Mass production of uniform spheroids using acoustic standing waves

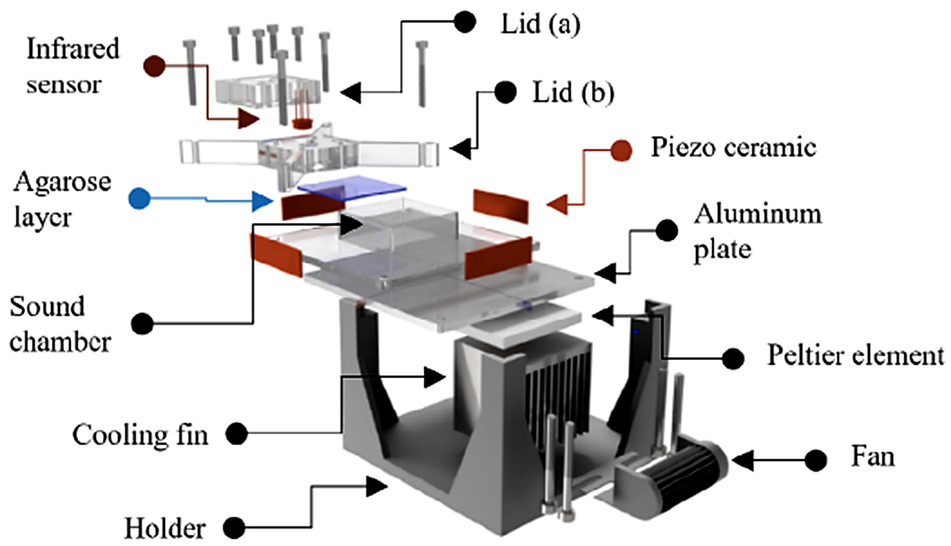
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INTRODUCTION/ OBJECTIVES

Organoids are important three-dimensional (3D) *in vitro* platforms for pharmacological, developmental or disease-related studies. We have developed a platform that enables the high-throughput production of uniform spheroids using standing acoustic waves [1]. Building up on this method, we introduce a strategy to assemble spheroids composed of different cell types into a layered architecture, thereby more closely recapitulating the the structural complexity of native tissues.

METHODS



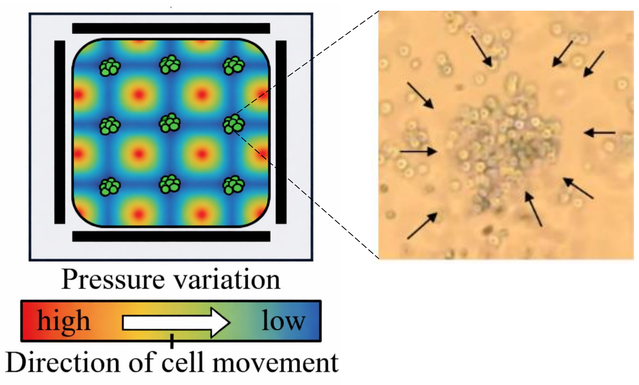


Fig. 1: Design and principle of the acoustic spheroid generation chamber. Top: Acoustic chamber whith four piezoelectric ceramics, a layer of agarose to prevent the cells from adhering to the bottom of the chamber, and a temperature control unit consisting of an infrared sensor, a Peltier element, an aluminium plate, cooling fins, and a fan. Bottom: Standing acoustic waves generated by piezoelectric ceramics create a pressure field within the chamber. The resulting acoustic radiation force drives cells towards regions of low pressure (blue), where they aggregate and form spheroids.

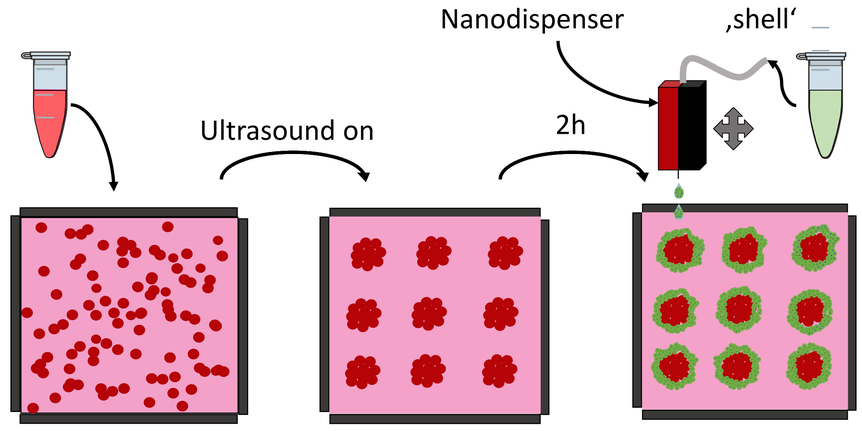
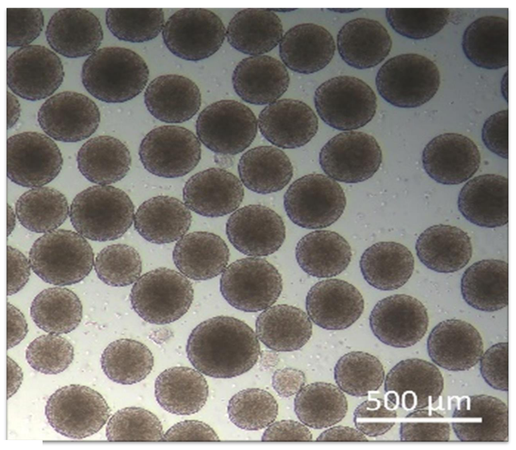
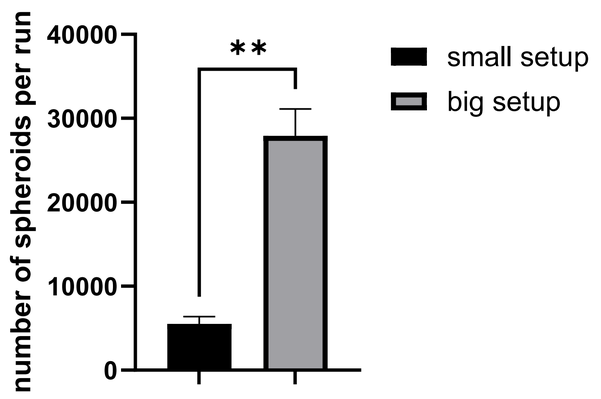


Fig. 2: Principle of generating layered multicellular spheroids. The cell type supposed to be in the core of the spheroids is introduced into the chamber, followed by the application of ultrasound to induce spheroid formation. After two hours, the cell type supposed to be in the shell of the spheroids is dispensed into the chamber using a nanodispenser. The subsequently added cells are directed toward the pressure nodes, where they accumulate and form a layer surrounding the initially formed spheroids.

RESULTS





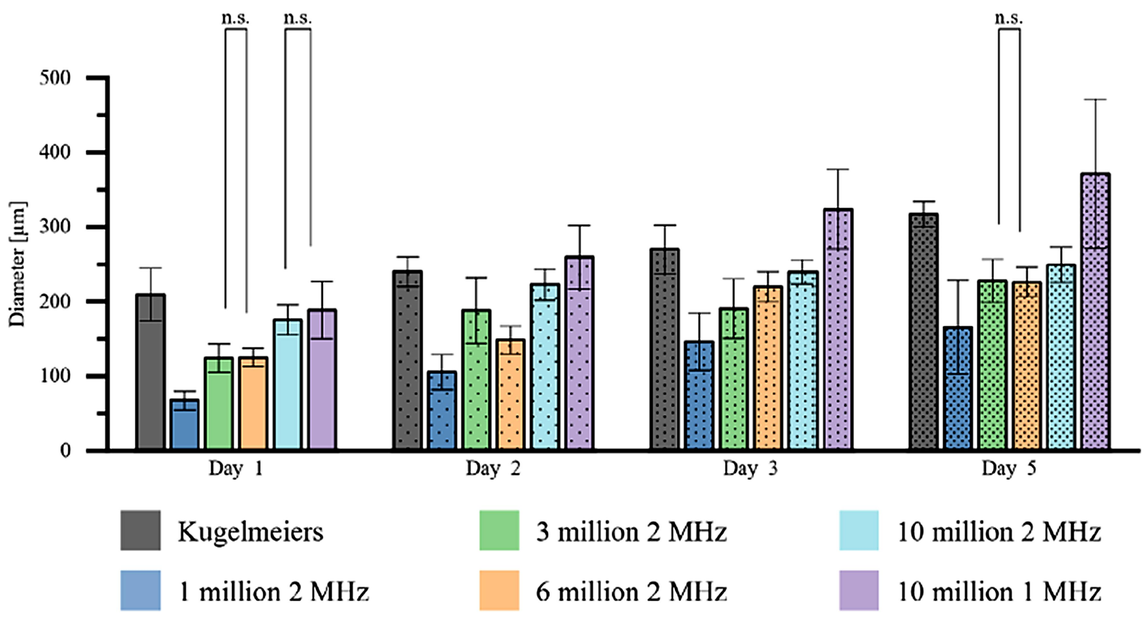


Fig. 3: Spheroids generated with the acoustic chamber. Top left: Spheroids generated out of 1 million cell using the 1 MHz acoustic chamber. Scale bar represents 500 µm. Top right: Number of spheroids generated at once by a small (30 mm x 30 mm) or a big (60 mm x 60 mm) chamber. Bottom: Size of the spheroids generated by the small setup (30 mm x 30 mm) depending on number of inserted cells and the acoustic frequency after one day, two days, 3 days and 5 days compared to spheroids generated using a ultra-low attachment plate from Kugelmeiers.

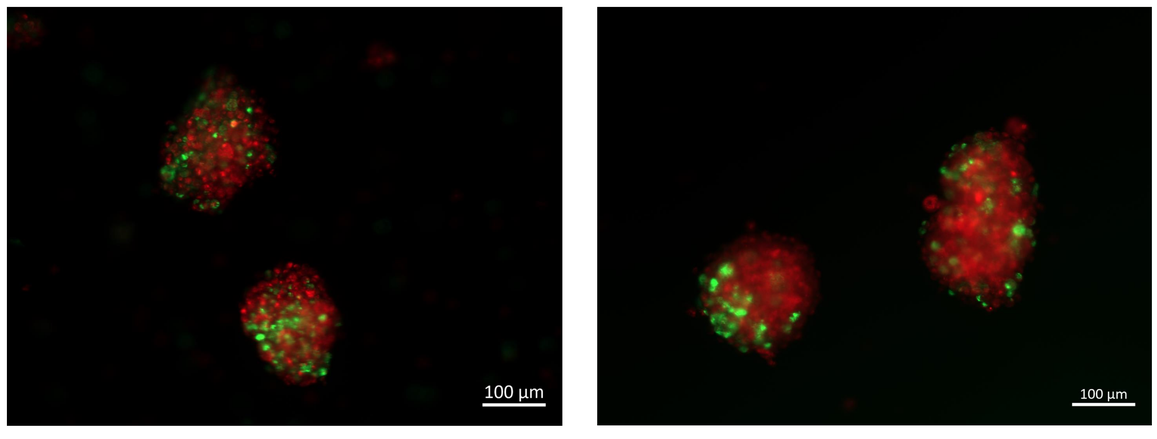


Fig. 4: Structured spheroids that have distinct layers of different cell types. The layered spheroids were generated using the principle of sequentially loading different cell types into the acoustic chamber as depicted in Figure 2. The red cells (MiaPaCa3, Vybrant DiI) were pipetted into the acoustic chamber first and the green cells (MiaPaCa3, Vybrant DiO) were dispensed into the running chamber after two hours using a nanodispenser. Scale bars represent 100 µm.

CONCLUSIONS

This method allows a mass production of uniform, round and dense spheroids and organoids which are highly requested for *in vitro* studies. Moreover, by sequentially filling in different cell types the structure and organization of these spheroids can be highly controlled allowing for vascularized and physiological microtissues.

Parts of the presented work were published in advance in the following original article:
[1] J. Hahn, E. Aksoy, S. Hamad, C. Kuckelkorn, A. Gómez Montoya, M. Ritter, K. Pfannkuche, H. Fischer (2025). Mass production of uniform embryoid bodies by acoustic standing waves. Small Methods 9:e01283. DOI: 10.1002/smtd.202501283.

The authors declare no conflict of interest.