

# Continuous separation of lipid particles from erythrocytes by means of laminar flow and acoustic standing wave forces

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Improved continuous acoustic particle separation (separation efficiency close to 100%) and separation of erythrocytes (red blood cells) from lipid microemboli in whole blood is reported.

## Introduction

It is a well-known fact that particles in fluid suspensions may be enriched at defined positions by means of forces generated by acoustic standing wave fields. Theoretical pioneers in this discipline were King,<sup>1</sup> Gorkov,<sup>2</sup> and Yosioka and Kawasima.<sup>3</sup> Their acoustic force theories have since been used by several groups of researchers in particle separation applications.<sup>4–9</sup> A new approach to continuous separation of particles in microfluidic channels was recently proposed by Nilsson *et al.*,<sup>4</sup> enriching particles in the pressure nodes of an ultrasonic standing wave in a continuously perfused microchannel. Earlier version of the system<sup>4</sup> reported particle enrichment in a double pressure node configuration, collecting enriched particles in the side outlets of a triple channel outlet. The current set-up described in this communication demonstrates improved particle separation efficiency, defined as the fraction of particles collected in the centre outlet. Also, successful separation of particles having different physical properties is reported, *i.e.* lipid vesicles were separated from erythrocytes. These accomplishments were attributed to the further miniaturisation of the separation device to a channel width of 350  $\mu\text{m}$ , a depth of 125  $\mu\text{m}$  and operation in a half wave length resonance mode, providing higher acoustic forces on the particles.

During cardiac surgery supported by a heart-lung machine a massive embolization of lipid particles occur in the brain when shed blood is returned to a patient *via* a filter.<sup>10</sup> The lipid particles are derived from triglycerides leaking from fat cells during surgery in adipose tissue. The embolization is associated with cognitive dysfunction observed after surgery.<sup>11</sup> The techniques currently available for blood wash do not meet the demand to remove these lipid particles. The most common method to wash blood is based on centrifuges which are burdened with a number of drawbacks, *i.e.* they only handle larger amounts of blood ( $\approx 0.5$  l), are harmful for the blood cells,<sup>12</sup> need specially trained personnel, are not continuous and display a limited lipid particle elimination.

The technology presented in this communication offers a solution to the embolization problem by employing the possibility of discriminating erythrocytes from lipid particles. In addition, when fully developed and implemented clinically, it reduces the demand for allogenic blood and reduces or eliminates blood transfusion related incompatibilities. The primary acoustic

radiation force equation<sup>13</sup> tells us that the acoustic force can move particles either towards a node or an anti-node of a standing wave depending on their densities and compressibilities. If the particles are red blood cells and lipid droplets in blood plasma, the erythrocytes gather in the pressure node (in the centre of the channel) while the lipid particles gather in the pressure anti-nodes (by the side walls), Fig. 1. At the end of the channel the red blood cells exit through the centre outlet while the lipid particles exit through the side outlets, separating the two particle types, Fig. 2.

## Experiments and results

The experiments were performed as reported in ref. 4 with the modification that a half wavelength standing wave was used. *In vitro* experiments were performed on a particle suspension composed of a 2% concentration of 5  $\mu\text{m}$  polyamide spheres (blood phantom), see ref. 4 for details.

All separations were performed at low Reynolds numbers, ( $Re < 40$ ). Considerably improved separation efficiencies (close to 100%)

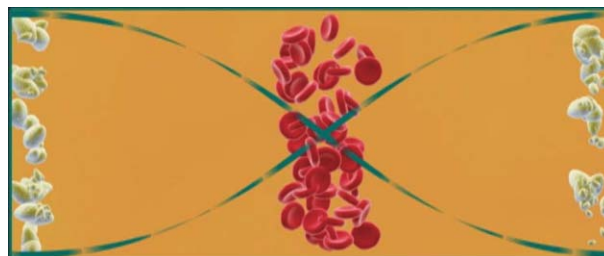


Fig. 1 Cross-section of channel with erythrocytes and lipid particles. When the ultrasound is turned on the two particle types are separated.

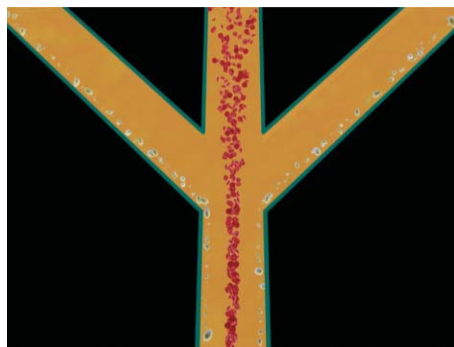


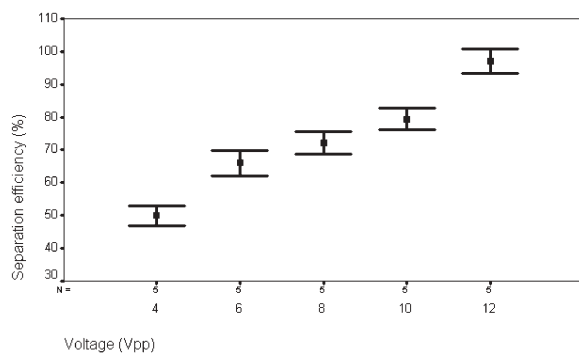
Fig. 2 If the main channel is split into three outlet channels the laminar flow properties makes it possible to collect erythrocytes and lipid particles separately.

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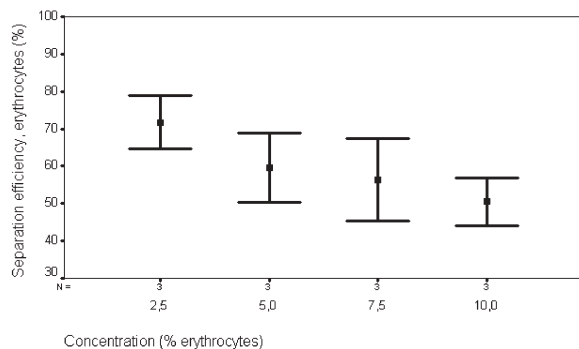
of the particles were collected in 1/3 of the original fluid volume, Fig. 3) as compared to earlier data were accomplished at lower actuation voltages ( $12 V_{pp}$ ), and at a four times higher flow speed.

To evaluate the systems ability to separate lipid particles from red blood cells a mixture of bovine blood, saline solution and a phospholipid-stabilized emulsion of triolein was used. The triolein emulsion was prepared according to ref. 14 with some modifications. A total amount of 320 mg triolein, tritium labelled and unlabelled, and 3.2 mg of phospholipids were sonicated in 7.2 ml of PBS. Following sonication, 0.8 ml of 20% BSA in PBS was added. The lipid emulsions were always used within 5 h from the time of preparation. The lipid content was measured by a scintillation counter (Wallac Guardian 1414 Liquid Scintillation Counter, PerkinElmer Life and Analytical Sciences Inc., Boston, MA, USA), according to standard scintillation counting protocol,<sup>15</sup> with the modification of using Ultima Gold (Packard Biosciences, Boston, MA, USA) as scintillation liquid. The lipid separation efficiency was defined as the fraction of lipid particles exiting through the side outlets.

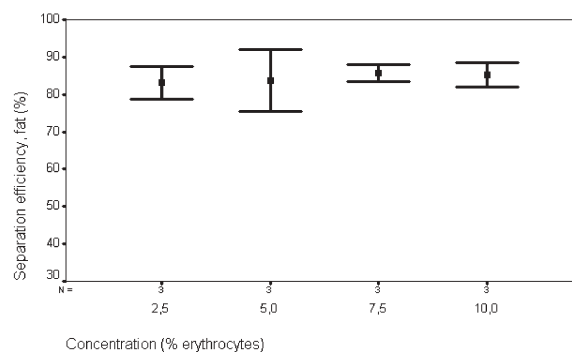
More than 70% of the erythrocytes were collected in 1/3 of the original fluid volume and more than 80% of the lipid particles were removed, Figs. 4 and 5. The applied voltage was  $10 V_{pp}$ , the flow rate  $0.3 \text{ ml min}^{-1}$ , the concentration of red blood cells 2.5% and the concentration of lipid particles 1%. The fraction of lipid particles removed seemed to be independent of the concentration of erythrocytes. It was not possible to quantify the systems ability to remove human lipids in human blood collected during cardiac



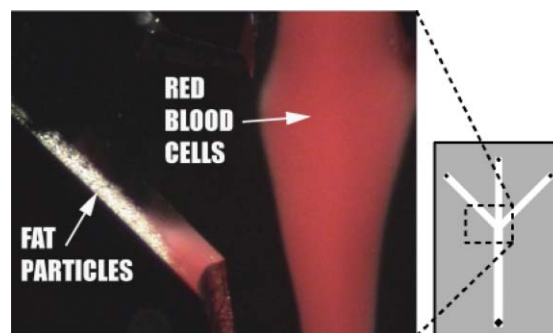
**Fig. 3** Separation efficiency of the  $350 \mu\text{m}$  separation channel *versus* applied voltage to the piezo ceramic element. Flow rate  $0.3 \text{ ml min}^{-1}$ .



**Fig. 4** Separation efficiency *versus* erythrocyte concentration. The total flow rate was  $0.3 \text{ ml min}^{-1}$  and a voltage of  $10 V_{pp}$  was applied to the piezo ceramic element.



**Fig. 5** Separation efficiency of lipid particles *versus* erythrocyte concentration. Flow rate  $0.3 \text{ ml min}^{-1}$ , actuation voltage  $10 V_{pp}$  and input lipid concentration 1%.



**Fig. 6** Human lipid particles separated from human erythrocytes at the trifurcation of  $350 \mu\text{m}$  separation chip with ultrasound turned on.

surgery since no reliable method was available. However, visual observations of the separation process performed on human blood could be made, displaying steady streams of lipid particles flowing along the side walls and a well defined band of red blood cells exiting the system through the centre outlet channel, Fig. 6.

The degree of hemolysis, red blood cell lysis, was measured using a HemoCue Plasma/Low Hb meter (HemoCue AB, Ängelholm, Sweden). No change in the degree of hemolysis was detected after the separation process compared to before, indicating that the process was harmless to the red blood cells.

## Conclusion

Separation efficiencies close to 100% could be reached for polyamide spheres using the new 350 micrometer separation device. Separation efficiencies of above 80% for triglyceride emulsions were obtained. Separation of micro emboli from human erythrocytes collected during surgery was visually confirmed. No hemolysis induced by the separation process was detected. To accomplish a system for full scale clinical use 100–200 separation channels have to be used in parallel to reach a reasonably high throughput.

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