Introduction
Bisphosphonates such as zoledronic acid (ZOL) are clinically used drugs in diseases associated with osteoclast-mediated bone loss. Besides their effect on osteoclasts there is evidence that their antiresorptive activity might be partly due to their effect on osteoblasts. Osteoblasts secretes two proteins, Osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand (RANKL), both of them are necessary for osteoclast differentiation (Lacey et al. 1999; Kong et al. 1999). This antiresorptive effect might be clinically useful in osseous implant integration and fracture healing. Clinical use has been systematic so far. However, local application of these potent substances might be superior regarding the therapeutic fence of their antiresorptive activity. Moreover regarding their potential effect on osseous implant integration and fracture healing local application allows aimed treatment and reduction of systemic side effects.

Materials and Methods
Implants were coated with Poly(D,L-Lactide) and different concentrations (10-150µM) of zoledronic acid (ZOL) kindly provided by Novartis Pharma AG (Basel, Switzerland). Primary human osteoblasts were taken out of human donor bone and cultivated in a standardized method. Cells were seeded (1E4 each well, 24 well plates) and cultured in MEM-Earle/HAM’sf12 (Biochrom AG, Berlin, Germany), 10% heat-inactivated horse serum, Penicillin/Streptomycin+b-Glycerol-P und L-Amino-2P on 96-well plates in a humidified incubator at 37°C with 5% CO2. Implants and cells were cocultured in a noncontact manner. Control groups were treated with uncoated implants, PDLLA coated implants and ZOL pure substance in equal concentrations. Cells were incubated for 144h in total. To analyze a possible effect of ZOL on osteoblast coating, cell proliferation, metabolism, and differentiation was investigated. Cell proliferation was measured 24, 48, 96 and 144 h after setting with alamar blue (BIOZOL, Munich, Germany).

Procollagen I amount synthesized in the supernatant by the osteoblasts after 144 hours in culture was analysed using the Procollagen-C assay (Metra CICP, Quidel, Germany). Primary human osteoblasts were taken out of human donor bone and cultivated in a standardized method. Cells were seeded (1E4 each well, 24 well plates) and cultured in MEM-Earle/HAM’sf12 (Biochrom AG, Berlin, Germany), 10% heat-inactivated horse serum, Penicillin/Streptomycin+b-Glycerol-P und L-Amino-2P on 96-well plates in a humidified incubator at 37°C with 5% CO2. Implants and cells were cocultured in a noncontact manner. Control groups were treated with uncoated implants, PDLLA coated implants and ZOL pure substance in equal concentrations. Cells were incubated for 144h in total. To analyze a possible effect of ZOL on osteoblast coating, cell proliferation, metabolism, and differentiation was investigated. Cell proliferation was measured 24, 48, 96 and 144 h after setting with alamar blue (BIOZOL, Munich, Germany).

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Results
Results showed a significant effect of released ZOL applied from the implant coating. There was no influence on cell viability of the uncoated implant or the PDLLA coating alone. Cell viability decreased in a time and dose dependent manner at concentrations higher than 100µM ZOL (coated implant) or 50µM ZOL (pure substance) (Figure 1).

Procollagen I synthesis was significantly increased in cultures treated with 50µM ZOL coated implants. Higher concentrations and exposure to pure substance showed a decrease in procollagen I. The ratio of osteoprotegerin (OPG) to total cellular protein increased significantly in the 10µM ZOL coated implant group and decreased when treated with higher concentrations. Receptor activator of nuclear factor-κB ligand (RANKL) decreased in a dose dependent manner when treated with different concentrations of ZOL coated implants (Figure 2).

Discussion
OPG acts as a soluble receptor antagonist for RANKL (Teitelbaum 2000) which has been proven to be a osteoclast differentiating factor (Hsu et al. 1999). The quantity of bone resorption is regulated by the balance between the expression of RANKL and its inhibitor OPG (Hofbauer et al. 1999). Bisphosphonates such as ZOL have been shown to stimulate OPG expression and secretion of osteoblastic cell lineages (Viereck et al. 2002). Changes in RANKL and OPG production due to the release of ZOL from the PDLLA coating may contribute to the inhibition of osteoclastic bone resorption. This study showed that there is a continuous release of ZOL out of the PDLLA coating. Cell proliferation was not affected significantly by coated implants with 10 to 100µM ZOL. However procollagen I synthesis was increased significantly when treated with 50µM ZOL coated implants. Exposure to 10 – 50µM ZOL coated implants caused osteoblasts rather to differentiate than to proliferate.

On the other hand higher concentrations or exposure to the pure substance showed a significant decrease in cell viability, proliferation, differentiation and protein synthesis. These findings might be useful in treatment of orthopaedic or traumatologic pathologies with ZOL coated implants like prostheses or osteosynthetic implants for better ingrowth or faster healing. However, further in vitro studies are required to analyse the effect of bisphosphonat coated implants on osteoclasts and other cell types involved in bone healing and in vivo studies with bisphosphonate coated implants need to be evaluated further on.