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The ideal timing of sirna plasmid transfection on primer chondrocyte cultures

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Abstract

Fibrocartilaginous repair tissue is found to be formed after the repair process of cartilage defects even after chondrocyte transplantation. The type I collagen compound of fibrocartilage can not fulfil the mechanical properties of hyaline cartilage which consists of mostly type 2 collagen. Gene therapy during the chondrocyte culturation stage may work on leading the cells to form a hyaline cartilage in-vivo. The ideal timing of the transfection in post transcriptional gene silencing of cultured chondrocytes must be displayed first. The standard chondrocyte cultures were preperared with this purpose. The cells were seeded in 6 main groups and also each main groups were taken in to the study in 3 sub-groups. Invert microscopic evaluation was performed after the passage of the cells on 0, 24th, 48th, and 72th hours. Non-targeting siRNA (p.GFP) ve GFP si RNA transfections were achieved. The efficiency of the transfection was determined with RNA isolation on protein acquisition, on 0, 48th and 72th hours. Expression of mRNA was evaluated with Bradford and semi-dry Western Blot analysis using anti-GFP and anti GAPDH anti-bodies. The ideal time, which resulted in better results, was reported by evaluating the expression on electrophoretic bands. One way ANOVA and Newman-Keuls multiple comparison tests were performed in the stastistical analysis of the comparison of the tranfection efficiency between each group. The discrepancies between groups were determined with Student's T Test. The most efficient silencing was obtained on 48th hour ($P<0.05$); The GFP expression decresed to 11.4% in post-transcriptional gene silencing performed group, while it was 59.7% in none silenced group, on 48th hour. The efficient gene silencing was achieved in 77% of the cells.

Biography

Nevzat Selim Gokay has completed his M.D. degree at the age of 24 years from Istanbul University Cerrahpasa School of Medicine. He has gained the title of Consultant Orthopaedic Surgeon at 2005 from Istanbul University Cerrahpasa School of Medicine. He has been working as an Assistant Professor in Namik Kemal University Department of Orthopaedics and Traumatology since 2008.