

R-12. The Effects of Chitosan on the Human Periodontal Ligament Fibroblasts *in vitro*

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I. Introduction

Periodontal therapy has dealt primarily with attempts at arresting progression of disease, however, more recent techniques have focused on regenerating the periodontal ligament having the capacity to regenerate the periodontium. The effect of chitosan (poly-N-acetyl glucosaminoglycan), a carbohydrate biopolymer extracted from chitin, on periodontal ligament regeneration is of particular interest.

The purpose of this study was to evaluate the effect of chitosan on the human periodontal ligament fibroblasts (hPDLFs) *in vitro*, with special focus on their proliferative properties, the synthesis of collagen and the activity of alkaline phosphatase (ALP).

II. Materials and Methods

1. Chitosan preparation

Chitosan solution (0.01, 0.1, 1, 2 mg/ml) was made by dissolving chitosan powder (Chitosan 100, Hanwha Co., Seoul, Korea) in phosphate-buffered saline (PBS).

2. Isolation and culture of hPDLFs

Fibroblast populations were obtained from individuals with a healthy periodontium.

3. Cytotoxic test

The cytotoxicity of chitosan on hPDLFs was evaluated using MTT assay.

4. Collagen synthesis test

Collagen synthesis was evaluated using reverse transcription-polymerase chain reaction (RT-PCR) of type I collagen.

5. ALP activity test

ALP activity was evaluated using ALP-10[®] kit.

6. Statistical analysis

Statistical differences were determined using a Kruskal-Wallis test and Mann-Whitney test.

III. Results

1. Chitosan-induced proliferative responses of hPDLFs reached a plateau at the concentration of 0.1 mg/ml ($p < 0.05$).
2. When hPDLFs were stimulated with 0.1 mg/ml chitosan, mRNA expression of type I collagen was up-regulated.
3. When hPDLFs were stimulated with 0.1 mg/ml chitosan, ALP activity was significantly up-regulated ($p < 0.05$).

IV. Discussion

Chitosan (0.1 mg/ml) enhanced the collagen synthesis in the early stage, and afterwards, facilitated differentiation into osteogenic cells.

V. Conclusion

The results of this *in vitro* experiment suggest that chitosan potentiates the differentiation of osteoprogenitor cells and may facilitate the formation of bone.