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CELL PROLIFERATION ASSAY AND SULPHATED GLYCOSAMINOGLYCAN PRODUCTION IN POLY(LACTIC-CO-GLYCOLIC ACID)-BASED SCAFFOLDS SEEDED WITH BONE MARROW MESENCHYMAL STEM CELLS FOR CARTILAGE TISSUE ENGINEERING

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ABSTRACT

Articular cartilage tissue is known to have poor capability of self-repair. Therefore, tissue engineering has significantly emerged as an alternative solution for articular cartilage restoration. Poly(lactic-co-glycolic acid) (PLGA) and fibrin have been widely used as biocompatible scaffolds materials to regenerate tissue. In this study, bone marrow mesenchymal stem cells (BMMSCs) were seeded onto the PLGA with (PLGA/Fibrin) or without fibrin (PLGA only). Macroscopic observation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and sulphated glycosaminoglycan (sGAG) assays were performed at week 1, 2 and 3 *in vitro*. Both PLGA/Fibrin and PLGA only groups showed similar hyaline-like appearance with white and smooth glistening surface. The MTT assay showed higher cell proliferation in the PLGA/Fibrin than the PLGA only group. The production of sGAG is higher in the PLGA/Fibrin group than in the PLGA only. This may suggest that PLGA/Fibrin may serve as prospective cell delivery vehicle and essential foundation for *in vitro* tissue engineered articular cartilage.

1.0 Introduction

Cartilage is an avascular tissue with only one cell type; the chondrocytes that have a limited self-repair capacity. Current available treatments for osteochondral defect include microfracture surgery, autologous chondrocyte implantation and osteochondral transplantation. The outcomes are, however, unsatisfactory and cartilage restoration remains a challenge in orthopaedics (1). Tissue engineering and regenerative medicine offers an alternative solution for tissue regeneration. Previous studies indicated that PLGA/Fibrin scaffold may be suitable for cartilage tissue engineering (2, 3, 4, 5). The combination between fibrin and PLGA scaffolds were able to facilitate cells growth, the production of matrix and early chondrogenesis of rabbit articular chondrocytes in *in vitro* (3) and *in vivo* (2). Fibrin is believed to be a good cell transporter. It provides homogenous cells distribution, minimal cell lost during seeding technique and promotes bone formation in PLGA (6). In terms of cell sourcing, BMMSCs is an interesting candidate as an alternative to chondrocytes. Abundant supply and ability to differentiate into numerous cell and tissue lineages make them as suitable cells source for tissue engineering (7). Therefore, we hypothesized that PLGA and fibrin would be an ideal BMMSCs carrier and enhances the in vitro cell proliferation and sGAG production.

2.0 Materials and Method

The research was approved by the IIUM Research and Ethical Committee. Approximately 100,000 BMMSCs suspended with or without fibrin were seeded onto PLGA. All constructs were cultured and evaluated for cell proliferation activity and the sGAG content (n=6) at each time point of 1, 2 and 3 weeks. Statistical analysis were carried out and the differences were considered significant when $p<0.05$.

3.0 Results

Macroscopic observation: At week 3, the PLGA/Fibrin construct exhibited slightly smaller size, firmer, smoother with glistening surface than the PLGA group.

MTT Assay: Cell proliferation was gradually increased until day-14 and declined by day-21 in both groups. PLGA/Fibrin showed a significant higher cells proliferation ($p < 0.05$) than PLGA at day-7, day-14 and day-21 with the p-value, 0.007, 0.005 and 0.012 respectively.

sGAG assay: The relative sGAG content increased from week 1 onwards with the significant highest sGAG production of 0.1% in PLGA/Fibrin at week 3. At week 1 and 2 the relative sGAG content in PLGA/Fibrin was 0.07% and 0.08% respectively, which was slightly higher than PLGA construct. However, no significant differences found between these two groups.

4.0 Discussions & Conclusions

With regards to new cartilaginous formation, the establishment of cell to cell communication and cell to matrix interaction is an indication of an early stage of chondrogenesis. Cell proliferation activity was indeed increased from day-1 to day-14 of culture but subsequently declined by day-21 in both groups. The reduction in cell proliferation activity might be due to less or no active cellular function towards the end of day-21 as previously described (3). Normalized by the dried-weight of each sample, it was found that the sGAG content in PLGA/Fibrin scaffold increased and inversely proportionate to the dried weight of the scaffold as reported elsewhere (2,3,4,5). Hence showing that PLGA/Fibrin scaffolds may resemble the nature of normal cartilage that exhibit stress shielding component due to the high water content. This study recommends that PLGA/Fibrin scaffolds seeded with BMMSCs may serve as a structural basis for tissue engineered cartilage.

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