Impact of dECM:MeHA Hydrogel Stiffness on Bovine Meniscus Cell Phenotype

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Human meniscus tears affect over 1 million individuals in the United States each year, but the avascularity of the meniscus limits its capacity to self-heal from injury. Therefore, an effective solution is required to restore the function and health of menisci. Biomaterials are a promising alternative to meniscectomies, by repairing the injured site, recruiting native cells for growth and repair, and salvaging the healthy meniscus instead of replacing it entirely. To gain insight on potential biomaterial treatments, we investigated the phenotypic effect of hydrogel stiffness on meniscus cell phenotype. To achieve this, a tunable hydrogel system was developed to promote meniscus cell adhesion, morphology, and proliferation, while controlling for the stiffness of the hydrogels to observe its phenotypic impact on juvenile bovine meniscus fibrochondrocytes (jBMFCs). This was achieved by using bovine decellularized extracellular matrix (dECM) as the medium for promoting cellular activity, and using methacrylate functionalized hyaluronic acid (MeHA) to adjust the stiffness of the hydrogels through polymeric crosslinking. dECM is a promising biomaterial for tissue repair because it contains essential proteins, such as collagen and RGD, which facilitate normal cell activity. Hyaluronic acid is a crucial component of the extracellular matrix, particularly for its role in wound healing; it was functionalized with methacrylate to provide a capacity for chemical crosslinking. The phenotype of the jBMFCs was characterized by the cells' adhesion, morphology, and proliferation within the dECM:MeHA hydrogels. To synthesize the dECM:MeHA hydrogels, 1.5% dECM solution was combined with various concentrations of MeHA (0.5-1.0%). The combined solution was crosslinked under ultraviolet light and heat (37°C). Following hydrogel synthesis, well-plate samples were seeded with jBMFCs for *in vitro* testing. After growth, they were compared to native meniscus tissue for cell adhesion and morphology in the dECM:MeHA hydrogel through actin staining and visualization, and their rate of proliferation was analyzed through Cell Counting Kit 8 assay (CCK-8). Unseeded dECM:MeHA hydrogel samples were analyzed for their mechanical properties, specifically compression, for future comparison to the biomechanics of native bovine meniscus tissue. The jBMFCs exhibited striated morphology along with greater adhesion and growth within the stiffer 1.5% dECM: 1.0% MeHA hydrogels compared to the softer 1.5% dECM: 0.5% MeHA hydrogels. This indicates the stiffer hydrogel promoted more favorable cell activity by mimicking the extracellular environment and mechanical properties of native bovine meniscus tissue through a higher concentration of crosslinked MeHA. This provides insight into regenerative biomaterial treatments for meniscus injuries, and preliminary data on the importance of biomaterial stiffness on cell activity and success.