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Culture of hepatic and stem cell spheroids in acoustic levitation induces different self-organisation dynamics

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Introduction

More than half of acute liver failures can be attributed to drug-induced liver injuries (DILI), causing major challenges for public health and drug development [1]. Currently, pharmaceutical tests of upcoming drugs mainly rely on animal models and two-dimensional (2D) human cell culture. However, both models suffer from serious limitations: variations between human and animal liver pathways cause some DILI to remain undetected in animal models, and the lack of long-term functionality of 2D cultures impairs reliability, costs, and convenience of *in vitro* tests [1].

A need for a shift in paradigm has emerged in recent years, calling for increasing functionality of human-based toxicity tests, for instance through the development of three-dimensional (3D) cultures [2]. With the development of organoids, organs-on-chip and microphysiological systems, the desired shift could be reached within years. These prementioned techniques can benefit from the field of acoustofluidics: acoustic manipulation of single cells can be used to form multicellular spheroids and organoids.

We have shown that in our microfluidics chip, viable and functional spheroids of hepatic cells can be cultured for long periods of time and submitted to different treatments via a continuous flux of medium [3]. Our robust technique allows self-organisation of the cells, which form standardized spheroids inside the microchip.

Theory and Experimental procedure

Acoustofluidics manipulation [4,5] allows the aggregation of a suspension of cells into monolayers, due to the acoustic radiation force (ARF) created by an ultrasonic standing wave of a few megahertz. Using this technology, we can create 30 to 50 large cell sheets (hundreds of micrometres long) in one PDMS (Polydimethylsiloxane) chip. A transparent side-wall allows optical microscopy. The ARF maintains the aggregates in acoustic levitation in the cell culture medium (no need of hydrogel or extracellular matrix) without any contact with the walls of the microfluidic chip.

Results and Discussion

We have shown previously that after a few hours, monolayers of mesenchymal stromal cells (MSC) spontaneously self-organize into spheroids [3]. We now successfully apply this method to other cell types, including the hepatic cell line HepaRG, cryopreserved primary hepatocytes and human umbilical vein endothelial cells (HUVEC). Except if stated otherwise, voltage is 3,5V and frequency is 2,2MHz, both stay constant during culture. In all cases, the self-organisation of cell sheets into spheroids is observed, see Fig. 1.

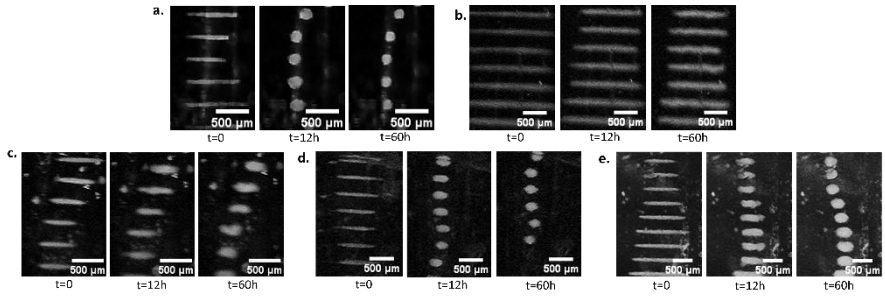


Figure 1: Optical imaging of the side-view of the chip during 60 hours of levitation of mesenchymal stromal cells (a), cryopreserved primary hepatocytes (b), HepaRG hepatic cell line (c), human umbilical vein endothelial cells HUVEC (d) and a coculture of HepaRG and HUVEC cells (e). (voltage: 3,5V / frequency: 2,2MHz)

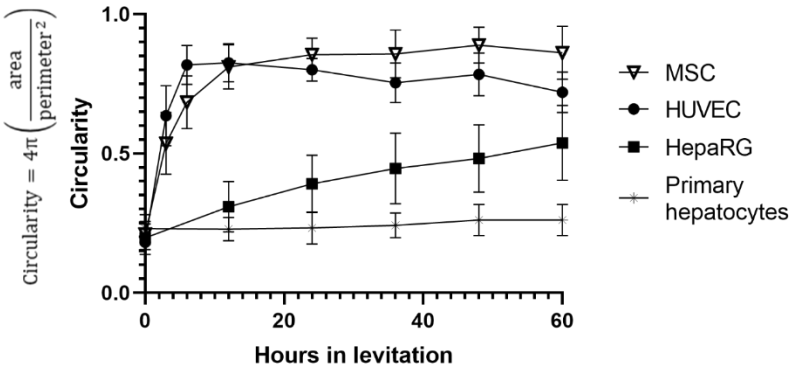


Figure 2: Circularity dynamic of different cell types levitated during 60 hours: human umbilical vein endothelial cells (HUVEC), mesenchymal stromal cells (MSC), HepaRG hepatic cell line and cryopreserved primary hepatocytes. (voltage: 3,5V / frequency: 2,2MHz)

The spheroids were levitated during several days without damaging cell viability, and kept at fixed positions while perfused with a flux of medium. Differences in shape of the spheroids and variation in the self-organisation dynamic depend on the cell type, and stay consistent between spheroids of the same cell type (see Fig. 2). The dynamic was not impacted by variations in the acoustic radiation force, thus suggesting a prominent role played by cell-cell interactions, see Fig. 3.

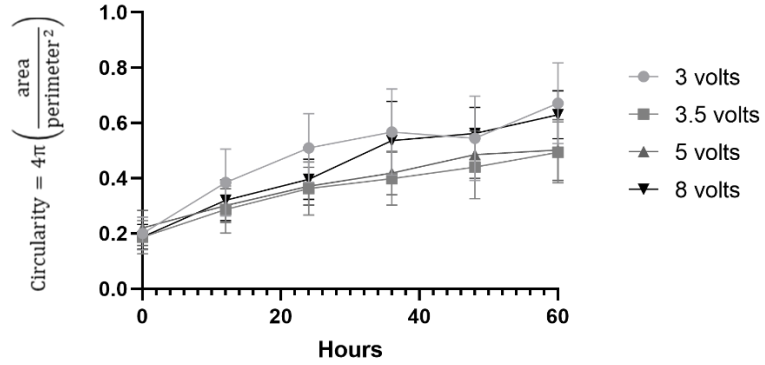


Figure 3: Circularity dynamic of HepaRG spheroids submitted to different voltages. (voltage: 3 to 8V / frequency: 2,2MHz)

Furthermore, dynamic was impacted by disruption of actin-mediated or E-cadherin-mediated self-organisation mechanisms, via treatment with ROCK inhibitor Y27632 or calcium chelator EDTA Ethylenediaminetetraacetic acid, see Fig. 4.

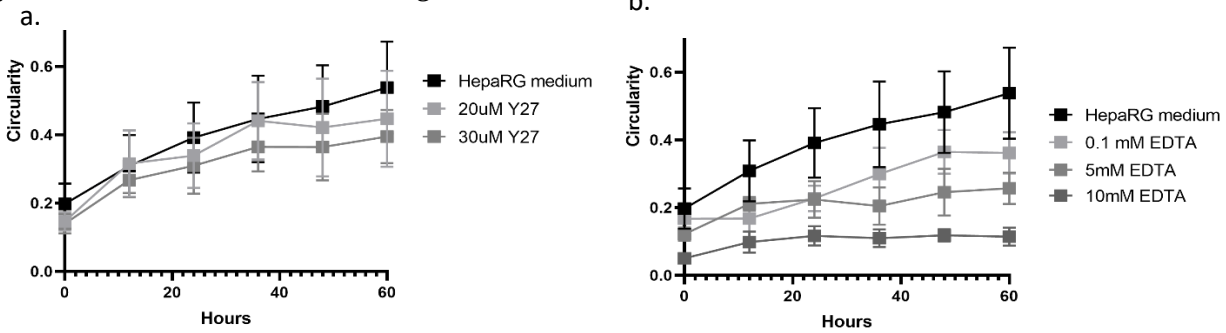


Figure 4: Circularity dynamic of HepaRG spheroids treated during 60 hours with ROCK inhibitor Y27632 (a) or calcium chelator EDTA Ethylenediaminetetraacetic acid (b). (voltage: 3,5V / frequency: 2,2MHz)

Conclusion

We can reproducibly create viable 3D spheroids, with a specific self-organisation dynamic for each cell type, and then culture them during several days. Self-organisation dynamic is not impacted by variations in the acoustic radiation force, but can be modified by altering cell-cell adhesion or actin-myosin interactions. This shows the prominent role played by cell-mediated forces compared to acoustic forces during this organisation.

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