In vitro intracellular signaling studies of calcium phosphate bone grafting materials predict in vivo tissue growth

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Introduction: Although autogenous bone grafts are currently the gold standard for bone reconstruction prior to dental implant placement in oral implantology, bone substitute materials are extensively studied in order to avoid harvesting autogenous bone. Compared to the bone substitutes, which are currently clinically available, there is a significant need for bone substitutes which degrade more rapidly. As a result, there has been an ongoing search for biodegradable bone substitute materials that degrade rapidly, but still stimulate osteogenesis at the same time, thereby resulting in complete substitution by newly formed functional bone tissue in view of placing dental implants in such augmented sites. This has led to the development of novel, bioactive, rapidly resorbable glassy crystalline calcium-alkali-orthophosphate materials. These materials have a higher solubility and biodegradability than β-tricalcium phosphate (TCP) and a deproteinized bovine bone material. Previously we were able to show that some of these calcium-alkali-orthophosphate materials had a stimulatory effect on osteoblast differentiation and osteogenesis *in vitro*[1-3] and *in vivo*.[3-5] This led to the hypothesis that these materials which stimulate osteogenesis are capable of upregulating intracellular signaling pathways, which enhance osteoblast differentiation and cell survival. This study evaluates the effect of two calcium-alkali-phosphate graft materials as compared to the currently clinically used materials β-tricalcium phosphate (β-TCP) and a bovine deproteinized bone xenograft (BioOSS) on the phosphorylation, i.e. activation of key signaling factors of the ERK differentiation pathway as well as of factors which downregulate apoptosis.

Methods: Test materials were two calcium-alkali-orthophosphates with a crystalline phase $Ca_2KNa(PO_4)_2$ and with a small amorphous portion containing either magnesium potassium phosphate (material denominated GB14) or silica phosphate (materials denominated GB9). These materials were compared to the currently clinically used materials β-tricalcium phosphate (β-TCP) and a bovine deproteinized bone xenograft (BioOSS). Specimens were prepared by compressing granules (grain size 40µm) followed by sintering to form 10-mm diameter discs, as described previously.[1, 2] Osteoblastic MC3T3-E1 cells were plated on the various test materials at a density of 4 x 10^5 per cm^2 and incubated for 90 min. Cells were then lysed, homogenized and centrifuged. 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 ERK1, phosphorylated ERK1/2, and phosphorylated JNK, phosphorylated p38, Bcl_2 and β-actin (Santa Cruz) overnight at 4 °C. This was followed by incubation with HRP-conjugated secondary antibodies for 1.5 h. Bands were then detected using an enhanced chemiluminescence kit (Amersham) and were quantified using a Fuji LAS-1000 with ScienceLab v 2.5 software (Fujifilm, Tokyo, Japan).

Results: After 90 min of incubation MC3T3-E1 cells displayed greatest phospho-Erk, phospho-jnk (Fig. 1), p38 and phosphorylated p38 levels when cultured on GB9 followed by cells grown on BioOss, GB14 and TCP.

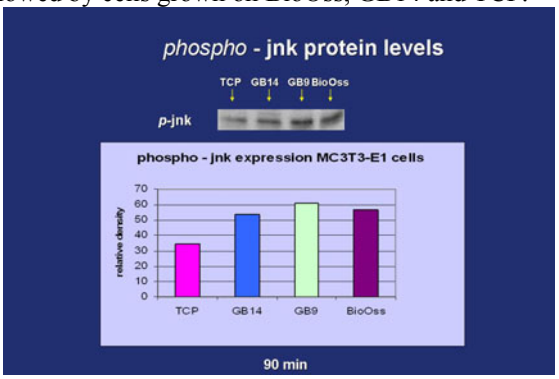

Figure 1: phospho-jnk protein levels after culturing MC3T3-E1 cells for 90 min on various bioceramics.

Moreover, greatest expression of BCl_2 protein was noted, when cells were cultured for 90 min on GB9. Osteoblasts grown on BioOss displayed the second highest BCl_2 levels. This was followed by BCl_2 expression on GB14. Considerably lower BCl_2 levels were expressed by cells grown on TCP.

Discussion / Conclusions: Of the various grafting materials studied, GB9 had the greatest stimulatory effect on the activation of key signaling factors of the ERK differentiation pathway as well as on expression of BCl_2 which downregulates apoptosis. Also BioOss exhibited a stimulatory effect on the activation of these signaling factors. These findings are in accordance with those of previous studies, in which GB9 showed a stimulatory effect on osteoblast differentiation *in vitro* and *in vivo*.[1-5] These findings also are in agreement with numerous studies which demonstrated the excellent osteoconductive properties of BioOss *in vivo*.

References:

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