In-vitro demonstration of phagocytic activity of macrophages in contact to beta-tricalcium phosphate bone substitute material using two-photon laser scanning microscopy

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Large bone defects need to be filled with a grafting material that acts as an osteoconductive scaffold for bone remodelling and new formation of bone tissue. Calcium phosphates are widely used substitutes for bone tissue engineering, since they are degradable over an appropriate period and consequently replaced by newly formed tissue. Osteoclasts resorb bone by changing the environmental pH-value below 7 and thus locally increase the solubility of the biomaterial. We hypothesise that also macrophages are actively involved in the resorption process of calcium phosphate scaffolds and addressed this question in in vitro culture systems by two-photon laser scanning microscopy (TPLSM). In the emission range of 495-540 nm autofluorescent signals originated from the endocytosed beta-TCP were detected in living cells. Imaging z-series of subsequent xy-frames in 1 µm z-steps were performed by TPLSM to visualise the uptake of beta-TCP. Representative image stacks of single cells containing β-TCP in were obtained for structural 3D reconstruction of ceramic particles inside of single cells. Thereby, the localisation and quantification of phagocytosed calcium phosphate within three different cell types over time were possible. Beta-tricalcium phosphate specimens with 10 mm diameter, 1 mm thickness were incubated with (1) macrophages, (2) interleukin-4 activated macrophages, and (3) osteoclasts for up to 21 days. Interestingly, macrophages degraded beta-tricalcium phosphate specimens even more efficiently than osteoclasts. 39 % of the macrophages were partially filled with ceramic material while this was only 18 % for osteoclasts, as shown by imaging with two-photon laser scanning microscopy. Thus, our studies show the previously unrecognised potential of macrophages to phagocytose ceramic particles, which is expected to have implication on osteoconductive scaffold design.