

## SOL-GEL BIOGLASS STIMULATE OSTEOGENESIS IN MOUSE PRIMARY CELL CULTURE

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**INTRODUCTION:** During the last decade, studies have been directed to find a substitute that can replace not only the structure of bone but also its functional capacities, in other terms to promote repair and regeneration by the host tissue itself. Bioactive glasses developed in the early 1970s<sup>1</sup>, are of particular interest due to their unique property of being osteoconductive and osteopductive at the same time, thus they can induce the differentiation of osteoblasts and stimulate the formation of bone tissue both in vivo and in vitro<sup>2,3</sup>. This bioactivity is related to their capacity of forming a direct chemical bond with the host tissue via an intervening layer of carbonated hydroxyapatite which is chemically and structurally similar to the mineral of bone. Although their osteogenic capacity is widely documented and their use in various clinical cases of bone loss have shown successful results, yet the mechanism behind their bioactivity remains largely unknown, and the cellular responses to these glasses have not yet been clearly elucidated. The aim of the present study was to evaluate at a molecular level the effects of bioactive glasses on the differentiation of osteoblastic cells, by studying the effect on the expression of major markers of the osteoblast phenotype.

**METHODS:** *Biomaterial tested:* The 58S bioglasses had a composition (in weight %): 60% SiO<sub>2</sub>, 36% CaO and 6% P<sub>2</sub>O<sub>5</sub>. 60S bioinert granules were used as control in our experiments.

*Culture model:* osteoblasts were enzymatically isolated from calvaria of 18 day old fetal swiss mice, as previously described by Nefussi et al.<sup>4</sup>. Briefly, calvaria were aseptically dissected and fragments incubated in phosphate buffered solution with 0.25% collagenase for 2 hr at 37°C. Then cells were dissociated from the bone fragments, and plated at 4x 10<sup>4</sup> cell/cm<sup>2</sup>. The glass granules were added on day 1 of culture. All cultures were incubated in DMEM supplemented with 10% fetal calf serum, β-glycerophosphate, and ascorbic acid, in a humidified atmosphere of 5% in air at 37°C.

Total RNA isolation and Northern Blot: total RNA was extracted at day 6, 12, 18, using a phenol/chloroform method (Tri reagent<sup>®</sup>). The concentration and purity of RNA were determined

by light absorbance at 260nm and by calculating A<sub>260</sub>/A<sub>280</sub> ratio respectively. The integrity was confirmed by electrophoresis on an agarose ethidium bromide gel. Twenty micrograms of total RNA from each sample was denatured, and loaded on a 10% formaldehyde gel for electrophoretic separation. Total RNA on the gel was transferred onto Hybond+ nylon membranes with 20X SSC solution for 24 hr, and fixed at 80°C for 2 hr. The hybridization was performed at 42°C using <sup>32</sup>P-dCTP labelled probes. Hybridized membranes were exposed to Biomax films and the mRNA profiles analyzed with visiomic program.

**RESULTS:** Phase contrast microscopy showed that 2 hours later, cells started to attach and spread on culture dishes. On day 3 to 4 of culture, the cells were confluent and began to form multicellular layers. Furthermore, the cells showed morphological changes one of which was the appearance of cellular condensations, these were the areas in which future bone nodule formation were to be seen. The first zones of mineralization were noticed around the granules especially in the cultures with the bioactive granules, at more advanced stages, true mineralized bone nodules were formed in both cultures.

The expression of characteristic osteoblastic markers was determined at day 6, 12, and 18 using Northern blot techniques. The mouse osteoblasts in both cultures, expressed the major phenotypic markers namely Cbfa1, PAL, BSP, Osteopontin and Osteocalcin. All the markers were detected at day 6 of cultures, while Cbfa1 decreased with time and the rest of markers increased or remained at the same level at advanced stages of the culture. An interesting finding was that the levels of mRNA of all these markers especially osteocalcin, was higher at all times, in cultures of sol-gel glasses when compared to control.

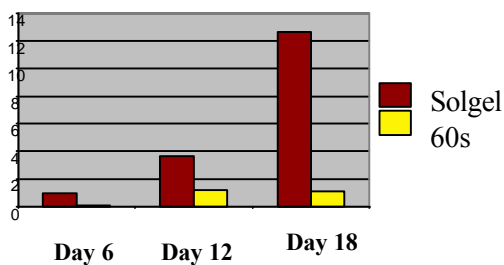


Fig. 1: Effect of sol gel bioglasses on the expression of osteocalcin, in primary mouse cultures

**DISCUSSION & CONCLUSIONS:** A line of evidence suggests that bioactive glasses can promote osteogenesis *in vitro*<sup>5</sup> and *in vivo*, however little is known on the cellular and molecular mechanisms underlying this effect. Our findings demonstrated that the cells maintained their phenotype in cultures of sol gel glasses, this was outlined by the expression of all phenotypic markers in a similar temporal pattern when compared to control. Furthermore the fact that the major osteoblastic markers were enhanced in cultures with bioactive glasses, indicates that these glasses has the capacity to stimulate the growth and osteogenic differentiation, by upregulating the major osteoblastic markers and the key transcription factor of bone (Cbfal).

**REFERENCES:** <sup>1</sup>L.L. Hench, R.J. Slinter RJ, W.C. Allen et al (1971) *J Biomed mater Res Symp* 2: 117-141. <sup>2</sup>L.L. Hench and J.K.West (1996) *Life Chemistry Reports* 13: 187-241. <sup>3</sup>H. Oonishi, S. Kushitani, E Yasukawa et al (1997) *Clin Orthop Rel Res* 334: 316-325. <sup>4</sup>J.R. Nefussi, Boy-M.L. Lefevre et al (1985) *Differentiation* 29: 160-168. <sup>5</sup>I.D. Xynos, M.V. Huckkanen, J.J. Batten et al (2000) *Calcif Tissue Int* 67: 321-329.

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