

MICROFLUIDIC CENTRIFUGO-PNEUMATIC SIPHON ENABLES FAST BLOOD PLASMA EXTRACTION WITH HIGH YIELD AND PURITY

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ABSTRACT

Centrifugal microfluidic plasma separation from whole blood is improved by minimized resuspension of cells. Plasma supernatant is removed by an air pressure mediated coating free siphon. Fluid dynamic characteristics are predicted by numerical simulation on a system level approach enabling reliable separation for a hematocrit range between 20 % and 60 %. Separation of 40 μ l of whole blood with 60 % hematocrit resulted in the following characteristics (compared to previous centrifugal microfluidic approach [1]): Separation time of 43 s (120 s), plasma yield of 88 %, cv = 4 % (< 83 %, cv = 6 %) and purity of 99.8 %, cv = 0.1 % (99.5 %).

KEYWORDS

Centrifugal microfluidics, pneumatic, whole blood, plasma separation

FUNCTIONAL PRINCIPLE

Plasma separation is essential for sensitive analysis due to inhibitory effects of hemoglobin and erythrocytes. Existing centrifugal methods (Fig. 1) require long phases of low deceleration after sedimentation to avoid resuspension of cells and they require hydrophilic surface treatment to enable capillary priming of siphons [1; 2]. By employing a centrifugo-pneumatic siphon, a gas bubble is enclosed and compressed (Fig. 2) when the blood sample is loaded into the sedimentation chamber. After sedimentation, the rotational speed is only slightly reduced so that the compressed air bubble expands and quickly displaces the plasma through an adjacent siphon.

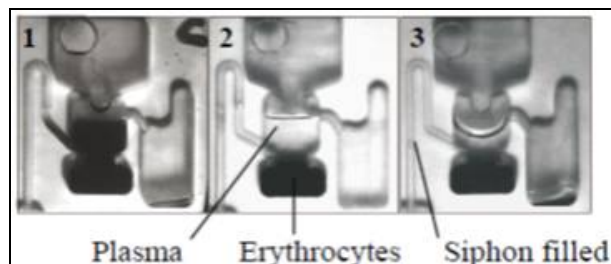


Figure 1: Benchmark – Live images of the state-of-the-art plasma separation process: (1) after loading of the blood sample, (2) after sedimentation and (3) during siphon priming [1]. The method employs capillary primed siphon valving that requires surface treatment.

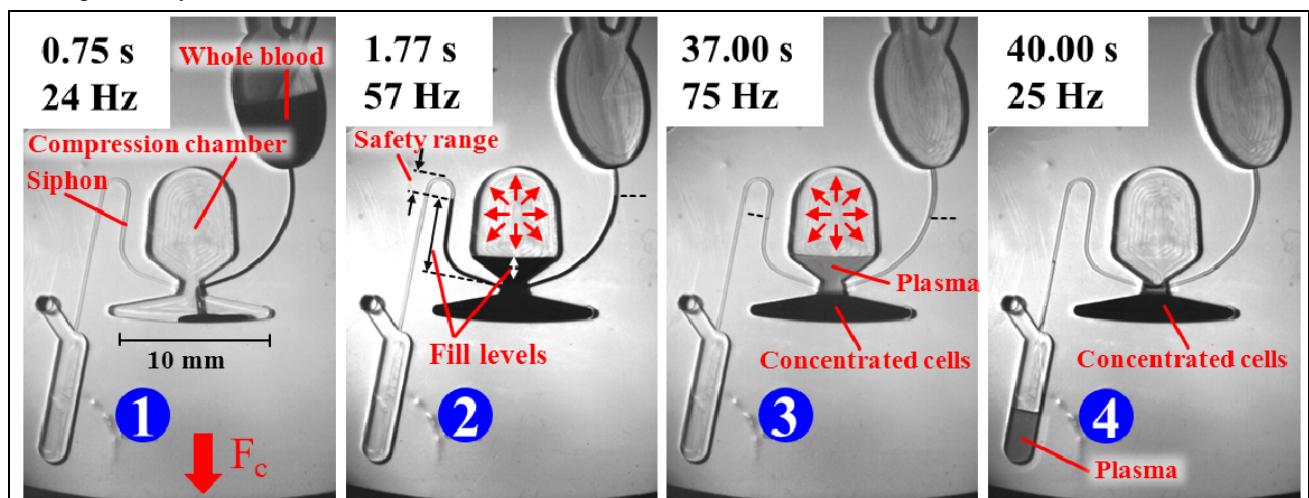


Figure 2: Live images of the new plasma separation process applying the centrifugo-pneumatic siphon: (1) loading of the blood sample, (2) bubble compression, (3) after cell sedimentation and (4) at the end of plasma collection. The centrifugo-pneumatic siphon enables fast valving without any surface treatment.

In contrast to the previous approach, the plasma supernatant is thereby removed at high centrifugation, which avoids resuspension of cells ensuring high purity of the plasma. A “safety range” of the siphon (Fig. 2 (2)) prevents premature siphon priming during the loading phase which occurs if high rotational frequencies are not reached fast enough. In this case, the whole blood is loaded into the sedimentation chamber immediately and primes the siphon already during the loading phase due to insufficient bubble compression.

MODELING AND SIMULATION

A network based simulation is performed to optimize the geometries and to derive a spin protocol (Fig. 4) that ensures reliable performance for hematocrit values between 20 % and 60 %. The microfluidic structure is broken down to single fluidic elements: a sample reservoir, sedimentation and compression chambers, a collection chamber and connecting channels with either radial or tangential orientation. The total pressure across each fluidic element is computed by $P_{total} = P_{centrifugal} + P_{Euler} + P_{viscous} + P_{inertial} + P_{capillary}$.

The enclosed air bubble is modeled as an ideal gas with $(p_0 + \Delta p) \cdot (V_0 - \Delta V) = p_0 \cdot V_0$ where $p_0 = 1013 \text{ hPa}$ is the ambient pressure, $V_0 = 60 \mu\text{l}$ is the initial bubble volume, ΔV is the volume change and Δp is the pneumatic overpressure that is used to prime the siphon.

EXPERIMENTS

All experiments were carried out in 4 mm thick PMMA disks with milled fluidic structures sealed with a pressure sensitive adhesive polyolefin foil (#900320, HJ Bioanalytik, Germany). Channel cross-sections were $250 \mu\text{m} \times 250 \mu\text{m}$ and chambers had a depth of 1.5 mm. 40 μl of human whole blood with hematocrit values between 20 % and 60 % were processed at spinning frequencies of 75 Hz for cell sedimentation and at 25 Hz for plasma collection, while the acceleration and deceleration were set to $32 \text{ Hz}\cdot\text{s}^{-1}$.

Live image acquisition was performed with a stroboscopic optical setup grabbing one image per revolution. In order to determine the hematocrit of the extracted plasma, the remaining blood cells were chemically lysed with Triton X-100. In a subsequent spectrometric measurement, the characteristic 576 nm band of hemoglobin was evaluated as a measure of the hematocrit.

RESULTS

Fig. 3 shows that experimental filling level dynamics during the plasma separation process are in good agreement with simulation. It was predicted by simulation that the safety range was maintained for hematocrit values between 20 % and 60 %. This was also proven experimentally.

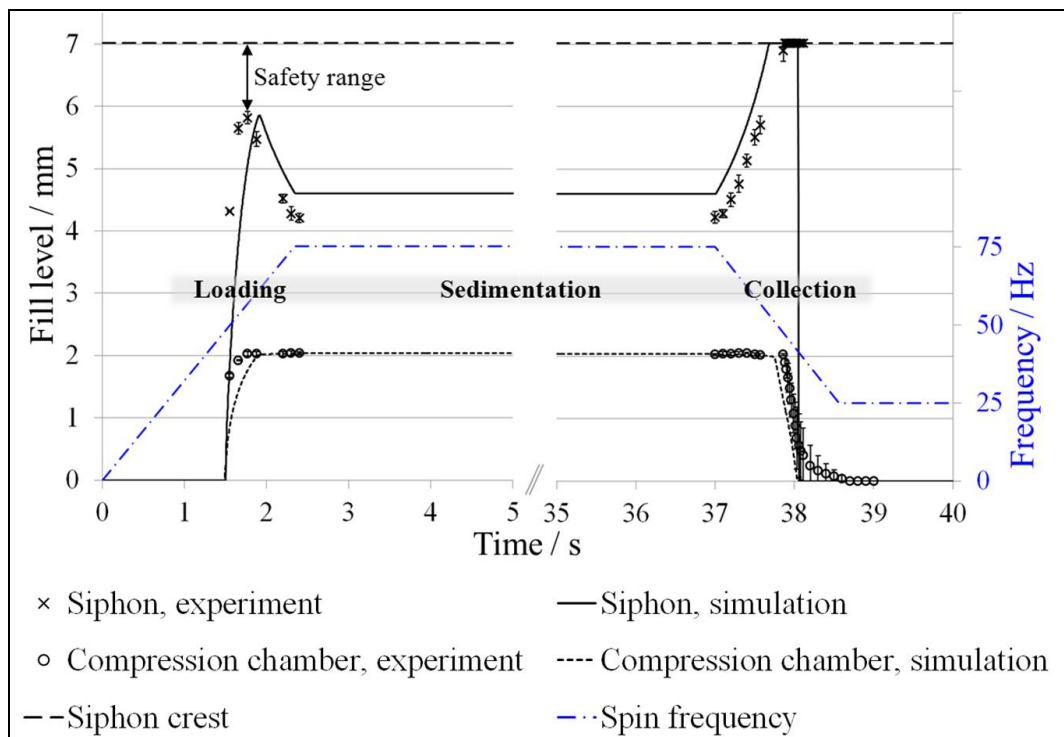


Figure 3: Optimized spin protocol and simulated and measured fill levels in the compression chamber and in the siphon. The safety range prevents premature siphon priming and thus ensures reliable performance. It was first derived from simulation and then proven experimentally. The high rotational frequency of 25 Hz during plasma collection prevents Euler force induced resuspension of cells ensuring high purity.

Fig. 4 schematically illustrates the optimized spin frequency protocol that has been derived from simulation and experimental data. It includes all effects that have to be considered for successful operation. Premature siphon priming due to insufficient bubble compression and Euler force induced resuspension of cells due to fast deceleration at low rotational frequencies has to be prevented. Undesired air entrapment in the siphon due to instability of the plasma-air interface at the siphon outlet may occur if the rotational frequency during plasma collection is too high. Tab. 1 summarizes the improved characteristics compared to the state-of-the-art method.

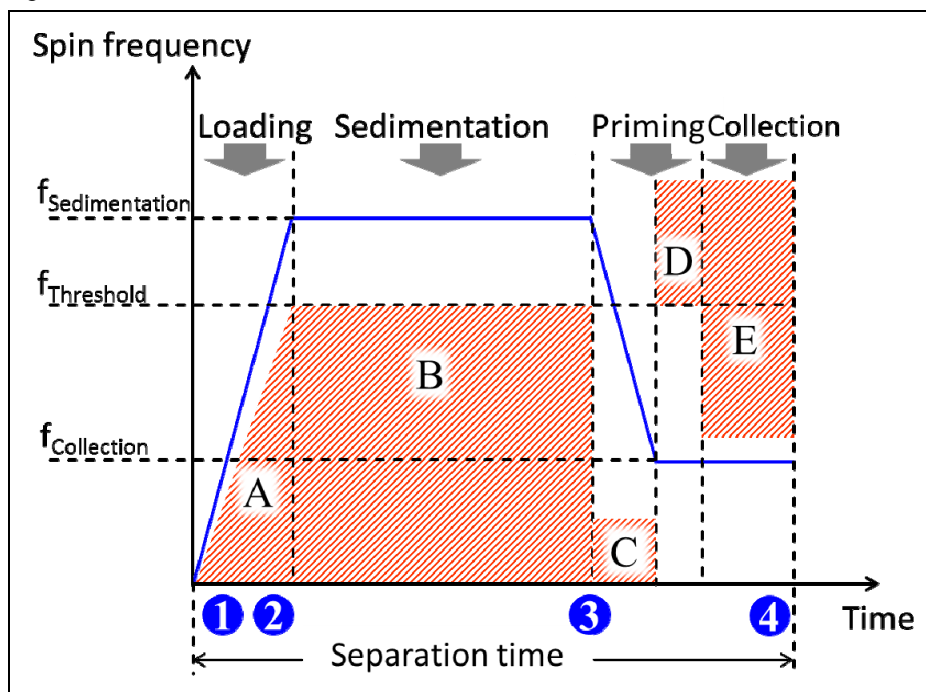


Figure 4: Optimized spin protocol for the plasma separation from whole blood. The protocol minimizes the risk of undesired effects which are: (A), (B) premature siphon priming due to insufficient bubble compression, (C) Euler force induced resuspension of cells, (D) no siphon priming and (E) air entrapment in the siphon. For fail safe operation, the frequency must not enter the shaded area. Numbers refer to the live images in Fig. 2.

Table 1: Comparison of the state-of-the-art plasma separation process with a capillary siphon valve to the new centrifugo-pneumatic process.

	Capillary siphon [1]	New centrifugo-pneumatic siphon
Surface coating required	Yes	No
Process duration	120 s	43 s
Yield	< 83 % (cv = 6 %)	88 % (cv = 4 %)
Purity	99.5 %	99.8% (cv = 0.1 %)
Hematocrit	< 52 %	60 %

CONCLUSION

Centrifugo-pneumatic siphon valving in centrifugal microfluidic systems significantly improves plasma separation from whole blood and can potentially also be used for the separation of other kinds of dispersions. Network modeling and simulation was demonstrated to optimize the fluidic layout of the plasma separation module and might have great potential to be applied for optimization of other centrifugal microfluidic modules, as well. Since the working principle is not based on capillary action, the centrifugo-pneumatic siphon makes surface treatment obsolete and thus enables low-cost fabrication and longtime storage of the microfluidic disk.

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