A Light-tunable Phantom Cell to Study Epithelial Mechanical Equilibrium

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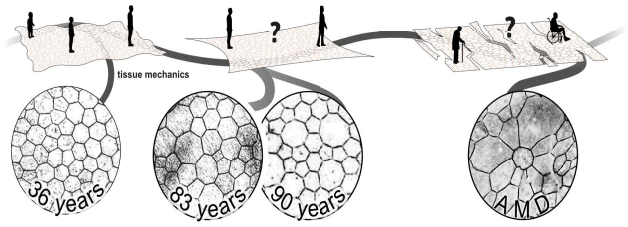
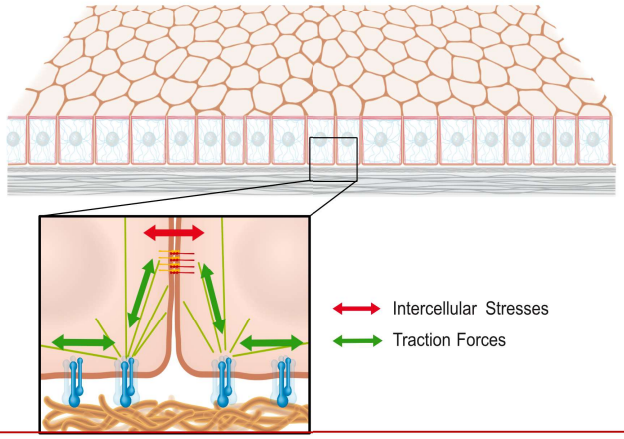
BACKGROUND & MOTIVATION

Age-related RPE changes



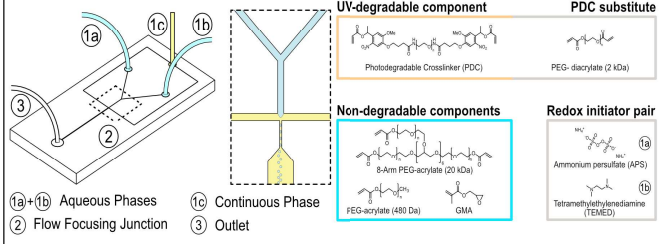
The retinal pigment epithelium (RPE) is a single, post-mitotic monolayer. As cells are lost with age, neighbors enlarge to fill gaps, increasing cell size heterogeneity and altering the force balance of the monolayer.

Epithelial force balance

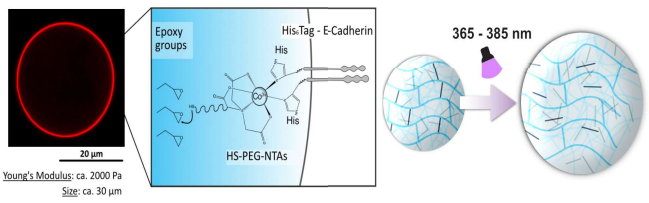


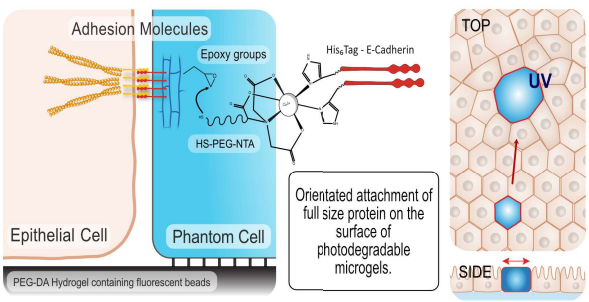
Epithelial integrity depends on the balance between traction forces (cell–ECM) and intercellular stresses (cell–cell). In the ageing RPE, this balance is disrupted, yet no existing tool simultaneously introduces a spatially defined, cell-sized mechanical perturbation and measures the monolayer's mechanical response.

THE PHANTOM CELL



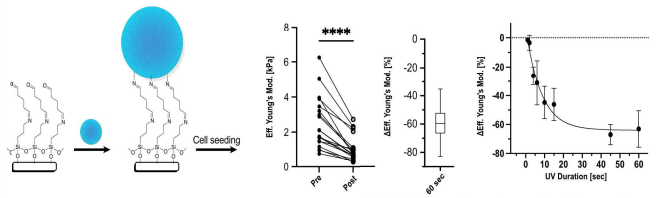
We developed a photodegradable PEG-based microgel (~30 µm) that acts as a "phantom cell". Microgels are produced via microfluidics, incorporating an o-nitrobenzyl photocleavable crosslinker for on-demand softening. Brief UV illumination (365-385 nm, 60 s) reduces microgel stiffness, mimicking the compliance change of a hypertrophying RPE cell during ageing. The microgel surface is functionalized with E-cadherin via oriented NTA-Co^{2+} coupling, enabling integration into the epithelial monolayer through adherens junctions.



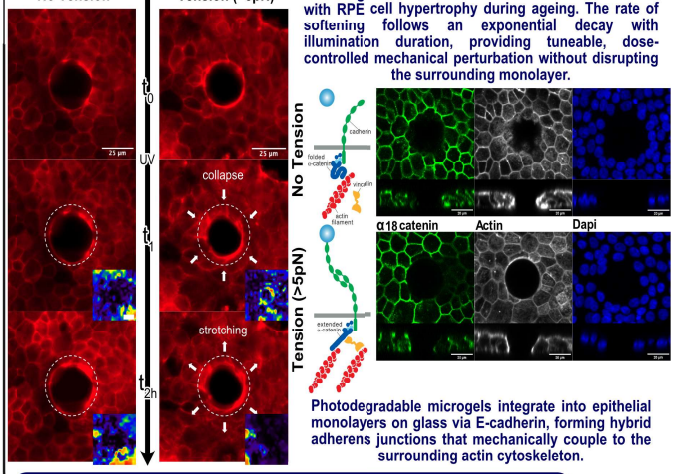


RESULTS

Uncoupling organizational and topographical changes



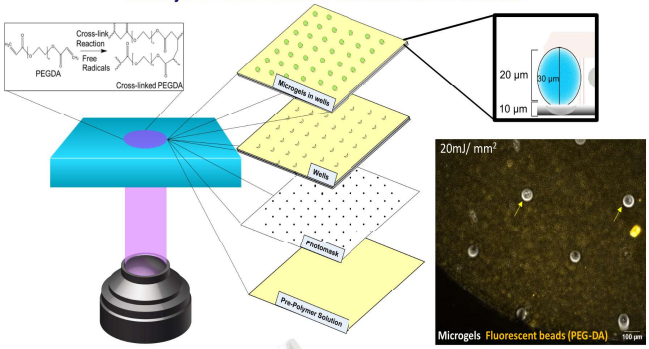
Upon brief UV illumination, microgel stiffness drops significantly from ~1-2 kPa to ~0.4-0.8 kPa upon UV illumination- a ~60% reduction ($p < 0.0001$). This softening mimics the stiffness reduction associated with RPE cell hypertrophy during ageing. The rate of softening follows an exponential decay with illumination duration, providing tuneable, dose-controlled mechanical perturbation without disrupting the surrounding monolayer.



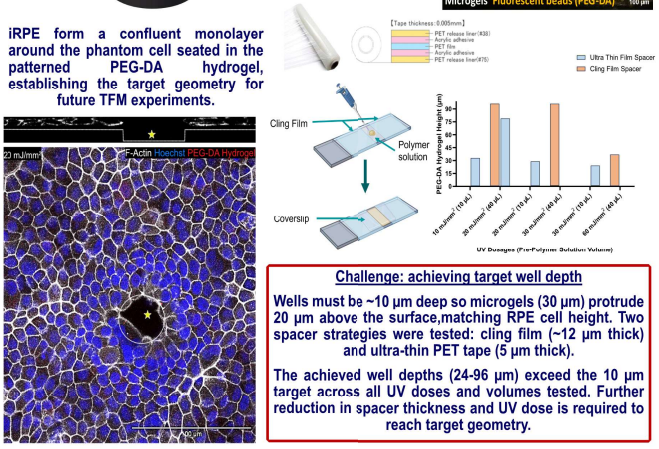
Photodegradable microgels integrate into epithelial monolayers on glass via E-cadherin, forming hybrid adherens junctions that mechanically couple to the surrounding actin cytoskeleton.

TOWARDS QUANTITATIVE FORCE READOUT

A thin PEG-DA hydrogel layer is photopatterned using maskless photolithography (PRIMO) to create defined wells that seat individual microgels, positioning them at cell height within the RPE monolayer for force readout via embedded fluorescent beads.



iRPE form a confluent monolayer around the phantom cell seated in the patterned PEG-DA hydrogel, establishing the target geometry for future TFM experiments.



Challenge: achieving target well depth

Wells must be ~10 µm deep so microgels (30 µm) protrude 20 µm above the surface,matching RPE cell height. Two spacer strategies were tested: cling film (~12 µm thick) and ultra-thin PET tape (5 µm thick).

The achieved well depths (24-96 µm) exceed the 10 µm target across all UV doses and volumes tested. Further reduction in spacer thickness and UV dose is required to reach target geometry.

SUMMARY & OUTLOOK

Light-tunable phantom cell enables controlled perturbation of epithelial mechanics.

Successful integration into epithelial monolayers through cadherin-based adhesion.

Optimize PEG-DA Hydrogel height further and quantify force propagation through actin and keratin networks.

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