IN-SILICO MODELING OF TUNABLE VISCOELASTIC HYDROGELS FOR THE STUDY OF CELL MECHANOTRANSDUCTION

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Introduction

Predicting hydrogel mechanical behaviour is essential to precisely control and guide cell behaviour [1]. Since hydrogel viscoelastic behaviour is strongly related to the interaction of liquid molecules with the solid network, different mathematical models have been proposed to simulate transport phenomena in gels, but they present limitations such as the lack of correlation with gel viscoelastic properties [2]. Here we propose an in-silico model to predict hydrogel viscoelastic behaviour resulting from tuning liquid phase viscosity [3]. The computational tool was used to design the experimental set up for the study of adipose derived mesenchymal stem cells (ADSC) mechanotransduction in response different viscoelastic stimuli. In particular, we focused on the definition of hydrogels with a relaxation time (τ_{rel}) shorter than the time required for the formation of cells focal adhesions ($\tau_{fak} \in [15-60]s$) [4].

Methods

5 mg/mL agarose hydrogels were fabricated with different dextran concentrations in the liquid phase (20, 30, 40 mg/mL) and modelled on Matlab 2022a as a homogeneous porous system. Dextran was considered as a spherical agglomerate linked to the water molecules. The liquid phase movement in the porous matrix was described using the reaction-diffusion equation (1), introducing an apparent diffusion coefficient D_{app} (2) which adapts the Einstein-Stokes coefficient D_0 through additional coefficients, which considers the obstacle imposed by the porous matrix and the correlation between diffusive and mechanical properties through the average mesh size ξ_{avg}. A modified Maxwell Standard Linear Solid (mSLS) lumped parameter model was used to describe hydrogel mechanics. mSLS equations in the time domain were implemented in Simulink (fig.1A) and used to fit the experimental data obtained from the hydrogel mechanical characterisation using the epsilon dot method, deriving the instantaneous and equilibrium elastic modulus (E_{eq}), and τ_{rel} [1]. ADSC (50.000 cell/cm^2) were cultured for 7 days on the hydrogels and on tissue culture plates (TCP) as control, coated with 50 mg/mL gelatin. YAP, lipidic and calcium deposits were labelled respectively thanks to immunostaining, Oil Red O and Alizarin Red. Confocal and bright field image analysis was used to quantity their distribution.

Results

Figs.1B-1C show a good correlation between experimental and computational data. Moreover, they also suggest that dextran mainly affect gel viscous properties (τ_{rel}), maintaining a constant $E_{eq.}$ Cell tests showed that YAP is localised in the cytoplasm in the case of 20 and 30 mg/mL dextran-agarose substrates (fig.1D), while the 40 mg/mL dextran gels induce the YAP translocation into the nuclei as in the case of TCP controls (fig. 1E).

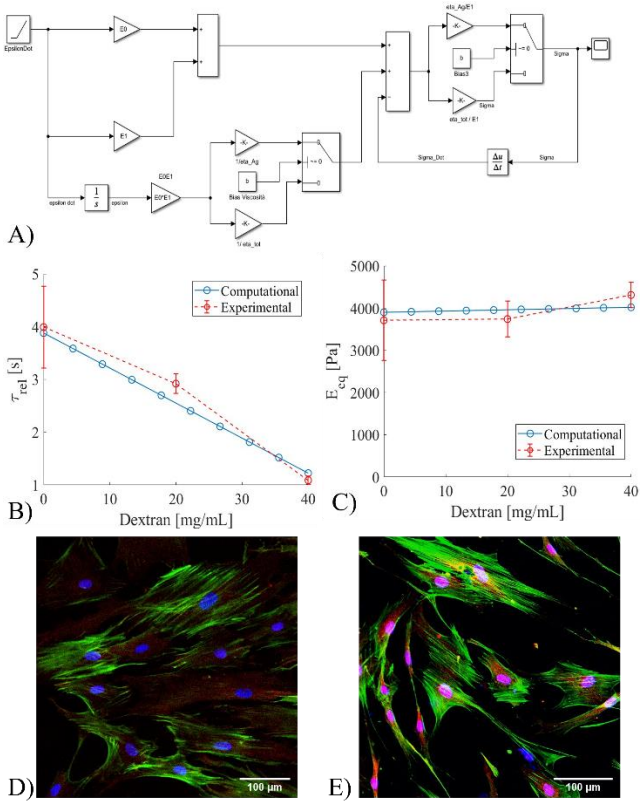

Figure 1. A)mSLS Simulink diagram; B) τ_{rel} and C) E_{eq} of dextran-agarose hydrogels. Confocal images of YAP (red) localization in ADSC on agarose-20 mg/mL dextran hydrogels (D) and on TCP control (E) (green-actina, blue-nuclei).

Discussion

Cell tests showed that ADSC on gels with dextran < 40 mg/mL preserve their stemness, while 40 mg/mL dextran gels induce ADSC adipogenic differentiation, and in the controls ($\tau_{rel} \rightarrow \infty$) ADSC produced calcium deposits compatible with the osteogenic differentiation.

Conclusion

The presented computational framework resulted effective in predicting gel mechanical behaviour, providing a useful tool for the identification of the substrates with $\tau_{rel} < \tau_{fak}$. Simulations with other materials combinations are ongoing to prove the versatility of the in silico tool for the design of hydrogels for regenerative medicine applications and advanced in-vitro models.

References

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