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Monolayer Culture Expansion of Annulus Fibrosus Cells for Intervertebral Disc Regeneration

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ABSTRACT

Objectives/Research Problem: Tissue engineering (TE) provides an alternative approach in regenerating human tissues for clinical transplantation and drugs development purposes. In principle, TE requires good cell source, compatible biomaterial scaffold and proper signalling factors. Regeneration of intervertebral disc (IVD) structure may be achieved by establishing well-balanced between the above three TE principles. Annulus fibrosus (AF) is a potential cell source for fabrication of IVD-engineered tissue. Anatomically, AF surrounds the inner nucleus pulposus region and forms the outer layer of IVD. Being one of the important IVD structures, much attention has been given to regenerate AF. Expansion of AF cells in monolayer culture is a vital step towards cellular incorporation onto 3D biomaterial scaffold. The present study attempted to evaluate monolayer culture expansion of AF cells at different passages.

Materials and Method: The rabbit's AF cells were harvested aseptically according to the IIUM approval (IIUM/IACUCApproval/2015/[5][23]). The cells were cultured from passage 0 (P0) until passage 3 (P3). At each passage, evaluation was performed in terms of cells morphology, viability, growth kinetics, sulphated glycosaminoglycan content, DNA content, standard cytology staining and immunocytochemistry.

Results and Discussion: The AF cells appeared spindle-shaped and adopted more fibroblastic traits in culture. At passage 1 (P1), AF demonstrated higher growth rate, lesser cell doubling time and significant sulphated glycosaminoglycan content when compared to other passages. No significant differences were observed in DNA content for all passages. Standard cellular staining and immunocytochemistry findings confirm the presence of pericellular matrix and cartilage specific marker i.e. collagen type II respectively. Presence of collagen type I was also noted, indicative of AF's fibrocartilaginous nature.

Conclusion: Current findings suggest expansion of AF at P1 may become a potential cell source for certain application in tissue engineering. Future improvement may be made by adding growth factors to promote the proliferation of AF cells.

KEYWORDS: Annulus Fibrosus, Growth Kinetics, Sulphated Glycosaminoglycan, Immunocytochemistry, Intervertebral Disc Engineering

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