Journal of Isfahan Medical School Original Article
Vol 30, No 188 2nd week, July 2012 Received: 10.1.2012 Accepted: 19.5.2012

Recognition of Cytokeratin 18 Marker by Flow Cytometry of Nucleus Pulposus Cells in Human Intervertebral Disc and Comparison of Proliferation and Morphology of these Cells in Chitosan-Gelatin and Alginate Scaffolds

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Abstract

Background: Low back pain is a major economical and social problem nowadays. Intervertebral disc herniation and central degeneration of disc are two major reasons of low back pain that occur because of structural impairment of discs. Intervertebral disc includes the annulus fibrosus, transitional region, and nucleus pulposus (NP). NP forms the central nucleus of the disc. Reduction of cell count and extracellular matrix, especially in NP, causes disc degeneration. Different scaffolds (natural and synthetic) have been used for tissue repairing and regeneration of intervertebral disc in tissue engineering. Most scaffolds have biodegradable and biocompatible characteristics and also prepare a fine condition for proliferation and migration of cells. Although no specific marker or method has been suggested for recognition of NP cells, some studies have used real time and immunocytochemical methods and reported high expression of cytokeratin 19, 18, 8, and others as markers for NP cells. This study aimed to recognize NP cells of human intervertebral disc by flow cytometry of cytokeratin 18 marker. It also compared the proliferation and morphology of these cells in chitosan-gelatin scaffold and alginate scaffold.

Methods: NP cells were derived by enzymatic hydrolysis of collagenase from NP tissue of patients undergoing open surgery for discectomy in Alzahra Hospital (Isfahan, Iran). Chitosan was blended with gelatin and glutaraldehyde was used for cross linking of the two polymers. Then, alginate scaffold was prepared. After approving the NP cells by flow cytometry of cytokeratin 18 marker, a cellular suspension with 4×10^5 cells was transferred to each scaffold and cultured for 21 days. Cell viability and proliferation were investigated by trypan blue and methyl thiazolyl tetrazolium (MTT) assay. A scanning electron microscope (SEM) was used to assert the porosity and to survey the structures of the scaffolds.

Findings: We can use flow cytometry of cytokeratin 18 markers for recognition of NP cells. MTT assay demonstrated that cell viability on the third day had significant difference with the first day in both scaffolds. There was also a significant reduction in cellular viability from day 3 to day 21. Results of cell count showed that mean difference between cell counts in alginate scaffold was significantly more than chitosan-gelatin scaffold ($P < 0.001$).

Conclusion: Flow cytometry of cytokeratin 18 can be used as a method for recognition of NP cells. Compared to chitosan-gelatin scaffold, alginate scaffold prepared a better condition for proliferation of NP cells. The results of this study suggested that alginate scaffold could be useful in in-vivo studies and treatment.

Keywords: Intervertebral disc, Tissue engineering, Degeneration, Scaffold, Chitosan, Gelatin, Alginate, Cytokeratin 18

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