EVENT ABSTRACT

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In vitro and in vivo gene expression studies of cartilage-like tissue engineered construct using a combination of transiently transfected human osteoarthritic chondrocytes and tissue engineering technique

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Introduction: Osteoarthritis is a state of degenerative changes which is common in the elderly. There is no known cure for this degenerative joint disease at the moment. The management of cartilage injuries and osteoarthritis remain a challenge in orthopaedic. Numerous attempts have been made to improve patients' clinical outcomes ranging from medical to surgical approaches [1]. The results range from excellent to unsatisfactory to a certain extent, but there is always room for improvement. Apart from those conventional treatments, research into tissue engineering is actively conducted to overcome cartilage anomalies. Reconstruction of functional cartilage-like tissue using tissue engineering principles in combination with gene transfer method have been studied [2]. These two methods have been advancing rapidly with the aim to regenerate and/or restore the normal tissue structure and function. Within experimental settings, it is noted that the chondrocyte loses its phenotype in monolayer cell culture model [3]. Cellular or replicative senescence takes place after serial passages. Cultured chondrocytes tend to adopt more fibroblastic traits than its initial chondrogenic properties. This phenomenon makes it difficult to reconstruct or engineer quality cartilage-like tissue in vitro. Cell source, biomaterial scaffold and signalling factors are important elements in tissue engineering. Transfecting the transcriptional factor, SOX9 gene into chondrocytes has been suggested as one potential option to sustain or enhance monolayer cultured chondrocytes [4]. The use of biocompatible 3D scaffold in culture and in vivo implantation model are necessary to validate the effectiveness of the approach. This study aimed to evaluate the formation of cartilage-like TECs using human osteoarthritic chondrocytes overexpressed or transiently transfected with SOX9 gene seeded onto PLGA with and without fibrin scaffolds by means of cartilaginous genes expression analysis. Basic research is always necessary to uncover the full potential of new cartilage treatment strategies. Materials and Methods: This study was approved by the IIUM Research Ethical Committee (IREC18); Ministry of Health (MOH) Malaysia (NMRR-12-1383-14531) and Institutional Animal Care and Use Committee (IIUM/IACUCApproval/2015/[5]/[22]). Six cartilage samples were obtained from consented patients after joint replacement surgery at the Hospital Tengku Ampuan Afzan, Kuantan, Pahang. The cells were isolated, cultured and transfected with pcDNA3-SOX9 using Lipofectamine 2000™ (Invitrogen, Life Technologies, USA). Prefabricated disc-shaped porous PLGA (50:50) (Evonik, Boehringer Ingelheim, Germany) with and without fibrin were used as scaffolds. The 'cells-scaffolds' TECs groups are "PFTC", "PFC", "PTC" and "PC" (P=PLGA, F=fibrin, TC=transfected chondrocytes, C=non-transfected chondrocytes). The TECs were cultured for 3-week and implanted subcutaneously at the dorsum of athymic mice for 4-week. Collagen I, II, IX, X, XI, SOX9 and aggrecan expression were evaluated using a qualitative two-step reverse-transcriptase PCR (Invitrogen, Life Technologies, USA). The housekeeping GAPDH and β-actin genes were used as internal controls. All primers were prepared by Integrated DNA Technologies (1st BASE, Singapore). All PCR product was separated using 1.5% agarose gel electrophoresis and stained with ethidium bromide. The DNA fragments were visualized by UV transillumination using gel documentation Alpha Imager HP System (Alpha Innotech, USA). Results: The presence of cartilaginous markers can be detected in all TECs with various expression intensity. Regardless of cells treatments and scaffolds types i.e. "PFTC", "PFC", "PTC" and "PC"; Collagen II, the cartilage-specific marker was down-regulated in vitro but re-expressed in vivo unlike Collagen X, the cartilage hypertrophic marker. Collagen I, SOX9 and aggrecan were steadily expressed in all TECs while collagen IX and XI are almost untraceable except in few samples. Presence of GAPDH and β-actin genes indicated the reliability of the analysis. Discussion and Conclusion: Tissue regeneration can be appreciated as a translation of embryonic development and morphogenesis. These processes are complex and yet to be fully understood especially when it involves molecular level discourses. Articular cartilage perhaps one of the most mysterious tissue in the human body. Cartilage development is influenced by transcriptional factor, SOX9. SOX9 helps regulate collagen II, the cardinal marker for cartilage-specific phenotype [7]. Although collagen IX is hypothetically associated with collagen II, in this study the expression is almost undetectable except for few samples unlike Collagen XI. The expression of other cartilaginous markers showed little if not much variation between the in vitro and in vivo constructs. However, implantation model facilitates the TECs' cartilaginous phenotype expression [4]. Collagen X expression seems lessen after implantation. Unlike the orthotopic implantation model, ectopic implantation in mice provides only minimal yet non-actual microenvironment for TECs maturation. Although minimal, the presence of natural in vivo mechanical cues seems better than the

static environment in in vitro culture. While motions are important for skeletal tissues formation, proper signalling molecule could directly influence cartilage-specific genes regulation. All in all, regardless of SOX9 transfection and 3D scaffold utilization, this study indicates that cartilage-like TECs have been successfully formed based on comparable genes expression analysis between all groups. An in-depth understanding of cartilaginous genes interaction is an important prerequisite for cartilage tissue engineering. Further study is required to deliberate this aspect accordingly.

Acknowledgements

IIUM, MOHE for MIRGS13-01-002-0003 and MOSTI for SF14-012-0062.

Keywords: Cartilage, Tissue Engineering, in vivo, in vitro, gene transfer, PLGA, SOX9

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: Shaban M, Shukri NM and Zulkifly AH (2016). In vitro and in vivo gene expression studies of cartilage-like tissue engineered construct using a combination of transiently transfected human osteoarthritic chondrocytes and tissue engineering technique. *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.2016.02.00010

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

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