Survival and apoptosis *in vitro* of osteoblastic cells in contact with synthetic bone grafts

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Introduction: Over the past decade, various bioactive calcium phosphate bone grafting materials have been developed to enhance osteoblast differentiation and osteogenesis. The effect of these materials on bone tissue signaling pathways involved in adhesion and differentiation has been extensively studied. This has led to the hypothesis that these materials are capable of enhancing cell survival. However, these studies did not examine apoptosis and cell survival. The goal of the present study was to evaluate anti- and pro-apoptotic protein expression of osteoblasts in contact with various calcium-alkali-phosphate graft materials in comparison to that on various currently clinically used materials, β-tricalcium phosphate (β-TCP), bioactive glass (BG45S5) and a deproteinized bone xenograft.

Methods: Test materials included four resorbable calcium-alkali-phosphate-based bone substitute materials: (1) a material which has the crystalline phase $Ca_2KNa(PO_4)_2$ and also a small amorphous portion containing silica phosphate (GB9); (2) a material which has the crystalline phase $Ca_2KNa(PO_4)_2$ and a small amorphous portion containing magnesium potassium phosphate (GB14); (3) a material which has the crystalline phase $Ca_{10}[K/Na](PO_4)_7$ (352i) and (4) a material which is similar to GB9 but also contains diphosphates (GB9/25). Osteoblastic MC3T3-E1 cells were plated on the materials at a density of 4 x 10^5 per cm^2 and incubated for pre-determined durations. After the cells were lysed, the supernatant was collected and protein concentrations were determined. Equal amounts of protein extracts were separated in 10-12% SDS polyacrylamide gels and transferred to poly(vinylidene difluoride) (PVDF, Millipore) membranes. Blots were incubated with primary antibodies against Akt, phosphorylated Akt, and Bad (Santa Cruz) overnight at 4°C. This was followed by incubation with HRP-conjugated secondary antibodies for 1.5h. Bands were then detected using an enhanced chemiluminescence kit (Amersham) and were quantified using a Fuji LAS-1000 with ScienceLab v2.5 software (Fujifilm, Tokyo, Japan). For TUNEL assay, MC3T3-E1 cells were cultured on various test materials for 3 days and subsequent exposure to an apoptotic challenge overnight. Then, cell viability was detected by fluorescent microscopy.

Results/Discussion: As shown in Fig. 1, GB9 displayed the smallest number of apoptotic cells followed by GB14, deproteinized bone and BG 45S5. Higher numbers of apoptotic cells were observed on GB9/25, and significantly more apoptotic cells were present on 352i, β-TCP and tissue culture polystyrene (PS). After 3 and 24h, cell cultured on GB9 displayed a significantly higher phosphorylated Akt to total Akt ratio compared to cells grown on all other materials (Fig. 2). For the same duration of cell culture, significant down-regulation of expression levels of the pro-apoptotic factor, Bad, was noted for cells grown on GB9 and GB9/25, β-TCP and deproteinized bone (Fig. 2).

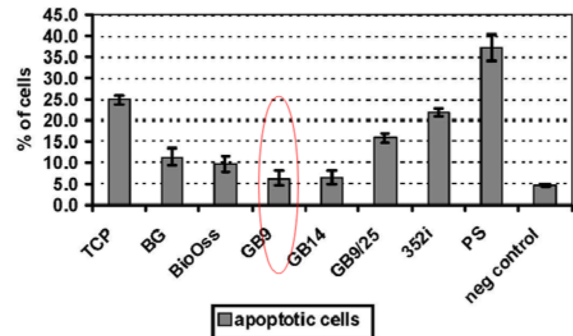

Figure 1. Percentage of apoptotic cells after culturing MC3T3-E1 cells on various test materials for 3 days and subsequent exposure to an apoptotic challenge overnight (TUNEL assays)

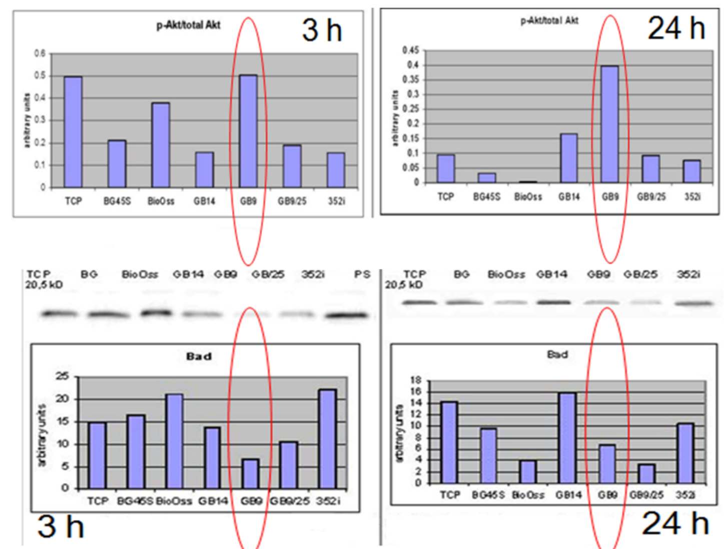

Figure 2. Phospho-Akt to total Akt protein expression ratio and Bad expression after 3 and 24h of cultivation of MC3T3-E1 cells on various test materials

Conclusion: The various bone substitute materials tested elicited a different effect on cell apoptosis. Cells cultured on GB9 displayed the greatest stimulation on the expression of anti-apoptotic factor and the greatest inhibition of the pro-apoptotic factor. These findings were in excellent agreement with the results of the TUNEL assay, which showed the smallest numbers of apoptotic cells on GB9.

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