Particle separation using ultrasound can be used with human shed mediastinal blood

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Background: **Shed mediastinal blood collected by cardiotomy suction has been shown to be a large contributor to lipid microemboli ending up in different organs. The aim of this study was to test the separation efficiency on human shed blood of a new separation method developed to meet this demand.** ***Methods:*** **Shed mediastinal blood collected from the pericardial cavity of 13 patients undergoing cardiac surgery with cardiopulmonary bypass was collected. The blood was processed in an eight-channel parallel PARSUS separator, and separation efficiency was determined.** ***Results:*** **Erythrocyte recovery, in terms of a separation ratio, varied between 68% and 91%. Minor electrolyte changes took place, where levels of sodium increased and levels of potassium and calcium decreased.** ***Conclusion:*** **This study demonstrates that PARSUS technology can be used on human shed mediastinal blood with good separation efficiency. The technology is, thereby, suggested to have future clinical relevance.** *Perfusion* (2005) **20,** 39–43.

Introduction

Despite the high incidence of a postoperative cognitive dysfunction, attempts made to decrease this complication after cardiac surgery have not eliminated this complication. Embolization during cardiopulmonary bypass (CPB) with lipid microemboli (LME) has lately been suggested as a main contributor to such a postoperative cognitive decline.[1–3] Brooker and coworkers found that shed mediastinal blood retransfused during surgery is a substantial contributor to lipid particles forming emboli in the capillaries of the brain,[4] thus defining a definite source for these lipid emboli.

Standard techniques for particle separation, centrifuges and filters, have shown to be inadequate in removing these lipid particles from shed blood. At best, they can remove 50% of particles forming LME.[3,5,6]

A new technology based on ultrasonic standing waves has been developed to meet this demand, and the technology has been denoted PARSUS (PARticle Separation using UltraSound). In a model utilizing porcine blood with radioactive triglycerides, this technology showed good results in terms of lipid particle removal, with an average efficiency of more than 80%.[7]

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The technology has not been tested on real human shed mediastinal blood. The purpose of this study was to test the technology on the substrate it has been developed for, i.e., shed mediastinal blood collected by cardiotomy suction during cardiac surgery, and measure efficiency in terms of red cell recovery. Furthermore, the safety of the method in terms of electrolyte changes was also investigated.

Methods

After approval from the local ethics committee, 13 patients undergoing cardiac surgery with CPB were included. Nine underwent coronary artery bypass grafting, and four aortic valve replacement.

Ten millilitres of shed mediastinal blood was collected from the pericardial cavity with a syringe soon after the initiation of CPB. At the same time, blood was drawn from the CBP circuit for blood gas determination.

Blood gases were determined with an ABL 700 blood gas analyser (Radiometer, Copenhagen, Denmark).

Microchip development

The microfluidic separation chip was manufactured by means of a standard silicon processing technique.[8] This microfabrication technique provides, in principal, perfect vertical side walls of the microchannels. Good accuracy of the side walls is a prerequisite for obtaining high-quality acoustic

resonance in the microfluidic device. The high fidelity of the microfabrication process enables the manufacture of a separation device based on a parallel microchannel array. In this study, a separator with eight parallel channels was used (Figure 1).

The acoustic forces exerted on the particles are mainly dependent on the density, compressibility and size of the particles. The acoustic properties of erythrocytes are such that the acoustic force field acts to displace the erythrocytes to the center of the channel, where the node of the standing wave is. At the end of each separation channel, a trifurcation divides the flow of fluid and particles according to their spatial distribution in the channel (Figure 1 and 2), as the flow is completely laminar (Reynolds number <20). Particles collected in the node of the standing wave (erythrocytes) are, thus, collected in the center outlet (Figure 2).

Study model

The flow in the microseparator was controlled with syringe pumps (SP260P, World Precision Instruments, Sarasota, FL, USA) and the flow rate was maintained at 0.5 mL/min for both center and side outlets by applying the syringe pumps after the structure. The ultrasonic transducer was operated with a 2-MHz sinusoidal signal, and tuned to obtain a standing wave.[7]

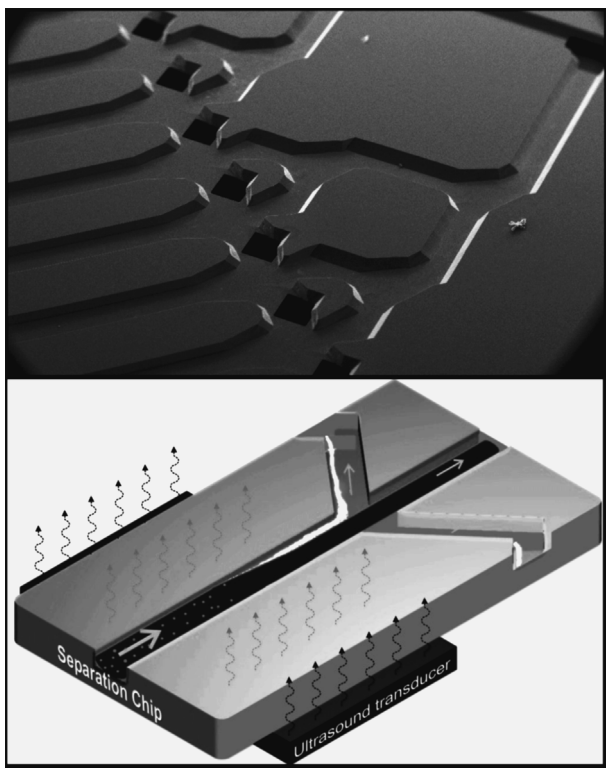

Figure 1 A scanning electron microscopy photo (top) of a structure consisting of eight parallel channels, which was used in the study. The parallel channels are a prerequisite for increased throughput. The picture below shows the principle with a trifurcation dividing flow after separation. Separation of erythrocytes (dark) and lipid particles (white) are depicted at the trifurcation. The orthogonal placement of the ultrasonic transducer is also depicted.

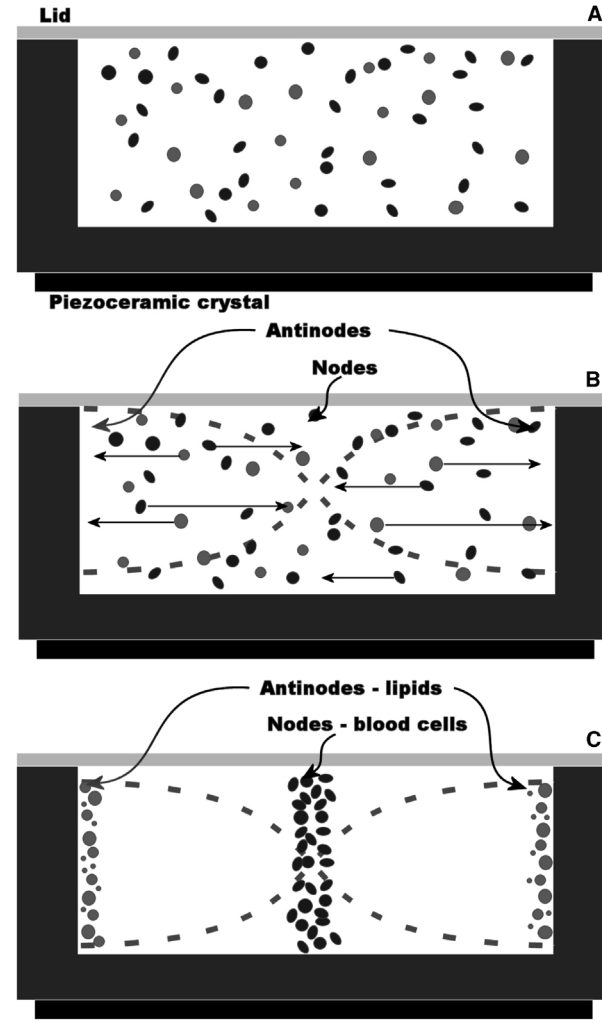

Figure 2 The principle of PARSUS separation. (A) Shows a cross-section of a microchannel with erythrocytes (darker spheres) and lipid particles (brighter spheres). (B) An ultrasonic standing wave is applied by the piezoceramic crystal and forces (arrows indicate examples of forces) start acting on the particles according to their physical properties. (C) Particles are displaced to either the node (erythrocytes) or the antinode (lipid particles).

The separation efficiency was measured as the sum of erythrocyte concentration (measured as hematocrit or concentration hemoglobin) collected from the center and the side outlets divided by erythrocyte concentration collected in the center outlet (flow rates were the same in both channels).

Statistics

Group values are expressed as the mean $\pm$1SD. Student's *t*-test was used to test group differences. Correlations were investigated with univariate

analysis, and univariate regression analysis was performed to test dependency. A p-value <0.05 was considered significant.

Results

Mean hematocrit of shed mediastinal blood before separation was $16.1 \pm 6.6\%$, and increased by 44.4% to $23.3 \pm 7.9\%$ after separation ($p < 0.00001$). Total hemoglobin levels increased 46.3% from 5.09 ± 2.19 g/dL before separation to 7.46 ± 2.64 g/dL after separation (Figure 3, $p > 0.00001$). The hematocrit in the waste outlet was $4.5 \pm 3.7\%$, and the hemoglobin was 1.24 ± 1.25 g/dL.

Separation efficiency, determined as erythrocyte recovery ratio, varied between 91.0% and 68.3% with a mean of 85.2%. If the separation efficiency was determined by hemoglobin levels, it varied between 94.9% and 69.0% with a mean of 87.8%, and depended only on concentration of erythrocytes before separation, if tested by univariate analysis (Figure 4 and 5).

Electrolytes were slightly affected by the process. Sodium increased slightly, and potassium levels decreased (Table 1). Sodium before separation varied between 128 and 143 mmol/L; after separation it was between 132 and 145 mmol/L. Potassium before separation varied between 2.2 and 7.8 mmol/L, and after separation it was between 2.0 and 6.3 mmol/L. Calcium levels also decreased (Table 1).

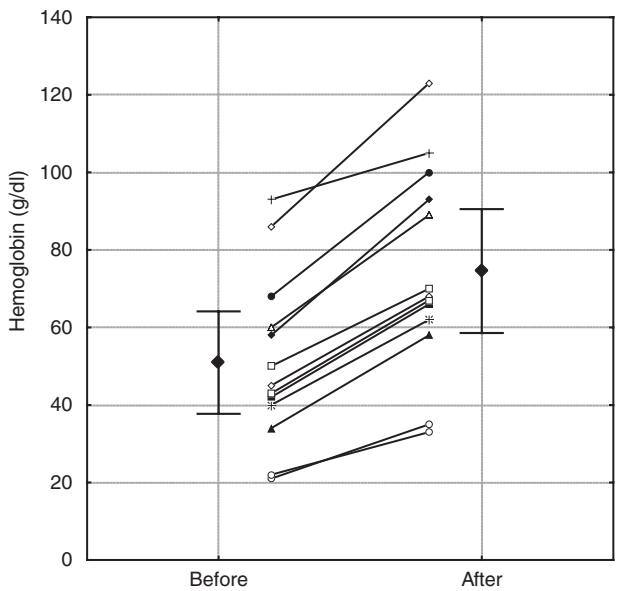

Figure 3 Hemoglobin (g/L) level for each sample before and after separation depicted as change and mean $1 \pm$SD ($p < 0.0001$). The after separation levels are determined in the middle (recovery) channel.

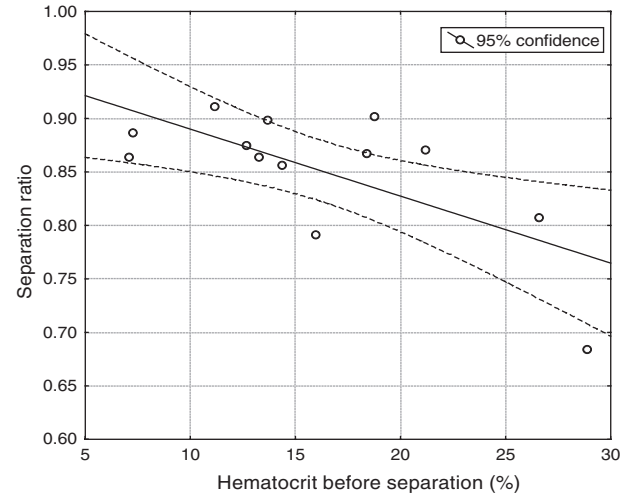

Figure 4 Separation efficiency for erythrocytes measured as recovery ratio determined from hematocrit levels plotted against the inflow hematocrit. $r = 0.68$, $p < 0.05$.

Discussion

This study demonstrates that PARSUS technology can be used to separate human cardiotomy suction blood with high efficacy and no adverse effects in terms of electrolyte changes. These two findings are important as they further strengthen the feasibility for the technology in processing human shed blood, which is the substrate for which the technology has primarily been developed.

The separation efficiency varied between 68% and 91% and depended on the hematocrit of inflow blood, where a lower concentration yielded a higher separation degree. At present, the technology seems

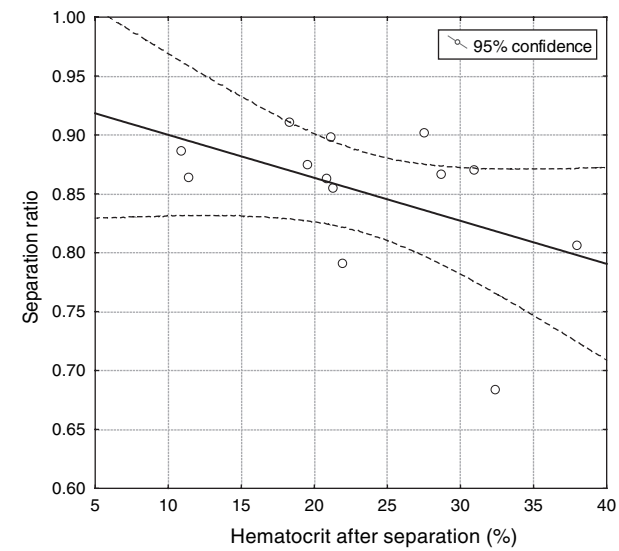

Figure 5 Separation efficiency for erythrocytes measured as recovery ratio determined from hemoglobin levels plotted against the outflow concentration of hemoglobin in the middle (recovery) channel. $p =$ n.s.

Table 1 Electrolyte levels from patient blood, before and after separation

	Mean ± SD
Na^+ (mmol/L)	
Patient	$127.5 \pm 2.36^{\dagger\dagger\dagger}$
Before	135.8 ± 4.50
After	$139.4 \pm 4.05^{\dagger\dagger\dagger}$
Waste	$139.4 \pm 4.05^{\dagger\dagger\dagger}$
K^+ (mmol/L)	
Patient	4.43 ± 0.39
Before	4.27 ± 1.47
After	$3.70 \pm 1.21^{\dagger\dagger\dagger}$
Waste	$3.69 \pm 1.24^{\dagger\dagger\dagger}$
Ca^{++} (mmol/L)	
Patient	$1.199 \pm 0.04^{\dagger\dagger\dagger}$
Before	0.84 ± 0.16
After	$0.73 \pm 0.12^{\dagger\dagger\dagger}$
Waste	$0.73 \pm 0.14^{\dagger\dagger\dagger}$
Glucose (mmol/L)	
Patient	6.49 ± 2.15
Before	5.42 ± 3.02
After	$4.24 \pm 2.53^{\dagger\dagger\dagger}$
Waste	5.26 ± 3.32
BE (mmol/L)	
Patient	$-0.78 \pm 1.84^{\dagger\dagger}$
Before	-6.13 ± 5.06
After	$-9.24 \pm 4.28^{\dagger\dagger\dagger}$
Waste	-8.09 ± 6.94

The after separation levels represent blood collected in the center channel, and waste levels represent the fluid collected from the side outlet of the structures. All comparisons are made against levels in shed blood before separation ($^{\dagger\dagger}p > 0.01$; $^{\dagger\dagger\dagger}p < 0.005$). BE = base excess.

to have limitations in separating high hematocrits of red blood cells. However, with up to 20% hematocrit in inflow blood, a separation ratio of 90% was achieved. Higher hematocrit levels are seldom found during perfusion, as the patient's blood is diluted by the priming solution in the heart–lung machine. Moreover, the shed blood is normally diluted with different fluids used in the surgical field. Therefore, in the clinical setting, hematocrits above 20–25% are rarely seen in shed blood.

Previously, the technology has been tested on phantom shed blood made from porcine blood.[7] In that study, mean separation efficacy was 79.8%, compared to the 85.2% achieved in this study. The difference in efficacy is small, and it is hard to draw any conclusion from it. Any actual difference in separation efficiency would be dependent on either density, size or compressibility as those factors determine the acoustic force exerted on a particle in a standing wave field.[8,9] For example, acoustic forces scale with the volume of the particle, and, thus, a particle twice as big will have twice the force exerted on it. Human erythrocytes have a volume that is approximately twice the volume of bovine erythrocytes.[10] The slightly improved separation efficacy in this study could, therefore, depend on this difference in cell volume. From these findings, it is reasonable to conclude that processing human blood with PARSUS technology is at least as, and maybe even more, efficient as processing porcine blood.

If we compare the erythrocyte recovery of 85–90% as described in this study to the recovery after centrifugal wash, which is used routinely to process scavenged blood from the surgical field in different types of operations, the recovery described could, at first glance, look inferior. However, we should not forget the intermittent nature of centrifugal-based cell savers compared to PARSUS technology, which is continuous. In an intermittent procedure, a full batch will have to be collected before starting a wash cycle. Any collected blood that cannot be processed due to too small an amount will be discarded. For example, if 300 mL of blood is collected and 400 mL is needed for a cell-saver wash, all of the blood will have to be discarded, and the recovery rate will be 0%. Greater bleeding will, of course, yield a higher recovery. In a 1000 mL bleeding, for instance, 800 mL can be processed, thus, leaving 200 mL that is not available for processing, yielding an 80% recovery. Given that the described technology is continuous and only a very small volume is lost during priming, a 90% recovery should be considered acceptable.

This study did not address the separation efficiency for removing LME from shed blood. In a previous study, using emulsified lipid particles, this separation efficacy was found to be between 66% and 94%. However, this model is not wholly representative for cardiotomy suction blood, as the lipid particles in human shed blood are larger and not emulsified. Intuitively, measuring the amount of triglycerides in the blood would seem to be a plausible method. However, we should not forget that there are endogenous lipid particles in the blood, such as chylomicrons, LDL- and HDL-particles, which also contain triglycerides and affect the results of such an analysis. Therefore, other methods for testing the LME removal from real shed human blood are needed. However, separation efficiency of erythrocytes and lipid particles are not independent of each other. Given the inherent properties of a standing wave field, a good separation of erythrocytes will yield a good separation of lipid particles.

Electrolyte changes in the blood before and after separation were also studied. Levels of sodium ions increased, whereas levels of potassium and calcium decreased from the determination of blood before separation. This could be attributed to the normal energy metabolism of the erythrocytes during handling and processing. For technical reasons, the

sample taken before and the two samples taken after processing differed approximately 2 hours in time. During this time, a normal metabolism and energy consumption will take place, which could be seen in the decreasing levels of glucose. Despite the long time the blood was outside the body, and that it was processed using our technology, the electrolyte changes must be considered reasonable. We should be able to conclude that electrolyte changes due to the process are within acceptable borders.

The presented device with eight parallel channels can handle approximately 60 mL/hour. The clinical demand for processing shed blood from cardiotomy suction will be at least 20 times higher, and, therefore, the throughput will have to be increased. This can be achieved by increasing the number of parallel channels.

Previously, it has been shown that the technology does not add to the hemolysis of the blood processed.[7] It was not the intent of this study to examine hemolysis in this early set up of the technology. A more comprehensive study on erythrocyte function will have to be performed on a full-scale device to conclude whether the technology is harmless or harmful for blood cells.

In conclusion, this study shows, for the first time, that human shed blood can be processed successfully with PARSUS technology. Neither efficacy for removing LME nor the rate of hemolysis were demonstrated in this study. This has, however, been demonstrated previously in a model with porcine blood, with good results.[7] Should the results of this study be transferable to the *in vivo* setting with human shed blood, a new technology for removing potentially hazardous LME from blood during surgery with CPB will be available. These parameters need to be demonstrated in real shed blood to justify the use of this technology in routine practice.

References

1 Stump DA, Rogers AT, Hammon JW, Newman SP. Cerebral emboli and cognitive outcome after cardiac surgery. *J Cardiothorac Vasc Anesth* 1996; **10**: 113–18.
2 Taggart DP, Westaby S. Neurological and cognitive disorders after coronary artery bypass grafting. *Curr Opin Cardiol* 2001; **16**: 271–76.
3 Whitaker DC, Newman SP, Stygall J *et al*. The effect of leucocyte-depleting arterial line filters on cerebral microemboli and neuropsychological outcome following coronary artery bypass surgery. *Eur J Cardiothorac Surg* 2004; **25**: 267–74.
4 Brooker RF, Brown WR, Moody DM *et al*. Cardiotomy suction: a major source of brain lipid emboli during cardiopulmonary bypass. *Ann Thorac Surg* 1998; **65**: 1651–55.
5 Kincaid EH, Jones TJ, Stump DA *et al*. Processing scavenged blood with a cell saver reduces cerebral lipid microembolization. *Ann Thorac Surg* 2000; **70**: 1296–300.
6 de Vries AJ, Gu YJ, Douglas YL *et al*. Clinical evaluation of a new fat removal filter during cardiac surgery. *Eur J Cardiothorac Surg* 2004; **25**: 261–66.
7 Jonsson H, Holm C, Nilsson A *et al*. Particle separation using ultrasound can radically reduce the embolic load to the brain after cardiac surgery. *Ann Thorac Surg* 2004; **78**: 1572–77.
8 Nilsson A, Petersson F, Jönsson H, Laurell T. Acoustic control of suspended particles in micro fluidic chips. *Lab Chip* 2004; **4**: 131–35.
9 Gröschl M. Ultrasonic separation of suspended particles – Part I: Fundamentals. *Acust Acta Acust* 1998; **85**: 432–37.
10 Weiser MG. Erythrocyte volume distribution analysis in healthy dogs, cats, horses, and dairy cows. *Am J Vet Res* 1982; **43**: 163–66.