Elucidating the Effects of Varying Ligand Type on Mechanotransduction of Mesenchymal Stem Cells using Hydrogels with Enhanced Conjugation Efficiency

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Transcriptional regulator Yes-associated protein (YAP) has been shown to be a mechanical rheostat of the cell, with increasing substrate stiffness triggering YAP translocation to the nucleus. Previous mechanotransduction studies of YAP have been conducted using fibronectin only. However, cells reside in an extracellular matrix comprised of different types and densities of biochemical ligands. Fibronectin binds to cells through αVβ3-integrin, while most other ECM proteins utilize different integrin subunits. It remains unknown how varying ligand type modulates YAP translocation in stem cells. Using polyacrylamide hydrogels with enhanced conjugation efficiency, here we examine the effects of varying ligand type on stem cell mechanotransduction using commonly used ECM proteins including fibronectin, collagen-I, collagen-IV, and laminin. Outcomes were measured by characterizing protein conjugation efficiency and distribution, YAP translocation, F-actin formation, focal adhesion formation, and osteogenesis of human mesenchymal stem cells. Different ligand types exhibit different incorporation efficiencies and protein distributions. While YAP nuclear translation is ligand density-dependent for all ECM types, translocation occurs at different ligand densities depending on ECM type. Using antibody blocking experiments, we further demonstrate that ligand type-induced YAP translocation is dependent on different integrin subunits (Figure 1). Finally, varying ECM type further modulates MSC osteogenesis even when nuclear YAP was observed across all ligand types. Together, these findings validate ligand type as an important parameter in modulating mechanotransduction and differentiation of stem cells, and provide valuable insights on how to choose optimal ligand type and density to induce desired mechanotransduction and stem cell fate.

Figure 1. Summary schematic: YAP mechanotransduction is mediated by specific ECM-integrin interactions.