Tuning Matrix Stress Relaxation in PEG-HA Hydrogels to Regulate the Spreading and Nascent Protein Deposition of Mesenchymal Stromal Cells

Alexandra N. Borelli[1,2], Mark W. Young[1,2], Sarah Mellett[1], Michael R. Blatchley[1,2], Varsha V. Rao[1,2], Kristi S. Anseth[1,2]

[1]Department of Chemical and Biological Engineering and the [2]BioFrontiers Institute.
University of Colorado, Boulder, 80303. USA

Statement of Purpose: Synthetic hydrogels have been utilized in tissue engineering to control both physical and biological properties of mesenchymal stromal cells (MSCs).[1,2] Previous work has investigated the encapsulation of human MSCs (hMSCs) in a hyaluronic acid (HA) guest host based adamantane-cyclodextrin viscoelastic hydrogel, crosslinked with methacrylate. The work reported the effect of the hydrogel to the hMSCs morphology and nascent protein deposition, however the effect of viscoelasticity or stress relaxation on MSCs has not been rigorously addressed.[3]

In this work, we engineered a covalent adaptable hydrogel, with varying degrees of stress relaxation, utilizing the hydrazone bond, formed between an aldehyde and hydrazide group. The resulting hydrogel was stabilized with a slow reacting strain promoted azide alkyne cycloaddition between an 8-arm poly(ethylene glycol) (PEG) functionalized with bicyclononyne and pendant azides on hyaluronic acid (HA). Rat mesenchymal stem cells (rMSCs) were encapsulated in hydrogels with different adaptability, controlled via the number of alkyl-hydrazone bonds present within the hydrogel, and rMSC morphology and nascent protein deposition (NPD) was measured as a function of the material properties.

Methods: Hyaluronic acid (HA) was functionalized with either an aliphatic aldehyde (HA-Ald) or hydrazide (HA-Hyd) following established protocols.[4] An 8-arm poly(ethylene glycol) (PEG, 40 kDa) was functionalized with bicyclononyne (PEG-BCN), following an established protocol.[5] Ald-Ph-PEG3-azide (PEG-Azide, 350 Da) was purchased from BroadPharm and used as a crosslinker, enabling the effective conversion of hydrazide functional groups into azide functionality on the HA-Hydrazone macromer. Macromers were dissolved in PBS at stoichiometric ratios for a total stock concentration of 3 wt% (HA) and 20 wt% (PEG). The ratio of the alky-hydrazone to benzyl-hydrazone bonds was used to yield hydrogels with varying degrees of stress relaxation (SR) over time. The stress relaxation of the resulting hydrogel was measured using a parallel plate rheometer (TA instruments, DHR-3).

Rat MSCs (Cyagen) were cultured in low-glucose DMEM (ThermoFisher Scientific) supplemented with Pen/Strep, fungizone and 10% FBS. rMSCs between passages 5-6 were used in all experiments and encapsulated in the network at a density of 3 million cells/mL. rMSC morphology and Yap localization was quantified by immunostaining the nuclei (DAPI), actin cytoskeleton (Rhodamine Phalloidin), and Yap (Yap antibody (63.7)) and imaged using a laser scanning confocal microscope (Zeiss LSM 710). The NPD was measured by immunostaining the cell membrane (HCS Cell Mask Blue Stain) and the deposited protein (incorporated HPG tagged with an Azide-647 fluorophore) and imaged using a laser scanning confocal microscope (Zeiss LSM 710).

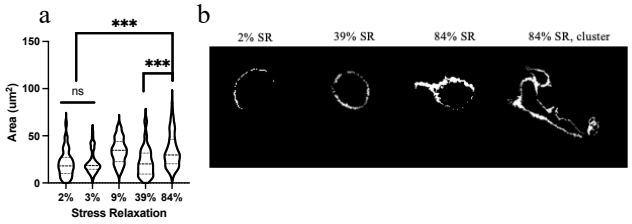

Figure 1. a) Total area of deposited nascent protein as a function of stress relaxation. b) Nascent protein deposition visualized as a function of stress relaxation.

Results: The final shear storage moduli of the hybrid network (HA-Hydrazone: PEG-triazole) was ~2000 Pa. The percent of stress relaxed over time was measured, and ranged from 92% to 2%, depending on the alkyl-hydrazone content, ranging from 100% to 0%, respectively. After 4 days in culture, the 92% SR condition had completely degraded, while the 84% SR condition showed significant cell spreading, with aspect ratios of 1.60, and Yap nuclear localization, with an average nuclear:cytoplasmic ratio of 6.10 ± 2.74. rMSCs in the 39%-2% SR formulations remained rounded, with nonsignificant cell spreading and Yap nuclear localization. Interestingly, small cluster formations were observed in the 84% SR condition by day 4, with morphologies not observed in any other condition.

Moreover, the rMSC NPD increased in hydrogels with higher levels of stress relaxation (Figure 1a, b). By day 4, rMSCs in the 84% SR condition had the highest levels of NPD, with an average total area for deposited protein of 34.2 ± 17.7 μm while rMSCs in the 39%-2% SR conditions deposited significantly less total protein area of 22.3 ± 15.3 μm or less (Figure 1a). Notably, the total area of deposited protein was seen to be the greatest from the small clusters, as compared to single cells, in the 84% SR condition, with an average area for deposited protein of 80.3 ± 37.9 μm (Figure 1b).

Conclusions: MSC NPD and morphology were studied as a function of the stress relaxation in PEG-HA hybrid networks. These findings may be important when designing therapeutically relevant viscoelastic biomaterials, as MSCs physical and biological properties can be tuned based on hydrogel formulations, enabling further control over cellular therapies.

Acknowledgments: This study was supported by the NIH (R01 DE016523). A. N. Borelli also thanks the support of a GAANN fellowship from DoEd.

References: [1] T. MW. Biotechnol. Bioeng. 2009; 103:655-663. [2] D. A. Foyt. Adv. Healthc. Mater. 2018; 7:1700939. [3] C. Loebel. Nat. Mater. 2019; 18:883-896. [4] Wang LL. J Biomed Mater Res A. 2018; 106:865-875. [5] DeForest CA. Nat. Mater. 2015; 14:523-531.