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Biomechanical stimulation and characterization of cartilage micropellets enabled by a unique fluidic-based device

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1. Introduction

Articular cartilage is a tissue with poor self-repair capacity, which is prone to progressive destruction after injuries or while aging. Novel therapeutic options include the development of cartilage engineering approaches for the repair of articular cartilage focal lesions. However, current approaches do not consider the mechanical stimuli, which are a key factor for the regulation of tissue homeostasis by chondrocytes. Our objective is to better understand the impact of mechanical forces on cartilage generation from mesenchymal stromal/stem cells (MSCs) and on extracellular matrix (ECM) production. Cartilage ECM is mainly characterized by the presence of type II collagen and aggrecan while Sox9 is the master gene. The cartilage micropellet is a relevant and widely used in vitro model to study cartilage growth, independently of the presence of an existing matrix (Johnstone et al 1998). This model is poorly investigated in terms of biomechanical stimulation and characterization because of its small size and imperfect round shape. That is why we have recently developed and validated a fluidic device both able to mechanically stimulate and characterize different types of microspheres (Petitjean et al 2021). Our objective is now to evaluate the ability of MSCs-derived cartilage micropellets to respond to mechanical stimulation applied with the custom-made device and characterize their mechanical properties.

2. Methods

2.1 Samples

Human bone marrow-derived MSCs were differentiated into chondrocytes by culture in micropellets with 10 ng/mL TGFβ3-containing inductive medium for 21 days.

2.2 Device

The fluidic-based device was designed for the concomitant culture of six micropellets – 800 μm in diameter – placed into the conical wells of a 3D-printed chamber. At the top of each conical well, a positive pressure with a sinusoidal, square or constant shape can be generated by two peristaltic pumps (15KS

series, Boxer, Ottobeuren, Germany) whereas atmospheric pressure is kept at the bottom. The difference of pressure, measured by the pressure sensor (ADP5161, Panasonic, New York, USA), cause the sinking of the micropellet into the cone (Figure 1, A), which is recorded by a camera (Mako, Allied Vision, Stadroda, Germany). All the system is controlled by two Arduino cards (Uno and M0 Pro, Arduino).

2.3 Mechanical stimulation

On day 21, groups of 6 cartilage micropellets were stimulated with square signals of pressure at different amplitudes (1.75, 3.5, 7.0 and 14 kPa), frequencies (0.25, 0.5, 1, 2 Hz) and durations (15, 30 and 60 min). The square signal was superimposed to a minimum pressure equivalent to the half of the amplitude pressure. Micropellets were then placed back in their tubes.

2.4 Biomolecular analyses

Micropellets were harvested at different time points after stimulation (0, 3, 6, 15 or 24 hours). Quantification of chondrocyte markers responsible for chondrogenesis (*SOX9*) and extracellular matrix secretion (*AGG* and *COL2B*) was performed by RT-qPCR (Dusfour et al 2020). Micropellet structure was assessed by histology.

2.5 Mechanical characterization

6 non-stimulated micropellets were characterized mechanically on day 21. As described in a previous study (Petitjean et al 2021), a multi-creep test was made by applying eight steps of pressure with an increment of 1.97 kPa ± 0.43 kPa, each maintained for 2 min. The displacement of the top and the bottom ends of micropellets was recorded by the camera. The last image at each step of pressure was used to determine the mechanical properties at equilibrium. A finite element model employing a neo-Hookean law was used to determine the Young's moduli and Poisson ratios of micropellets by inverse analysis. This model was also used to evaluate the internal deformation of micropellets during mechanical stimulation.

3. Results and discussion

First, we wanted to determine the impact of mechanical stimulation on the expression of chondrocyte markers. Twenty-four hours after 30 min of mechanical stimulation at different amplitudes and frequencies, upregulation of the three markers *SOX9*, *AGG* and *COL2B* was observed. Expression of the three genes was higher at 1 Hz frequency, although only *SOX9* expression reached significance. Gene expression was also higher at the 3.5 kPa amplitude but was not significant. We hypothesized that 24h could not be the optimal time point for gene expression analysis after stimulation. Therefore, analysis of chondrocyte markers was performed at different time points from 0 to 24h after stimulation. Upregulation of *SOX9*, *AGG* and *COL2B* was higher at early time points after mechanical stimulation (Figure 1, B). Upregulation of *SOX9* and *AGG* peaked at 3h while expression of *COL2B* was already high at 0 and 3h after stimulation and was the highest after 6h. Finally, we did not observe structural alteration 24h after stimulation.

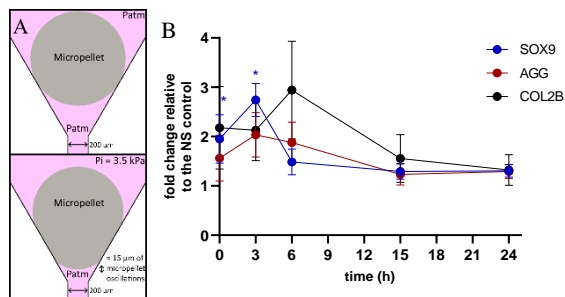


Figure 1. A - Scheme of the sinking of a micropellet in a cone. B - Expression of chondrocyte markers at 5 time point after mechanical stimulation (3.5 kPa, 1 Hz during 30 min) of 21-day cartilage micropellets (NS: non stimulated normalized to 1). *: $p < 0.05$

The finite element model showed that during mechanical stimulation, micropellets were compressed in the upper part while, in the lower part, they were stretched in the vertical direction and compressed in the others. Compression induced a decrease of micropellet volume (determinant of the deformation gradient tensor $J < 1$) in the upper part and an increase in the lower part ($J > 1$). This suggests that mechanical stimulation could increase fluid flow inside the cartilage micropellets. Moreover, for the 3.5 kPa amplitude of stimulation, the von Mises strain could reach 40% but stayed under 20% for 79.9% of the volume of the micropellets.

As shown in Figure 2, the finite element model perfectly reproduced mechanical behavior of 21-day cartilage micropellets at equilibrium. The Young's modulus determined by inverse analyze was $47.6 \pm$

26.2 kPa and consistent with the literature (Dusfour et al 2020). The system was sensitive enough to calculate the Poisson ratio that was estimated to be 0.46 ± 0.02 .

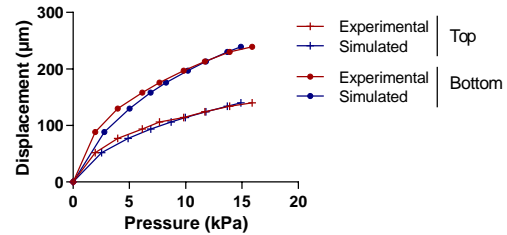


Figure 2. Fit of the experimental (red) and simulated (blue) displacements of the top (cross) and bottom (point) ends of a cartilage micropellet into a conical well of the device during a multi-creep test.

4. Conclusions

The interest of our device lies in the ability to mechanically stimulate and characterize six MSC-based cartilage micropellets, allowing the molecular and mechanical characterization on the same group of samples. Our data demonstrates the interest of fluidic-based compression for reproducible stimulation of cartilage micropellets and set the basis for further longitudinal studies on the long term.

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