EVENT ABSTRACT

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Effects of SRY (Sex Determining Region Y)-Box 9 (SOX9) and Telomerase Reverse Transcriptase (TERT) genes transfection in chondrocytes seeded on three-dimensional scaffolds: Gross observation and cell proliferation assay

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Introduction: The use of genes for treating diseases has been researched for decades. The therapeutic gene delivers by means of gene transfer approach, acts as a biological repair agent in the targeted cell. The potential of gene therapy in human is still controversial. Numerous clinical trials on gene transfer have been studied. It is noted that the positive outcomes seem to outweigh the risks of the procedure. Some people opine that the actual potential of gene transfer is yet to be uncovered and thus research into this aspect should go on. In cartilage tissue engineering related studies, SOX9 [1], transforming growth factor beta (TGF-β) [2] and insulin-like growth factor-I (IGF-I) [3] are among the targeted genes use to enhance cartilage regeneration. In this present study, SOX9 and TERT genes were used as signalling factors and transfected into cultured chondrocytes. SOX9 is well-known as master transcriptional factor for chondrogenesis and important for cartilage development. While TERT gene controls the elongation of telomere length at the end of chromosome during cell proliferation and essential for maintaining cell's life-span. These signals seem necessary to promote cells quality for cartilage tissue engineering. This preliminary study utilises tissue engineering triad namely cell, scaffold and signalling factors. The authors evaluate the formation of in vitro 3D tissue constructs engineered from the SOX9 and TERT genes post-transfected chondrocytes seeded on PLGA based scaffolds using gross observation and cells proliferation activity. Materials and Methods: Institutional research approval was obtained (IIUM/IACUC/Approval 2015/[5]/[24]). The harvested rabbits' chondrocytes (n=2) were expanded in monolayer culture with an initial seeding of 5000 cells/cm2. The chondrocytes were transfected with SOX9 and/or TERT genes via lipofection method (Lipofectamine® 3000, Invitrogen, Carlsbad, CA, USA). An approx. 100,000 cells were seeded on the prefabricated PLGA (Sigma-Aldrich Co., St. Louis, MO, USA) with and without fibrin scaffolds. The resulting constructs were cultured for three-week. The following groups: (1) non-transfected chondrocytes (control), (2) SOX9-transfected chondrocytes, (3) TERT-transfected chondrocytes and (4) SOX9/TERT-transfected chondrocytes; each seeded on PLGA and PLGA/fibrin scaffolds were examined. This experiment setting allows comparison between eight "cells-scaffold" construct groups. All groups were evaluated for gross morphology and cells proliferation activity using (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) at each time point of 1-, 2-, and 3-week. Results: All eight "cells-scaffold" construct groups demonstrated reduction in size as the incubation time increased (Fig. 1). Only PLGA/fibrin constructs had a glassy, hyaline-like cartilage appearance throughout the in vitro culture, regardless of cells treatment. While both transfected and non-transfected chondrocytes seeded on PLGA/fibrin showed an increasing growth pattern in culture, the chondrocytes seeded on PLGA alone showed a decreasing growth pattern from day-1 to -21 (Fig. 2). The SOX9/TERT-transfected chondrocytes seeded on PLGA/fibrin exhibited a growth pattern comparable to that of control group; the non-transfected chondrocytes seeded on PLGA/fibrin. Discussions and Conclusion: In this study, the ability of cells to proliferate in the scaffolds can be a direct indication of the cells viability status. Transfection or gene transfer method is often associated with cells toxicity. Despite the harmful effect, this study showed the transfected chondrocytes have comparable growth pattern with the non-transfected chondrocytes particularly when seeded onto PLGA/fibrin scaffold. Unlike SOX9 or TERT single gene transfection, SOX9/TERT co-transfection may have synergistic effect in maintaining cell proliferation activity in the PLGA/fibrin scaffolds. The prefabricated PLGA in this study acts as a temporary 3D scaffold for cellular attachment and growth. While the present viable cells in the scaffold secrete cartilaginous extracellular matrix, the PLGA will gradually degrade over time, leaving the newly formed natural tissue intact. Introduction of the 3D structure plays an important role to mimic the internal microenvironment of the human body [4]. It is noted that the cellular growth pattern seems better in PLGA/fibrin than PLGA alone as indicated by previous findings [5]. The presence of fibrin seems to enhance surface properties of the PLGA and facilitate cells proliferation [5, 6]. Moreover, stable macroscopic structure shown by PLGA/fibrin constructs can be one of the deciding factors for future in vivo implantation [5]. The glassy, cartilage-like appearance of PLGA/fibrin constructs is comparable with the previous finding [6]. Other essential

assessments involving construct's weight measurement, histology analysis, genes expression, biochemical assessments and in vivo implantation are currently underway. Additional number of samples is required to complete the data collection. With support from the ongoing encouraging outcomes, it is hoped that the combination of SOX9 and TERT genes transfection and 3D tissue engineering scaffolds can facilitate the formation of good quality cartilage in vitro and in vivo.

Acknowledgements

IIUM and MOSTI for SF14-012-0062.

Keywords: Chondrocytes, Tissue Engineering, gene transfer, TERT, 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: Oral **Topic:** Gene Therapy, Reprogramming and Pluripotency

Citation: Nazir NM, Zulkifly AH, Khalid KA, Zainol I, Zamli Z and Shaban M (2016). Effects of SRY (Sex Determining Region Y)-Box 9 (SOX9) and Telomerase Reverse Transcriptase (TERT) genes transfection in chondrocytes seeded on three-dimensional scaffolds: Gross observation and cell proliferation assay. *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.2016.02.00027

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

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