Effect of β-Tricalciumphosphate coated with Zoledronic Acid on human osteoblasts and osteoclasts in vitro

Wildemann B1, Kadow-Romacker, A2; Greiner, S3; Schmidmaier G2

1Julius Wolff Institut & Center for Musculoskeletal Surgery, BCRT, Charité-Universitätsmedizin Berlin, Germany
2Department for Orthopedic and Trauma Surgery, University Clinic of Heidelberg, Germany
Britt.wildemann@charite.de

Introduction
Today, large bone defects often were filled with synthetic bone grafts as guiding structures, which are ideally resorbable with an osteoconductive surface to promote new bone formation. A combination of a bone graft material with bisphosphonates might be advantageous for an optimal balance of bone resorption and stimulation of bone formation. The present study investigates the effect of β-tricalciumphosphate (β-TCP) bone grafts coated with zoledronic acid (ZOL) on osteoblast-like cells and osteoclast-like cells.

Material & Method
β-TCP blocks (ChronOS®, Synthes, USA; porosity: 60-80%, pore size: 100 to 500 μm, diameter: 14 mm and a thickness: 5 mm) were coated with Poly(D,L-lactide) (PDLLA; Boehringer Ingelheim, Germany) and different concentrations of incorporated Zoledronic acid (Novartis, Switzerland). Human primary osteoblast-like cells or peripheral blood mononuclear cells (PBMC) were cultured separately on the blocks for 12 days, and PBMC also for 21 days. PBMC were cultured with MCSF (5 ng/ml) and RANKL (20 ng/ml) to stimulate osteoclastogenesis. Cell vitality: Alamar blue (Biozol, Germany)
Alkaline Phosphatase: 1.3 mg 4-Nitrophenyl phosphate disodium salt hexahydrate in 1 ml 0.1 M AP-Puffer; pH 10.5 (Sigma, Germany)
Osteoblasts: ELISA for Collagen-Type 1 (COL-1) & Osteocalcin (OC); Osteoclasts: ELISA for TRAPiso-5b (all from Tecomedical, Germany)
Compression test: To measure compressive strength of coated and uncoated β-TCP, compression tests with a material testing machine (Zwick, Germany) were performed. The magnitude of elastic modulus (E-module) was correlated to the resistance of the material against deformation.
Statistics: Kruskal-Wallis-Test followed by Mann-Whitney-Test and Bonferroni-Holm correction for multiple comparisons.

Results
Osteoblast-like cells (POB)
No significant effect on the POB cell vitality and total protein content was seen after cultivation on β-TCP blocks with and without ZOL. The quantity of COL-1 and osteocalcin was significantly increased after cultivation with the PDLLA-coated β-TCP compared to uncoated control (Fig. 1a-b). ZOL application had no effect on COL-1 synthesis but stimulated significantly the osteocalcin production at higher concentrations (1.2 % and 2%).

Osteoclast-like cells (OLC)
The influence of β-TCP coated with ZOL on the fusion of PBMC to OLC and the long-term survival of the cells was investigated after 12 and 21 days of cultivation (Fig. 2). A significant inhibition of the cell vitality was detected after cultivation with PDLLA and all ZOL concentrations for 12 d, compared to uncoated controls. After cultivation for 21d, a reduction in cell vitality was detected for the 1.2% and 2% ZOL-coated β-TCP. For the shorter cultivation period (12 days), TRAPiso-5b was significantly reduced in the PDLLA group in comparison to the control. Moreover, in all ZOL-groups no TRAPiso-5b was measured. The long-term cultivation (21 days) showed a significant decrease in TRAPiso-5b synthesis only in the 1.2% and 2.0% ZOL groups. There were no significant changes in the amount of TRAPiso-5b in the PDLLA-group.

Discussion
The results of the present study show that coating of β-TCP with ZOL has stimulating effects on osteoblast-like cells. Additionally an inhibition of osteoclasts was seen. The inhibitory effect on osteoclast like cells on day 12 might be explained by the initial release of the bisphosphonate from the coating. The early inhibition of osteoclasts is beneficial to inhibit scaffold resorption in the early phase of graft incorporation. The inhibitory effect, however, must be reversible at the later time point to allow the degradation of the material and ensure a remodeling of the graft. In this study, results from the longer cultivation time point (21 d) showed less inhibitory effects on the OLC regarding TRAPiso-5b synthesis and cell vitality. Therefore, releasing BP’s from the coating might be more beneficial compared to other methods where the substance is incorporated into a scaffold by binding to hydroxyapatite crystals and will be released mainly by scaffold degradation [1-3]. The combination of this bone grafting material with bisphosphonates might therefore be effective in the treatment of large bone defects.

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Fig. 1: (a) Production of collagen-1 and (b) osteocalcin by osteoblasts after cultivation on β-TCP coated with Zoledronic acid for 12 days.

Fig. 2: Effect of ZOL-coated TCP on osteoblast-like cells after 12 days (a, c) or 21 days (b, d) in culture.