II-4. Effects of Chitosan on Human Periodontal Ligament Fibroblasts In Vitro and on Bone Formation in Rat Calvarial Defects

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Background

The purpose of this study was to evaluate the effect of chitosan on human periodontal ligament fibroblasts(hPDLF) in vitro and on bone formation in rat calvarial defects in vivo.

Methods

Fibroblast populations were obtained from individuals with a healthy periodontium and cultured in α minimum essential medium(MEM) for the control group. For the experimental groups, cells were cultured in α-MEM containing chitosan at concentrations of 0.01, 0.1, 1, or 2 mg/ml. The 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide(MTT) assay, reverse transcription-polymerase chain reaction(RT-PCR) and the assay of alkaline phosphatase(ALPase) activity were performed.

Eight mm calvarial critical-sized defects were created in 30 male Sprague-Dawley rats. The animals were divided into three groups of 10 animals each. The defects were treated with either chitosan/absorbable collagen sponge(ACS) or ACS alone in the experimental groups or were left untreated(surgical controls). The animals were sacrificed at 2 or 8 weeks post-surgery and the treatment outcomes were evaluated using histological and histomorphometric parameters.

Results

The chitosan-induced proliferative responses of the hPDLF reached a plateau at a concentration of 0.1 mg/ml(P <0.05). When the hPDLF were stimulated with 0.1 mg/ml chitosan, both the mRNA expression of type I collagen and the ALP activity were significantly up-regulated(P <0.05). The surgical implantation of chitosan/ACS

enhanced the new bone formation at 8 weeks post-surgery and the amount of new bone formation of the chitosan/ACS group was significantly greater than that of both the ACS alone group and the surgical control group(P <0.01).

The new bone area and defect closure in the chitosan/ACS group were significantly greater than those in the ACS control and sham surgery control groups at 8 weeks(P <0.01). However, the chitosan/ACS group exhibited significantly less bone density than both the ACS control and the sham surgery control group at 8 weeks(P <0.01).

Conclusions

Chitosan(0.1 mg/ml) enhanced the type I collagen synthesis and facilitated the differentiation into osteogenic cells. Chitosan reconstituted with ACS has a significant potential to accelerate the regeneration of bone in rat calvarial critical size defects.

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