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CARTILAGINOUS PROPERTIES OF NUCLEUS PULPOSUS CELLS SEEDED ON POLY(LACTIC-CO-GLYCOLIC ACID)-BASED SCAFFOLDS

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ABSTRACT

Tissue engineering and regenerative medicine has the potential of improving the quality of life for many people. One particular area that presents an exciting challenge is the regeneration of intervertebral discs (IVD). To achieve this, it is necessary to identify suitable cells, biomaterial scaffolds and biologically active signalling factors for *in vitro* and *in vivo* IVD generation. This present study aimed to evaluate the cartilaginous properties of nucleus pulposus (NP) cells seeded on poly(lactic-co-glycolic acid) (PLGA) with or without fibrin scaffolds by measuring sulphated glycosaminoglycan (sGAG) content and collagen type II (COL-II) gene expression. The analyses were performed at week 1, 2, and 3 *in vitro*. The sGAG content was higher in the PLGA/Fibrin than in the PLGA only group. The expression of COL-II was only detected at week-2 culture in PLGA/Fibrin group. This study indicates that PLGA-based scaffolds may have the potential for intervertebral discs tissue engineering.

1.0 Introduction

Degenerative discs disease (DDD) may be resulting from excess mechanical loading, smoking, and changes in cell nutrition (1). It is manifested by deterioration of biomechanical loading and changes of extracellular matrix (ECM) in the intervertebral discs (IVD). The available treatments such discectomy and spinal fusion may cause post-surgical morbidity to the patient (2), and thus explaining why tissue engineering field may serve as promising approach to treat DDD (3). Tissue engineering requires suitable cell source, biomaterial scaffold and signalling molecules for tissue regeneration (4). The IVD is located between two vertebral bodies and surrounded by ligaments and muscles (5). The IVD consists of the outer layer of annulus fibrosus that enclosed the inner region of NP layer. The NP layer contains high water content and proteoglycan. This is to maintain tissue hydration and biomechanical properties of the IVD. The use of PLGA and Fibrin was reported to facilitate cell proliferation, ECM production and provide homogenous cell distribution in the 3D environment (6). PLGA was also reported to be mechanically stable scaffold for tissue engineering (7), while fibrin enhances cell attachment ability to the scaffold. Therefore, the study aimed to evaluate the cartilaginous properties of NP cells seeded on PLGA with or without fibrin scaffolds.

2.0 Materials and Method

This research was approved by the IIUM Research and Ethical Committee. A total of 100,000 NP cells were seeded into each PLGA/Fibrin and PLGA only scaffolds. The sGAG

content (n=5) and COL-II gene expression (n=3) analyses were performed at each time point of 1, 2, 3 weeks. The sGAG content were normalized to the dried weight of each scaffold, respectively. Non-parametric statistical analysis was carried out on the sGAG content analysis and the differences were considered significant when $p<0.05$. The analysis for gene expression was carried out using the two-step reverse transcriptase polymerase chain reaction (RT-PCR).

3.0 Results

The sGAG production in PLGA/Fibrin group was higher than in the PLGA only. After 1, 2 and 3 weeks of culture, the relative sGAG content in PLGA/Fibrin group were 0.091380 (±0.0634)%, 0.1010 (±0.0199)% and 0.12160 (±0.0604)%, respectively and in PLGA group, the content were 0.08180 (±0.0206)%, 0.08760 (±0.0181)% and 0.1003 (±0.0657)%, respectively. Statistically, there was no significant increment of sGAG content in PLGA/Fibrin group (p=0.449), while the content in PLGA group had significantly increased after 3 weeks (p=0.022). COL-II marker was only observed in week 2 culture in PLGA/Fibrin group but, collagen type I (COL-I) was steadily expressed throughout the culture for both groups.

4.0 Discussions & Conclusions

Incorporation of cell with PLGA/Fibrin may have the potential to restore the structure and function of degenerated IVD. In this study, although PLGA/Fibrin scaffold produced higher sGAG content than PLGA, it was not statistically significant. The total sGAG content was increased with the decrement of dried weight of the construct. This indicates that PLGA/Fibrin may have the ability to absorb water more than PLGA. This condition corresponds the nature of native NP cells, thus helps to improve cell adhesion, proliferation and ECM production. Collagen type I was steadily expressed throughout the in vitro culture, whereas collagen type II marker was only observed in week 2 culture of PLGA/Fibrin. This may be because immature cartilage expressed collagen type I rather than collagen type II (8). In conclusion, PLGA/Fibrin scaffold shows some potential for tissue engineering application. It is hoped that further improvement and analysis can be carried out to gain more information on the cell-scaffold interactions before it can be used to treat DDD.

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