Viscoelastic Cell Memory: Can mesenchymal stem cells sense and remember viscoelastic signals?

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INTRODUCTION

In several mechanobiology studies, cell behaviour and differentiation are associated with **the stiffness of culture substrates.** In particular, it has been shown that cells 'remember' the elastic properties of past mechanical environments [1,2]. Considering that tissue also posses viscoelasticity, the aim of this study is to verify whether mesenchymal stem cells (MSCs) are also able to 'remember' time dependent mechanical properties.

ENGINEERING HYDROGEL VISCOELASTICITY

Gels with different agarose concentration (0.5, 0.8 and 1% w/v) were prepared and tested to identify **one able to mimic the mechanical properties of the stem cell niche** (≅ 3 kPa [2]).

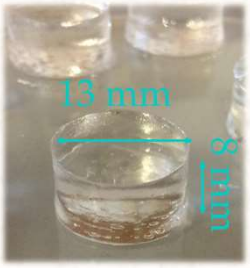

Figure 1: Agarose Gels

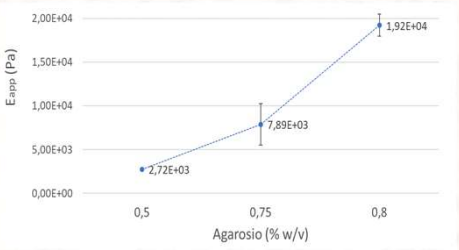

Figure 2: Apparent moduli (E_{app}) of agarose gels

Then, 0.5% w/v agarose gel were fabricated using aqueous solutions with increasing dextran concentrations (0, 3, 5% w/v), and hence viscosities, in order to obtain **hydrogels with the same degree of crosslinking but different viscoelastic properties**. Mechanical properties were investigated, using the epsilon-dot method [3]. A significant decrease of the instantaneous elastic moduli (E_{inst}) and of the relaxation time (τ), while the equilibrium elastic modulus (E_{eq}) did not show significant variations.

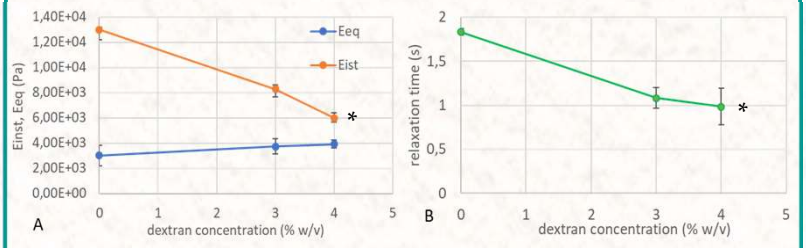

Figure 3: A) Instantaneous (E_{inst}) and equilibrium elastic moduli (E_{eq}) and (B) characteristic relaxation time (τ) of agarose samples as a function of different dextran concentrations. (= p<0.05, 1-way ANOVA)*

FUTURE STUDIES

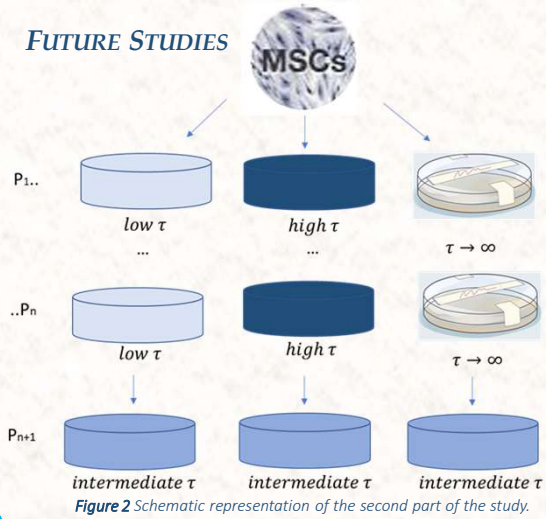

Figure 2 Schematic representation of the second part of the study.

Stemness: surface markers (pos: CD105 - CD90, neg: CD45 - CD34)

Differentiation: gene expression - YAP (migration in the nucleus), RUNX2, PPARγ, adipogenic (oil red)/ osteogenic (alizarin red)/ chondrogenic (alcian blue) differentiation stainings

Morphology: elongation, branches number

PRELIMINARY MSC STUDIES

Bone marrow MSCs were seeded at two different cell densities (r1= 5000 and r2=10000 cells/cm²) on the gels with and without a 5% w/v gelatin coating and on 96-well multiwell plates (as control). Cell viability was assessed with Alamar blue (Fig. 4) at day 1, 3 and 7.

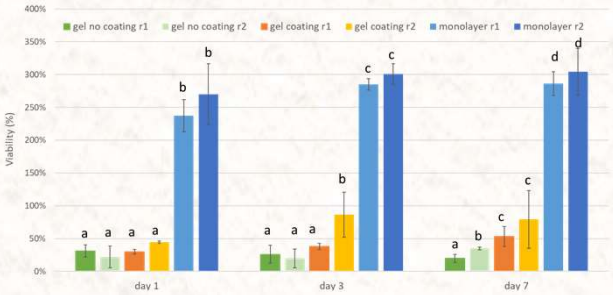

Figure 4: MSC Viability. *Viability of MSCs grown on agarose hydrogels with and without gelatine coating, and on standard culture plates. Different letters indicate statistical differences within the same day (p<0.05)*

Finally, samples were stained with DAPI (nuclei) and rhodamine-conjugated phalloidin (actin). Results showed a significant lower viability and a slower adhesion respect to the controls, suggesting that because of gel softness cells needs more time to adhere and spread, probably maintaining their 'quiescent' state for a longer time.

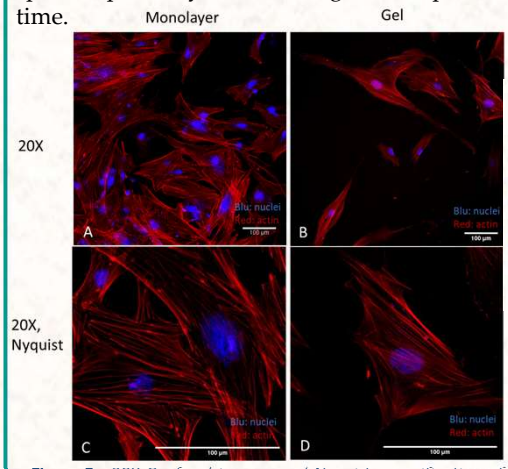

Figure 5: 20X Confocal images and Nyquist magnification of MSCs cultured on the monolayer (A,C) and on the gels (B, D)

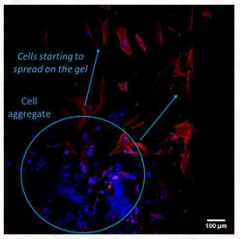

Figure 6: *10X Confocal images of MSCs cultured on the gels, showing a cell aggregate from which yhr cells start to adhere on the gel.*

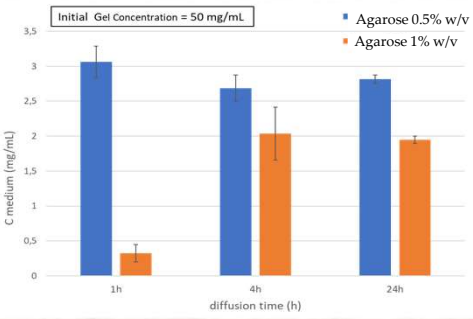

Figure 7: *Dextran diffusion from the gels to the medium*

Diffusion test with Fluorescein isothiocyanate (FITC) - labelled Dextran were performed, demonstrating that the dextran diffusion is very low and in the 0.5% w/v gel occurs mainly within the first hour.

CONCLUSIONS

In this preliminary study, **hydrogels with tunable viscoelastic properties** were **optimised for MSC culture**. Further studies, such as experiment with longer culture times, are necessary to *better understand MSC behaviour on the gels*. This will allow the investigation of the 'viscoelastic memory' of the cells and to identify optimal substrates to preserve their undifferentiated status in culture. The ultimate goal is to develop more physiologically relevant in-vitro models to study cell responses to mechanical alterations of their environment.

References:

[1] Patrick C. Baer et al., St. Cel. Int., 2012.
[2] Chun Yang et al., Nat. Mat., 2014
[3] L. Cacopardo et al., JMBBM, 2019
[4] Tirella et al, JMBR, 2013

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