2D Mechanical memory of periosteal cells influences their 3D condensation and early chondrogenic commitment in microtissues



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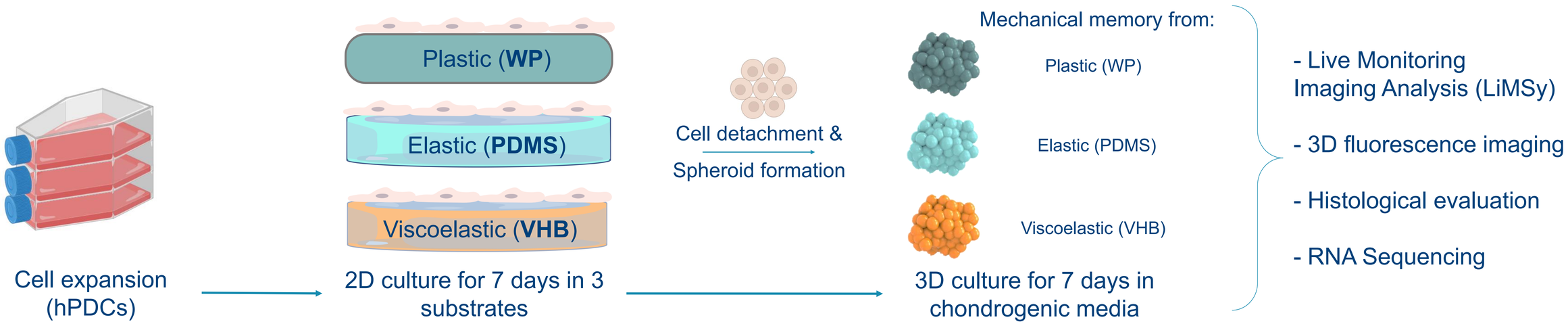
INTRODUCTION

- Cells develop a **mechanical memory** depending on the **stiffness of the substrate** they are cultured on. This memory is observed through changes in the cytoskeleton [1]. In addition to the stiffness, substrates with fast stress relaxation have also been found to affect cellular function and differentiation [2], but whether cells acquire memory when cultured on such viscoelastic substrates is not well understood.
- **Cellular aggregation**, a key process encountered in both developmental biology systems as well as tissue engineering applications, is a prerequisite for the initiation and continuation of chondrogenic differentiation [3].
- In this study, human periosteum-derived cells (**hPDCs**) were cultured on **plastic** (Well Plate, WP), **elastic** (Polydimethylsiloxane, PDMS) and **viscoelastic** (VHB) substrates. All substrates were coated with polydopamine to increase cell attachment.

AIM

Our aim is to **explore the influence of the mechanical memory** of human periosteum-derived cells (**hPDCs**) on their capacity to aggregate, undergo condensation, and initiate **successful chondrogenic differentiation**.

EXPERIMENTAL DESIGN



RESULTS

2D Cell Culture

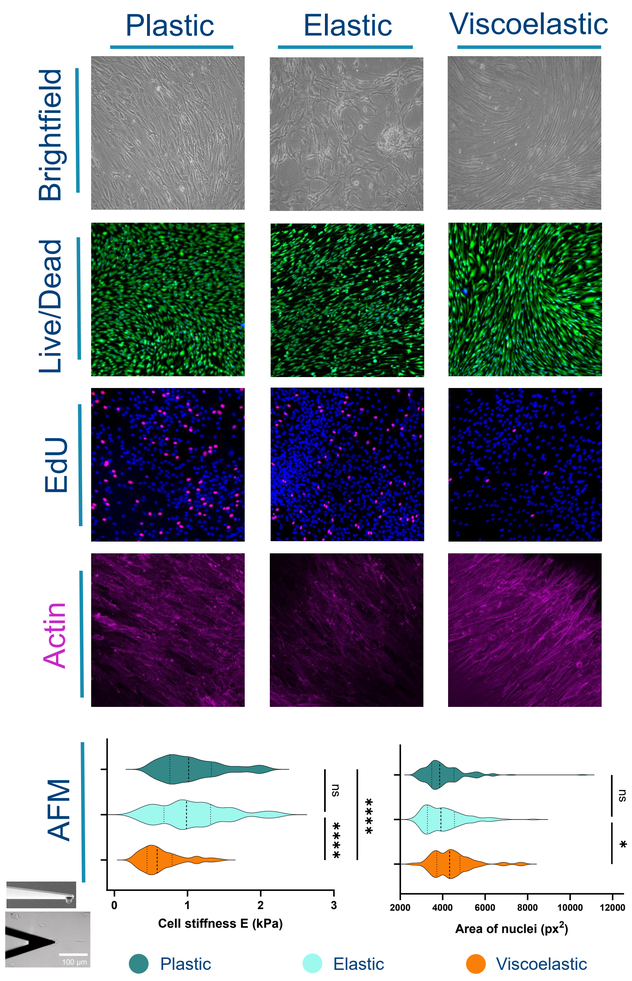


Figure 1. 2D Brightfield microscopy, Live/Dead staining, EdU staining, Atomic Force Microscopy and measurement of nuclei area of hPDCs cultured for 7 days in plastic, elastic and viscoelastic substrates. One-way ANOVA analysis was performed. Results were considered significant when p-values were below 0.05 (*), 0.01 (**), 0.001 (***) or 0.0001 (****).

3D Cell Culture

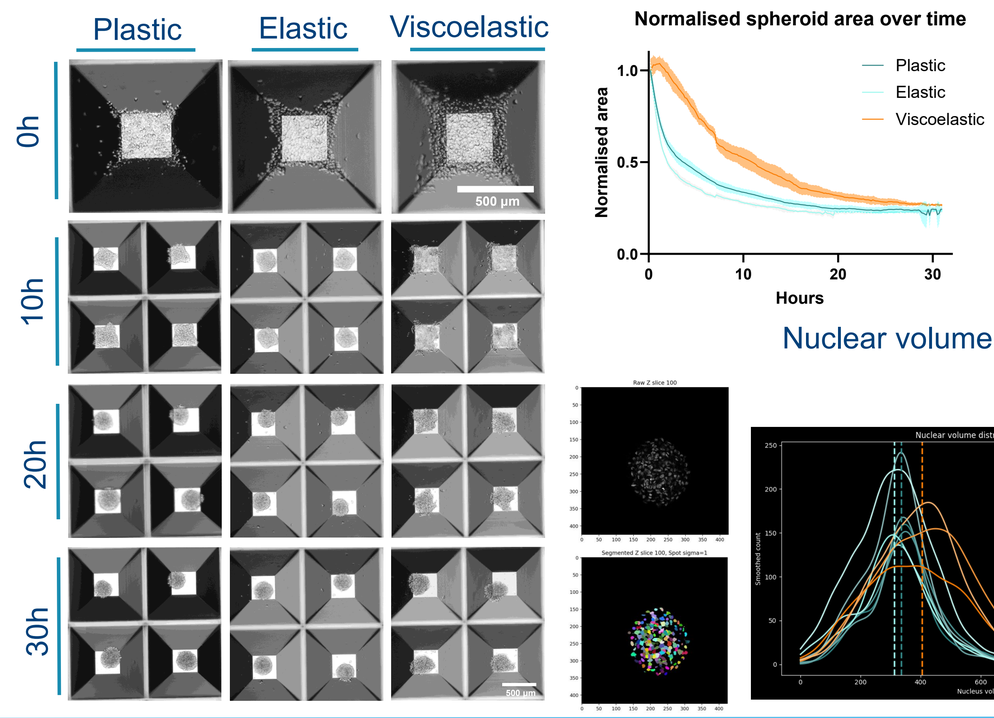
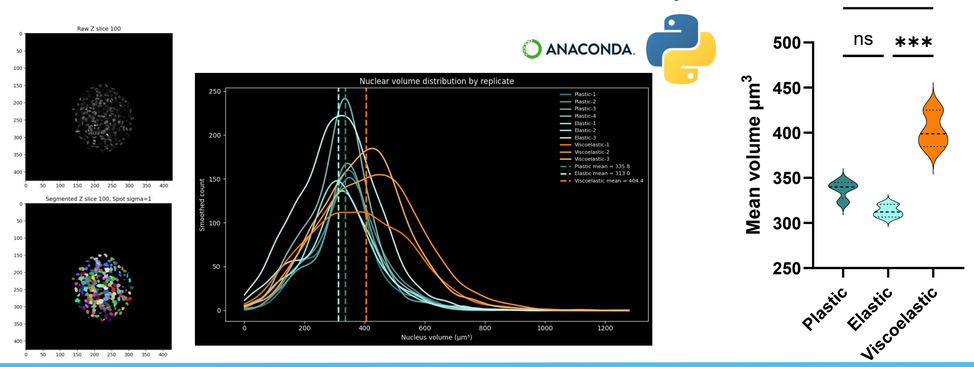


Figure 2. Timepoints of fusion of plastic- , elastic- , and viscoelastic- cultured hPDCs. Measurements were taken every 10 minutes for 30 hours of fusion with 6 replicates at each timepoint. Sketched area represents the SD at each timepoint. Nuclear volume distribution at Day 1 spheroids is also depicted.



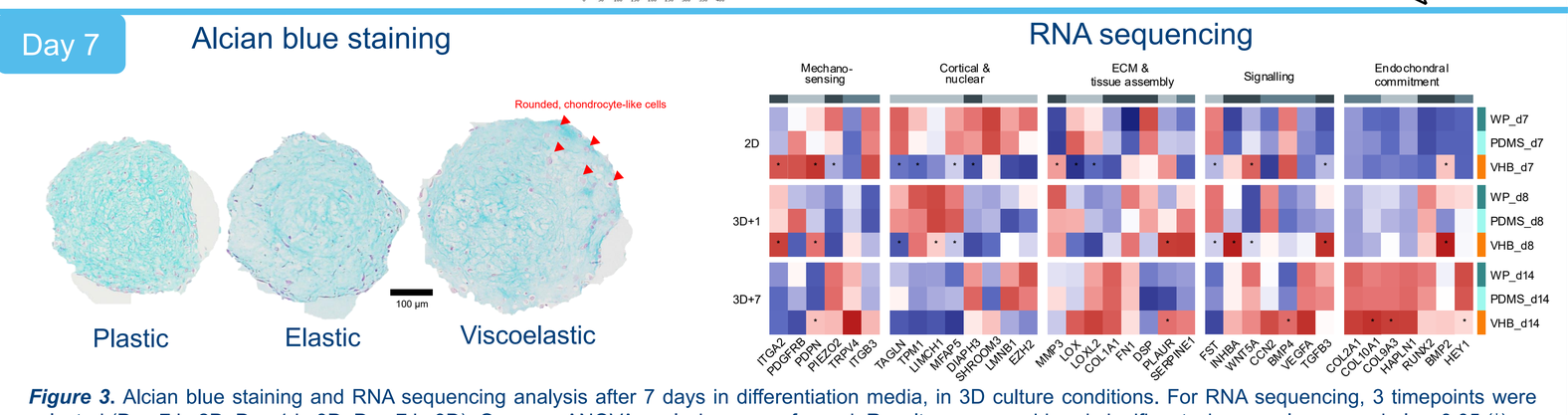


Figure 3. Alcian blue staining and RNA sequencing analysis after 7 days in differentiation media, in 3D culture conditions. For RNA sequencing, 3 timepoints were selected (Day 7 in 2D, Day 1 in 3D, Day 7 in 3D). One-way ANOVA analysis was performed. Results were considered significant when p-values were below 0.05 (*).

CONCLUSION

Priming periosteal cells on viscoelastic substrates resulted in:

- ➢ Softer cells with larger nuclei and liquid-like properties.
- ➢ Slower aggregation kinetics leading to diverse condensation kinetics.
- ➢ Differential gene expression, with early signs of accelerated chondrogenic commitment.
- ➢ Mechanical memory presence even after 7 days of substrate removal.

REFERENCES & CONTACTS

References:

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