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# Novel acoustofluidic approach using a Bulk Acoustic Wave resonator to control the topology and culture conditions of brain derived spheroids

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## Introduction

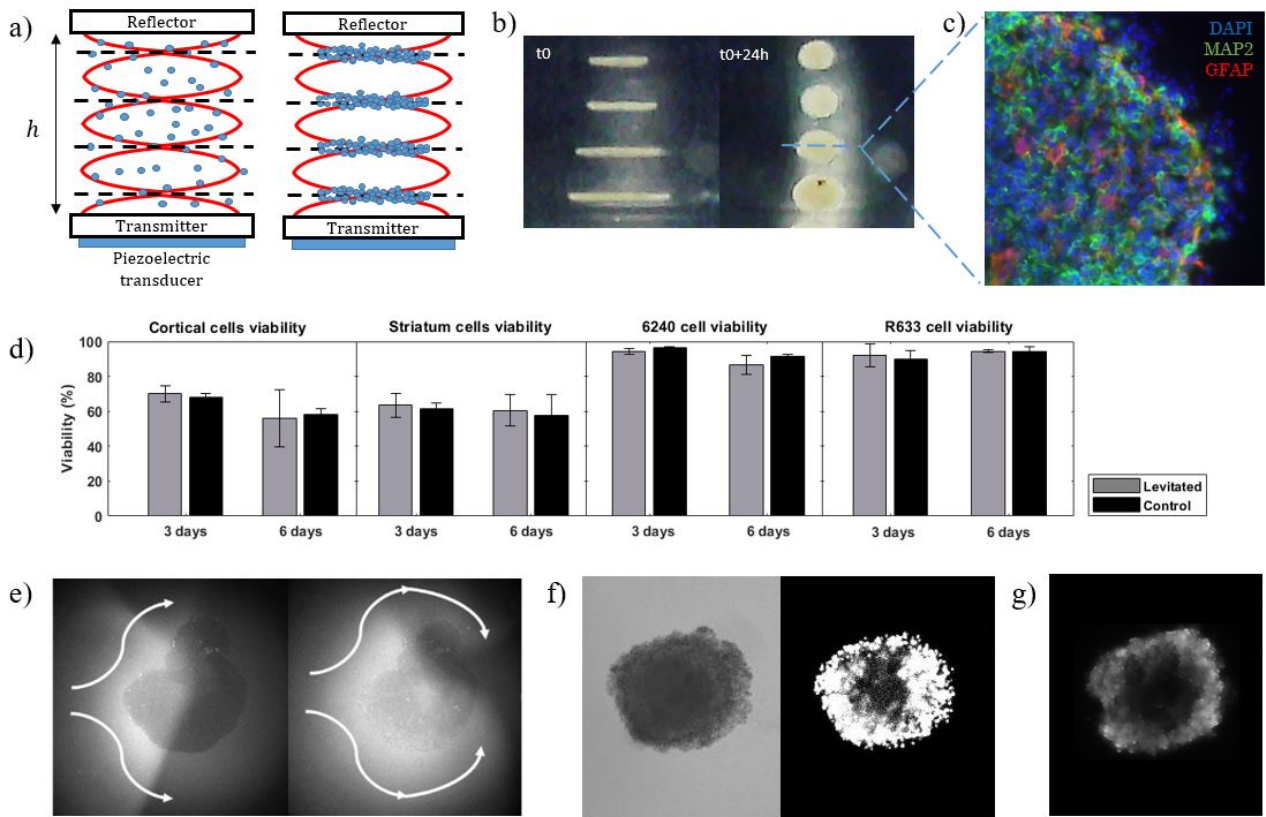
For the last decade, organoids have proven to be a good candidate to study developmental stages as they self assemble, recapitulating some of the tissue properties. Recent studies showed that guiding their self assembly by tuning both mechanical and signaling properties of their environment improves 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, a new scaffold-free approach, 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 and exhibited accelerated differentiation compared to classical 2D culture conditions [2]. We also demonstrated that varying the acoustic frequency in a multi-node configuration allows for the spatial manipulation of cell spheroids giving the possibility to merge them, leading to the formation of "assembloids" composed of contiguous spheroids [3]. Here, we first demonstrate the possibility of growing brain derived spheroids made of mouse primary neurons or patient derived glioblastoma cells for a week in constant acoustic levitation. Furthermore, we introduce an innovating method combining the fine tuning and shaping of acoustic radiation force fields with controlled microfluidic flows to structure concentric assembloids of different types of cells, recapitulating more faithfully the topology that can be found in vivo.

## Theoretical background & experimental setup

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 in the middle of the fluid (Figure 1.a). At these positions, the gravity is counterbalanced by the Acoustic Radiation Force (ARF) created when the resonance condition is respected ( $h = \frac{1}{2}\lambda_{ac}$ , with  $h$  the height of the cavity and  $\lambda_{ac}$  the acoustic wavelength). Thanks to this phenomenon, acoustic levitation can 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 higher 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 around a single aggregate and structure assembloids in a concentric manner.

## Results

With the specific setup allowing for monitoring of multiple cell spheroids growth in acoustic levitation, we were able to observe their self-organization from sheet to sphere in 24 hours as previously shown [2] for all types of brain derived cells (Figure 1.b). The spheroids were recovered after few days of culture and analyzed to evaluate their survival. On Figure 1.c, a cryosection of a cortical spheroid cultivated by acoustic levitation 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. For the structuration of assembloids, a feasibility assessment was conducted with the injection of fluorescent liquid in the BAW resonator already containing a neuronal spheroids. We observed that laminar flows were moving forward into the chamber without mixing with culture medium and wrapping up the spheroid by hydrodynamic effect (Figure 1.e). The enfolding effect due to hydrodynamic flows was then even enhanced by the axial component of the ARF. As a proof of concept of the structuration of concentric



**Figure 1:** (a) Schematic of the multi-node acoustic wave resonator (b) Time lapse snapshots showing the self-organization of cortical primary neurons after 24 hours of culture (c) Cryosection of a spheroid of cortical primary neurons cultivated for 6 days in levitation, stained with DAPI (nucleus), MAP2 (dendrites and cell body) and GFAP (astrocytes) (d) Viability results of spheroids after 3 and 6 days of culture in levitation with different cell types. From left to right, cortical and striatal mouse primary neurons (E14), 6240 and R633 patient derived cancer cells. For mouse primary neurons, a quantification algorithm of healthy DAPI staining was used to assess the survival of the spheroids. For patient derived cancer cells, viability was assessed with trypan blue exclusion test. (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 24 hours of culture in acoustic levitation. (g) Cryosection of the cortico-striatal assembloid, fixed after 24 hours of culture.

assembloids 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 cryosectioning and staining protocol, showing that the concentric topology was conserved (Figure 1.g). Cortico-striatal assembloids cultivated for 6 days showed high viability and on-going analysis aim to verify the stability of the topology and establish connection patterns between the different neuronal types.

## Conclusion

In this study, we succeeded in growing brain derived spheroids from mouse primary neurons and patient glioblastoma cells for a week in constant acoustic levitation 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 architecture with controlled topology, offering the possibility to build ordered neuronal networks like the cortico-striatal neuroanatomic pathway or co-culture of neurospheres and tumoroids.

## References

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