A LOW-COST AND EASY TO USE SORTING DEVICE FOR THE SEPARATION OF EXTRACELLULAR VESICLES FROM COMPLEX FLUIDS

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ABSTRACT

We propose a simple microfluidic device able to separate extracellular vesicles (EVs and exosomes) from a complex sample using a low cost and ready-to-use hydrodynamic filtration lab on chip. We have shown the efficient separation of biofunctionnaliszed nanoparticles mimicking platelets derived EVs (diameter 700 nm) from synthetic samples and from diluted whole blood. Separation yield and recovery yield have been measured > 96% and 100% respectively.

KEYWORDS: Hydrodynamic filtration, Sorting, Extracellular vesicles

INTRODUCTION

Identifying and characterizing extracellular vesicles (EVs) in biofluids could lead to unprecedented diagnosis/prognosis assays for a wide set of pathologies. Owe to their complexity in size, origin, membrane markers, there is currently no ideal technology available yet to quantify and characterize cell-derived microvesicles, which have high potential in clinical diagnostics and early treatment of life threatening diseases such as cancer, cardio vascular diseases… A major bottleneck lies in standard separation technics, which rely on heavy equipment and complex and denaturing methods. In that goal, we propose a microfluidic device (Lab-On-Chip) enabling to separate particles from a complex sample efficiently, easy to use and in a single step.

THEORY

Hydrodynamic filtration is a simple and passive method introduced by Yamada and Seki [1] and revisited by Fouet et al [2], aiming at separating particles according to a defined size. This method answers an essential requirement: it is less prone to clogging than filter-based geometries, since the minimal dimensions of channels can be more than one order of magnitude superior to the critical sorting size. As illustrated in Figure 1A, it is based on the exclusion of the particles whose diameter is larger than a defined value, called the cut-off radius R_c defined by the flow ratio between the main and lateral channel.

EXPERIMENTAL

Choosing the appropriate dimensions of the channels, we designed and fabricated chips (Figure 1B) [3] enabling the separation of vesicles at tunable cut-off (150-900 nm) using an original technology based on polymer dry film lamination and photolithography. The proof-of-concept was done using 3 fluorescent calibration nanoparticles (NP140, NP480 and NP920 nm in diameter) biofunctionnalized (Figure 1C) with proteins (ovalbumin and casein). The size range covers EVs dimensions, and the protein biofunctionnalization allows to capture them on a dedicated biochip presenting corresponding ligands. Once validation with synthetic solution (nanoparticles diluted in PBS + 0.01% Tween20), we injected diluted blood (10 x dilution) spiked with a combination of NP480 and NP920. Their concentration was ranging from 2.10^6 to 1.10^8 particles /mL. Filtered solutions were then studied by Tunable Resistive Pulse Sensing (TRPS) or direct counting (Malassez cells).

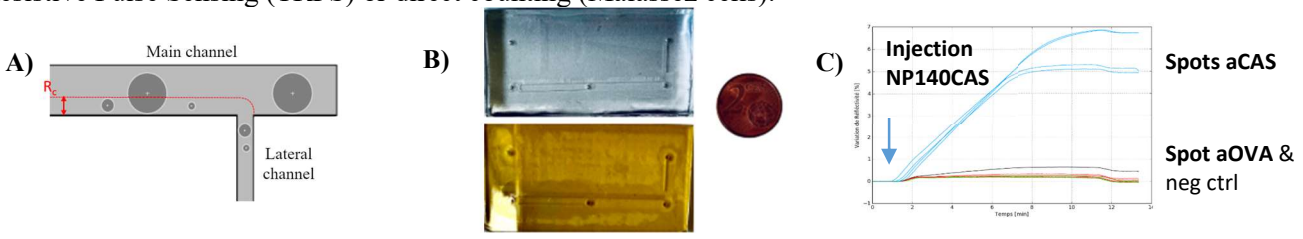

Figure 1: Microfluidic approach using hydrodynamic filtration. A) Principle of hydrodynamic filtration. B) Lab-on-chip device. C) Immunocapture of biofunctionnalized beads on dedicated biochips.

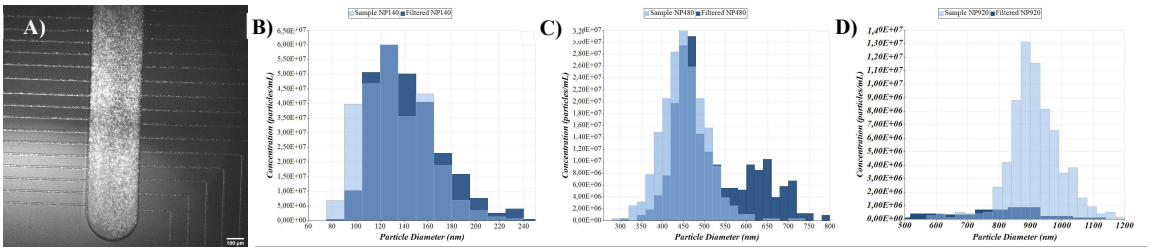

Figure 2: A) Image tracking of filtration by microscopy focus on lateral channels. Comparison between size distribution and concentration in injected NP sample (light blue) and filtered (dark blue) by TRPS. A) NP140 distribution. B) NP480, distribution of filtered NP480 was larger than sample due to agglomeration of NP480. C) NP920 distribution.

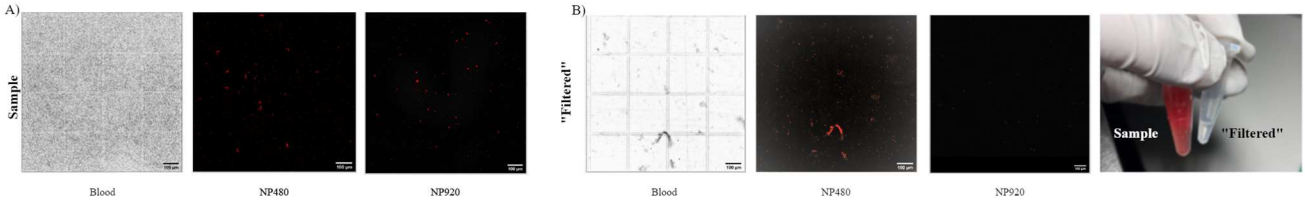

Figure 3: Direct cell counting. A) Counting blood cells and NP in sample. B) Counting blood cells and NP in filtered.

RESULTS AND DISCUSSION

We demonstrated, first in PBS, that particles of diameter 140 and 480 nm were filtered while 920 nm particles remained flowing in the unfiltered sample. With our device, we were able to collect 35 µL of processed sample (i.e. filtered) within 30 minutes, which is highly compatible with the requirements of the following analysis step. For each separation, we compared the injected sample with "filtered" solution and showed that NP140 and NP480 concentration are identical in injected and filtered solution (Figure 2). We also observed the quasi-total depletion of NP920 in the filtered sample. From measurements, we deduced a 100% recovery yield and a 96-98% filtering yield. The separation of particles spiked in more complex media (diluted blood and plasma) occurred also efficiently proving this method is a good candidate for processing natural samples (Figure 3).

CONCLUSION

We did the proof-of-concept of on-chip nanoparticles size-separation in the 100-900 nm range where flow cytometers cannot separate. First results, based on calibrated biofunctionnalized nanoparticles mimicking EVs, showed high efficiency separation. We demonstrated that the use of a biological fluid, such as blood or plasma, did not affect the separation results. We are currently running experiments, with another design, with NP140 mimicking exosomes. We are also improving the performances of the devices (filtration flow rate, filtered volume) and are coupling it to an immuno-trapping device in order to provide an integrated EVs sorting and capture bio-analytical system.

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REFERENCES

[1] Yamada, M., & Seki, M. (2005). Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics. Lab on a Chip, 5(11), 1233-1239.

[2] Fouet, M., et al. "Filter-less submicron hydrodynamic size sorting." Lab on a Chip 16,4 (2016): 720-733.

[2] Courson, R, et al. "Low-cost multilevel microchannel lab on chip: DF-1000 series dry film photoresist as a promising enabler." RSC Advances 4,97 (2014): 54847-54853.

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