

Poly(lactic-co-glycolic acid), atelocollagen and fibrin hybrid scaffold seeded with annulus fibrosus cells enhances the formation of cartilaginous tissue engineered construct in vitro

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Introduction: A systematic analysis of the Global Burden of Diseases Study 2013 indicated the top 10 leading causes of YLDs included low back pain (LBP) [1]. Degeneration of intervertebral disc (IVD) is one of the common causes leading to LBP. Most available treatments act as temporary measures to lessen the pain but not treating LBP. One of the potential alternative modalities to treat IVD injuries is through the reconstruction of IVD-like tissue using tissue engineering technique. Anatomically, the IVD comprises centrally located nucleus pulposus (NP) and outer annulus fibrosus (AF) layer. Disruption of AF layer can cause IVD degeneration. The degeneration usually progresses and becomes more severe with age. In this study, the poly(lactic-co-glycolic acid) (PLGA) was chosen as the scaffold base due to its good mechanical properties and biocompatibility towards human tissues. The incorporation of atelocollagen and fibrin has been reported to facilitate and enhance cell proliferation, ECM production and cartilaginous tissue formation in vitro [2]. The cells from the AF layer was used because the AF is easy to access and culture in vitro. The AF cells are mainly fibrochondrocytes and may be trained towards chondrogenic lineage [3]. The objective of this study was to evaluate the in vitro formation of 3D tissue engineered constructs (TECs) using rabbits' annulus fibrosus (AF) cells seeded on PLGA based scaffolds.

Materials and Method: The AF layer was aseptically dissected from the IVD (L1-L5) of the New Zealand White rabbits (n=3). The cells were seeded onto 6 well-plates with the initial seeding of 5,000 cells/cm² at primary passage (Po). All cells were subcultured into passage 1 and trypsinized when they reach 80-90% confluency. The PLGA scaffolds were fabricated using solvent casting and salt leaching method. The scaffolds were then incorporated with atelocollagen (Koken, Japan) and/or freshly prepared fibrin accordingly. The AF cells were seeded onto PLGA only scaffolds (AFP), PLGA with atelocollagen scaffolds (AFPA), PLGA with fibrin scaffolds (AFPF) and PLGA with atelocollagen and fibrin scaffolds (AFPAF) at density of 1 x 10⁵ per scaffold. The resulting "AF-scaffolds" TECs were cultured for three weeks in an incubator with 5% CO₂ and 95% humidified atmosphere. The TECs were evaluated by comparing the groups in terms of the cellular morphology and attachment using scanning electron microscopy (SEM) (Jeol, Japan), cells viability using a modified MTT cells proliferation assay on day 4, 7, 14 and 21, cartilaginous sulphated Glycosaminoglycan (sGAG) matrix production using K-Assay® sGAG assay kit and DNA content using PicoGreen® DNA quantification assay at each time point of week 1, 2 and 3.

Results: Grossly, the TECs with fibrin maintained the initial size and exhibited smooth, whitish as well as glass-like appearance similar to that normal cartilage. While the TECs without fibrin increased in size and exhibited sponge-like appearance. In terms of SEM, cellular attachment and proliferation are indicated by the presence of densely packed cellular structure on the surface of the constructs. Most cells maintained its oval and/or fibroblastic shape with some filopodial-like processes indicative of proliferating cells. In MTT assay, all TECs exhibited increment in cells proliferation from day 4 to 21 indicative of favourable cells viability. Cumulative number of viable cells yielded from all TECs thru the assay from the highest to lowest can be summarized as AFPAF>AFPA>AFPF>AFP. For sGAG production, AFPF has the highest cumulative sGAG content but shows no statistical difference when compared to the other TECs. The level of sGAG content from the highest to lowest can be summarized as AFPF>AFPA>AFPAF>AFP. All groups showed comparable trend of DNA content. Although there are no significant differences between the groups but AFPAF has the highest cumulative DNA content when compared to the other TECs. The level of DNA content from the highest to lowest can be summarized as AFPAF>AFPA>AFPF>AFP.

Discussion and Conclusion: The cellular attachment and proliferation in constructs suggested the biocompatibility of the biomaterials used in this study. The oval shape of cells serves as the manifestation of fibroblastic phenotype [4]. Positive cell viability throughout the three-week for all TECs groups suggested cells proliferation activity. The data suggested that atelocollagen and fibrin provide proper environment for the cell attachment to grow and proliferate. This natural biomaterial also reported to contribute to have better biocompatibility in maintaining viability of cells in constructs. Other than that, the incorporation of atelocollagen with other biomaterial can prevent 'flowing out' of cells from the constructs and help in solving the leakage problem of cell-based therapies. The incorporation of atelocollagen and fibrin, the construct can maintain the ability of producing type II collagen and proteoglycan which necessary to retain its chondrocyte phenotype and ECM production. A lower sGAG content in AFPAF than AFPF and AFPA perhaps is because of actively proliferating cells. This study shows

that PLGA, atelocollagen and fibrin hybrid scaffold seeded with AF cells promotes the formation of tissue engineered IVD construct in terms of cellular morphology and attachment, cell viability, sGAG production and DNA content. It is hoped that this work can shed some lights and serves as a step towards realizing the reconstruction IVD-like tissue in vitro. The results may facilitate for further studies in this area towards a better LBP management in the future.

Acknowledgements

IIUM, MOHE for FRGS13-016-0257 and MOSTI for SF14-012-0062.

Keywords: DNA, Fibrin, in vitro, PLGA, Annulus fibrosus, Atelocollagen, sGAG, MTT cell proliferation assay

Conference: 6th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting (6th MTERMS) 2016 and 2nd Malaysian Stem Cell Meeting, Seberang Jaya, Penang, Malaysia, 17 Nov - 18 Nov, 2016.

Presentation Type: Poster **Topic:** Biomaterials and Tissue Regeneration

Citation: Mohamed Amin MI, Mohamad MY, Zulkifly AH and Shaban M (2016). Poly(lactic-co-glycolic acid), atelocollagen and fibrin hybrid scaffold seeded with annulus fibrosus cells enhances the formation of cartilaginous tissue engineered construct in vitro. *Front. Bioeng. Biotechnol. Conference Abstract: 6th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting (6th MTERMS) 2016 and 2nd Malaysian Stem Cell Meeting*. doi: 10.3389/conf.FBIOE.2016.02.00023

Received: 08 Dec 2016; **Published Online:** 19 Dec 2016.

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