DESIGNING SILK FIBROIN-BASED MATRICES WITH IONIC LIQUIDS FOR TISSUE ENGINEERING STRATEGIES USING HUMAN ADIPOSE STEM CELLS

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Silk (SF) is an attractive biomaterial to be used in tissue engineering applications because of its excellent mechanical properties and biocompatibility [1, 2]. In this work, the cellular response of silk hydrogels produced through dissolution of this protein in ionic liquids (ILs) was investigated. For that, degummed fibers obtained from the cocoons of mulberry silkworm (Bombyx mori) were dissolved in an IL and the solution was gelified in ethanol, followed by IL removal from gels using soxhlet extraction. The fabricated hydrogels were characterized biochemically and biophysically by detecting amino acid composition, FTIR, SEM and mechanical testing (DMA). For in vitro assessment, human adipose stem cells (hASCs) were seeded in the hydrogels and cultured for different time periods. The resulting hydrogels have a rubbery consistency, homogeneous surface and viscoelastic behavior. Additionally, no differences on amino acid composition were found, indicating that the silk composition was kept. Confocal images confirmed cell attachment and alignment of actin filaments within the hydrogel matrix with well-develop nuclei. The MTS assay demonstrated the metabolic activity of hASCs in contact with hydrogels up to 28 days. Furthermore, the results of DNA quantification showed that hASCs are able to proliferate during studied period. These results indicated that (i) the efficiency of IL removal resulted in hydrogels with minimal cytotoxicity; and (ii) positive cellular response of the materials surface for the adhesion and proliferation of hASCs. SEM observations corroborated with the results obtained from MTS and DNA suggested that cells are able to migrate at different levels within the structure. These findings indicated that silk hydrogels produced using ILs may be potential candidates for tissue engineering strategies, namely cartilage regeneration.

References:

^[1]Silva, S.S., A. Motta, M.R.T. Rodrigues, A.F.M. Pinheiro, M.E. Gomes, J.F. Mano, R.L. Reis, and C. Migliaresi. Biomacromolecules, 2008. **9**(10): p. 2764-2774.

^[2]Altman, G.H., F. Diaz, C. Jakuba, T. Calabro, R.L. Horan, J.S. Chen, H. Lu, J. Richmond, and D.L. Kaplan. Biomaterials, 2003. **24**: p. 401-416

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