Multiscale mechanical characterization of viscoelastic hydrogels for designing regenerative tissue scaffolds

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Introduction

Developing 3D bioprinted constructs requires precise mechanical characterization to mimic native tissue microenvironments. While local stiffness influences cell behavior, conventional bulk testing cannot resolve microscale heterogeneities like crosslinking and porosity gradients. Atomic force microscopy (AFM) offers the necessary resolution; however, standard Hertzian analysis overlooks the inherently viscoelastic nature of hydrogels. By applying Kelvin-Voigt (1) and standard linear solid (SLS) (2) models to AFM data, we decouple elastic and viscous contributions. This framework provides a more accurate estimation of the intrinsic elastic modulus and a realistic assessment of scaffold mechanics.

$$E_{\mathrm{app}}(r) = E_0 + \frac{\eta}{L}\cdot r \quad (1) \qquad E_{\mathrm{app}}(r) = E_0 + \frac{E_1\cdot\eta^*\cdot r}{E_1\cdot L+\eta^*\cdot r} \quad (2)$$

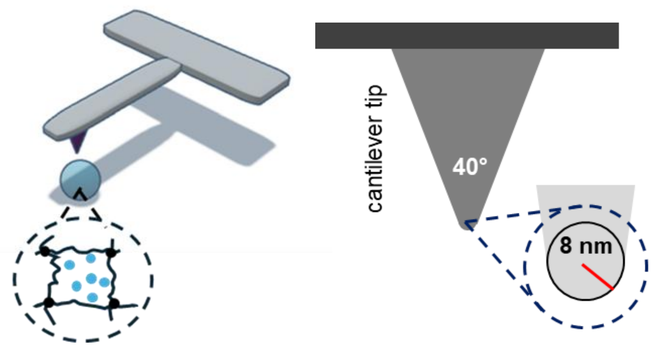


Figure 1. Sketch of AFM cantilever equipped with conical tip probing a generic hydrogel.

Materials and methods

We focused on four hydrogel formulations: alginate, alginate reinforced with nanofibrillated cellulose (Cellink-RGD), GelMA, and GelMA-A. Constructs were prepared via drop-casting, alginate and Cellink-RGD were crosslinked using a 3% w/v $CaCl_2$, GelMA was crosslinked with 405 nm UV light, and GelMA-A using both methods sequentially.
Nanoscale mechanical characterization was performed using a sharp conical tip (radius 8 nm, spring constant 0.3 N/m) (**Fig. 1**). Indentation speeds varied from 0.5 to 15 µm/s with a 5.5 nN force limit.

Results

The apparent Young's modulus exhibited a non-linear, monotonic increase as a function of the indentation rate (**Fig. 2**), reflecting a transition from time-dependent viscoelasticity to time-independent elastic response as probing speeds exceeded the polymer relaxation time. Specifically, the addition of nanocellulose in Cellink-RGD and the dual-crosslinking in GelMA-A resulted in a 17-fold and 34-fold increase in stiffness, respectively, compared to pure alginate and GelMA. This stiffening positively correlated with effective viscosity, as reinforced networks dissipate more energy through internal friction and polymer chain motion during rapid deformation. The SLS model effectively captured the transition from viscous-dominated regimes at low frequencies to elastic-dominated dynamics at higher frequencies, demonstrating a robust consistency in capturing intrinsic polymer network dynamics.

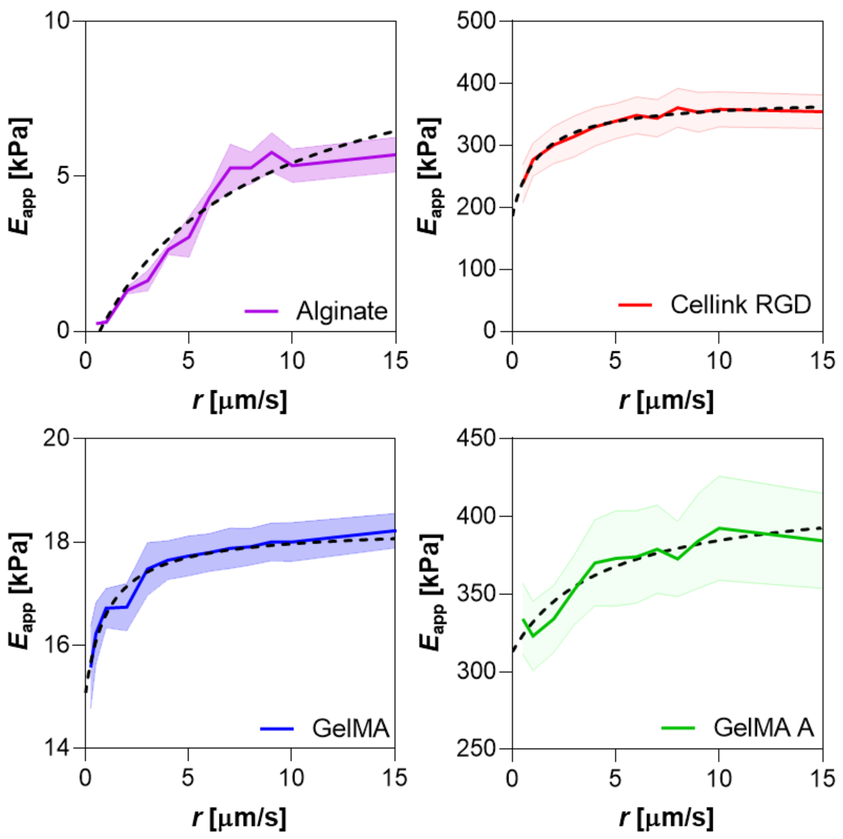


Figure 2. Apparent Young's modulus of alginate, Cellink-RGD, GelMA, and GelMA-A as a function of indentation rate.

Validation in erythrocytes and zona pellucida

To validate our model, we analyzed the rate-dependent responses of erythrocytes and the zona pellucida (ZP). AFM testing on erythrocytes confirmed that reaction forces increase with indentation rates due to dissipative processes in the cytoskeleton. While healthy cells maintain flexibility through efficient energy storage, pathological erythrocytes exhibit increased effective viscosity and stiffness, reflecting a reduced ability to rearrange under stress (**Fig. 3**, top). Similarly, porcine and equine ZP act as viscoelastic networks, showing a 5-fold stiffening within physiological penetration speeds. Porcine ZP displayed significantly higher stiffness and viscosity than equine ZP, highlighting that viscous damping is the predominant mechanism for resisting sudden structural failure (**Fig. 3** bottom).

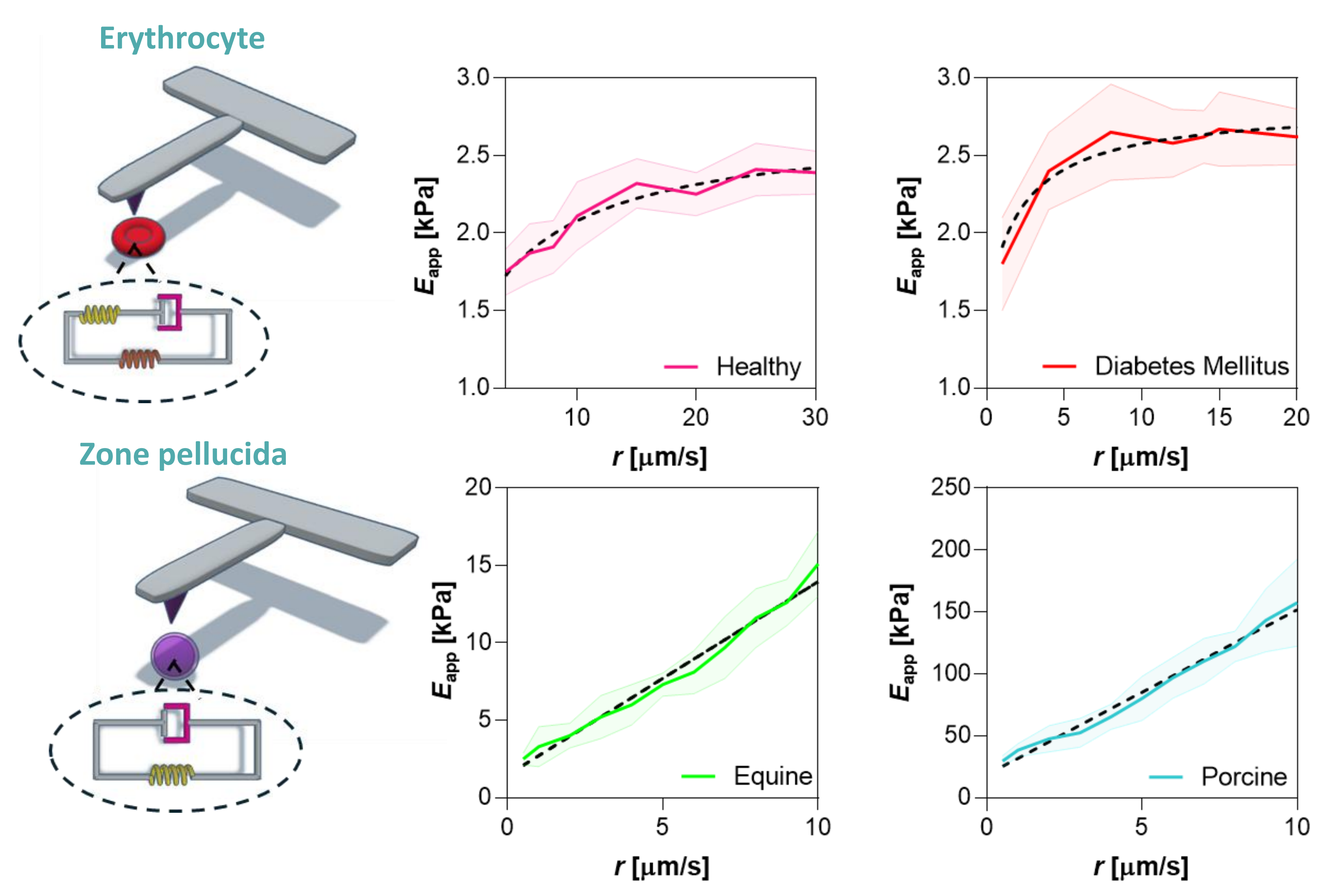


Figure 3. Sketch of AFM cantilever tip probing an erythrocyte (top) and an oocyte (bottom); the insets show the scheme of SLS and Kelvin-Voigt models, respectively, used to fit experimental data. Apparent Young's modulus of healthy vs. pathological erythrocytes (top) and porcine vs. equine zone pellucida (bottom) as a function of indentation rate.

References

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