The Effect of Peptide Affinity in Neural Stem Cell Mechanosensing

Jessica Stukel, Rebecca Kuntz Willits.
The University of Akron.

Statement of Purpose: Cell mechanosensing is an essential aspect of tissue engineering to understand how cells sense and respond to their environment. Increasing our knowledge of neural stem cell (NSC) mechanosensing has the potential to improve scaffold design with applications involving change in tissue stiffness due to traumatic brain injury or neurodegenerative disease. Our lab is working towards this goal by investigating the combined effect of varying scaffold stiffness and peptide affinity, with linear or cyclic Arg-Gly-Asp (RGD), to the NSC response. As the affinity for cyclic RGD is increased over linear RGD [1], we hypothesize that increased peptide affinity will lead to increased tension and alter differentiation. Focusing on cell:RGD bonds allows for more specific evaluation of integrin involvement in mechanotransduction and how tension affects downstream pathways such as those for adhesion, proliferation, and differentiation.

Methods: Maintenance of human induced pluripotent NSC pluripotency was confirmed by immunocytochemistry (ICC) and flow cytometry, labeling for nestin (NES) and SOX1. Upon withdrawing fibroblast growth factor-2 (FGF2), NSCs differentiated towards neurons as analyzed via ICC and PCR for TUJ1, MAP2, and GFAP, markers for early neurons, mature neurons, and astrocytes, respectively. To confirm NSC adhesion to RGD, NSCs were cultured on fibronectin-coated glass in maintenance or differentiation media, inhibited by soluble RGD, and labeled for f-actin, vinculin, a protein in focal adhesions, and integrin subunits. To vary the substrate stiffness, poly(ethylene glycol) (PEG) hydrogels containing 4, 5, 7, or 9% (wt/wt) PEG were crosslinked with UV light. The shear modulus was measured via oscillatory shear rheometry with 1% strain at 10 rad/s. Next, PEG hydrogels were crosslinked with 0.1, 1, or 2.5mM acryl-PEG-RGDSP (linear RGD) or acryl-PEG-c(RGDfK) (cyclic RGD). NSC adhesion to the hydrogels was analyzed after 1 day in differentiation media by nuclei-labeling the cells with Hoechst 33342 and obtaining tiled images of the scaffold. Nuclei were manually counted in 3 regions of interest per scaffold with the ImageJ Cell Counter; 3 scaffolds were imaged for each sample type. Next, NSCs were cultured on each hydrogel sample for 7 days in differentiation media and gene expression was analyzed via PCR. Future work includes analyzing protein expression at 1, 4, and 7 days.

Results: NSCs maintained in maintenance media expressed NES and SOX1 as confirmed via ICC, flow cytometry, and PCR. After 7 days without FGF2, NSCs differentiated primarily to neurons with 60 times greater MAP2 expression by ICC than NSCs in pluripotent media ($p<0.05$), with low GFAP expression. Next, NSC interaction with RGD was confirmed via ICC. Multipotent NSCs and those differentiated towards neurons expressed integrin subunits $\alpha5$ and αv. Addition of 0.5mM soluble RGD caused the cells to cluster together rather than binding to fibronectin (Figure 1). The hydrogel shear modulus, measured by oscillatory shear rheometry, was 0.49±0.056, 1.30±0.19, 4.83±0.92, and 14.89±0.79 kPa for 4, 5, 7, and 9% PEG hydrogels without bound RGD. Because the addition of RGD will reduce the modulus, total peptide content (RGD and RAD control) will remain constant and the RGD concentration will be varied. Cell adhesion to PEG hydrogels was lowest on 4% PEG gels with 2.5mM RGDSP, but adhesion was constant on all other scaffolds (Figure 2). Further manipulation of the peptide concentration, however, demonstrated that reduced concentrations of 0.1 and 1mM RGD increased adhesion on 4% PEG gels. After 7 days, gene expression for *TUJ1* and *GFAP* on 4% PEG gels was greater for 2.5mM RGDSP compared to 1mM RGDSP. Differences in gene expression for varying peptide and PEG concentrations are being evaluated.

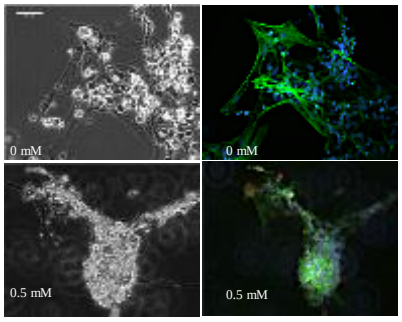

Figure 1. NSCs on fibronectin were binding through RGD as noted by differences in morphology after addition of soluble peptide (actin=green, nuceli=blue) (scale bar = 50μm).

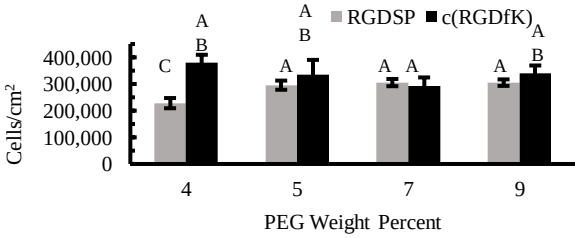

Figure 2. NSC adhesion on PEG gels with 2.5mM RGDSP. Groups with the same letter are not significantly different ($p<0.05$).

Conclusions: NSC neuronal differentiation was confirmed upon withdraw of FGF2. Culture with 0.5mM soluble RGD showed that cells altered their morphology demonstrating that the NSCs use integrins for cell adhesions to the substrate, further motivating our hypothesis. Additionally, NSCs adhered to PEG scaffolds with a range of peptide concentration and affinity. Current work includes examining adhesion on an expanded stiffness range and further analyzing peptide concentration, as this affects peptide spacing. Future work will demonstrate the time dependent differentiation of NSCs over the range of stiffness with both RGD peptides to more clearly investigate the impact of mechanotransduction on differentiation.

References: [1] Xiao, Y, Biophys. J., 1996, 71: 2869-2884.