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CARTILAGINOUS MARKERS EXPRESSION IN HUMAN ARTICULAR CHONDROCYTES OVEREXPRESSED WITH SOX9 GENE

Norhamiza Mohamad Sukri[1], Nurul Syamimi Mat Jusoh[1], Hanisah Mohd Mohadis[1], Noorhidayah Md Nazir[1], Rozlin Abdul Rahman[1], Muhammad Aa'zamuddin Ahmad Radzi[1], Ahmad Hafiz Zulkifly[2], Abbdurezak Abdullahi Hashi, Mohd Yusof Mohamad[1], Munirah Sha'ban[1,*]

[1]Department of Biomedical Science, Kulliyyah of Allied Health Sciences and [2]Department of Orthopaedics, Traumatology & Rehabilitation, Kulliyyah of Medicine, International Islamic University Malaysia Kuantan Campus, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, MALAYSIA

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*Corresponding author email:
munirahshaban@iium.edu.my
/munirahshaban@gmail.com

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ABSTRACT

SOX9 is an essential transcription factor for chondrocyte differentiation and cartilage formation. The limited regenerative capacity of cartilage has led to the development of various treatment modality including gene transfer approaches. In this study, we transiently overexpressed or transfected *SOX9* gene in articular chondrocytes derived from consented patients after joint surgery. The aim was to achieve optimum transfection effect by optimizing the lipofection procedure using various DNA concentrations. Chondrocytes were overexpressed with 2.0µg, 4.0µg and 6.0µg of pcDNA3-*SOX9*. The transfection effect on chondrocytes was assessed through the expression of collagen type II, aggrecan, *SOX9* and collagen type I using the two-step reverse-transcriptase polymerase chain reaction (RT-PCR). Results showed that at low DNA concentration, the *SOX9* transfection up-regulates all cartilage-specific markers expression, but down-regulates collagen type I. Hence, optimum transfection effect can be achieved using low DNA concentration. The ability to sustain the cartilage-specific markers indicates potential implications in cartilage engineering.

1.0 Introduction

Tissue engineering has given opportunity to regenerate cartilage. Nowadays, cartilage tissue engineering can be merged with gene transfer approaches such as transient delivery of *SOX9* gene using lipofection procedure (1). Optimization of this non-viral gene delivery procedure is necessary to identify optimum condition for efficient transfection in cell culture. Therefore, this study aims to optimize lipofection conditions for the delivery of *SOX9* gene into human articular chondrocytes using various concentration of DNA.

2.0 Materials and Methods

With the approvals from the IIUM Research Ethics Committee (IREC), and National Medical Research Register (NMRR), human articular cartilage samples were obtained from consented patients after joint surgery. Chondrocytes were cultured and assigned to five different groups; positive control (C+), negative control (C-), 2 µg (D1), 4 µg (D2) and 6 µg (D3) of pcDNA3-*SOX9* expression vector. Freshly digested cells were used as normal control. Cells at passage 1 (P1) were transfected with Lipofectamine 2000 (Invitrogen) according to manufacturer's protocol. Transfected chondrocytes were expanded until passage 3 (P3). Expression of collagen type I, collagen type II, aggrecan, and *SOX9* from each passage were evaluated by the two-step RT-PCR. The housekeeping genes, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were used as internal controls.

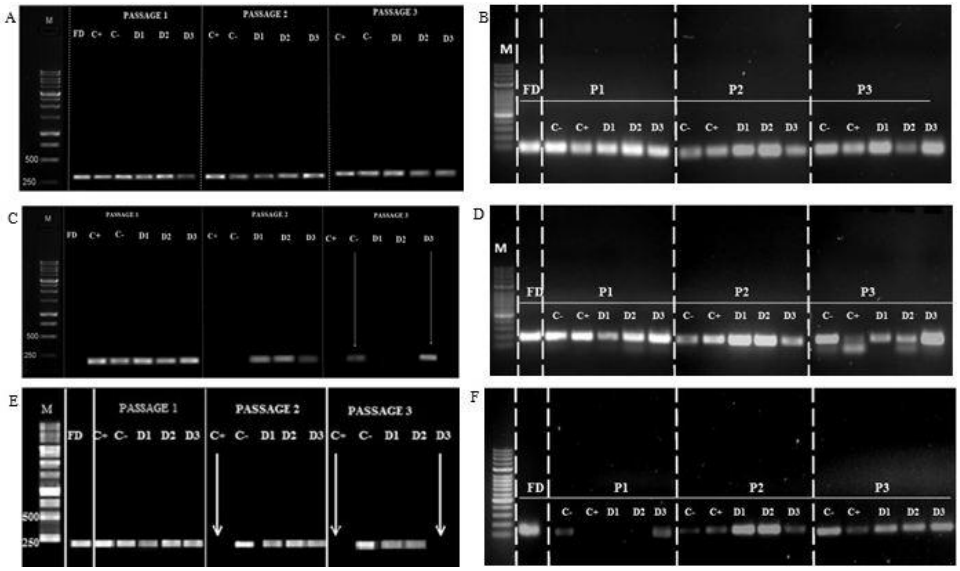

Fig. 1 Gel electrophoresis results following the two-step RT-PCR analysis of the mRNA expression of (A) β-actin, (B) GAPDH, (C) Collagen type I, (D) Aggrecan, (E) Collagen type II, (F) *SOX9* for fresh digested sample, non-transfected chondrocytes, and transfected chondrocytes. **Note**: M (A,C,E) =1kb (1st Base DNA ladder), M (B,D,F) = 100bp DNA Ladder (GeneDirex), [FD] = fresh digested samples [C+] = control positive i.e. cells transfected with no DNA, [C-] = control negative i.e. non-transfected cells, [D1] = cells transfected with DNA concentration 1 (2 µg), [D2] = cells transfected with DNA concentration 2 (4 µg), [D3]= cells transfected with DNA concentration 3 (6µg).

2.0 Results

Collagen type II expression was detectable in almost all groups throughout passages, with the most intense band showed in D2. Collagen type I was not expressed in D1 and D2 for P3 and was only expressed in D3. The increased band intensity for aggrecan in the transfected cells from P1 to P2 demonstrated that 2 µg and 4 µg of DNA may be good enough for optimum transfection effect. Results are shown in Figure 1.

4.0 Discussion and Conclusion

Collagen type II and aggrecan expression in transfected cells with low DNA concentrations demonstrate efficient transfection and confirm the master role of *SOX9* in chondrogenesis [1,2]. In this study, *SOX9* overexpression suppressed collagen type I expression. It is a good indication since collagen type I often associated with dedifferentiation [3] and cartilage degeneration [4]. It can be concluded that optimum transfection efficiency can be achieved with low DNA concentrations and may have potential implications in cartilage tissue engineering.

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