APPLYING DISSOCIATIVE CHEMISTRY TO OBTAIN GRADIENT HYDROGELS

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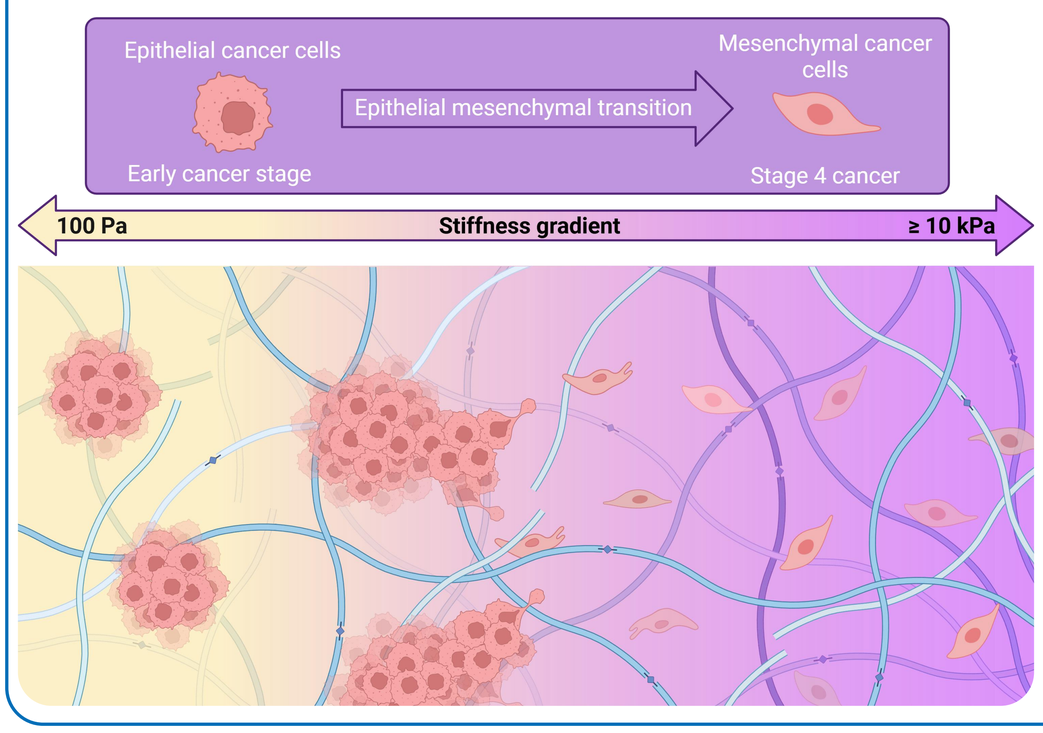
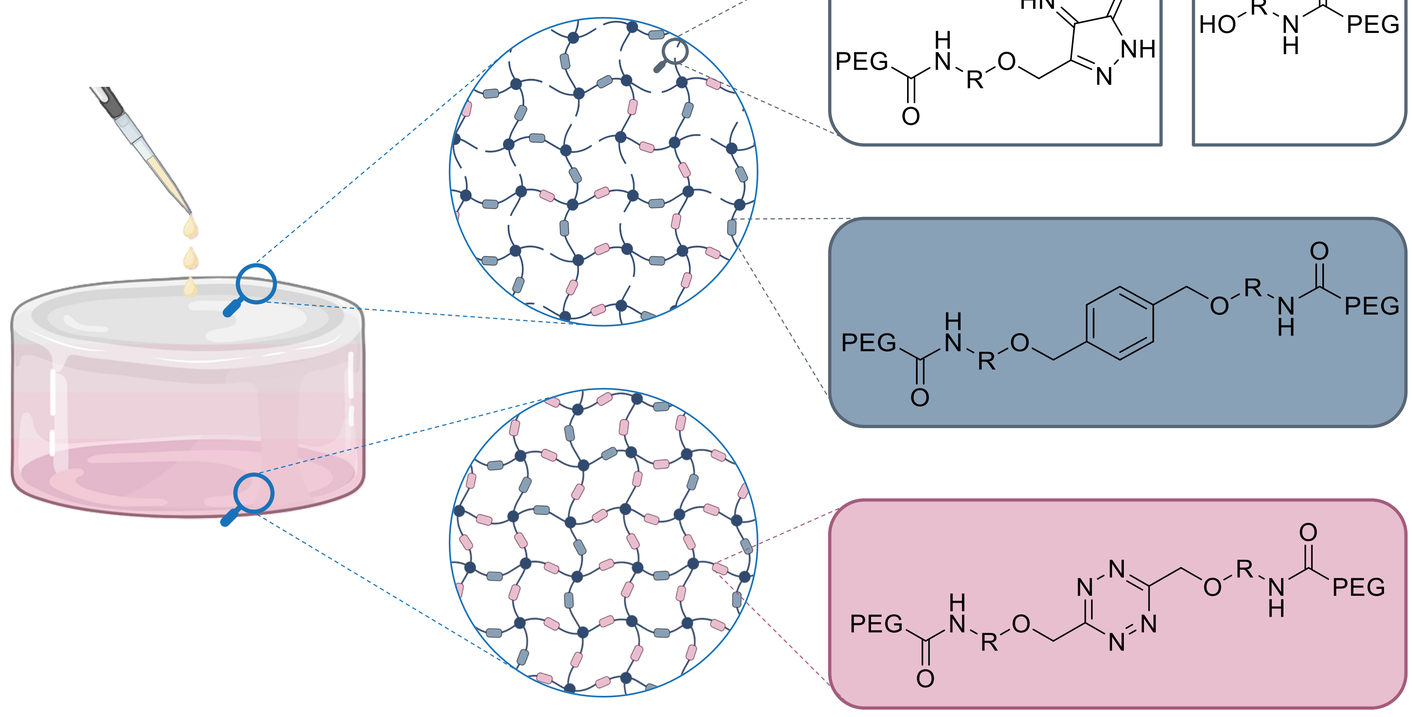
Context

Gradients in the extracellular matrix (ECM) are critical regulators of tissue development, physiological function, and disease progression. In pancreatic ductal adenocarcinoma (PDAC), progressive changes in ECM mechanics correlate with disease stage. Studying PDAC *in vitro* requires models that mimic the mechanical anisotropy of the native tumor microenvironment. Here, we present an innovative approach to fabricate hydrogels with tunable stiffness gradients using tetrazine–isonitrile click-to-release chemistry. Tetrazine moieties serve as degradable crosslinkers, which are selectively cleaved by the isonitrile cleaving solution. Diffusion of the cleaving solution through the hydrogels results in spatially controlled reductions in crosslink density. This dissociative bioorthogonal strategy enables the generation of mechanical gradients in hydrogels that mimic key features of the PDAC tumor ECM.

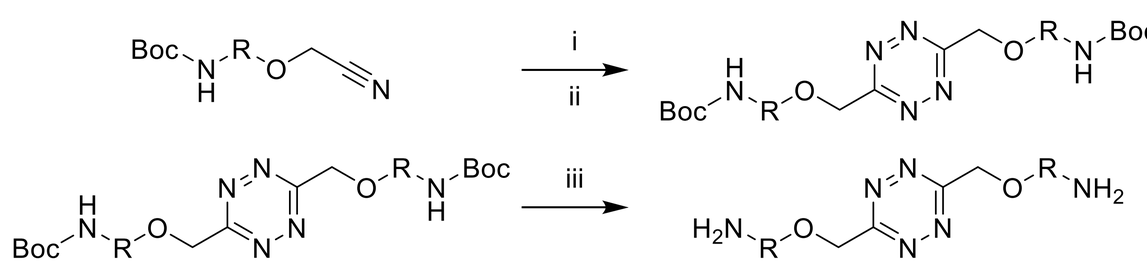
Gradient hydrogel formation

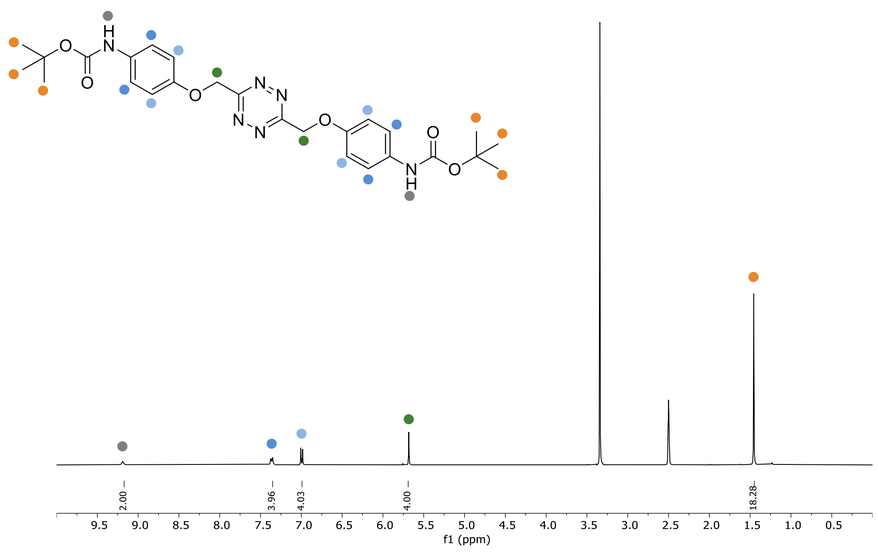




Tetrazine: degradable crosslinkers

For this project, the starting reagent requires a protected primary amine on one side and an acetonitrile linked to an ether functionality on the other.



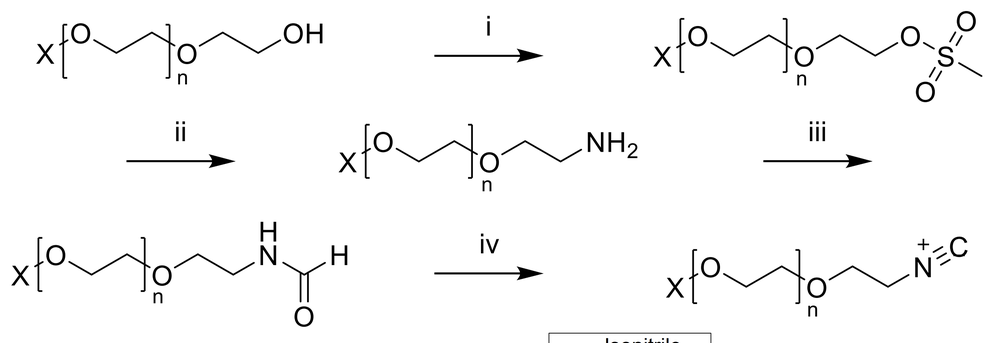


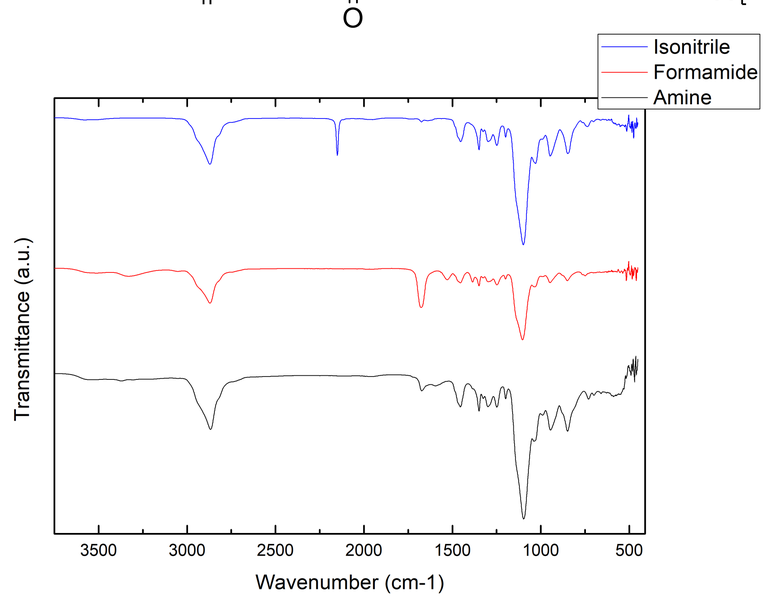
≈70% Yield

i: 3-mercaptopropionic acid and hydrazine hydrate in ethanol, ii: acetic acid and sodium nitrite in water, iii: trifluoroacetic acid in DCM

Isonitrile: cleaving solution

The number of repeats and the X functional group can be modified to change the diffusion constant. This will influence the formation speed and shape of the final gradient.





≈85% Yield

i: Triethylamine and methanesulfonyl chloride in DCM, ii: Ammonia and ammonium chloride in water, iii: Ethyl formate, iv: Triethylamine and phosphoryl chloride in THF

Outlook

- First, compression testing and swell tests of the homogeneous hydrogels will be performed.
- Afterward, these tests will be repeated with the anisotropic hydrogels.
- Finally, the optimized hydrogels will be tested *in vitro* using 3D PDAC tumor models and in combination with chemotherapy.

Acknowledgements

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