

1. Can the changes in the gel architecture that result in making gel relaxation time close to that of viscoelastic tissues be made simultaneously while keeping the gel Young's modulus constant? Explain.

Different changes could be made in the gel architecture to allow tuning the stress relaxation time close to the ones observed in viscoelastic tissues while maintaining the gel Young's modulus constant:

1. Lowering the molecular weight of alginate hydrogel crosslinked with calcium and introducing PEG spacers in the gel architecture increase the rate of stress relaxation.
  2. Any reduction in the initial gel's Young modulus caused by a decrease in the gel's molecular weight can be compensated by increasing ionic calcium crosslinking within the gel.
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2. Discuss the main features of osteogenic differentiation of stem cells sitting on modified gels of greater stiffness.

At higher gel stiffness (17 kPa vs. 9 kPa), almost no adipogenic differentiation is observed, while stiff alginate gels (~17 kPa) that relax stress fast show significant osteogenic differentiation.

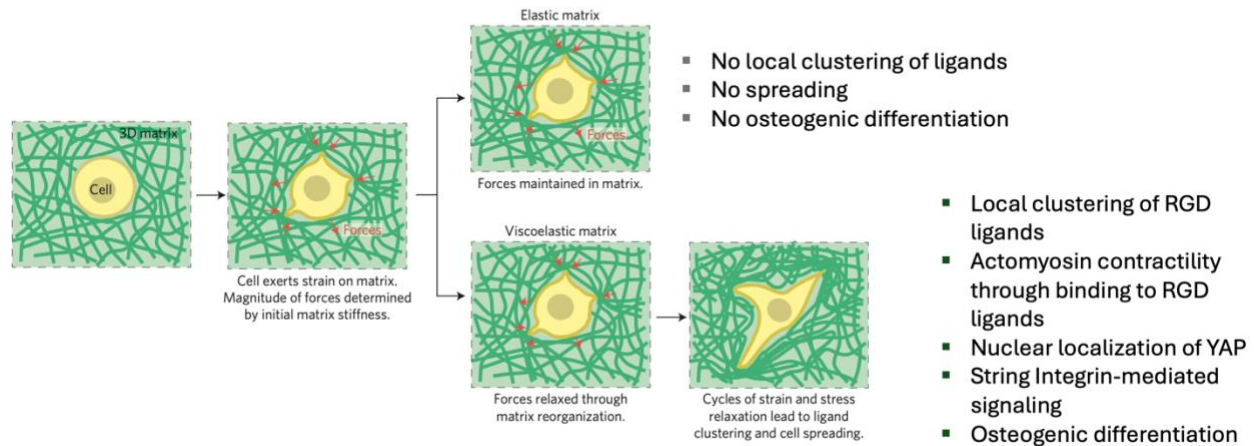
In addition, osteogenic differentiation in modified gels of greater stiffness and fast-relaxing gels forms an interconnected, mineralized, collagen-I-rich matrix and results in bone-forming activity.

3. What changes in the gel architecture result in making gel relaxation time close to that of viscoelastic tissues?

Lowering the molecular weight of the alginate gel, crosslinked by calcium, which decreases entanglement and connectivity, coupled with small PEG pacers which provide a steric spacing of crosslinking zones in the alginate gel, increase the rate of relaxation time. The relaxation time was successfully reduced from  $T_{1/2}$  of about  $10^3$  seconds (High-MW) to  $\sim 10^2$  seconds (Low-MW\_PEG) (closer to rates observed for viscoelastic tissues such as liver, brain and fracture haematoma).

4. Sketch the main steps of cell/ECM interaction in the cases of elastic and viscoelastic matrices.

# Cell/ECM interaction Steps in elastic and viscoelastic matrices



5. Do stem cells sitting on the modified gels significantly increase their spreading and proliferation? Explain.

Faster rates of stress relaxation with modified gels increased stem cell spreading and proliferation:

Experiments showed that the increase of spreading and proliferation of MSCs in alginate gels was due only to a decrease in rates of stress relaxation as observed in two alginate gels with two initial moduli (9 kPa and 17 kPa), and two RGD concentrations (150  $\mu$ M and 1,500  $\mu$ M RGD) (Supplementary Fig. 7: Chaudhuri, O., Gu, L., Klumpers, D. *et al.* Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nature Mater* **15**, 326–334 (2016). <https://doi.org/10.1038/nmat4489>)