



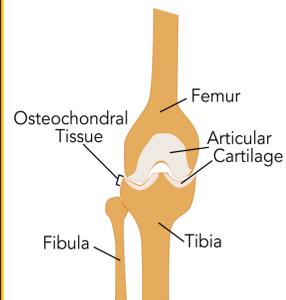
Evaluating *In Vitro* Cytocompatibility of Encapsulated Mesenchymal Stem Cells in a Chondroitin Sulfate Crosslinked Poly(*N*-isopropylacrylamide)-Based Hydrogel

RICE

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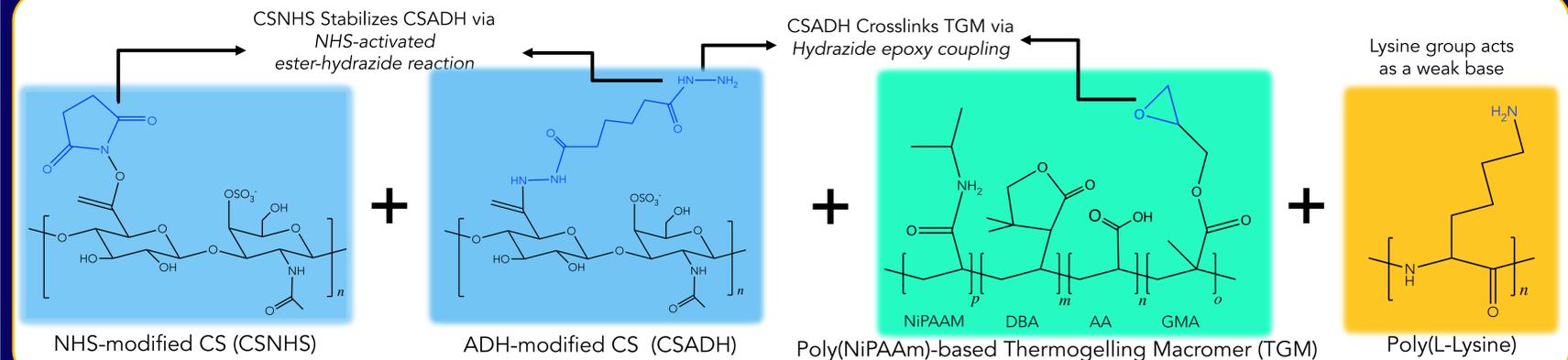
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Motivations



- Osteochondral defect unable to heal: low cell density and avascularity
- Hydrogels show potential to minimize invasiveness, localize delivery, degrade over time
- Our hydrogel design:**
 - Crosslinked Poly(*N*-isopropylacrylamide) hydrogel, combats issues with shrinking leading to decreased site of regeneration
 - Also crosslinked with chondroitin sulfate, which has shown capabilities of inducing chondrogenic behavior¹
 - Poly(L-Lysine) promotes chondrogenic differentiation and the lysine group acts as a weak base

Hydrogel Chemical Structure

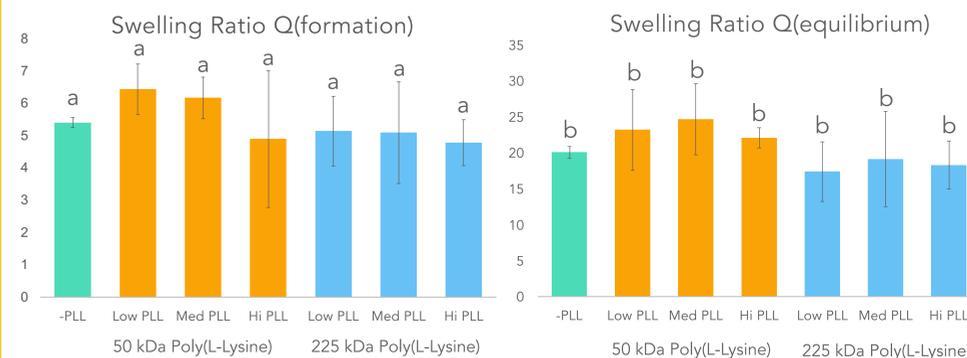


Study Groups

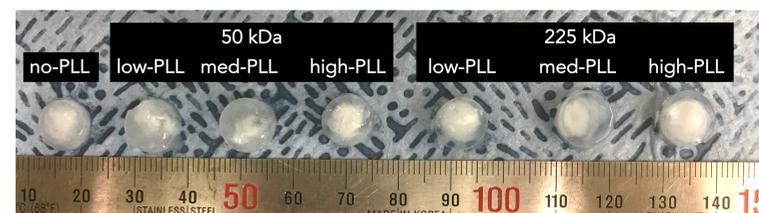
No Poly(L-Lysine)	
50 kDa Poly(L-Lysine)	1 µg of PLL/100 µL of hydrogel (low PLL)
	10 µg of PLL/100 µL of hydrogel (med PLL)
	20 µg of PLL/100 µL of hydrogel (only used for swelling study, high PLL)
225 kDa Poly(L-Lysine)	1 µg of PLL/100 µL of hydrogel (low PLL)
	10 µg of PLL/100 µL of hydrogel (med PLL)
	20 µg of PLL/100 µL of hydrogel (only used for swelling study, high PLL)

Swelling Behavior of PLL-Loaded Hydrogel

- The effects of PLL on swelling are negligible (n=3)
- Q(formation), Q(equilibrium): mass of hydrogel normalized by the dry mass immediately after formation and 24 hours after being swollen in PBS, respectively
- According to Tukey's HSD test, no groups within Q(formation) or Q(equilibrium) show statistically significant difference in swelling ratio magnitude



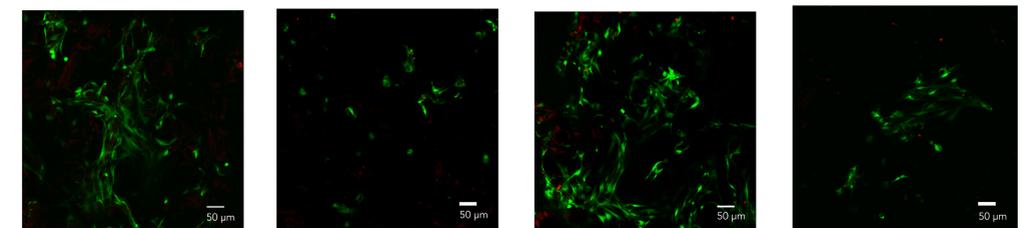
- Gels synthesized in 6 mm diameter cylindrical Teflon molds
- After swelling appeared approximately equal in diameter and integrity



In Vitro Cytocompatibility of PLL-Loaded Hydrogel

Confocal Imaging Day 7

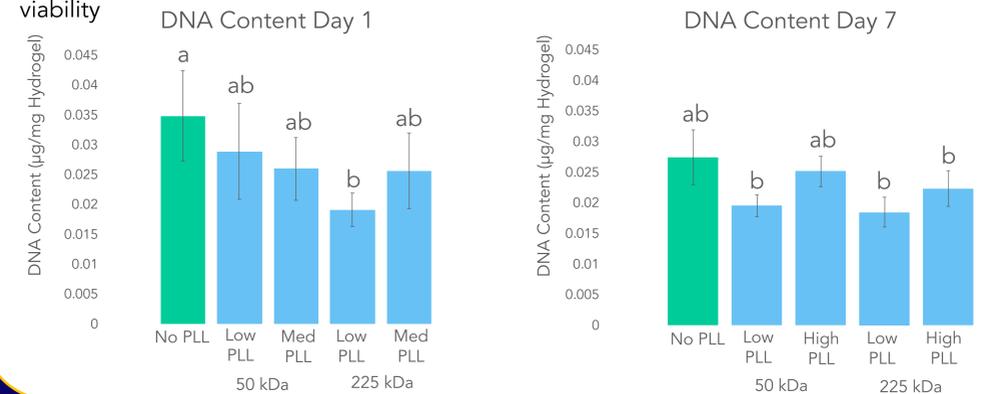
- Mesenchymal stem cells were able to spread within the hydrogels



No Poly(L-Lysine) | 1 µg of PLL/100 µL of hydrogel, 50 kDa PLL | 1 µg of PLL/100 µL of hydrogel, 225 kDa PLL | 10 µg of PLL/100 µL of hydrogel, 225 kDa PLL

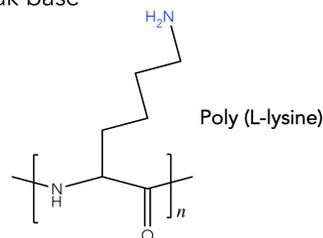
Pico Green Assay: Measuring DNA content

- According to Tukey's data, no groups at day 7 show statistically significant differences in their cell viability



Poly(L-Lysine) (PLL) pH Effects

- Low concentrations promote mesenchymal stem cell adhesion, spread, proliferation, and chondrogenic differentiation²
- Lysine group has the potential to decrease the acidity of the hydrogel (due to carboxylic acid groups), as a weak base



References & Acknowledgments

- Steinmetz N. & Bryant S. (2012). Chondroitin sulfate and dynamic loading alter chondrogenesis of human MSCs in PEG hydrogels. *Biotechnology and bioengineering*. 109. 2671-82. 10.1002/bit.24519.
- Lu H., Guo L., Kawazoe N., Tateishi T., and Chen G. "Effects of Poly(L-Lysine), Poly(Acrylic Acid) and Poly(Ethylene Glycol) on the Adhesion, Proliferation and Chondrogenic Differentiation of Human Mesenchymal Stem Cells." *Journal of Biomaterials Science, Polymer Edition* 20, no. 5-6 (January 2009): 577-89. <https://doi.org/10.1163/156856209X426402>.

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Conclusions & Future Directions

Addition of Poly-L Lysine had no effect on the crosslinking of the gels nor were we able to see significant improvement with respect to cell viability

Future Directions:

Test other PLL loading concentrations to determine if there is an effect in PLL dosage on hydrogel properties and encapsulated cell behavior.