

## **Triple click chemistry for crosslinking, stiffening, and annealing of gelatin-based microgels**

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### **Supplementary information**

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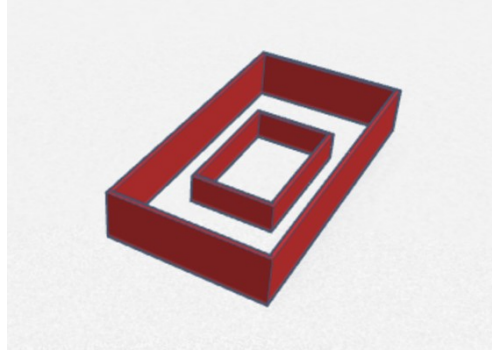
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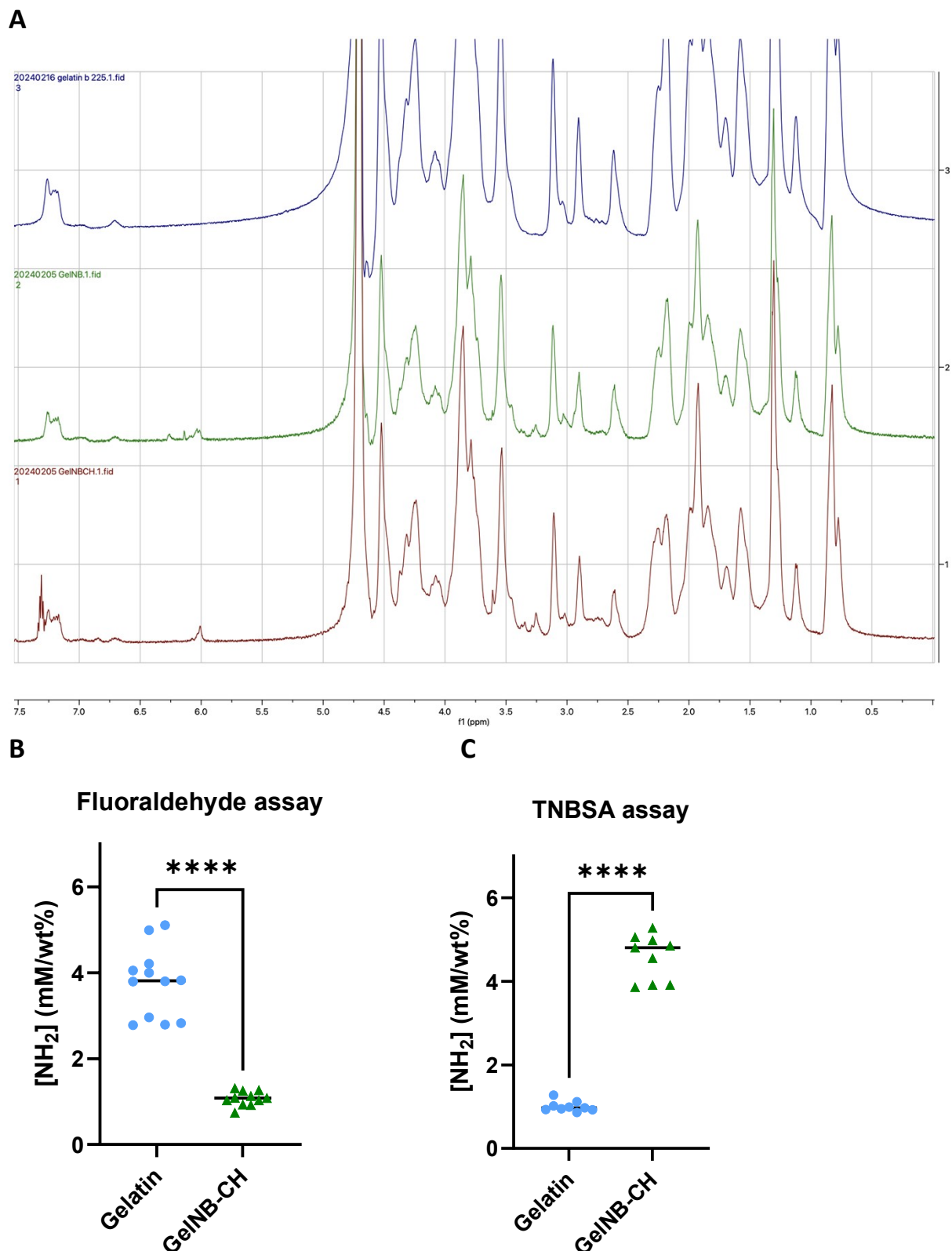
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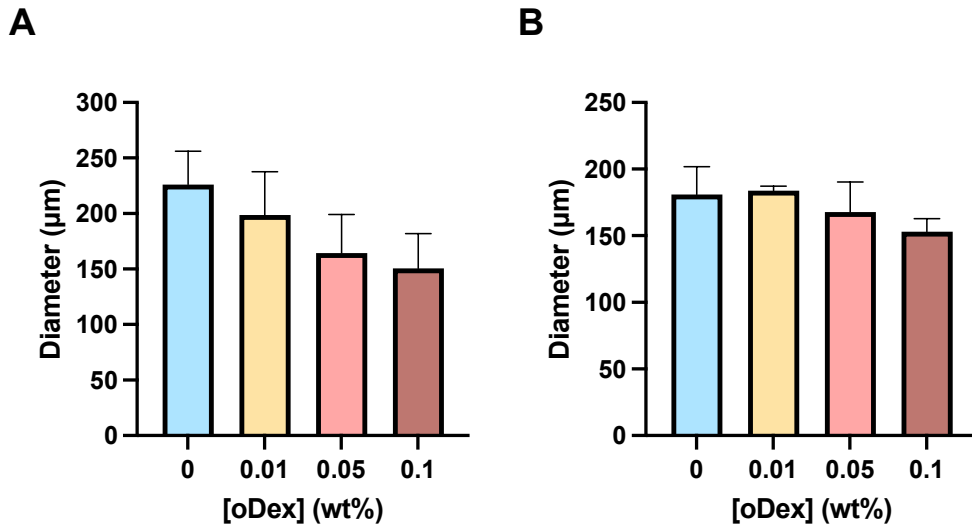
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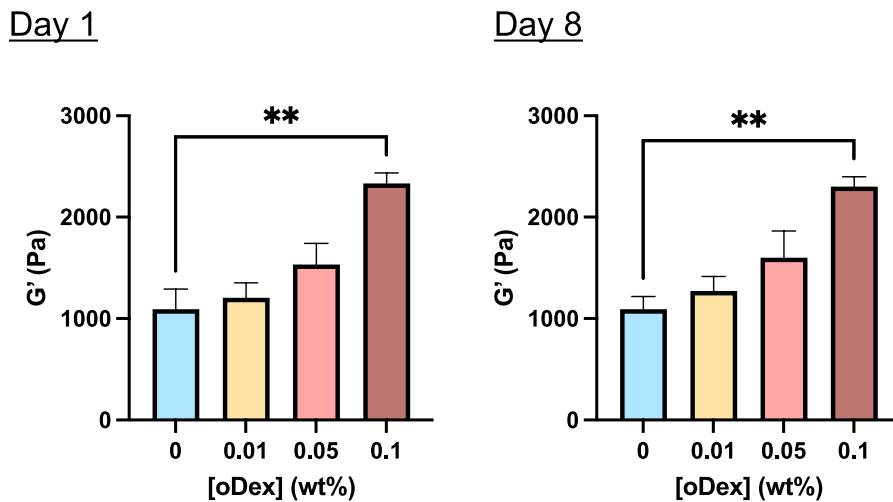
**Figure S1.** Screenshot of the STL file for 3D printing of mold/reservoir to create TC-MAP gel for cell culture. TC-MAP gel was deposited in the center mold (width x length x height = 5.6 mm x 9.6 mm x 4 mm), while the outer space served as a reservoir for cell culture media (width x length x height = 11 mm x 22 mm x 7 mm). Wall thickness = 0.25 mm.



**Figure S2.** (A) NMR spectra of gelatin, GeINB, and GeINB-CH. The peak at 6 ppm is assigned to the vinyl protons on the norbornene group, and the peak at 2.2 ppm is assigned to glutamic acid and hydroxyproline.<sup>7</sup> (B) Fluoraldehyde assay results for amine concentrations on gelatin and GeINB-CH. (C) TNBSA assay results for acid concentrations on gelatin and GeINB-CH (\*\*\*\*  $p < 0.0001$ ).



**Figure S3.** Changes of microgel diameters as a function of oDex concentration. (A) Microgels prepared by microfluidic droplet generator. (B) Microgels prepared by the emulsion method. Data represents the average diameters of three independent experiments (N=3, Mean  $\pm$  SEM). There is no significant statistical difference between any stiffened and non-stiffened microgel size.



**Figure S4.** MAP gel bulk stiffness on D1 and D8. Data represents the average moduli of three independent experiments (Mean  $\pm$  SEM). \*\*: p < 0.01.