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Sulfated hydrogels as primary intervertebral disc cell culture systems

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INTRODUCTION: Intervertebral disc (IVD) degeneration is a key contributor for low back pain, a leading cause of disability worldwide1. During degeneration, IVD aging is accelerated, leading to progressive structural changes, including blood vessel and nerve ingrowth that promote discogenic pain2. In vitro studies require novel biomaterials that mimic the IVD extracellular matrix (ECM) to provide mechanical support and a reservoir of cytokines and growth factors. As proteoglycans with their attached sulfated glycosaminoglycans (GAGs) are one of the major components of the ECM, the ECM's sulfation state could be a key factor for IVD cell-fate3. Thus, we aim to explore human NP cell fate using a novel sulfated alginate model with varying degrees of sulfation (DS).

METHODS: Primary human NP cells were expanded, mixed with solutions of i) 2.5% of standard alginate, ii) 0.1 DS, and iii) 0.2 DS alginate (4 x 106 cells/ml) and casted in 27 µl cylindrical-shaped carriers (4 mm diameter, 2 mm height). Carriers were cultured for two weeks for phenotype recovery and were collected with the culture media on day 0, 7 and 14.

RESULTS: A significant decrease of cell density ($p<0.05$) was observed in 0.2 DS alginate after 7 and 14 days of culture. Similarly, cell viability was significantly reduced ($p<0.05$) in 0.2 DS alginate after 7 days of culture (N=4). In addition, cell metabolic activity tended to be decreased in 0.2 DS alginate compared to standard alginate after 14 days of culture. Surprisingly, ECM remodeling factors such as MMP2 and TIMP1 were slightly upregulated in the 0.1 DS group (N=1), whereas catabolic cytokines were downregulated in the 0.1% DS group.

DISCUSSION & CONCLUSIONS: We demonstrate significant cellular differences between 0.2 DS alginate vs standard alginate and 0.1 DS alginate. Particularly, a significant decrease in cell density, metabolic activity and viability were observed in the 0.2 DS alginate after 7 days of culture. According to the secretome, the sulfated alginate group seems to possess increased catabolic ECM remodeling with lower secretion of catabolic factors, suggesting less responsive NP cells to ECM structural changes. Overall, standard alginate seems to be the best option for NP cell 3D culture models.

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