Acetylcholine Improved Osteoblasts Migration and Maturation

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INTRODUCTION: Improper restoration of the tendon-to-bone interface (TBI) can lead to inflammation and secondary tears in patients recovering from rotator cuff repair surgery. Implantable scaffolds have aimed to improve the regenerative capacity of the TBI by introducing bioactive compounds and micro-architecture that is conducive to tendon adhesion [1]. Recent studies in murine models have shown that local supplementation of acetylcholine (ACh) to the TBI can significantly enhance the early stage of bone-tendon insertion healing by reducing inflammation [2]. However, the effect of ACh on osteoblast cells migration and differentiation remains largely unknown. This study aims to elucidate the effect of ACh on osteoblasts proliferation, migration, and bone formation.

METHODS: Human-like osteoblasts (Saos-2) were cultured in Opti-MEM growth media (10% FBS, 1% P/S) until confluent. Cells were then transferred to 24-well plates, with each well containing a starting concentration of 3.10^4 osteoblasts. After an incubation period of 3 days, the media from each well was collected and replaced with ACh-containing media (with concentrations ranging from 10^{-3} to 10^{-9} M). Osteoblast activity and differentiation was analyzed over the following 14 days using AlamarBlue, ROS staining, alkaline phosphatase activity, and gene expression for osteocalcin and Runx-2. Osteoblast migration after Ach treatment was analyzed using the scratch test at 0, 4, 8, and 10 hours. Statistical analyses were performed with Prism 7 (GraphPad Software Inc). Statistical differences between groups were determined by a Student's t-test. Significance was accepted at $p < 0.05$.

RESULTS: Saos2 cells treated with Ach at concentrations ranging from 10^{-8} and 10^{-4} M resulted in an increase of cellular proliferation. At the highest dose of 10^{-3} M, Saos2 proliferation started to decrease after 5 days of culture, due to cytotoxicity of ACH at this concentration. Ach had a positive impact on osteoblasts migration, as early as 4 hours post-treatment. The greatest overall effect was observed at concentrations of 10^{-5} M.

DISCUSSION: We have determined the positive effect of Ach supplementation on human osteoblasts proliferation, migration and bone nodules formation.

CONCLUSION/CLINICAL SIGNIFICANCE: Ach can be incorporated in scaffolds for rotator cuff repair applications. Ach will enhance cell migration, proliferation and differentiation which will enhance the repair of the tendon-to-bone interface.

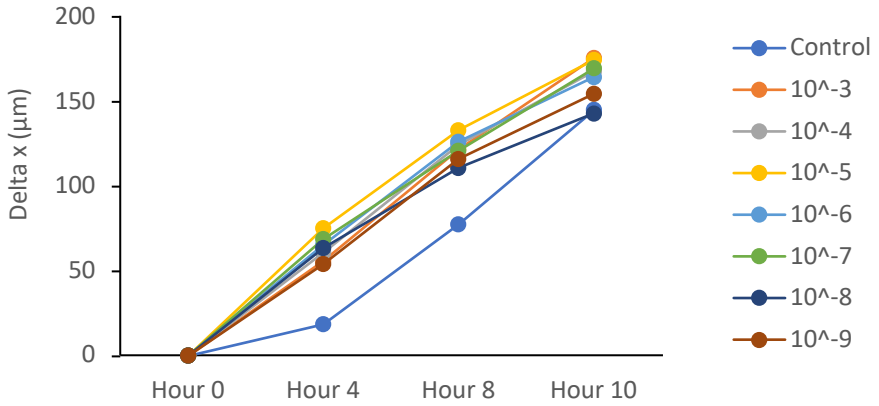

Figure: Saos2 migration after treatment with increasing concentrations of acetylcholine.

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