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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 (Article)

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Abstract

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Background:: This study aimed to observe the cartilaginous matrix production in SRY (sex determining region Y)-box 9 (SOX9)- and/or telomerase reverse transcriptase (TERT)-transfected chondrocytes from 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. © 2019, The Korean Tissue Engineering and Regenerative Medicine Society.

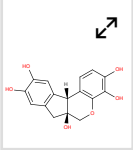
SciVal Topic Prominence

Topic: Telomerase | Telomere | Human telomerase

Prominence percentile: 95.698

Chemistry database information

Substances



Author keywords

Cartilage | Chondrocytes | Glycosaminoglycan | Proteoglycan | Tissue engineering

Indexed keywords

Engineering controlled terms: Cartilage | Cell culture | Genes | Molecular biology | Monolayers | Polymerase chain reaction | Scaffolds (biology) | Tissue engineering

Engineering uncontrolled terms: Cartilage regeneration | Chondrocytes | Glycosaminoglycans | Proteoglycans | Real time polymerase chain reactions | Telomerase reverse transcriptase | Three-dimensional culture | Threedimensional (3-d)

Engineering main heading: Body fluids

EMTREE drug terms: alcian blue | biological marker | eosin | fibrin | glycosaminoglycan polysulfate | hematoxylin | molecular scaffold | polyglactin

EMTREE medical terms: ACAN gene | animal cell | animal experiment | animal tissue | Article | biochemical analysis | cartilage matrix | cell culture technique | chondrocyte | COL1A1 gene | COL2A1 gene | controlled study | down regulation | gene | gene expression | genetic marker | genetic transfection | in vitro study | monolayer culture | New Zealand White (rabbit) | nonhuman | priority journal | quantitative analysis | real time polymerase chain reaction | SOX9 gene | TERT gene | three dimensional culture | upregulation

Chemicals and CAS Registry Numbers:

alcian blue, 12040-44-7; eosin, 17372-87-1, 51395-88-1, 548-26-5; fibrin, 9001-31-4; glycosaminoglycan polysulfate, 63449-40-1; hematoxylin, 517-28-2; polyglactin, 26780-50-7, 34346-01-5

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