Collagen I and Collagen II Immunohistochemistry Analyses On *In Vitro* 3D Poly(Lactic-Co-Glycolic Acid) Seeded With Intervertebral Disc Cells With and Without Fibrin Scaffold

*Muhammad Azri Ifwat Mohamed Amin, Noor Faizatul Husna Shuib, Noorhidayah Md Nazir, Mohd Yusof Mohamad, Muhammad Aa'zamuddin Ahmad Radzi & Munirah Sha'ban**

Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia

ABSTRACT

Objectives/Research Problem: Successful formation of 3D tissue constructs requires appropriate combination of the three established tissue engineering principles. The principles include quality cell source, biocompatible material scaffold and suitable biological signalling factors. This preliminary study aims to evaluate in vitro tissue constructs engineered from poly(lactic-co-glycolic acid) (PLGA) seeded with intervertebral disc (IVD) cells namely annulus fibrosus (AF), nucleus pulposus (NP), and a combination of AF:NP (1:1) with and without fibrin using specific cartilaginous markers i.e. collagen I and II immunohistochemistry staining.

Materials and Method: Porous PLGA discs (7.0mm dia. X 3.0mm height) were fabricated using solvent casting and salt leaching method. The cells were harvested from rabbits' IVD, cultured and seeded onto the pre-fabricated PLGA-based scaffolds. The resulting six "cells-scaffolds" construct groups were cultured for 3-weeks. The immunohistochemistry procedure and microscopic observation were performed at week 1, 2 and 3.

Results and Discussion: Minimal cartilaginous tissue formation is noted in all constructs at week 1 until week 3. This can be appreciated by the presence of cartilage-isolated cells in lacunae embedded within extracellular matrix (ECM) ground substance. Cellular and ECM distribution are better in PLGA+Fibrin+AF:NP group than the other groups. Presence of brownish precipitation in most of the constructs after the immunolocalization of collagen I and II indicates positive results. It demonstrates that certain constructs have cartilaginous properties.

Conclusion: The combination of PLGA and fibrin has the potential in facilitating early chondrogenesis of in vitro constructs engineered from AF, NP, and the combination of AF:NP (1:1) cells. The minimal cartilaginous tissue formation may be due to inefficient cells seeding. While it is apparent that human factor is unavoidable, there is much evidence in biomedical research and other fields that certain methodology can be refined to prevent errors before one's experiment is compromised. In this case, the cells seeding method should be observed and improved for future IVD tissue engineering research.

KEYWORDS: Annulus Fibrosus, Nucleus Pulposus, Collagen II, Collagen I, Intervertebral Disc Tissue Engineering

*CORRESPONDENCE: munirahshaban@iium.edu.my