The composition of silica-collagen-calcium phosphate nanocomposites manipulates the ratio of bone forming cells to bone resorbing cells in a co-culture

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Introduction: The development of innovative 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. Own previous studies on the templating activity of collagen fibrils during the silica sol-gel process demonstrated the suitability of the system to form macro porous scaffolds with low strength as well as compact meso porous xerogels with bone-like mechanical properties. A three component material was created by introducing different calcium phosphate phases, in the first place hydroxyl apatite (HAP). The presence of HAP enhances the bioactivity, the ability of a sample to adopt calcium ions from the surrounding medium to be precipitated as an apatite surface layer. We have studied how the composition dependent bioactivity manipulates the ratio of human osteoblasts to osteoclasts in a co-culture without addition of RANKL and M-CSF.

Methods: Suspensions were prepared by stirring bovine tropocollagen type I fibril lyophilisate for 24 h at 4°C in 0.1 M Tris/HCl buffer solution (pH 7.4). For the preparation of the three component materials an appropriate amount of HAP powder (d_{50}=1.6µm) was suspended in the collagen suspension by vigorous stirring for 24 h. Tetraethoxysilane (TEOS) was chosen as the silica precursor and was hydrolyzed for 24 h at 4°C. The resulting orthosilicic acid was added to collagen suspensions. The mixtures were transferred to molds and were allowed to stand for gel formation followed by stabilization for 3 days. Gentle drying of the hydrogels in order to obtain monolithic xerogels was performed at 37°C and 95% RH in an Espec SH-221 climate chamber. Samples were sterilized by gamma-irradiation. Disc-like composite xerogel samples B30 (70% silica, 30% collagen) and B30H20 (50% silica, 30% collagen, 20% HAP) were seeded with human bone marrow stromal cells (hBMSC) and cultivated up to 42 days – with or without addition of osteogenic supplements (ascorbic acid 2-phosphate, dexamethasone, ß-glycerophosphate, 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 (TRAP) isoform 5b were determined for osteoblastic and osteoclastic differentiation, respectively.

Results: A co-culture of human BMSC and monocytes that differentiate towards osteoblasts and osteoclasts, respectively, was established right on the material. No RANKL or M-CSF was added, which allows to study the influence of the material onto 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 (B30) maintains the critical level of Ca^{2+} and allows usual differentiation and proliferation of hBMSC to osteoblasts as well as fusion of monocytes into osteoclasts. Increased bioactivity (B30H30) resulting in a decreased Ca^{2+} level reduces the proliferation rate of hBMSC/osteoblasts. On the other hand, a higher number of active osteoclasts is formed, which is supported by enhanced TRAP 5b activity.

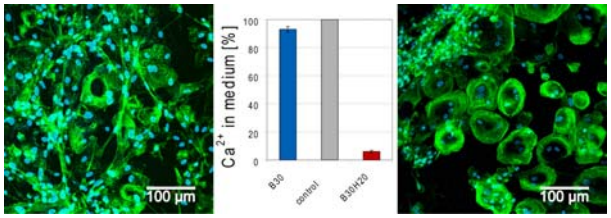

Figure 1. CLSM images of osteoblast/osteoclast co-culture on B30 (left) and B30H20 (right) samples after 28 days. The chart in the middle visualizes the corresponding Ca^{2+} concentration in the medium.

Conclusions: Calcium is not only a structural component of the three components composite. Because of its impact on the bioactivity and the subsequent cellular responses, Ca^{2+} has to be considered as a factor, too. 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, the material B30 might be useful in order to support fracture healing in bones suffering from osteoclast hyperactivity, like osteoporosis.
Material B30H20 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.

References:

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