Influence of extracellular calcium concentration on the bone matrix synthesis and mineralization in cultures of human primary osteoprogenitor cells

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Introduction:
The proper ratio of organic and inorganic components within the bone matrix is an extremely important factor that both influences development and functionality of bone tissue. Focusing on the influence of extracellular calcium concentrations on the bone matrix formation and mineralization we were looking for suitable methods to better control the process of human bone tissue generation in vitro.

Materials and Methods:
For the experiments primary human osteoprogenitor cells were used which were derived either from a heterotopic ossification or from trabecular bone. Cells were grown in ZKT-I medium (in-house formulation*) containing 0.89 mmol/l Ca2+ or in ZKT-I modifications with twice and four times increased Ca2+ concentrations. All media were additionally supplemented with human serum, dexamethasone, ascorbate-2-phosphate, and β-glycerophosphate. Cell growth and vitality, type I collagen synthesis, alkaline phosphatase (AP) activity, Ca2+ consumption and Ca2+ accumulation in the extracellular matrix (ECM) were monitored over a period of 31 days.

Results:
Increased Ca2+ concentrations in the media resulted in an accelerated collagen matrix mineralization and a higher mineralization level. Scanning electron microscopy carried out in the middle of cultivation showed different stages of mineral crystal formation dependent on the Ca2+ concentration in the extracellular environment. Moreover, the increase of Ca2+ content in culture media caused reduction of collagen I synthesis and lowering of AP activity. Therefore, bone-forming cells produced more ECM with a lower degree of mineralization using medium with a low Ca2+ content. Less ECM with a higher degree of mineralization was generated in medium with a Ca2+ content of about 1.8 mmol/l. An even higher extracellular Ca2+ concentration of 3,3 mmol/l was associated with non-physiological mineralization or calcification as well as a decline of cell growth and intensification of apoptosis.

Discussion and Conclusions:
Ca2+ concentrations in culture media have a substantial impact on the ECM synthesis and mineralization. We believe that the reduction of collagen matrix formation and decrease of AP activity are two of numerous regulatory reactions which counteract the non-
physiological mineralization. In the context of bone tissue engineering media with lower or slightly increased Ca2+ content may be used to influence and to improve the ratio of organic and inorganic components of artificial bone tissue.