

## Self-organization of human mesenchymal stem cells into spheroids trapped in acoustic levitation.

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Today, three-dimensional (3D) cell cultures tend to replace 2D conventional methods because of their more relevant tissue-mimicking characteristics. Indeed, the 3D cell architecture (spheroid, organoid, etc) and the microenvironment is closer to In Vivo physiological behavior [1, 2]. Especially, the mechanic conditions of the environment will favor some cell behaviors. For example, the human mesenchymal stem cells (hMSC), which are adherent cells, are classically grown in 2D petri dishes until they reach the confluence.

Here we present a new approach based on multi-node acoustic levitation to grow the cells without interactions with solid walls. Once the cells are suspended in their culture medium, they can be moved toward the acoustic pressure nodes where they are trapped and maintained in acoustic levitation in perfectly straight monolayers. The monolayers of cells mimic the classical confluency of the cells in 2D culture but reach the confluency much faster. Interestingly, by maintaining the monolayers of MSCs in culture in levitation over a 24-h period, the MSCs spontaneously self-organized from cell sheets to cell spheroids with a characteristic time of about 10 h (see Fig.1) [3].

This approach of 3D cell culture is based on the use of the acoustic wave coupled with microfluidic. We designed a standing wave cavity to generate an important acoustic radiation force (ARF) while keeping a good optical access, which allows the observation and characterization of the self-organization dynamics. This new 3D cell culture method has been validated on MSCs over 24h experiments. The MSCs viability has been checked. They also maintained the expression of their cell surface markers. Interestingly, they also show a higher differentiation capacity compared to standard 2D culture conditions.

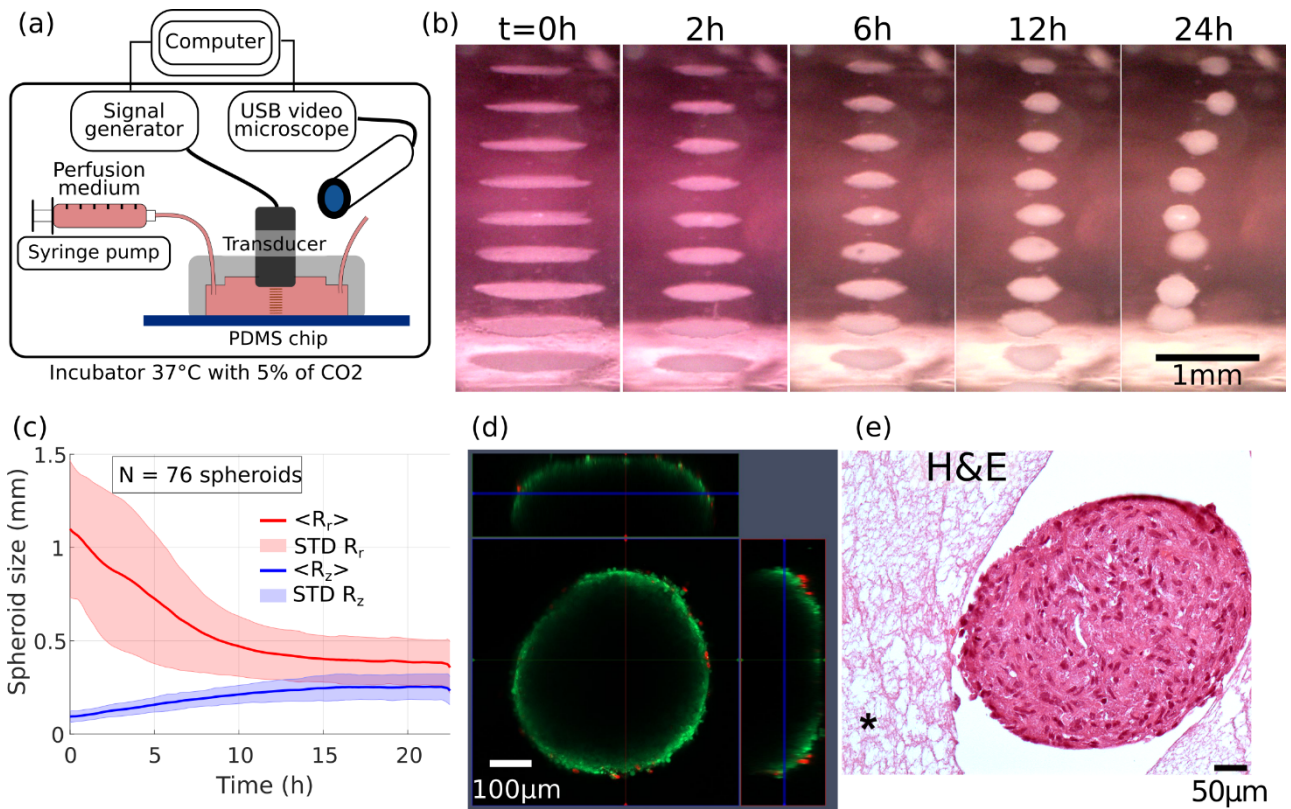
In our acoustofluidic system, the rapid formation of spheroids mimics the high confluency of 2D cultures and spheroid formation owing to the acoustic confinement (aggregation induced by the axial and transverse components of the ARF). This approach opens the path to a better understanding of the self-organization phenomena and can be applied to many other different types of cells. It also demonstrates the use of acoustic levitation as a new method of long-term, reproducible cell culture process for scaffold-free tissue engineering purposes such as organoid formation.

### References

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**Figure 1:** Self-organization of hMSCs cells sheets into spheroids shapes. (a) Global setup (b) Time lapse snapshots of the MSC self-organization. (c) Statistic time evolution of the axial and radial dimensions of the cells aggregates. (d) Confocal and fluorescence imagery of a spheroid. (e) Histology of MSC spheroids, stained with H&E for a general structure evaluation.