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# Matrix Production in Chondrocytes Transfected with Sex Determining Region Y-Box 9 and Telomerase Reverse Transcriptase Genes: An *In Vitro* Evaluation from Monolayer Culture to Three-Dimensional Culture

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monolayer to three-dimensional (3D) culture.

## Methods:

The genes were transferred into chondrocytes at passage-1 (P1) via lipofection. The post-transfected chondrocytes (*SOX9-*, *TERT-* and *SOX9|TERT*) were analysed at P1, P2 and P3. The non-transfected group was used as control. The 3D culture was established using the chondrocytes seeded in a disc-shaped PLGA/fibrin and PLGA scaffolds. The resulting 3D "cells-scaffolds" constructs were analysed at week-1, -2 and -3. The histoarchitecture was evaluated using haematoxylin and eosin, alcian blue and safranin o stains. The quantitative sulphated glycosaminoglycan (sGAG) content was measured using biochemical assay. The cartilage-specific markers expression were analysed via real-time polymerase chain reaction.

## Results:

All monolayer cultured chondrocytes showed flattened, fibroblast-like appearance throughout passages. Proteoglycan and sGAG were not detected at the pericellular matrix region of the chondrocytes. The sGAG content assay indicated the matrix production depletion in the culture. The cartilage-specific markers, *COL2A1* and *ACAN*, were downregulated. However, the dedifferentiation marker, *COL1A1* was upregulated. In 3D "cells-scaffolds" constructs, regardless of transfection groups, chondrocytes seeded in PLGA/fibrin showed a more uniform distribution and produced denser matrix than the PLGA group especially at week-3. Both sGAG and proteoglycan were clearly visualised in the constructs, supported by the increment of sGAG content, quantitatively. Both *COL2A1* and *ACAN* were upregulated in *SOX9|TERT*-PLGA and *SOX9|TERT*-PLGA/fibrin respectively. While, *COL1A1* was downregulated in *SOX9|TERT*-PLGA.

## Conclusion:

These findings indicated that the *SOX9|TERT*-transfected chondrocytes incorporation into 3D scaffolds facilitates the cartilage regeneration which is viable structurally and functionally.

**Fig. 3**

**Fig. 4**

**Fig. 5**

**Fig. 6**

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## Ethics declarations

### Conflict of interest

The authors have declared that there is no conflict of interest.

### Ethical statement

Animal ethical approval was granted by the Institutional Animal Care and Use Committee of International Islamic University Malaysia (IACUC-IIUM) (Reference No. IIUM/IACUC/Approval 2015/[5]/[24]).

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## Keywords


- Cartilage
- Chondrocytes
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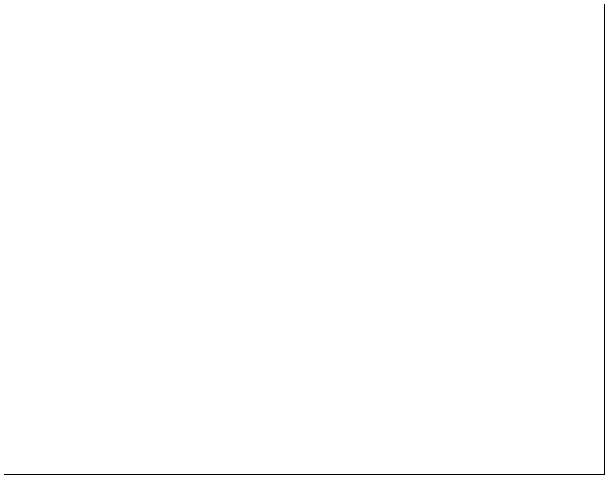

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