Acoustofluidic based device for extracellular vesicles isolation

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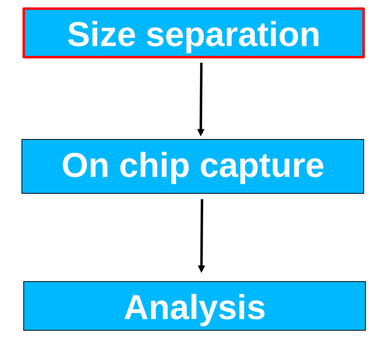
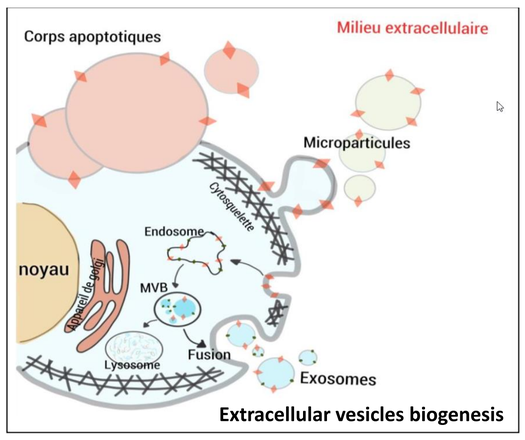
INTRODUCTION

Extracellular vesicles[1] (EVs, among them exosomes and microvesicles) and subcellular species can be found in all biological fluids and present an increasing potential for biological applications. Succeeding in separating these vesicles from their physiological environment and analysing different subsets is of primary importance.
The real challenge, for this kind of particles, is to find a reliable method, which works in an easy and a simple way, and that is reproducible and fast. For this purpose acoustofluidic particles-sorting devices have been demonstrated[2,3]. We propose a combination of several complementary approaches to isolate, detect and analyse biological microparticles. First, we developed an acoustofluidic device enabling to separate particles from a complex sample by using stationary acoustic waves. Secondly, we propose an other device, which consists in a fluidic chamber integrating a home-made gold biochip[4], with a tailored design and allowing the capture of vesicles subsets on specific biofunctionalized spots of antibodies and receptors. Then different analytical technics could be used, firstly in solution to characterize sorted vesicles, by tuneable resistive pulse analysis (TRPS), and then on the biochip to characterize the size and phenotype of the captured material, by atomic force microscopy (AFM) and mass spectrometry (MS) respectively. These methods allow obtaining important information about these nano-vesicles which have high potential and relevancy in clinical diagnostics and early treatment of numerous diseases.



EXTRACELLULAR VESICLES



Extracellular vesicles biogenesis

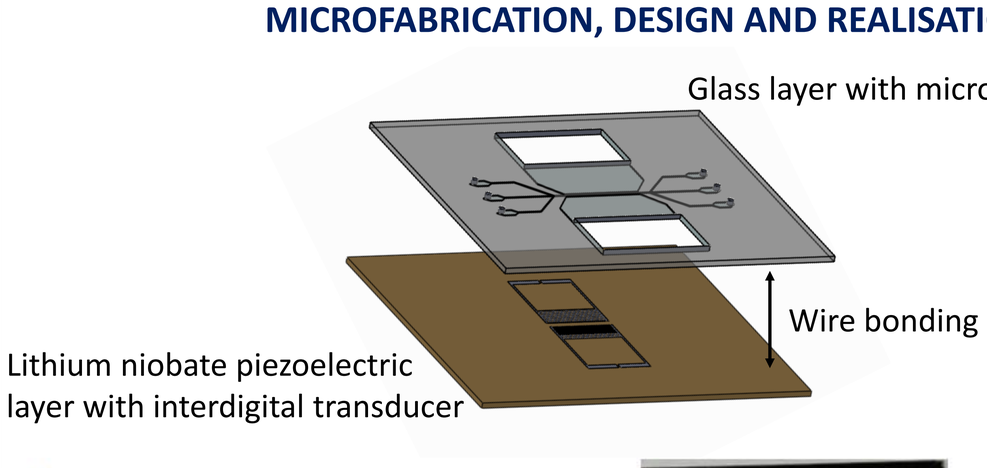
Extracellular vesicles are subdivided in 3 groups:
exosomes (30-100 nm), **microvesicles** (50-200 nm) and **apoptotic bodies (**500-5000 nm).
Each group is generated by different pathways, have specific content (DNA, RNA…) and display different kinds of proteins.

Isolation technics	time	price	consuming volume	recovery yield	resolution and LOD
Flow cytometry	Long (hours)	Expensive (more than 10 k euro)	High (ml)	-	LOD 300 nm
Ultracentrifugation	Long (hours)	Expensive (more than 1 k euros)	High (ml)	about 50%	medium resolution
Steric exclusion chromatography	quick	Low cost	High (ml)	less than 50%	good
Polymer assisted centrifugation	quick	Low cost (at short time)	Medium(µl/ml)	less than 50% with some contaminant	medium resolution

Isolation methods usually used as prior steps for EVs analysis

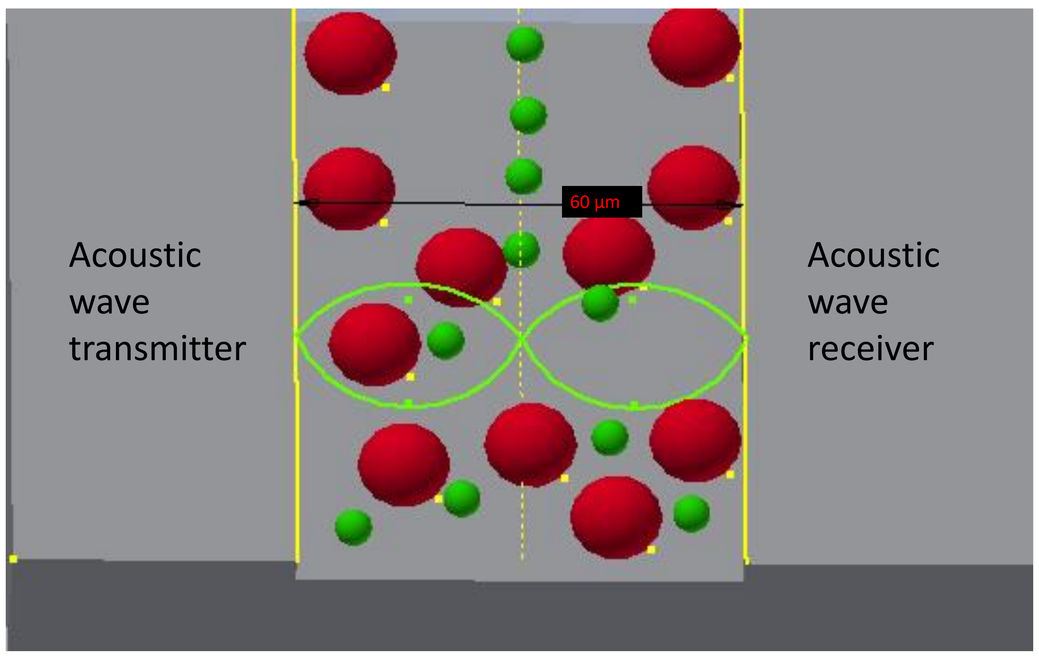
EVs could be used as biomarkers only if there is a standardized isolation method, which is able to separate fastly, reduce the sample volume in order to make it safe for healthy donors, and maintain a high yield. One of the best candidate is acoustic particle sorting.

MICROFABRICATION, DESIGN AND REALISATION



Design of the device (left), and picture of the device after microfabrication (right)

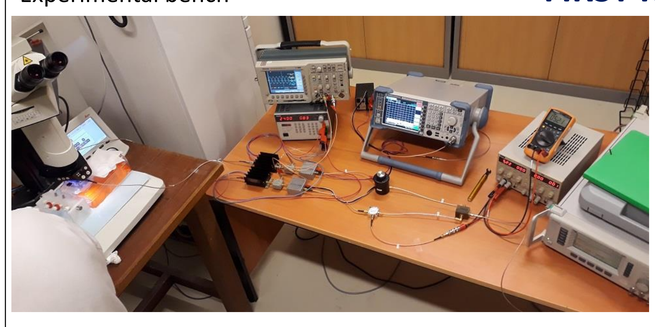
Particle sorting by SSAW principle

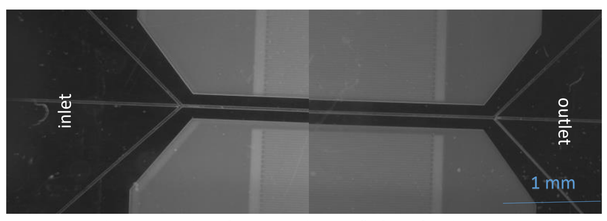


To be used as particle sorting, several conditions should be reached :
- Stationary acoustic wave (SSAW)
- the particle flow should be laminar
- Particle diameter < λ/50
- The system should be modular
- Work at 40 Mhz

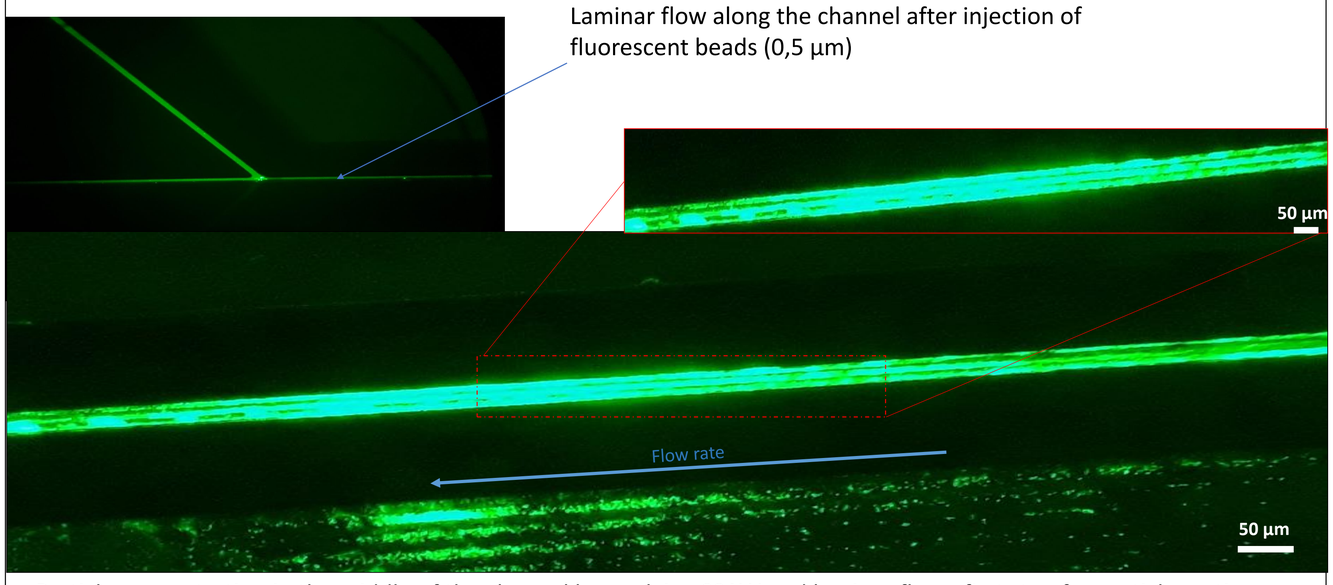
FIRST RESULTS

Experimental bench





Microscopic observation of the device



Particle concentration in the middle of the channel by applying SSAW and laminar flow : first step for particle sorting

Acknowledgements and reference

We thank Renatech national network for its support on microtechnological facilities
We thank Emmanuel DORDOR from common service of mechanics for his advice and his work
We thank Franck Vaudrin-Lardet from common service of electronics for his advice and his work

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CONCLUSION & PERPECTIVES

The purpose of this project is to develop an acoustofluidic device, which allows micro/nanoparticle sorting by size, and to couple this device with other microfluidic device allowing EVs immuno-capture and analysis. Our device was able to manipulate particles in the microfluidic channel (60 µm*60µm) with SSAW and to guide different kind of particles in different outlets, depending on particle size. Proof of concept of this (pre)-analytical solution is obtained with biosynthetic beads used as calibrants in various media (ideal solution to biological fluid). Then, this solution will be adjusted to such biological particles as extracellular vesicles (exosomes and microparticles) contained in several types of complex body fluid, to get specific information allowing diagnostic and knowledge on different diseases.

This project was supported by the EUR EIPHI program (Contract No. ANR-17-EURE-0002)