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INTEGRATED MICROFLUIDIC PLATFORM FOR SINGLE ORGANOID CULTURE AND SECRETED EXTRACELLULAR VESICLE ISOLATION

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ABSTRACT

In this abstract, we present a versatile microfluidic platform enabling the isolation of extracellular vesicles (EVs) secreted by a single organoid. Our automated device combines both the on-chip culture of a single organoid and an integrated micropump downstream allowing the continuous secretion sampling. Leveraging an immunomagnetic capture protocol, we achieve selective and efficient EVs isolation from whole secretions. This work is paving the way for an integrated companion test assay based on EVs secretion monitoring from organoids.

KEYWORDS : Extracellular vesicles – Organoid-on-chip – Microphysiological systems

INTRODUCTION

Understanding extracellular vesicles (EVs) as mediators of intercellular communication holds great promise for clinical applications. However, the challenge of isolating EVs from bio-fluids impedes progress in this field, highlighting the necessity for physiologically relevant in vitro tools. Currently, studies of EVs secreted by organoids are carried out using conventional methods of cell culture and EVs isolation, requiring the pooling of several organoids and mass analysis, which masks the heterogeneity within the population [1]. To address these challenges, we have been developing a microfluidic platform for (i) the culture and secretion collection of a single organoid and (ii) the specific isolation of EVs from its whole secretion.

EXPERIMENTAL

The microfluidic cartridges consist of two layers of COC (cyclo-olefin copolymer) which enclose a hyperelastic membrane, forming a network of pneumatically actuated valves (Fig. 2) and are operated automatically by in-house software [2]. Experimental steps are summarized in Figure 3. Firstly, the organoid is hydrodynamically trapped [3], then it is perfused at 1 μ L/min with exo-free medium thanks to an integrated peristaltic-type micropump. Afterwards, EVs are isolated from the collected secretions using anti-CD63 and anti-CD81 magnetic beads (Dynabeads, Thermofisher). Finally, from the isolated EVs, RNA is extracted (miRNeasy kit, Qiagen) and the miR profile analyzed by qRT-PCR (TaqMan, Thermofisher).

RESULTS AND DISCUSSION

Firstly, the fluidic function of single organoid culture and secretion sampling was validated using both native pancreatic islets and endoC β H5-derived spheroids. The spheroids were maintained in culture in our microfluidic system for 10 days with a remarkable viability rate of 97% (Fig. 4A-B). Furthermore, the functionality of both the islets and spheroids was assessed by glucose-stimulated insulin secretion (GSIS) tests conducted at multiple time points (Fig. 4C). PIV experiments and COMSOL Multiphysics simulations confirmed minimal shear stress (less than 1Pa) from the integrated peristaltic pump experienced by the trapped organoid, which remained below the critical values documented in literature (30kPa)[4]. Subsequently, the efficacy of the EVs microfluidic isolation from secretions was confirmed, achieving 60% capture efficiencies. Finally, the complete protocol was applied to NCI-H295R-derived spheroids. PCR analysis of EVs-derived miRNA enabled the detection of miR-139-5p and miR-483-5p, associated with tumorigenicity in adenocortical cancer [5]. Overall, these innovative results demonstrate the feasibility and relevance of using microfluidic tools to study EVs secreted by a single organoid.

CONCLUSION

As a summary, this work presents a novel, fully automated microfluidic platform tailored for precise isolation of EVs from single organoid secretions. This innovative fully integrated microfluidic approach holds great promise for advancing pathology research and streamlining drug screening processes.

Word Count: 500

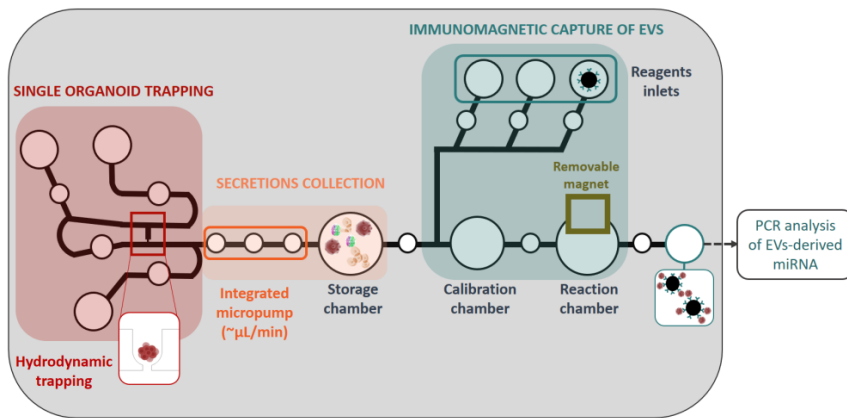


Figure 1: Conceptual diagram of the microfluidic cartridge showing (i) the hydrodynamic trapping zone of the organoid, (ii) the integrated micro-pump allowing continuous collection of secretions from said organoid and (iii) the immunomagnetic capture zone for selective isolation of EVs from the secretions

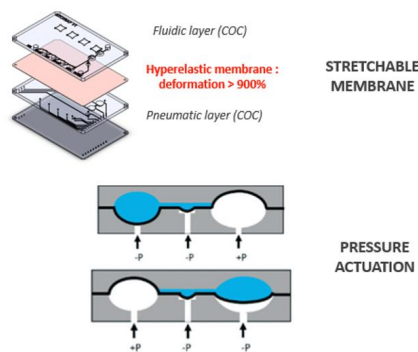


Figure 2: Structure of the microfluidic cartridge. Schematic view of the pneumatic actuation of the system creating a valve network.

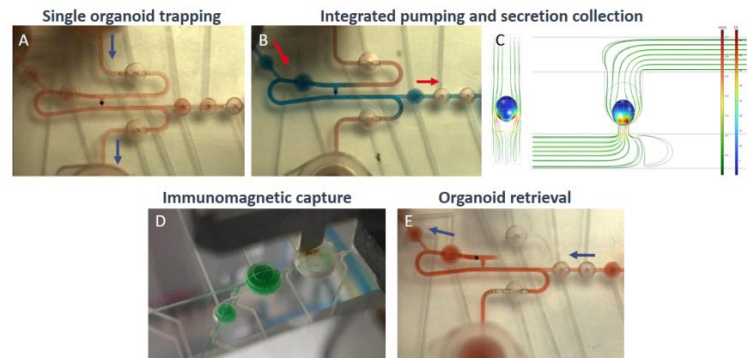


Figure 3: Experimental stages. (A) Hydrodynamic trapping of the organoid, (B) Integrated pump actuation to collect secretions, (C) Numerical simulation results. Current line: velocity field (mm/s), surface: shear rate (1/s), (D) Magnetic beads retention thanks to a removable magnetic arm, (E) Organoid retrieval at the end.

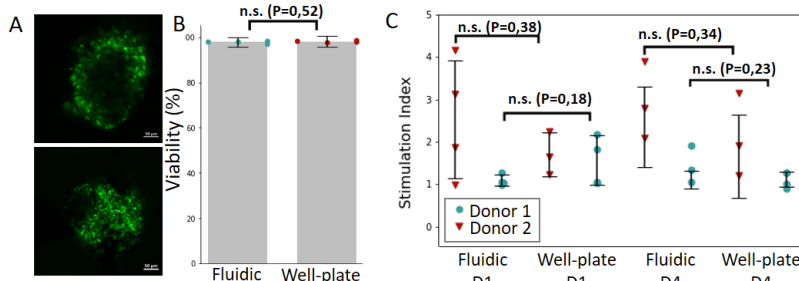


Figure 4: Validation of the functionality of the microfluidic platform for the culture of a single organoid and sampling of its secretions. (A-B) Viability results for EndoCBH5 spheroids after 10 days of static and fluidic culture, (C) Comparison of stimulation index obtained in static and fluidic conditions for human pancreatic islets from two donors after both 1 and 4 days of static and fluidic culture.

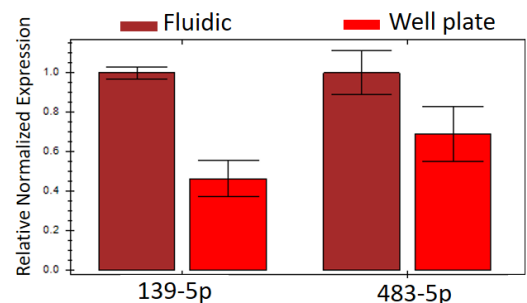


Figure 5: Validation of the overall integrated microfluidic platform. PCR results of EVs-derived miRNA secreted by a single NCI-H295R spheroid cultured both in fluidic and in static – Results normalized by RNU48 gene.

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