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Differentiation of stem cells derived from human infrapatellar fat pad for cartilage engineering: Characterization of cells undergoing chondrogenesis

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Background: Hyaline cartilage repair is a challenge in orthopedics. Current techniques result in suboptimal long-term outcomes due to inability of cartilage to regenerate. Human infrapatellar fat pad (hIPFP)-derived mesenchymal stem cells (MSC) can differentiate into multiple tissue lineages, including cartilage and bone but the molecular mechanisms regulating *in situ* chondrogenesis are yet to be completely defined. The aim of this study was to investigate chondrogenesis in MSC-derived from hIPFP tissue and to assess temporal changes in gene expression using microarray analysis.

Methods: Human IPFP-derived MSC were maintained in chondrogenic medium supplemented with TGFβ3 and BMP6 for 7, 14 and 28 days. Endpoints included histology, immunohistochemistry (IHC), quantitative real-time PCR (qPCR). Gene expression profiling was performed using an illumina-based microarray technique to study temporal changes in expression of a broad range of genes during chondrogenesis.

Results: Over 28 days, clusters of chondrocytes formed that were predominantly surrounded by collagen and aggrecan in the extracellular matrix (ECM). Production of collagen type II and aggrecan was confirmed using IHC. Between days 14 and 28, collagen type 2A1 (COL2A1) and aggrecan gene expression increased substantially. SRY-related transcription factor (SOX9) gene expression was also increased. Normalized microarray data highlighted 608 differentially expressed genes during the time course. Ten chondrogenic genes were up-regulated (2- to 87-fold) through to days 14 and 28; this included COL2A1, COL10A1, COL9A1, COL11A1, COL9A2, COL11A2, COL1A1, COMP, SOX9 and COL3A1. We found that up-regulated genes (2-fold or more) show significant expression (Enrichment score) of ECM structural constituents at days 7, 14 and 28 during chondrogenesis.

Conclusion: Therefore, we have successfully demonstrated *in vitro* production of hyaline-like cartilage from IPFP-derived MSC in micromass culture. Microarray has provided novel information concerning genes involved in chondrogenesis of hIPFP-derived MSC. Our approach offers a potentially useful strategy for generating clinically relevant cartilage for therapeutic use.

Keywords: Hyaline cartilage; mesenchymal stem cells (MSC); human infrapatellar fat pad (hIPFP) tissue; chondrogenesis; micromass culture; microarray, extracellular matrix (ECM).

Biography

Raed Felimban is a Ph.D. candidate in the department of orthopaedics at St Vincent's Hospital, University of Melbourne in Australia. He received Master Degree in Laboratory Medicine in 2010 from the Royal Melbourne Institute of Technology (RMIT) University, Melbourne, Australia. From 2003 to 2008, he was with King Abdul-Aziz Medical City Hospital (JCI, CAP, and AABB accredited) Jeddah, Saudi Arabia, as a medical Technologist (Hematology-Flowcytometry). Since 2009, he has been Teacher Assistant at King Abdul-Aziz University. He is a student member of The Australasian Society for Stem Cell Research (ASSCR). His major current research interest is in adult mesenchymal stem cells (MSCs) tissue engineering.

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