen insufflation of the membrane oxygenator, it was measured every 30 min using a pH meter (F-55; HORIBA, Kyoto, Japan).

2) Hold up and pressure gradient

To evaluate the grade of blood clotting in the circuit, the difference in the liquid level (the "hold up," HU) (Fig.2) between inside and outside of the venous reservoir filter was measured every 15 min (Fig.1), as was the pressure gradient (PG) across the membrane oxygenator. HU was measured in mm using a ruler. PG was measured using a digital pressure sensor (AP-34A; KEYENCE, Osaka, Japan), and the units were converted from kPa to mmHg. The PG (mmHg/L) per 1 L of flow was also calculated.

Fig.2

3) Red blood cell reduction rate

To confirm the protective effects of albumin on red blood cells (RBCs), the RBCs were counted every 30 min, and the percentage reduction from the initial count was calculated (RBC reduction rate; RRR). The RBC count was measured using an automated multichannel blood cell analyzer (XT-1800i, Sysmex, Kobe, Japan).

2. Albumin concentration

To confirm that albumin reduced the grade of blood clotting in the circuit under alkalemic conditions, albumin concentrations (g/dL) were measured at the initiation of CPB using a microplate reader (Infinite M200; Tecan Austria GmbH, Grödig, Austria).

3. Prothrombin time, activated partial thromboplastin time, and soluble fibrin monomer complex

To investigate whether the blood clot in the circuit was caused by echinocytes, the prothrombin time (PT; s), activated partial thromboplastin time (APTT; s), and soluble fibrin monomer complex (SFMC; μg/mL) were measured at the initiation and completion of circulation using an automated blood coagulation analyzer (Coapresta 2000, SEKISUI MEDICAL, Tokyo, Japan).

4. Electron microscopic analysis

The membrane oxygenator surface that resulted in a PG because of a blood clot in the circuit under alkalemic conditions (100 mL $NaHCO_3$, 400 mL lactated Ringer's solution, and 200 mL heparinized bovine blood) was analyzed using scanning electron photomicrography. The membrane oxygenator was fixed with 2.5% glutaraldehyde for 1 h after washing with saline and the sample was analyzed

using a SEMEDX Type N scanning electron microscope (Hitachi, Tokyo, Japan).

5. Statistical analysis

The aim of this study was to confirm the *in vitro* findings of a previous study. Accordingly, this was a single-run experiment, and the effects of the control, $NaHCO_3$ 100, $NaHCO_3$ 200, Alb 50, Alb 100, and Alb 200 conditions on blood pH, HU, PG, and RRR were analyzed using comparisons of two regression slopes. These analyses were performed using Microsoft Excel, and a P value of <0.05 was considered statistically significant.[10,11)]

RESULTS

The results for blood pH, HU, PG, and RRR are shown in Fig.3. Because of the alkaline conditions, the blood pH slope increased with time across all six conditions (control, $NaHCO_3$ 100, $NaHCO_3$ 200, Alb 50, Alb 100, and Alb 200), but there were no significant differences in their slopes ($P > 0.05$). The blood pH of the control condition only increased to 8.29, but the pH increased above 9.0 in the other five conditions (Fig.3a).

Fig.3

There were significant differences between the HU slopes of all six conditions ($P < 0.05$) (Fig.3b). Notably, increasing albumin concentration reduced the HU. In the $NaHCO_3$ 200 experiment, a circulation of 2 L/min could not be maintained due to the increase in HU, and the experiment was terminated after only 60 min.

PG did not differ significantly between the three albumin concentrations (Alb 50, Alb 100, and Alb 200), but there were significant differences with the other three conditions ($P < 0.05$) (Fig.3c).

The RRR results for both $NaHCO_3$ 100 and $NaHCO_3$ 200 differed significantly from

those of all the other five conditions ($P < 0.05$); however, there were no significant differences between the RRR results for the control and Alb 50, Alb 100, and Alb 200 conditions (Fig.3d).

The albumin concentration was 0.6 g/dL in the control, $NaHCO_3$ 100, and $NaHCO_3$ 200 conditions, 1.6 g/dL in Alb 50, 2.6 g/dL in Alb 100, and 4.1 g/dL in Alb 200.

PT and APTT were prolonged at a level higher than the detection limit (PT >100 s; APTT > 300 s). SFMC was 0.4 μg/mL or lower for all conditions, indicating that the blood's coagulation ability was not enhanced.

On electron microscopy, several echinocytes were observed on the membrane oxygenator surface (Fig.4).

Fig.4

DISCUSSION

Blood does not readily coagulate during CPB because of the application of continuous anticoagulant therapy. However, if a blood clot occurs, the venous reservoir filter and membrane oxygenator can become obstructed, and it may become necessary to replace parts during surgery,[1,4] although a blood clot in the circuit that appears soon after initiating CPB may reversibly disappear with time.[4]

In this study, we hypothesized that blood clotting in the circuit may be caused by echinocytes that form because of alkalization of the priming fluid, as previously reported.[6] We therefore investigated blood clotting in the circuit soon after initiating CPB under six different priming conditions.

In the control condition, there was no $NaHCO_3$ but the pH was elevated to 8.29 by oxygen insufflation of the membrane oxygenator alone. A blood clot did not

form in the circuit in this condition. This suggests that, without $NaHCO_3$ in the priming fluid, oxygen insufflation of the membrane oxygenator alone may not induce echinocyte-forming alkalemic conditions. In contrast, a blood clot did appear in the circuit in both the $NaHCO_3$ 100 and the $NaHCO_3$ 200 conditions. Although the pH change was similar under these two conditions, the blood clot in the circuit was more prominent in the $NaHCO_3$ 200 condition.

The cytoskeletal lining of the RBC membrane maintains a wide extension in a planar form through the binding of component proteins (e.g., spectrin and actin); this lines the cytoplasmic side of the lipid bilayer by coupling with the RBC transmembrane Band 3 protein (Band 3).[12,13] The Band 3 conformation (inward- or outward-facing) depends on the Donnan equilibrium ratio of Cl^-, HCO_3^-, OH^-, and H^+ concentrations between the inside (i) and outside (o) of the RBC membrane, as represented by the following equation: $[Cl^-]_i/[Cl^-]_o = [HCO_3^-]_i/[HCO_3^-]_o = [OH^-]_i/[OH^-]_o = [H^+]_o/[H^+]_i$.[14-16] Previous studies have reported reductions in this ratio with increased pH, and that higher concentrations of HCO_3^- outside the RBC membrane increase the amount of Band 3 with an inward-facing conformation.[16,17] This conformation induces a contraction of the membrane cytoskeleton and induces the echinocyte shape.[16,17] Echinocytes have previously been shown to be less deformable than discocytes,[18,19] and so the obstruction of pores in the venous reservoir filter and membrane oxygenator may occur because of echinocytes. However, it is also possible that a change in the electrostatic attraction on the surface of the echinocyte membrane could potentially result in a blood clot in the circuit.[20] Considering that PT and APTT were markedly prolonged in the $NaHCO_3$ 100 and 200 conditions with a very low SFMC, and that platelet aggregation ability was not enhanced under the alkalemic conditions

in our previous study, it is unlikely that blood clotting in the circuit with alkalemia was caused by enhanced blood coagulation ability or platelet aggregation.[9,21,22] We also morphologically demonstrated an increase in the number of echinocytes under the alkalemic conditions used in our previous study,[8] an observation consistent with the present findings.

Thus, the greatest degree of blood clotting observed in the circuit under the $NaHCO_3$ 200 condition may have been due to an accumulation of echinocytes, perhaps because of a decrease in the Donnan equilibrium ratio resulting from an increase in the concentration of HCO_3^- outside the RBC membrane due to the high alkalemia induced by this condition.[16,17]

In the Alb 50, 100, and 200 conditions, the albumin was combined with 100 mL $NaHCO_3$. Although the change in pH was similar in all the alkalemic conditions, blood clotting in the circuit resulted in only slight increases in HU and PG. The increase in HU was inhibited as the concentration of albumin increased. In an aqueous solution, albumin is present as a negatively charged colloid. It has been demonstrated that albumin prevents changes in Band 3 conformation by elevating the pH alkalization-reduced Donnan equilibrium ratio, resulting in the prevention of shape transformation to form echinocytes.[17] Because the isoelectric point of albumin is pH 4.9, its negative charge was enhanced under alkalemic conditions; this negative charge in the albumin surrounding the RBC membrane may have repelled HCO_3^-.[17] In our previous studies, we reported that the presence of albumin under alkalemic conditions inhibited the appearance of echinocytes,[8] and this was morphologically confirmed in the present study. Furthermore, RRR was inhibited more strongly by Alb 50, 100, and 200 than by $NaHCO_3$ 100 and 200, suggesting that albumin has a protective effect against

hemolysis or pore obstruction of the venous reservoir by echinocytic transformation. These findings therefore confirmed the previous reports that the addition of albumin can inhibit the appearance of echinocytes in the circuit, perhaps due to an increase in the Donnan equilibrium ratio between the inside and outside of the RBC membrane.[8,16,17,23)]

CONCLUSION

Blood clotting in the circuit soon after initiating CPB may be caused by echinocytes that appear under alkalemic conditions. The addition of albumin to the priming fluid may inhibit this echinocyte-induced clotting.

CONFLICTS OF INTEREST

This was a cooperative study in which three institutions participated: Nara Medical University, JMS Co., Ltd., and Tenri Hospital. The first author performed the study at the research facility of JMS Co., Ltd., using materials and instruments provided by the facility. All remaining authors have declared no conflicts of interest.

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FIGURE LEGENDS

Fig. 1 The experimental recirculation circuit comprised a venous reservoir, roller pump, and membrane oxygenator.

Fig. 2 Blood clotting in the circuit results in a difference in the liquid level (the "hold up," HU) between the inside and outside of the venous reservoir filter.

Fig. 3 The results for blood pH, hold up (HU), the pressure gradient (PG), and red blood cell reduction rate (RRR). Increases in blood pH were similar under all the conditions tested (excluding the control condition), regardless of the volumes of $NaHCO_3$ and albumin added. However, HU and PG increased, and RRR was greatest with $NaHCO_3$ 200. The addition of albumin inhibited these changes to a greater extent than did $NaHCO_3$ 100. The increase in HU was inhibited as the albumin concentration increased.

Fig. 4 A representative scanning electron photomicrograph of the membrane oxygenator surface resulting in a pressure gradient due to a blood clot in the circuit under alkalemic conditions. Many echinocytes can be seen on the membrane oxygenator surface.

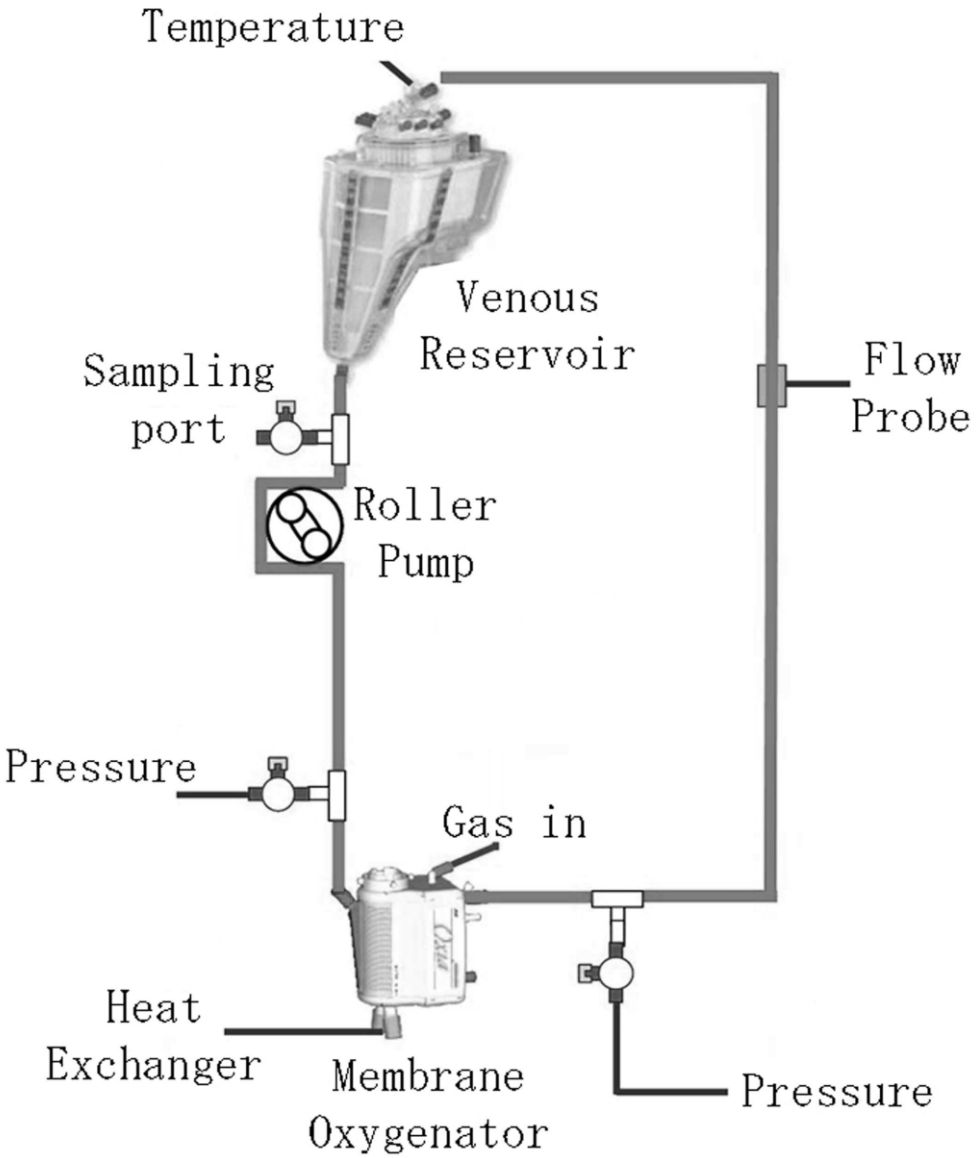


Figure 1 Experimental recirculation circuit

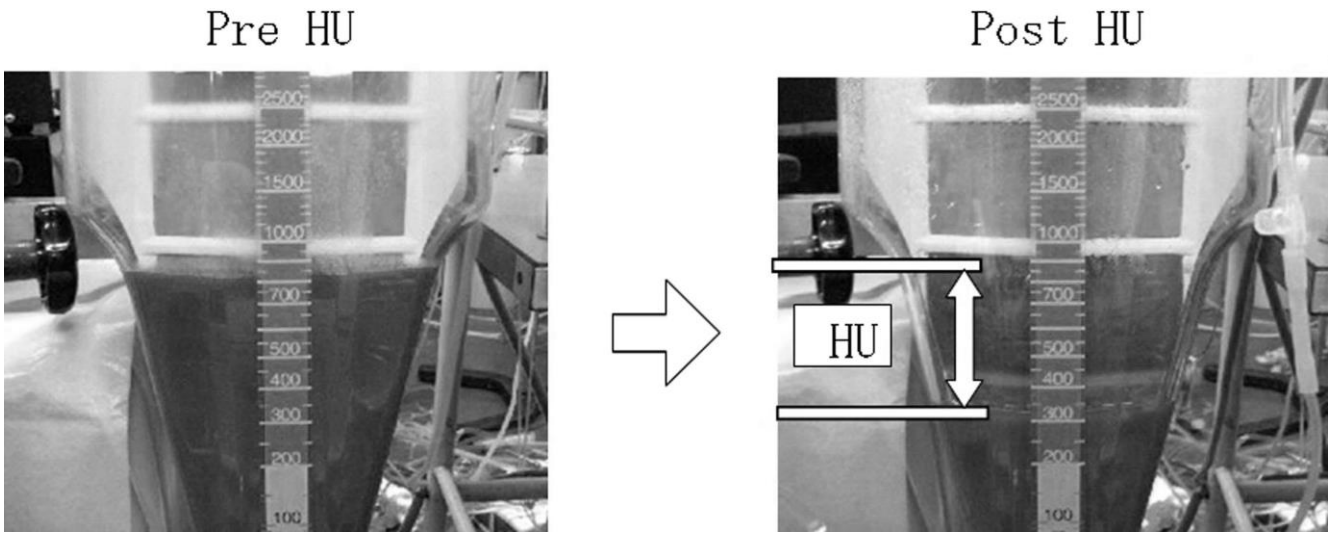
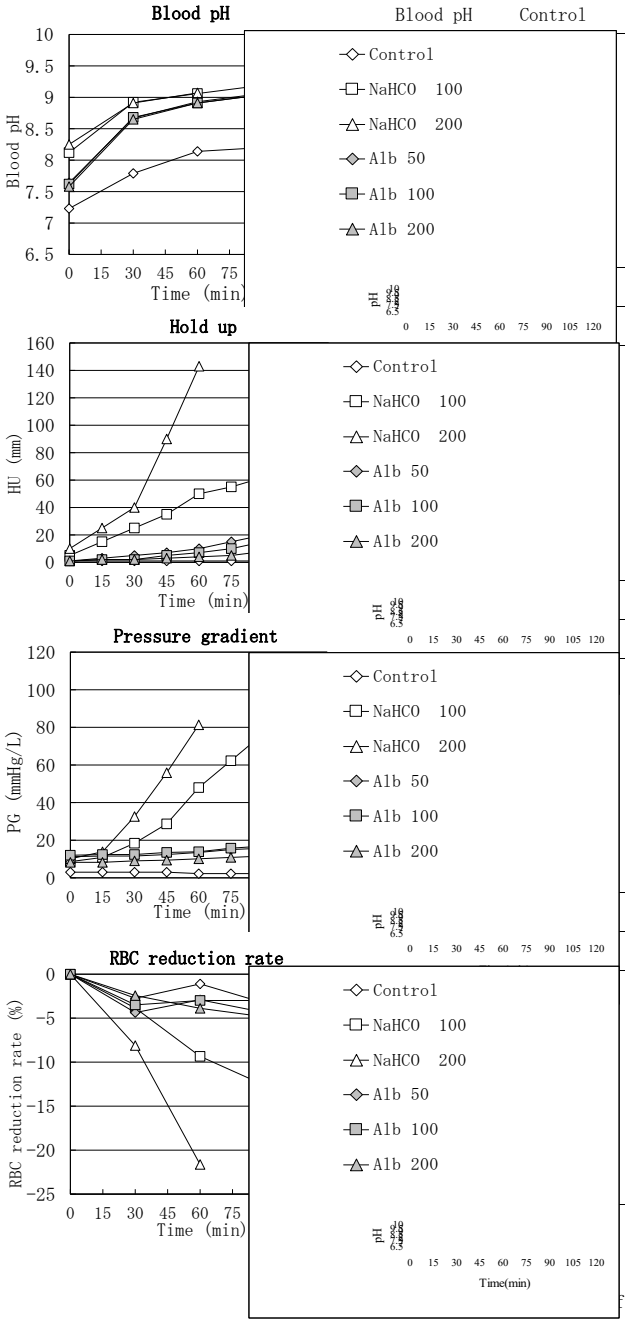


Figure 2 Hold up

a



Blood pH	Control	$NaHCO_3$ 100	$NaHCO_3$ 200	Alb 50	Alb 100	Alb 200
		n. s.	n. s.	n. s.	n. s.	n. s.
			n. s.	n. s.	n. s.	n. s.
				n. s.	n. s.	n. s.
					n. s.	n. s.
						n. s.

b

	$NaHCO_3$ 100	$NaHCO_3$ 200	Alb 50	Alb 100	Alb 200
Hold up	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
			$p < 0.05$	$p < 0.05$	$p < 0.05$
				$p < 0.05$	$p < 0.05$
					$p < 0.05$

c

	$NaHCO_3$ 100	$NaHCO_3$ 200	Alb 50	Alb 100	Alb 200
Pressure gradient	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
			$p < 0.05$	$p < 0.05$	$p < 0.05$
				n. s.	n. s.
					n. s.

d

	$NaHCO_3$ 100	$NaHCO_3$ 200	Alb 50	Alb 100	Alb 200
RBC reduction rate	$p < 0.05$	$p < 0.05$	n. s.	n. s.	n. s.
		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
			$p < 0.05$	$p < 0.05$	$p < 0.05$
				n. s.	n. s.
					n. s.

fferent alkaline conditions

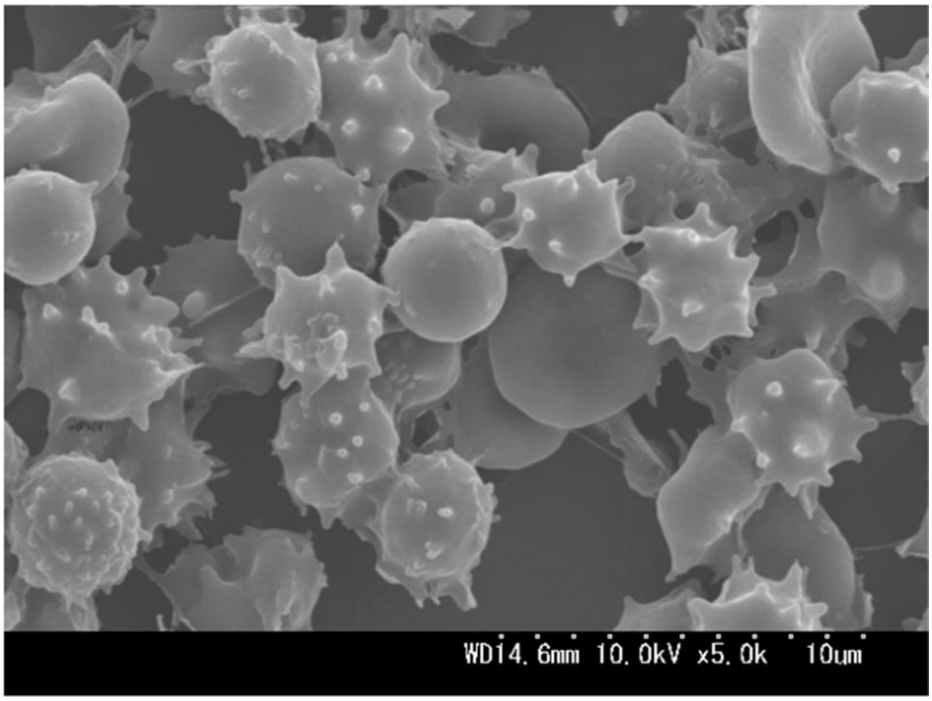


Figure 4 Echinocytes on the membrane oxygenator surface

Table 1 Priming fluid

Priming fluid (mL)	Control	$NaHCO_3$ 100	$NaHCO_3$ 200	Alb 50	Alb 100	Alb 200
7% $NaHCO_3$ solution	0	100	200	100	100	100
25% Albumin solution	0	0	0	50	100	200
Lactated Ringer's solution	1,000	900	800	850	800	700
Total Volume	1,000	1,000	1,000	1,000	1,000	1,000

$NaHCO_3$: 7% $NaHCO_3$; Alb: 25% albumin solution