The Effects of Viscoplasticity on Cells Embedded in 3D RGD-Free Agarose Hydrogels

Lorenzo Mio[1], Martina Conti[2], Francesco Brun[3], and Pasquale Sacco[4].

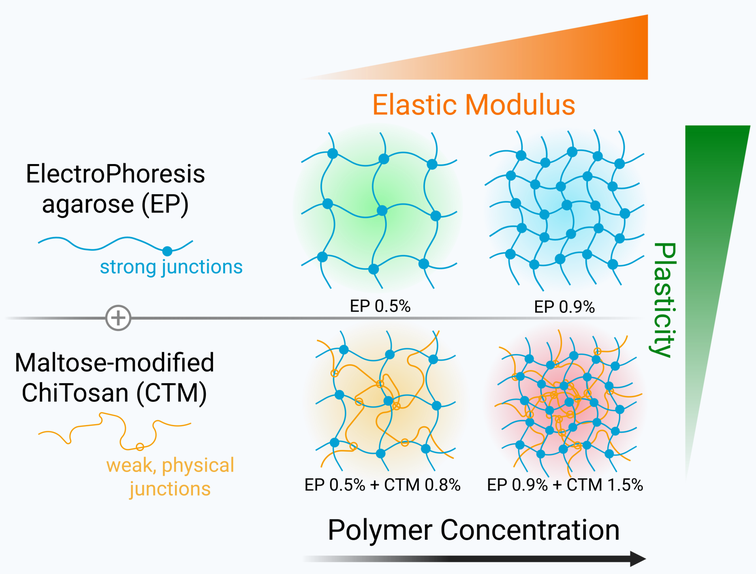
[1]Department of Medicine, Surgery and Health Sciences, University of Trieste, Piazza dell'Ospitale 1, 34129 Trieste, Italy. lorenzo.mio@phd.units.it



INTRODUCTION

Viscoplasticity is a material's time-dependent response to stress that combines elastic behavior with viscoplastic deformation.

The use of electrophoresis agarose (EP) and maltose-modified chitosan (CTM) allows for the preparation of hydrogels with finely tuned elastic and viscoplastic behavior.

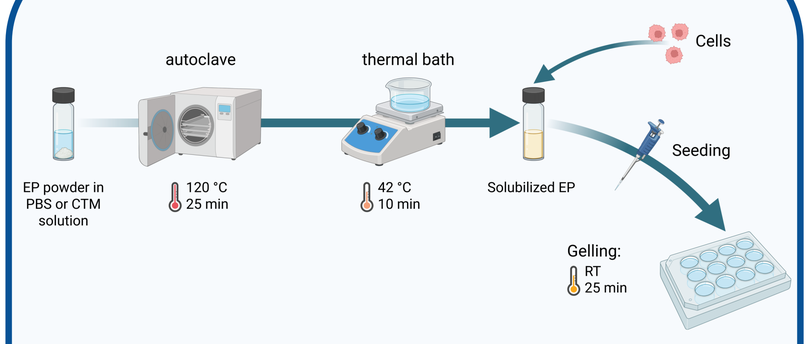


Osteosarcoma–derived cells (MG63) were embedded in these hydrogels at a final concentration of $1x10^6$ cells/mL. Then, their viability, proliferation, changes in volume, RUNX2 localization and ALP activity were investigated.

AIM OF THE STUDY

Assessing the effects of viscoplasticity on cells embedded in 3D-confined, RGD-free microenvironments.

PREPARATION



For experiments with cells, agarose and CTM were initially prepared at 1.25x the final desired concentration. DMEM HG was used as culture media.

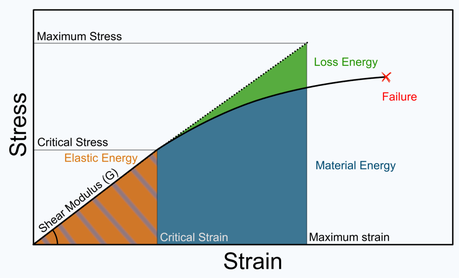
CONCLUSIONS

Cells embedded in 3D, RGD-free microenvironments show lower viability, proliferation, volume, RUNX2 nuclear localization (at day 1) and lower growth of ALP activity in stiff, elastic hydrogels.

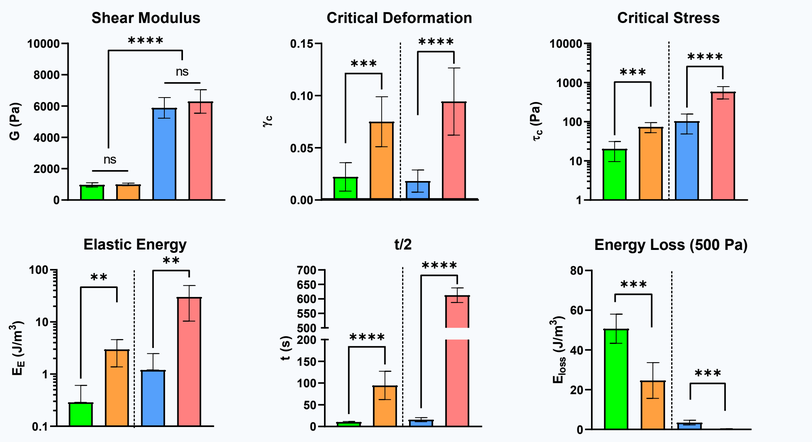
RESULTS

MECHANICAL CHARACTERIZATION

Mechanical characterization on cell-free hydrogels.
Applied rheological tests: frequency sweeps and long stress sweep test.



A model from a previous work[1] was employed to derive various mechanical quantities from the long stress sweep test.



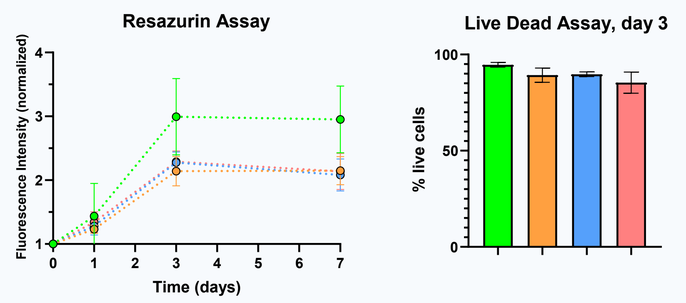
EP 0.5% n = 6 · EP 0.5% + CTM 0.8% n = 6 · EP 0.9% n = 8 · EP 0.9% + CTM 1.5% n = 6

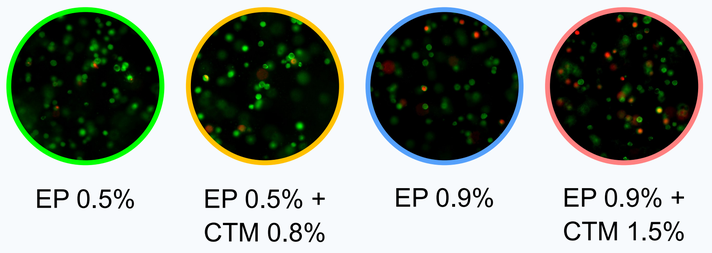
[1] Piazza et al., 2023, *Adv. Funct. Mat.*, DOI: 10.1002/adfm.202307224

EFFECTS ON ENCAPSULATED CELLS

Cell Viability and Proliferation

Cells show good viability and proliferation after seeding in every condition.

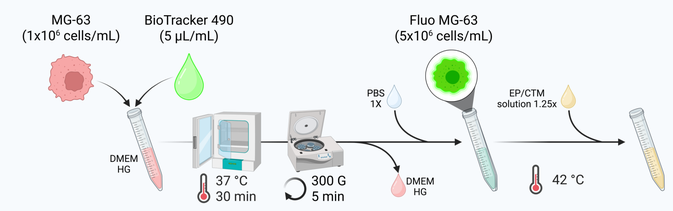




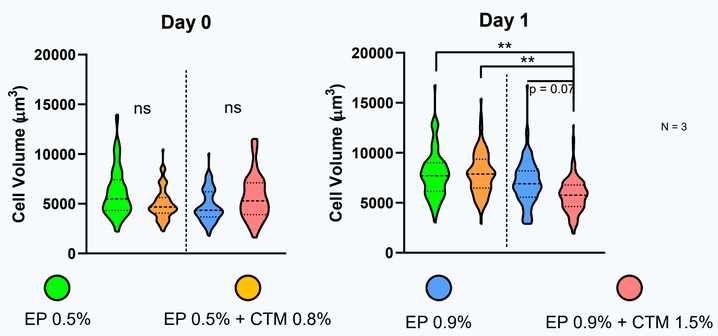
EP 0.5% · EP 0.5% + CTM 0.8% · EP 0.9% · EP 0.9% + CTM 1.5%

Higher plasticity is associated with both a greater proliferation and a better viability.

Cell Volume



Cells were visualized after 3 hours of incubation using a confocal microscope. Cell Volume was calculated with a MathLab script.

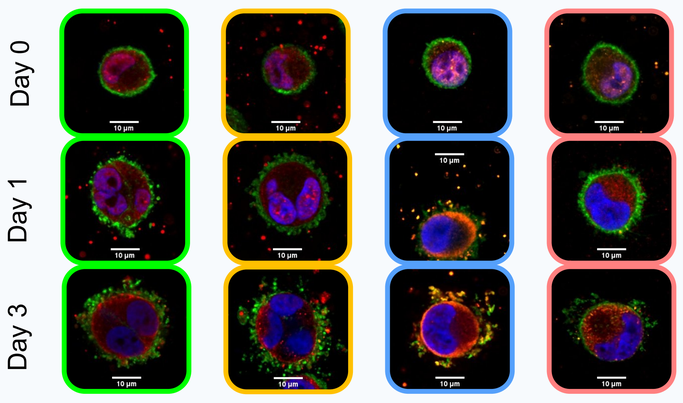


EP 0.5% · EP 0.5% + CTM 0.8% · EP 0.9% · EP 0.9% + CTM 1.5%

At day 1, cell embedded in the stiffer, less viscoplastic hydrogel (EP 0.9% + CTM 1.5%) are smaller.

RUNX2 Localization

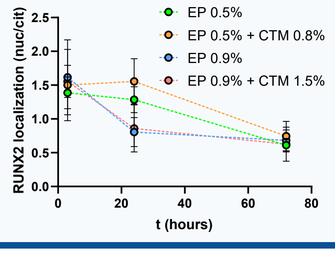
RUNX2 localization was investigated via immunostaining (DAPI and phalloidin) after 3 hours, 1, and 3 days of culture.



EP 0.5% · EP 0.5% + CTM 0.8% · EP 0.9% · EP 0.9% + CTM 1.5%

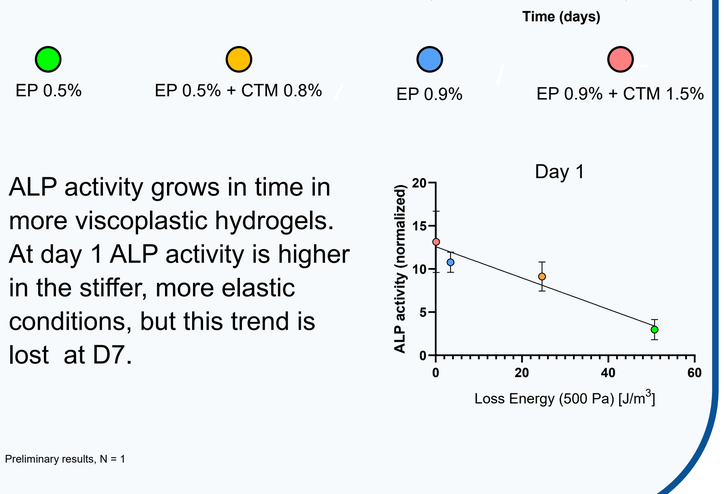
At day 0, RUNX2 is localized in the nucleus in every condition, while at day 1 only in soft hydrogels. At day 3 RUNX2 is more citoplasmatic.

Preliminary results, N = 1



ALP Activity

The ALP activity of the embedded cells was investigated during the first week of culture.



EP 0.5% · EP 0.5% + CTM 0.8% · EP 0.9% · EP 0.9% + CTM 1.5%

ALP activity grows in time in more viscoplastic hydrogels. At day 1 ALP activity is higher in the stiffer, more elastic conditions, but this trend is lost at D7.

Preliminary results, N = 1

AFFILIATIONS

[2]Istituto Officina dei Materiali (IOM-CNR) Area Science Park, Trieste I-34149, Italy.
[3]Department of Engineering and Architecture, University of Trieste, Via A. Valerio 6/1, 34127 Trieste, Italy.
[4]Department of Life Sciences, University of Trieste, Via L. Giorgieri 5, 34127 Trieste, Italy.

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