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DETERMINISTIC LATERAL DISPLACEMENT FOR THE SORTING OF EXTRACELLULAR VESICLES FROM COMPLEX BIOLOGICAL SAMPLES

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ABSTRACT

Extracellular Vesicles (EVs) are secreted by cells and found in biofluids, with concentration correlated with oncogenic signals, making them attractive biomarkers for liquid biopsy. The current gold-standard method to isolate EVs requires an ultracentrifugation (UC) step among others. The costs and complexity of this technique are forbiddingly high for many researchers, as for clinical implementation. This paper reports a microfluidic method for EVs isolation based on Deterministic Lateral Displacement (DLD) size sorting. We improved the design from our initial device and demonstrated the capacity to isolate EVs from cell culture media, with main population size distribution consistent with UC technique.

KEYWORDS: Extracellular Vesicles, Deterministic Lateral Displacement, Size Sorting, Microfluidic

INTRODUCTION

EVs are lipid nanoparticles secreted by cells for communication purpose that circulate in biological fluids as blood. They convey information from a parental cell to another cell through surface proteins and their DNA/RNA content. Thus, they are considered as promising biomarkers in the field of liquid biopsy. EVs isolation is a technological challenge currently addressed using UC. Even though this method is widely used, it has a significant number of limitations, so the International Society of Extracellular Vesicles (ISEV) has encouraged the development of more reproducible methods for EVs isolation in 2018 [1]. Previous works [2] have demonstrated the ability to sort EVs using nanoscale DLD arrays but required restrictive preparation steps. Our approach aims at sorting nanometric particles using micrometer-sized pillar array thus decreasing the fluidic resistance and significantly increasing the flow rate. Improving our initial DLD device [3], in terms of DLD design, fabrication and fluidic packaging, has allowed the sorting of submicronic biological particles, as EVs, as we report here.

EXPERIMENTAL

Silicon DLD arrays, fabricated using silicon microfabrication technologies, were integrated into a polymer-based fluidic cartridge, specifically equipped to withstand inlet pressures as high as several bars, necessary to overcome the large fluidic resistance of DLD micrometer-scaled channels. First, a low-speed centrifugation (300 g, 10 min) was performed to remove THP-1 cells from the culture media, then the supernatant was injected into the DLD device at a flow rate of 180 $\mu\text{L}/\text{h}$ under a five bars pressure. The analysis of particle distribution was performed using Nanoparticle Tracking Analysis (NTA, NS300, Malvern Panalytical) at 22°C.

RESULTS AND DISCUSSION

The DLD technique consists of a size sorting system using a tiled pillar array, which geometrical parameters define a cut-off diameter (D_c). Particles smaller than D_c are not deviated by the pillar array as opposed to larger particles (Figure 1 (a)). Our design features pillars diameter and gap of 1.5 μm , leading to an estimated D_c of ~ 345 nm. This D_c has been targeted to collect the most abundant EVs population, presumably distributed between 50 and 300 nm in diameter, while the gap size has been chosen to minimize hydraulic resistance. A Scanning Electron Microscopy (SEM) picture of the array and an optical picture of the integrated fluidic system are shown in Figure 1 (b) and (c). The separation performance was compared to results obtained on similar samples processed by UC. Both samples were then compared in terms of size and concentration using a NTA analysis, which is considered as the standard method for EVs characterization (Figure 2). Data shows multi-modal populations in both purification methods with a main population around 130 nm. These results highlight the potential of our DLD system as an interesting way of offering a reproducible and standardized EVs isolation system from complex biological samples.

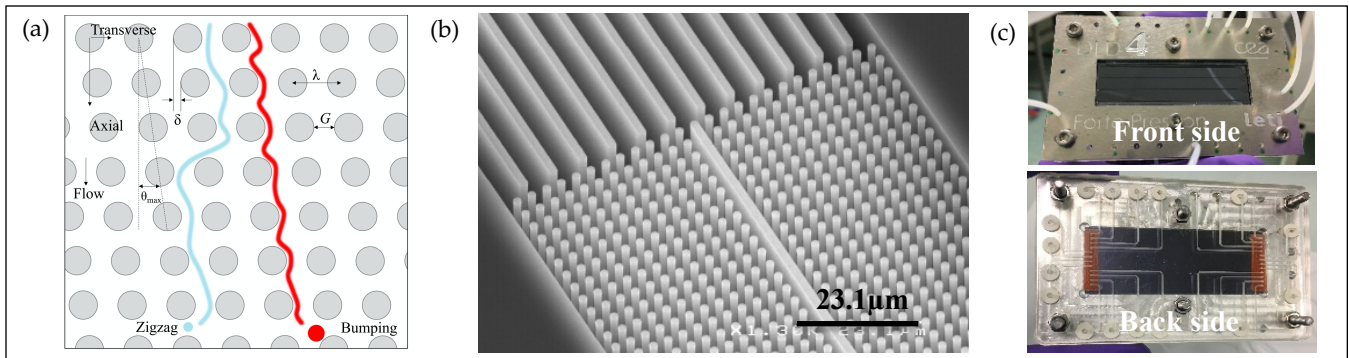


Figure 1: (a) Scheme of DLD size sorting principle; (b) SEM picture of DLD silicon chip; (c) Pictures of DLD chip device composed of silicon chip and a microfluidic cartridge for holding the chip and connection with tubings.

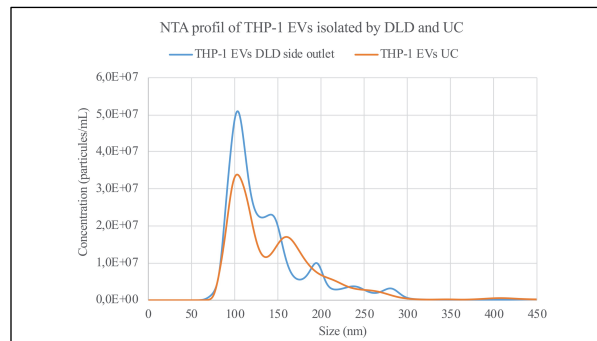


Figure 2: Comparison of NTA profile size VS concentration of EVs isolated from THP-1 cells by conventional ultracentrifugation (orange curve) and with our DLD microfluidic device (blue curve).

CONCLUSION

We have reported an original strategy enabling the fast and easy-to-operate isolation of EVs as promising biomarkers. Through a specific DLD design and optimization of hydraulic resistance, we have demonstrated the capacity to isolate EVs from THP-1 cell culture medium. The characterization data obtained with NTA show similar size distribution trend for the main population subset as compared with UC. These encouraging results pave the way for a non-destructive size-based microfluidic technique for the isolation of EVs from complex samples.

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