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Acoustic levitation: a new process to stimulate stem cells for regenerative medicine

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1. Introduction

Acoustic levitation may constitute an alternative to conventional processes such as bioreactors for applying controlled mechanical stimulations on stem cells, as it is non-contact method with easily defined boundary conditions (Argyri et al, 2023). Indeed, cells are particularly sensitive to their mechanical environment and therefore response continuously by mechanotransduction process (Zhang & Habibovic, 2022), leading potentially to their differentiation. This dependence to external stimuli has brought mechanobiology as a key field for regenerative medicine. However, previous studies used acoustic levitation to obtain a spatial distribution of cells in thin layers to produce spheroids (Jeger-Madiot et al., 2021) or multilayered tissues such as epithelial tissue within a fluidic environment (Tait et al., 2019) rather than to directly stimulate cells. To date, no work has focused on the cyclic loading of cells within droplets for biological and medical purposes.

This project aims to better understand the mechanical response of gel and hydrogel droplets during acoustic levitation through the development of a dedicated setup combined with simulations to establish the macro-micro link between the external stimuli and cellular local mechanical environment in the forthcoming stem cell culture.

2. Methods

2.1 Biomaterial fabrication

Sterile alginate gels with different concentrations from 1% to 12% (weight/volume in water) were prepared, as well as hydrogels based on umbilical cord and rat tail collagen using a sterile protocol. The viscosity of samples was measured using a TA Instrument AR2000 imposed-stress rheometer at different shear rates and temperatures, i.e. 20°C and 37°C.

2.2 Acoustic levitation setup

The establishment of an acoustic field, through the superposition of standing waves, enables droplets to be placed in specific nodes, called pressure nodes, and held in stable levitation. A tec5 ultrasonic levitator with a standard operation frequency of 58 kHz was used. Since the applied pressure (resulting in a force counteracting the effect of gravity) depends on the input voltage, cyclic compressions can be applied by varying this value at controlled frequencies. Using an Allied Vision camera equipped with a microscopic lens, pictures were captured using umbroscopy method, providing close-up images of the droplets for simplified Matlab processing, as their shape and dimensions.

2.3 Tests with alginate droplets

Using a 2-20 μL micropipette, droplets with a volume of 9 μL were generated in spherical-ellipsoidal shape with a diameter around 3 mm. The evaporation of the droplet was observed and quantified over a 30-minute period at a given temperature and humidity. The experiment was repeated for different input voltage values, to study the effect of mechanical stimulation on the droplets.

2.4 Finite element modeling

Finite element modeling of the studied object was made using Matlab/GIBBON/FEBio to understand the mechanical environment within the drop. Two models were described. The first one was based on theoretical values of pressure issued from Yarin et al., with prescribed boundary conditions at the surface of the droplet. Based on an initially spherical configuration, the final shape resulting from the pressure field and the application of gravity was computed, using a hyperelastic material whose parameters were extracted from preliminary experiments. The other model was built on experimental results (shape and dimensions of the droplet) to identify by an inverse approach (least square method on the node-to-node distance between experimental and simulated droplet) the pressure field required to obtain this final configuration, the material properties considered as known.

3. Results and discussion

Different gels at various concentrations were characterized. The viscosity, ranging from 38.2 mPa to 46.2 Pa, was observed to evolve non-linearly with respect to concentration. Experimental preliminary results highlighted the evaporation phenomenon (Fig. 1), with a change of volume and shape of the droplets since the reduction in volume modified the distribution and values of the acoustic radiation pressure on its surface.

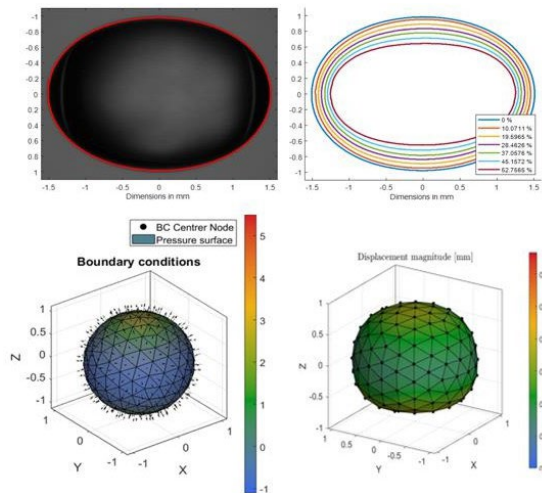


Figure 1. (up) Experimental observations of droplets and characterization of the volume loss over time from their shapes (bottom) simulation of the droplet shape after loading using a theoretical pressure field (first model)

Finite element analysis of the droplet (assumed initially spherical) for theoretical values of acoustic radiation pressure enabled us to analyze the strains and stresses fields within the droplet (Fig. 1). This led to a link between the prescribed mechanical macro-environment and the local cellular microenvironment. Comparison between the experimental results and the simulated geometries enabled to estimate the acoustic radiation pressure values at the surface, based on an inverse method rather than theoretical values, and therefore to accurately identify the mechanical boundary conditions around the droplet. Further enhancement of the FE simulations will involve a better consideration of gel material's properties (including viscous effects) to improve droplet response imitation.

Further experiments will be performed to attempt to minimize the evaporation and hence the volume variation during the levitation, to study the impact of cyclical loading on gels and hydrogels droplets. One of the next critical steps will be to automate the deposition and harvesting of single droplets before moving on to biological tests, to study the cellular response to controlled and known mechanical stimulations.

4. Conclusions

The present contribution constitutes a first proof-of-concept to cyclically stimulate droplets made of biocompatible gels by using acoustic levitation. Initial experiments and the development of a simulation model have highlighted various factors influencing the droplet behavior, so that the boundary conditions will be precisely monitored during future biological tests involving stem cells. The proposed *ad hoc* device may constitute an innovative tool to give new insights into mechanobiological knowledge with applications in the field of regenerative medicine.

Conflict of Interest Statement

None.

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