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Chloé Dupuis, Xavier Mousset, Guillaume Viraye, Pierre-Ewen Lecoq, Mauricio Hoyos, et al.. Towards concentric topology in organoids: Structuration and culture of brain derived spheroids in a Bulk Acoustic Wave resonator. 26th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2022), Oct 2022, Hangzhou, China. hal-03865434

HAL Id: hal-03865434

<https://hal.science/hal-03865434>

Submitted on 22 Nov 2022

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Towards concentric topology in organoids: Structuration and culture of brain derived spheroids in a Bulk Acoustic Wave resonator

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ABSTRACT

New tools and methods to control the way organoids self-organize or differentiate have recently arisen as it becomes more and more necessary to cultivate and analyze them in a reproducible manner. Here, we propose a new method using acoustic levitation to grow brain organoids from both primary neurons and patient derived glioblastomas and structure them with a concentric topology, reproducing the architecture found in vivo.

KEYWORDS: Acoustic Levitation, Neurosciences, Organoids

INTRODUCTION

For the last decade, organoids have proven to be a good candidate to study developmental stages as they self-assemble, recapitulating some of the tissue properties. Recent studies showed that guiding their self-assembly by tuning both mechanical and signaling properties of their environment improves differentiation and repeatability [1]. Alternatively, many techniques from bio-printing to microfluidic organ-on-a-chip are emerging with increased repeatability and control over both the topology and chemical environment of the engineered tissue. To unify this two parallel visions, the acoustic levitation, arises as a way to both structure organoids and let them self-organize. We previously showed that human mesenchymal stem cells (hMSC) self-organized into spheroids in just 24 hours of acoustic levitation [2] and that varying the acoustic frequency in a multi-node configuration allows for the spatial manipulation of cell spheroids and their merging in "assembloids" composed of contiguous spheroids [3]. Here, we first demonstrate the possibility of growing in acoustic levitation brain-derived spheroids, made of mouse primary neurons, or patient-derived glioblastoma cells for a week. Furthermore, we introduce an innovating method combining acoustic radiation force with controlled microfluidic flows to structure concentric assembloids of different types of cells, recapitulating more faithfully the topology found in vivo.

THEORY & EXPERIMENTAL

In Bulk Acoustic Wave (BAW) resonators filled with water or liquid medium, spherical objects such as particles or cells can be attracted towards precise positions (acoustic nodes) in the middle of the fluid by the Acoustic Radiation Force (ARF) (Figure 1.a). Acoustic levitation can thus be used to create large aggregates of cells in a contact-less and label-free manner and with enhanced cell-cell adhesion. In this work, we designed two different BAW resonators - millifluidic chips of PDMS bonded on a glass slide - for specific experimental needs: a larger one to cultivate multiple spheroids with a lateral optical access based on previous studies [2]; a smaller one with a particular geometry which allows us to control fluid flows and structure concentric assembloids.

RESULTS AND DISCUSSION

Using these setups, we were able to observe the self-organization of the spheroids from cell sheets to spheroids in 24 hours as previously shown [2] for all types of brain derived cells (Figure 1.b). On Figure 1.c, a cryosection of a cortical spheroid grown in acoustic levitation for 6 days is shown, with specific staining to identify particular compartments of neuronal cells like dendrites and somas (MAP2). Different techniques were used to assess the health of the spheroids and the survival rates are plotted on figure 1.d. We observed that for all types of cells, the spheroids cultivated in acoustic levitation had similar survival rates than control spheroids. The feasibility assessment of structuration of assembloids was conducted with the injection of fluorescent liquid in the BAW resonator already containing a neuronal spheroids. We observed that the fluorescent liquid moved into the chamber without mixing with the culture medium and wrapped up the spheroid by hydrodynamic effect (Figure 1.e). The hydrodynamic enfolding effect was then even enhanced by the axial component of the ARF. With the combination of those two phenomena, a first aggregate of striatal neurons was formed at the center of the BAW resonator chamber. Then, cortical neurons, stained with a fluorescent marker, were injected in the chamber and

surrounded the first aggregate, as expected from previous experiments. The resulting concentric assembloid was cultivated for 24 hours under a microscope and the same self-organization than monocellular spheroids was observed (Figure 1.f). Finally, the assembloid was recovered and analyzed with the same cryo-sectioning and staining protocol, showing that the concentric topology was conserved (Figure 1.g). Cortico-striatal assembloids cultivated for 6 days showed high viability. On-going analysis aim to verify the stability of the topology and establish connection patterns between the different neuronal types.

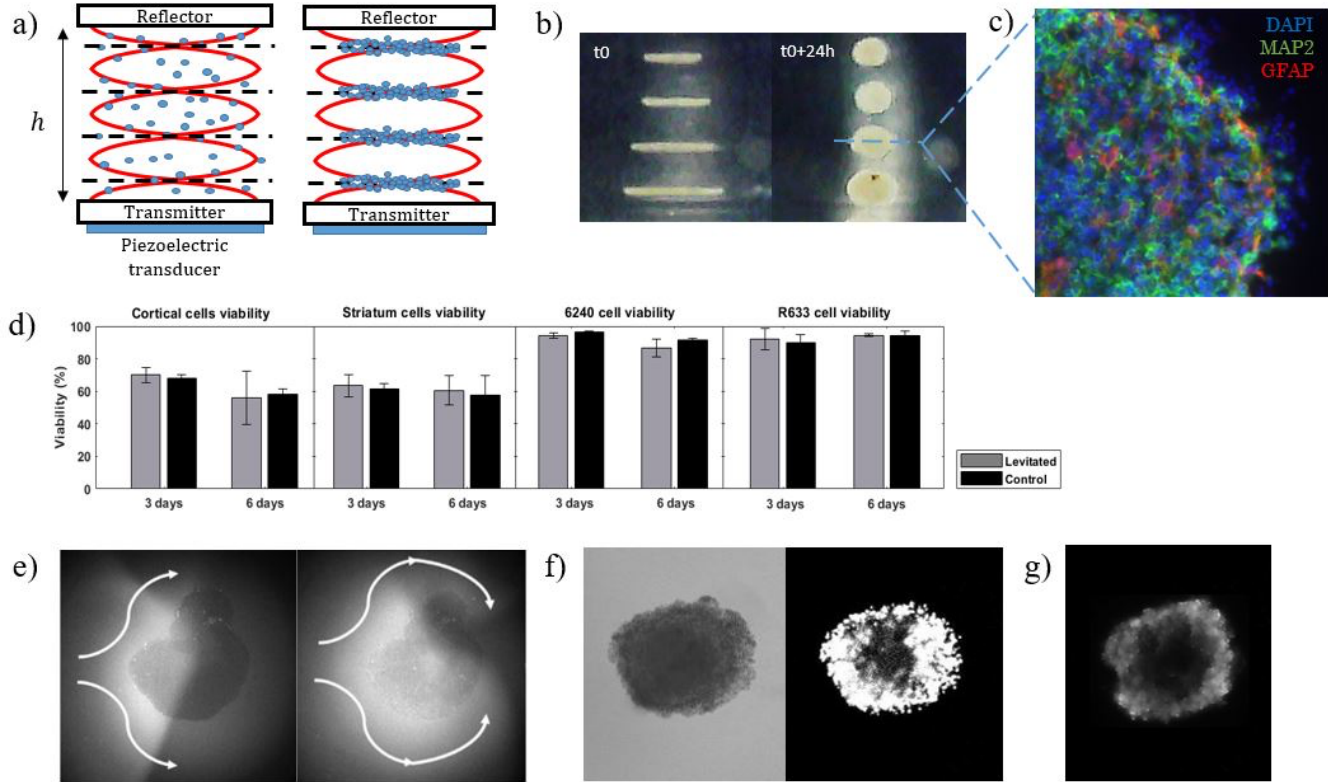


Figure 1: (a) Schematic of the multi-node acoustic wave resonator (b) Time-lapse snapshots showing the self-organization of cortical primary neurons after 24h of culture (c) Cryosection of a spheroid of cortical primary neurons cultivated for 6d in levitation, stained with DAPI (nucleus), MAP2 (dendrites and cell body) and GFAP (astrocytes) (d) Viability results of spheroids after 3d or 6d of culture in levitation with different cell types. (e) Demonstration of acoustofluidic enfolding of a cell aggregate with fluorescent solution. (f) Striatal neurons core surrounded with cortical neurons stained with CM-DIL after 24h of culture in acoustic levitation. (g) Cryosection of the cortico-striatal assembloid, fixed after 24h of culture.

CONCLUSION

In this study, we succeeded in growing in constant acoustic levitation brain-derived spheroids from mouse primary neurons and patient glioblastoma cells for a week, with similar viability than control spheroids. Moreover, we demonstrated a new method to structure concentric assembloids thanks to the combination of hydrodynamic and acoustic forces. This proof of concept is a step towards complex organoid architectures with controlled topology, offering the possibility to build ordered neuronal networks like the cortico-striatal neuroanatomic pathway or co-culture of neurospheres and tumoroids.

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