In vitro effects of Mg/Sr–substituted hydroxyapatite on osteoblast activity

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Stoichiometric hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) has been used as a model for the apatite of human bone tissue for many years. Besides calcium, phosphate and carbonate, mineral bone contains many inorganic compounds such as sodium, fluoride, chloride, magnesium, strontium, zinc, copper and iron.

This work investigates in vitro effects of magnesium and strontium substitutions in the apatite lattice on the behaviour of human osteoblasts. We compared powders of Mg and Sr substituted apatites (MgSrHA) with those of stoichiometric HA. Powders were prepared as-synthesized and not sintered, which is the most critical condition in terms of reactivity towards cells.

Human osteoblasts were seeded in 24 well plates at the density of 15,000 cells/cm^2 in DMEM supplemented with 250 µM ascorbic acid and 10% FCS. Treatments with powders were applied 24 hours after seeding. Dose-response (range 0.1-10 mg/ml) curves of HA and MgSrHA powders were performed with MTT assay after 3 days of treatment. At 10 mg/ml, viability level of HA-treated osteoblasts was lower than that of MgSrHA-treated cells (respectively 35% and 50% of untreated cells). At 3 mg/ml viability values were comparable and proliferation was slightly hindered (80% of untreated). No sign of cell death, evaluated with calcein-propidium iodide assay on living cells, was detectable at any of the tested doses. MgSrHA and HA did not affect cell proliferation (evaluated by cell counting and cell protein content) after 3 or 7 days of treatment with 0.3 and 1 mg/ml. At these dosages, alkaline phosphatase (ALP) activity was not affected after 3 days, but was slightly lower than that of untreated cells at 0.3 mg/ml after 7 days. Calcium deposition was measured with red alizarin after 7, 14 and 21 days of treatment with 0.3 or 1 mg/ml of powders in the presence of 100 nM dexamethasone and 10 mM β-glicero phosphate. Calcium deposition increased in a time-dependent way and was highly stimulated in cells treated with both powders.

These preliminary results suggest that MgSrHA has no toxicity effects and seems to have a better biocompatibility than stoichiometric HA. Further investigations are needed to elucidate the cell-biomaterial interactions.

Key words
Bone regeneration, synthetic apatite, biomaterial, osteoblast