Enhanced Osteoblast Proliferation by Bioactive Glass Particles is Associated With Modulation of the Immediate-Early Gene c-Jun

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Introduction: Bioactive glass promotes repair of periodontal and skeletal defects in dogs [1,2]. Consil® Bioglass® (Nutramax Laboratories, Inc.; Edgewood, MD) is a particulate synthetic bone graft ceramic (90-710 µm) composed of elements found naturally in bone [3]. Consil® particles stimulate bone formation due to formation of a calcium-phosphate layer mimicking hydroxyapaptite found in bone mineral. The Consil® surface serves as a scaffold onto which new bone can regenerate. The mechanism by which bioactive glass particles transduce cellular signals to enhance osteoblast proliferation is unclear. Previous studies suggest that cell proliferation and differentiation may be attributed to specific signal transduction pathways. In the present study, we hypothesized that Consil® mediates osteoblast proliferation by transducing signals through the MAPK gene pathway.

Methods: Human osteoblasts (NHOsts), MG-63 cells, and canine osteoblasts from trabecular bone ($1x10^5$ cells/ml) were incubated with (a) control media alone or (b) Consil® Bioglass® particles (500 µg/ml). Cellular RNA was analyzed by RT-PCR for phenotype and the immediate-early gene c-Jun. GAPDH was used as the housekeeping gene. The Quant-iT™ DNA kit assessed total cellular DNA. Secreted osteocalcin was measured by EIA. FITC immunofluorescence was used to visualize osteoblast immunostaining for type I collagen. Secreted type I collagen was analyzed by EIA and Western blot using a polyclonal goat anti-type I antibody. Data was analyzed using SigmaStat with multiple comparisons by one-way analysis of variance (ANOVA, Tukey post-hoc) at $p<0.05$ level of significance.

Results/Discussion: Cells incubated with Consil® remained 100% viable and exhibited the osteoblast morphology when viewed with phase-contrast microscopy. Enhancement of osteoblast proliferation after 7 days of culture with Consil® was indicated by an increase in total DNA compared to cells cultured with media alone ($p<0.001$ for all cell types) [Figure 1, A-C]. The osteoblast phenotype was confirmed by RT-PCR analysis. Following exposure to Consil®, cells continued expression and production of osteocalcin, alkaline phosphatase, and type I collagen at the gene level. Osteocalcin levels secreted from Consil® incubated cells remained similar to controls (95-100% of controls). Western blot analysis confirmed the molecular weight of secreted type I collagen while immunostaining confirmed similar levels of secreted type I collagen between Consil® cultured and control cells (90-100% of controls).

RT-PCR of the immediate-early gene c-Jun in NHOsts and MG-63 cells after 4 hrs of incubation with Consil® showed no detectable change in gene expression [Figure 2, A-B, respectively]. There was a profound drop in c-Jun expression for both control and Consil®-incubated cells at 24 hrs in NHOsts. However, there was a profound decrease in gene expression of c-Jun in only MG-63 cells incubated with Consil® but not in controls. Levels of c-Jun expression recovered by day 7.

Figure 1. Total DNA levels in (A) canine osteoblasts, (B) NHOsts, and (C) MG-63 cells.

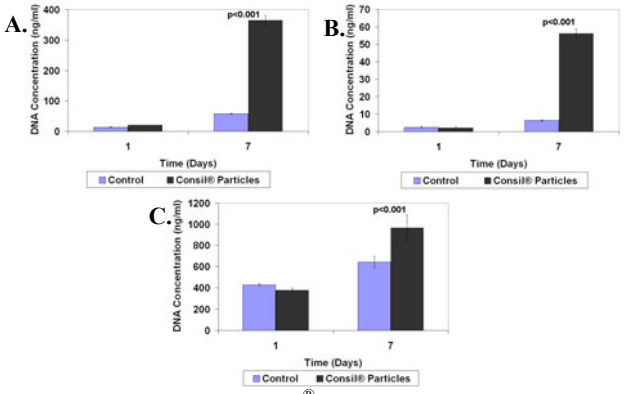
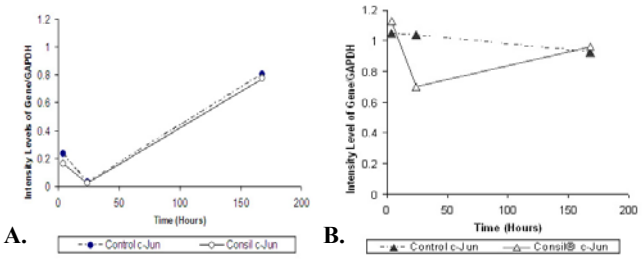


Figure 2. Effect of Consil® on c-Jun expression in (A) NHOsts and (B) MG-63 cells.



Conclusions: The present study demonstrates that Consil® particles significantly enhance osteoblast proliferation while maintaining the production of extracellular matrix components. The induction of proliferation is associated with time-dependent changes in expression of specific signal transduction genes known to modulate proliferation. Our observation indicates that bioactive glass particles may transmit cues which stimulate cell division critical to bone healing and repair.

References:

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