Cells sense and respond to mechanical stimuli from their external environment through a process called mechanotransduction. Focal adhesions are integrin-containing, multiprotein structures through which mechanical force is transmitted between the extracellular matrix and the interacting cell. Cells convert the transmitted force into biological responses including migration, proliferation and differentiation. The Garcia lab has previously engineered an integrin-specific hydrogel system resulting in changes in mesenchymal stem cell (MSC) gene expression, secretome, and ultimately regenerative capacity in a murine bone repair model. However, the mechano-biological mechanism driving this cell response to varying hydrogel biophysical and biochemical properties has yet to be studied.

Here we have developed relationships between various hydrogel properties and cellular responses (cell adhesion, YAP localization, cell area, and cell shape). We engineered PEG-based hydrogel systems with two different polymerization chemistries, malemide and norbornene, to explore the effect of hydrogel chemistry on MSC cell adhesion and spreading. Using rheology, we demonstrated that hydrogel mechanical properties can be tuned by altering the weight percent of PEG macromer, while adhesion ligand type and density had no effect on hydrogel mechanical properties. PEG-4MAL gels were used for the remaining studies because that chemistry resulted in improved spreading and cell adhesion over norbornene hydrogels. By varying the density of RGD, the adhesive ligand for αvβ3 integrin, on the hydrogels, we showed that higher RGD densities resulted in greater YAP nuclear localization. We sought to understand the mechano-biological signaling pathway involved in YAP nuclear localization by inhibiting ROCK and FAK, proteins critical in mechanosensing via focal adhesion complexes. The inhibition of ROCK, and FAK decreased cell spread area, increased cell circularity and decreased YAP nuclear localization. Taken together this data demonstrates that external signals from PEG-based hydrogels as well as the intracellular signaling cascades involving ROCK and FAK can modulate YAP mechanosensing in MSCs.