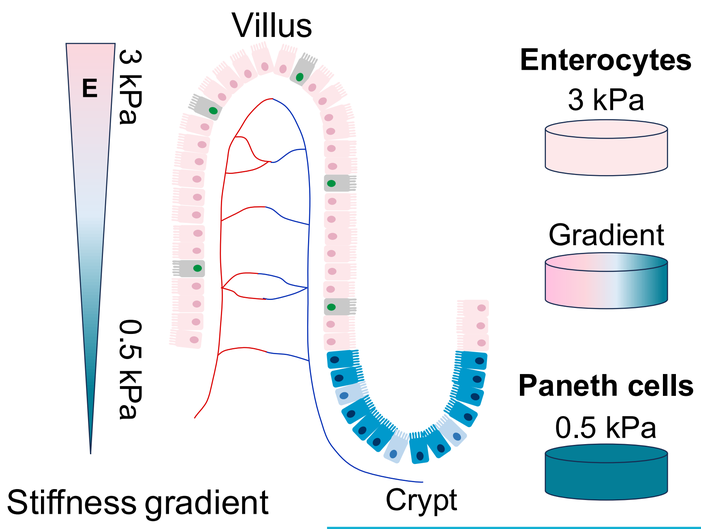
Mechanically tuned hydrogels driving physiologically relevant intestinal cell differentiation for predictive in vitro drug absorption studies

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Introduction

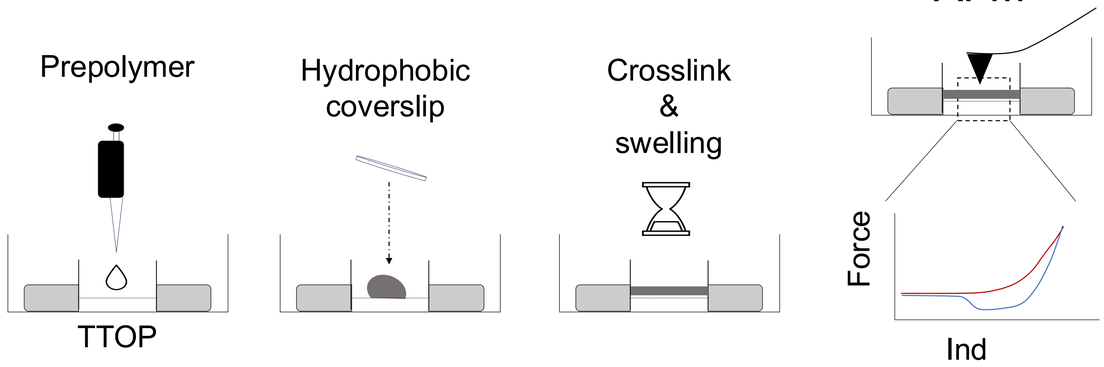
The small intestine is the **primary site of absorption** for orally delivered drugs. The recapitulation of the intestinal microenvironment could **enhance the biomimicry and predictivity of in vitro models for drug absorption studies**. The small intestine epithelium is organized in protrusions called villi and invaginations called crypts, and acts as a **selective barrier regulating molecular uptake.**

Hypothesis

We hypothesized that mechanically-tuned substrates could **drive Caco-2 cells sub-differentiation** through **passive mechanical stimuli**, if cultured in a **bicompartmental environment** provided by the bicompartmental TTOP insert (True Tissue On Platform), to enhance *in vitro* drug absorption predictivity.

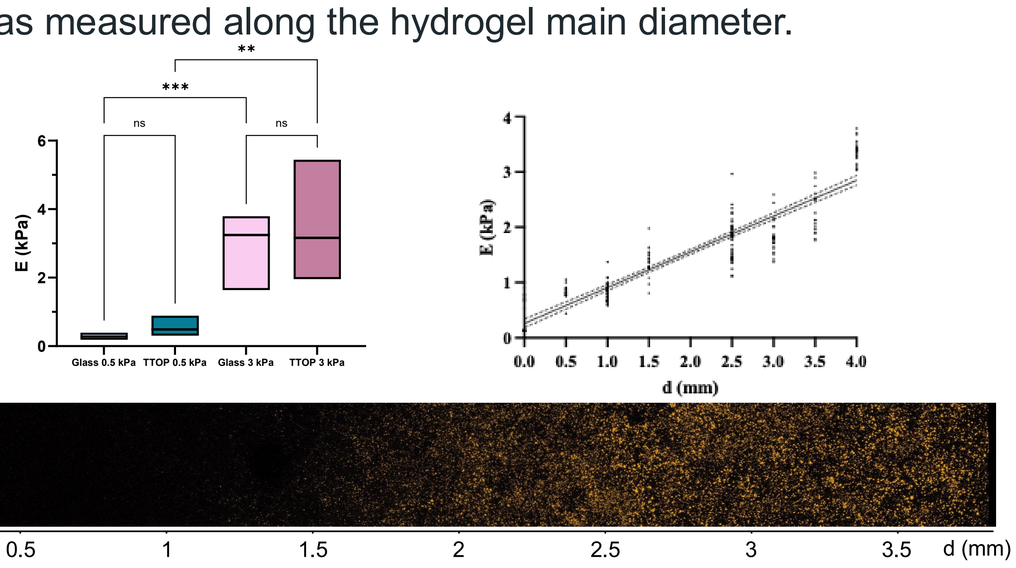
Protocol

Polyacrylamide (PAAm) hydrogels with **stiffnesses of 0.5kPa and 3kPa** were synthesized onto polycarbonate (PC) membranes hosted in bicompartmental TTOP inserts according to the following protocols. **Gradient hydrogels spanning 0.5-3 kPa** were obtained by combining the two formulations.



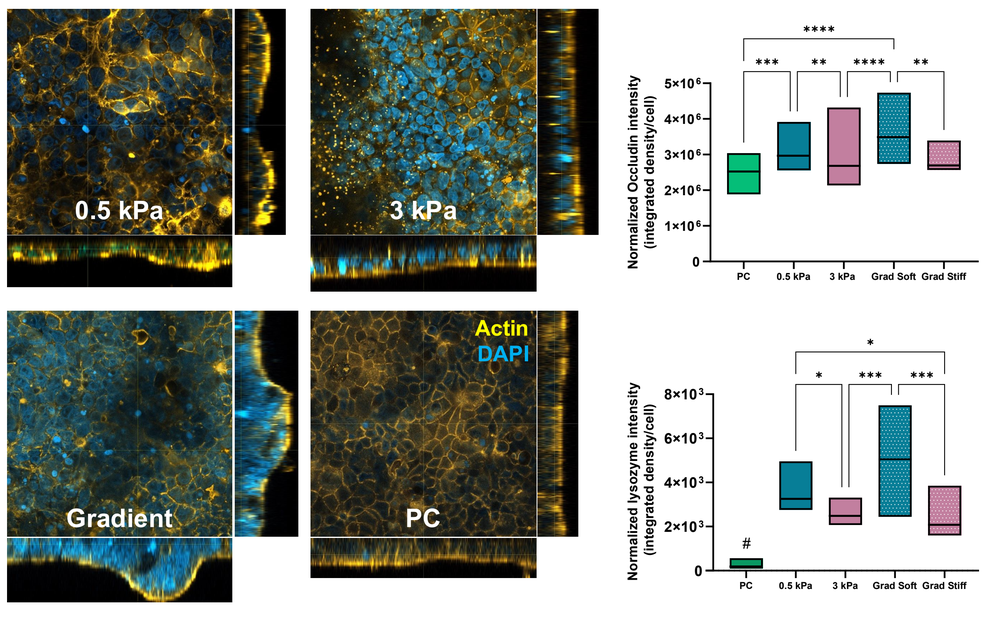
Mechanical properties

Hydrogel **stiffness was measured with AFM** and results on glass coverslips and PC membranes were compared. Gradient hydrogels stiffness was measured along the hydrogel main diameter.



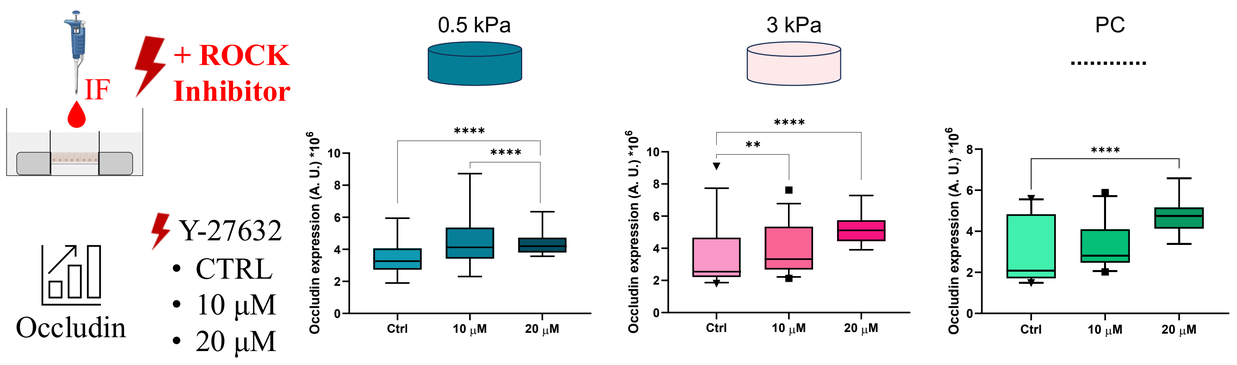
Morphology and sub-differentiation

Caco-2 cell sub-differentiation was assessed through **occludin** (tight junction protein) and **lysozyme** (Paneth cell specific marker) staining, revealing higher levels of expression on soft substrates and soft region of gradient hydrogels.



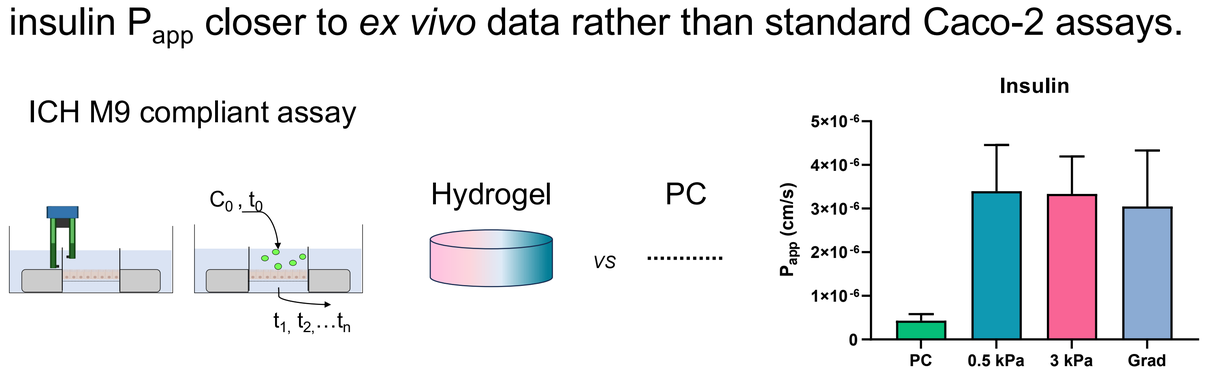
Mechanotransduction

ROCK/RhoA pathway is modulated by soft hydrogels, promoting cell-cell adhesions.



Drug absorption

Mechanically-tuned hydrogels modulate intestinal barrier function with insulin P_{app} closer to *ex vivo* data rather than standard Caco-2 assays.



Conclusion

Hydrogel stiffness directed Caco-2 sub-differentiation into enterocytes or Paneth cells, depending on the mechanical properties of the substrate. The **bicompartmental environment was essential for promoting polarization and enhancing cell heterogeneity**. This scalable approach enables the generation of **multiple cell subtypes in a single sample**, improving the physiological relevance of drug permeability assays.

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