Silica-collagen-calcium phosphate composite materials for bone replacement. In vitro-manipulation of the ratio of bone-building to bone-resorbing cells in a co-culture model

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Statement of Purpose: The development of novel materials suitable for bone replacement and tissue engineering is still a major concern in orthopaedic surgery. Among bio-inspired materials, composites consisting of both organic and inorganic phases, have been recognized as promising candidates to accomplish a wide range of requirements. The templating activity of collagen fibrils during the silica sol-gel process was used to prepare compact microporous (1…4 nm) xerogels with bone-like mechanical properties. A three-component material was created by introducing different calcium phosphate phases, as for instance hydroxyapatite (HAP) or ß-tricalcium phosphate (ß-TCP). The presence of different calcium phosphate phases manipulates the bioactivity in various ways, and finally the cross-talk between the bone-building and bone-resorbing cells as well. We have studied how the composition dependent bioactivity manipulates the ratio of human osteoblasts to human osteoclasts in a co-culture model without external addition of RANKL and M-CSF.

Methods: Suspensions were prepared by stirring collagen type I fibril lyophilisate in 0.01 M Tris/HCl buffer solution (pH 7.4). For the preparation of the three-component materials an appropriate amount of HAP or ß-TCP was suspended in the collagen suspension by vigorous stirring for 24 h. Orthosilicic acid obtained from hydrolyzed tetraethoxysilane (TEOS) was added to the collagen suspensions. The mixtures were transferred into molds and were allowed to stand for gel formation and stabilization for three days. Gentle drying of the hydrogels in order to obtain monolithic xerogels was performed at 37°C and 95% relative humidity in an Espec SH-221 climate chamber. Samples were sterilized by gamma-irradiation. Disc-like composite xerogel samples 70% silica / 30% collagen, 50% silica / 30% collagen / 20% HAP, and 50% silica / 30% collagen / 20% ß-TCP were seeded with human bone marrow stromal cells (hBMSC) and cultivated up to 42 days. Osteogenic differentiation was induced on day three by addition of ascorbic acid 2-phosphate, dexamethasone, ß-glycero-phosphate, and 1,25-dihydroxy-vitamin D3. On day 14, monocytes isolated from human buffy coat were added and both cell species were cultivated for another 28 days without addition of the ostoclastogenesis stimulating factors RANKL and M-CSF. Proliferation analysis was done by PicoGreen® DNA-kit and lactate dehydrogenase (LDH) activity kit. Alkaline phosphatase (ALP) activity and tartrate resistant acid phosphatase 5b (TRAP 5b) were determined for evaluation of osteoblastic and osteoclastic differentiation, respectively. The calcium content was measured by means of a calcium sensitive electrode.

Results: A co-culture of human BMSC and monocytes that differentiate towards osteoblasts and osteoclasts, respectively, was established directly on the material. No RANKL or M-CSF was added, which allows to study the influence of the material on the expression of these factors by the osteoblasts as well as to both osteoblast and osteoclast formation. The xerogel is able to manipulate the ratio between cell types by its different levels of bioactivity. The composition of the xerogel regulates the Ca^{2+} concentration in the cell culture medium. A lower bioactivity or a release of Ca^{2+} maintains the critical level of Ca^{2+} and allows usual differentiation and proliferation of hBMSC into osteoblasts as well as fusion of monocytes into osteoclasts. Increased bioactivity resulting in a decreased Ca^{2+} level reduces the proliferation rate of hBMSC/osteoblasts, whereas a higher number of active osteoclasts is formed, which is supported by enhanced TRAP 5b activity.

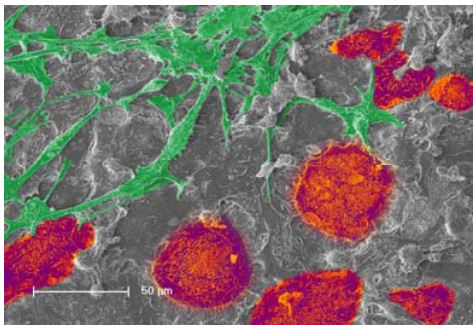

Figure 1. SEM image of osteoblasts (green) and osteoclast (red) in co-culture on a composite sample after 28 days.

Conclusions: Calcium is an important functional component of the composite material. Because of its impact on the bioactivity and the subsequent cellular responses, Ca^{2+} has to be considered as a biological factor. The stability and the mechanical properties of the material are mainly provided by the silica as well as the collagen phase. Because of the ability to manipulate the ratio of the osteoblasts to the osteoclasts in favor of the aforementioned bone forming cells, compositions with lower bioactivity might be useful in order to support fracture healing in bones suffering from osteoclast hyperactivity, like osteoporosis. Compositions with enhanced bioactivity might be appropriate in cases where faster cellular resorption and subsequent remodeling of the material is necessary. Both may open new directions for therapeutic strategies in bone disease.

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