

Histology Staining On *In Vitro* 3D Poly (Lactic-Co-Glycolic Acid) Seeded With Annulus Fibrosus, Nucleus Pulposus, and a Combination of Annulus Fibrosus: Nucleus Pulposus (1:1) Cells With and Without Fibrin Scaffold

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ABSTRACT

Objectives/Research Problem: Poly(lactic-co-glycolic acid) (PLGA), an FDA-approved, synthetic copolymer has been widely applied in clinical settings as one of the suturing materials. It has been used as cartilage tissue engineering scaffolds because it is bioabsorbable and safe for human use. PLGA can be prepared alone or in combination with other biomaterials such as fibrin to enhance the surface-adhesion properties. Fibrin helps to hold the cells inside the scaffolds and thus minimizes cells lost. This condition facilitates homogeneous cells distribution and extracellular matrix (ECM) production. This study aimed to evaluate in vitro constructs engineered from PLGA seeded with intervertebral disc (IVD) cells namely annulus fibrosus (AF) cell, nucleus pulposus (NP) cell, and a combination of AF:NP (1:1) with and without fibrin using histology staining.

Materials and Method: All cell groups were cultured until passage 1 (P1) prior to seeding step onto the pre-fabricated PLGA scaffolds. Approx. 1.0×10^6 cells were used per scaffold. The resulted "cells-scaffolds" constructs were cultured for 3-weeks. The microscopic evaluation using H&E, Alcian Blue and Safranin O staining was performed on all constructs at weeks 1, 2 and 3.

Results and Discussion: The overall results suggested minimal formation of cartilaginous tissue at week 1 until week 3 in all groups. Formation of cartilaginous tissue is indicated by the presence of cartilage-isolated cells in lacunae spaces. PLGA+Fibrin seeded with AF:NP demonstrated better cellular and ECM distribution than the other PLGA based constructs. Glycosaminoglycan (GAG) accumulation is noted in most constructs using Alcian Blue staining. However, the presence of proteoglycan-rich matrix was not detected in most constructs using Safranin O staining. This may be due to the immature nature of the in vitro tissue constructs. They have yet to produce cartilage specific proteoglycan-rich matrix.

Conclusion: This preliminary study showed that PLGA+Fibrin has the potential to promote early formation of in vitro cartilaginous constructs engineered from the IVD cells. It is hoped that the findings provide a useful information for future research in IVD tissue engineering.

KEYWORDS: Annulus Fibrosus, Nucleus Pulposus, Intervertebral Disc Tissue Engineering, PLGA, Fibrin

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