

Biocompatibility evaluation of four different BioglassTM-HA using primary culture of osteoblasts

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INTRODUCTION: Hydroxiapatite (HA)-based materials are considered to be potentially useful as bone implant materials, particularly those reinforced with glass to improve mechanical strength (1). However, the effects of glass-reinforced HA on bone cells depends on the material composition and need further investigation. In the present study we examined the response of rat primary culture osteoblast to HA, reinforced with 5% and 10% of BioglassTM and sinterized at 1200°C and 1300°C.

METHODS: Primary cultures of osteoblasts were prepared from sequential enzymatic digestion of calvarie obtained from 1-5 days old Wistar rats. Culture medium containing each of the four different BG-HA powders was put in contact with osteoblasts that were plated in 5×10^4 cell density at passage 2. After 72 h of incubation cell viability and proliferation was evaluated by MTT assay. The alkaline phosphatase production was measured by NBT-BCIP assay and secreted collagen by SIRCOL assay. The apoptosis index was investigated by nuclei staining with propidium iodide (2).

RESULTS: The viability and proliferation of osteoblasts cultured in the presence of reinforced HA were similar to control. Alkaline phosphatase production decreased around 5% in the presence of all BGTM-HA, when compared to control. On the other hand, collagen secretion enhanced approximately 20% above control in all conditions (Fig 1). A slight increase in apoptotic osteoblasts was observed when cells were in the presence of the biomaterials (Table 1).

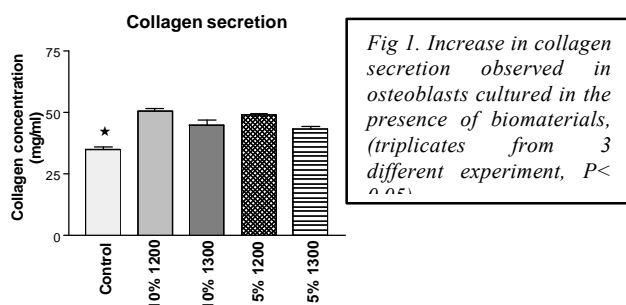


Fig 1. Increase in collagen secretion observed in osteoblasts cultured in the presence of biomaterials, (triplicates from 3 different experiment, $P < 0.05$)

BG TM -HA	Apoptotic cells (%)
10% 1200	18 %
5 % 1200	19,5 %
10% 1300	19 %
5% 1300	20%
CONTROLE	16%

Table 1. Apoptosis. After staining osteoblasts with propidium iodide, apoptotic cells were counted as well as the total number of the cells in the field. Comparing to control, a slight increase was found on the proportion of apoptotic cells, when in the presence of BGTM-HA. (Results reflect counting six fields in three different experiments.)

DISCUSSION AND CONCLUSION: Bioactive glasses and calcium phosphates have a recognized good material-tissue interaction (3). Our studies of viability and proliferation corroborated this statement. The observed increase in collagen secretion may be due to the presence of BioglassTM contents, since we already demonstrated that bioactive glass enhance the collagen production (4). Previous studies showed that ceramics particle induces macrophage apoptosis (2) and that 1,5% more apoptotic osteoblasts were present in ceramic perimplant region compared to other regions *in vivo* (5). These findings are in accord with our results of a slight increase in the percentage of apoptosis. Together our data indicate that BGTM-HA would be an interesting material for medical application, and substantiate its *in vitro* studies.

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