

## Enhancement of Osteoblast Proliferation by Bioactive Glass is Accompanied by Selective Modulation of c-Jun, MAPK, and Integrin Gene Expression

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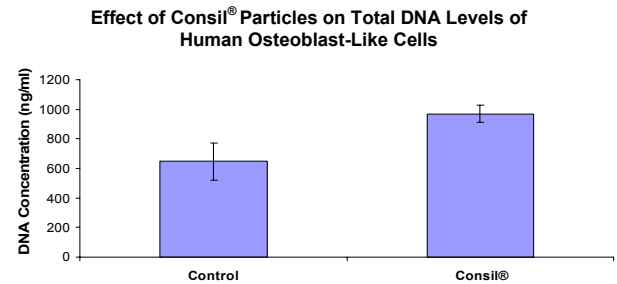
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**Introduction:** Bioactive glass has been used to treat periodontal and skeletal osseous defects. A modified form of bioactive glass (Consil<sup>®</sup> Bioglass<sup>®</sup>) has been shown to promote bone repair in animals.<sup>1</sup> Bioglass<sup>®</sup> is a synthetic bioactive bone graft ceramic in particulate form (90 to 170  $\mu\text{m}$ ) composed of silica ( $\text{SiO}_2$ ), calcium and sodium salts ( $\text{CaO}$  and  $\text{Na}_2\text{O}$ ), and phosphates ( $\text{P}_2\text{O}_5$ ). These components are naturally found in bone.<sup>2</sup> Implantation of bioactive glass particles results in a surface modification that stimulates bone formation. A calcium phosphate layer develops, allowing particles to bond directly to bone and connective tissues. The layer mimics hydroxyapatite (HA) found in bone mineral and serves as a scaffold onto which new bone can grow and regenerate. How bioactive glass particles transduce signals to enhance osteoblast proliferation is still unclear. Previous studies suggested the role of integrins, the immediate-early gene c-Jun, and the mitogen activated protein kinases (MAPKs) in regulating cell proliferation and differentiation. We hypothesized that signals transmitted through this gene pathway are involved in the growth promoting effect of Consil<sup>®</sup> Bioglass<sup>®</sup> particles.

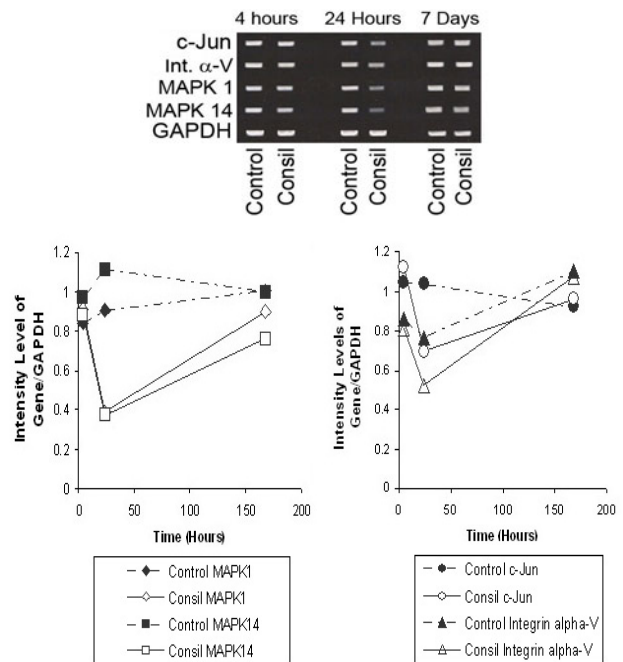
**Methods:** Human osteoblast-like MG-63 cells ( $1 \times 10^5$  cells/ml) were incubated with control media alone or Consil<sup>®</sup> Bioglass<sup>®</sup> particles (500  $\mu\text{g}/\text{ml}$ ). At various timepoints, cellular RNA and DNA were isolated with the TRIzol<sup>®</sup> (Life Technologies<sup>™</sup>) reagent method. The RNA was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) to assess c-Jun, MAPK, and integrin expression. Electrophoretograms of cDNA bands were scanned using GAPDH as the housekeeping gene. Total DNA levels were determined using the Quanti-iT<sup>™</sup> DNA kit (Molecular Probes). The data was analyzed using the SigmaStat program with multiple comparisons by one-way analysis of variance (ANOVA, Tukey post-hoc test). Differences were considered statistically significant when  $p < 0.05$ .

**Results / Discussion:** Osteoblast-like cells incubated with Consil<sup>®</sup> particles remained viable and exhibited osteoblast morphology when viewed by phase-contrast microscopy. Osteoblast proliferation was enhanced after 7 days in culture with Consil<sup>®</sup> particles, as indicated by the significantly higher DNA content compared to control levels ( $p < 0.001$ ). There was no detectable change in the expression levels of integrin  $\alpha$ -V, MAPK 1 and 14, and the immediate-early gene c-Jun four hours following incubation with Consil<sup>®</sup> particles. After 24 hours of incubation with Consil<sup>®</sup> particles, transcript levels of these genes decreased more profoundly compared to untreated controls. The gene levels returned to control levels in the presence of Consil<sup>®</sup> particles by day 7. In

contrast, integrins  $\alpha$ -2,  $\alpha$ -3, and  $\beta$ -1 expression did not change.



**Effect of Consil<sup>®</sup> Particles on Signal Transduction Gene Expression in Human Osteoblast-like Cells**



**Conclusions:** Our results indicate that Consil<sup>®</sup> particles induce time-dependent changes in expression of certain signal transduction genes associated with cell proliferation. This observation suggests that bioactive glass particles may provide cues that enhance cell division leading to bone tissue regeneration.

### References:

- Deforge DH. Evaluation of Bioglass/PerioGlas (Consil) synthetic bone graft particulate in the dog and cat. *J Vet Dent.* 1997 Dec;14(4):141-5.
- Bendall SP, et al. Effect of bioactive glass particle size on osseous regeneration of cancellous defects. *J Biomed Mater Res.* 1998 Sep 15;41(4):527-33.

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