Junction Functionality-Dependent Stiffness and Solute Transport in Biocompatible Hydrogels

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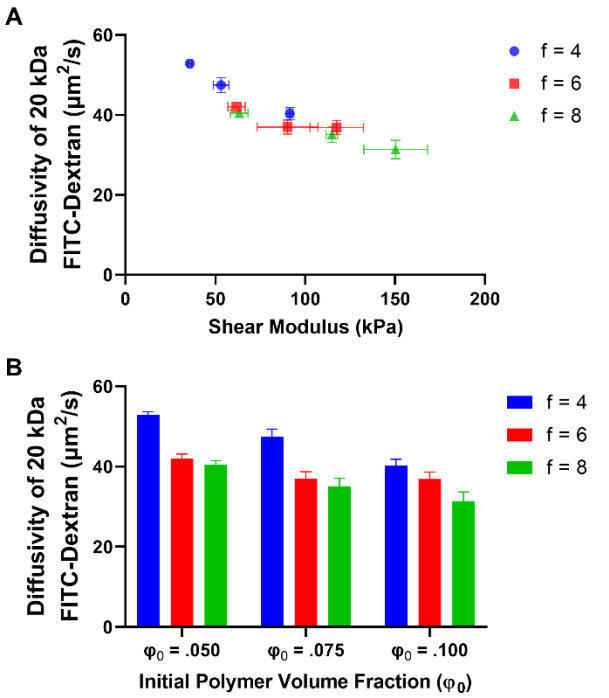
Statement of Purpose: As multi-arm poly(ethylene glycol) (PEG) macromers are increasingly being used to create cell-encapsulating hydrogels, more information about their material properties is needed to contextualize their effects on cell culture. Specifically, four-arm and eight-arm PEGs have become popular precursors to cell-encapsulating hydrogels, but structural comparison of four-arm and eight-arm PEGs have largely been limited to discussion of crosslink densities, swelling ratios, and hydrogel stiffness. However, the doubling of junction functionality from four-arm to eight-arm PEG hydrogels changes the geometry of the network, which our recently updated models predict to have a strong effect on solute transport within the hydrogels.[1] Here, we investigate how changing the number of arms per precursor macromer in a PEG hydrogel affects both stiffness and solute diffusivity. We anticipate that these results will lead to more nuanced design of cell-encapsulating hydrogels, with control mechanisms for both hydrogel-mediated stiffness and solute transport that enable precise control of hydrogel-encapsulated cell-environment and cell-cell signaling.

Methods: Multi-arm PEG macromers (4-arm, 10 kDa; 6-arm 15 kDa; 8-arm, 20 kDa; JenKem Technology USA) were end-functionalized with norbornene carboxylic acid, purified, and confirmed >95% functionalization by ^{1}H-NMR. The resulting PEG-norbornenes (PEGNB) were each dissolved in phosphate-buffered saline (PBS) at 5%, 7.5% and 10% volume fraction with a 1:1 stoichiometry of norbornene groups to crosslinking dithiothreitol (DTT) and 1 mM lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate (LAP). Hydrogels were formed from these solutions by 30 seconds of UV exposure (3 mW/cm^2). The resulting 9 formulations of hydrogels (4, 6, 8 arms with 5%, 7.5%, or 10% initial polymer volume fraction) were swollen to equilibrium then subject to compressive testing to measure stiffness and fluorescence recovery after photobleaching (FRAP) experiments to measure solute transport.

Compressive testing with a dynamic mechanical analyzer (TA Instruments) to 20% strain was evaluated using a Neo-Hookean model to calculate a shear modulus for each hydrogel formulation, representing its stiffness.

Since both solute size and hydrogel network structure affect solute diffusivity within a hydrogel, FRAP experiments were performed using a 20 kDa FITC-Dextran with a hydrodynamic radius of 3.3 nm. Three hydrogel samples per formulation ($n = 3$) were incubated in 10 μM of the FITC-Dextran in PBS for 24 hours before a FRAP experiment was performed within each sample using a confocal laser scanning microscope (Zeiss). Diffusion coefficients were calculated from FRAP experiments using a well-established spatial Hankel analysis method.[2] To provide context on the FRAP experiments, partition coefficients for each hydrogel were calculated from the fluorescence intensity of the source solution and each hydrogel's supernatant solution after 24 hours.



Results: The nine PEGNB hydrogel formulations made with three junction functionalities ($f = 4, 6, 8$) and three initial polymer volume fractions ($\varphi_0 = 0.050, 0.075, 0.100$) all formed intact hydrogels. Compressive testing and FRAP studies with 20 kDa FITC-Dextran showed that the physical properties (stiffness and diffusivity) of the hydrogels are highly coupled, with increasing stiffness corresponding to decreasing solute diffusivities (A). This result agrees with previous studies and suggests that more than two structural parameters need to be simultaneously manipulated to decouple stiffness and diffusivity in hydrogels. However, the data relating the diffusivity of FITC-Dextran to the hydrogel's junction functionality provides new insight into the influences of hydrogel structure on physical properties. Specifically, the experimental evidence that diffusivity decreases with increasing junction functionality (B) validates our geometry-based hypothesis[1] that mesh size, which increases with increasing junction functionality, is an inadequate predictor of solute diffusivity within hydrogels. Instead, mesh radius, which incorporates the geometric influences of junction functionality on network structure, positively correlates with solute diffusivity, providing a physical rationale relating junction functionality to solute transport in hydrogels. These results enable more precise and accurate control of hydrogel physical properties for the design of hydrogel scaffolds for tissue engineering applications and hydrogel-based drug delivery devices.

References: 1. (Richbourg NR, et al. Macromol Chem Phys. 2021; 222: 2100138.) 2. (Jönsson, P, et al. Biophys J. 2008;95:5334-48.)