IMPROVEMENT OF COLLAGEN HYDROGEL AS SCAFFOLD FOR TISSUE ENGINEERING APPLICATIONS BY FORMATION OF COLLAGEN-POLYMER HYBRID CONSTRUCTS IN PLASTIC COMPRESSION

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Plastic compression (PC) is a simple, yet effective method to improve the mechanical properties of hydrogel-based scaffolds [1]. In this approach, excess fluid present in hydrogels are removed by rapid expulsion to result in dense and mechanically robust structure. However, the scaffolds formulated via PC process are often still weak, especially when cell-seeded, due to extensive cell-mediated contraction. We propose that this can be improved by incorporating a rigid supporting substrate as a part of the scaffold, i.e. generating a bio-synthetic hybrid construct [2]. In this study, we have employed collagen as the major hydrogel scaffold and electrospun PLGA as a sheet between two layers of collagen hydrogels. Initial concentration of collagen hydrogels was very low, e.g. varied from 0.4 mg/mL to 1.6 mg/mL, but it substantially increased after PC, e.g. ca. from 90 to 320 mg/mL. TEM images have shown that the cross-sectional structure of hybrid scaffold is composed of two thin layers of collagen (ca. 5 μm) and a thicker central PLGA layer (ca. 80 μm). Magnified morphological views of the scaffold by SEM revealed very dense nanofibrillar constructs; as expected, decreasing the initial composition of collagen in hydrogel formula led to lower fibrous densities. The scaffolds were then seeded by 3T3 fibroblast cells, and were incubated for 1, 4, and 7 days in vitro for further SEM, metabolic activity (MTS assay), and staining (CLSM) studies. All the scaffolds supported cell adhesion and proliferation, but the scaffold with lower initial component of collagen showed more favorable morphological and cell activities. A similar trend was observed in CLSM images of DAPI-Phalloidin stained cells, where the highest proliferation was associated with the lowest collagen content based on cell nuclei counting. Lastly, nanomechanical properties of collagen sheets after PC characterized by AFM showed that the elasticity moduli of the scaffolds after PC were inversely proportional to the initial concentration of collagen. We propose that too high collagen concentration after compression tends to present unfavorably mechanical and biological properties by the loss of too much fluid within hydrogels. This study has shown that initially low collagen content in the formula of collagen hydrogel for PC could be rather more attractive not only for lower costs, but also for improved mechanical properties, cell-scaffold interaction, and higher metabolic cell activities.

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