Introduction
Particle-induced osteolysis is the primary cause of aseptic loosening of total joint replacements. A consensus has emerged that the predominant process is one of cytokine production in response to phagocytosis of implant wear particles resulting in increased proliferation and differentiation of osteoclast precursors into mature osteoclasts. The increased bone mineral density in patients receiving bisphosphonates has been attributed to inhibition of osteoclasts and induction of their apoptosis (1). Previous studies have shown that bone resorption is modulated by osteoclasts interfering with bisphosphonates. In addition, bisphosphonates may have direct effects on osteoblast function. Bisphosphonates have been shown to enhance the recruitment of osteoblasts from among human bone marrow stromal cells (2), stimulate osteoblastic proliferation and maturation (3,4) and inhibit apoptosis of the osteoclast in vitro (5). Further, in vivo studies showed enhanced net bone growth into implant porosities (6) and during distraction osteogenesis (7) as well as a pronounced thickening of peri-prosthetic cortical bone after bisphosphonate treatment (8). In a particle-induced murine calvarial osteolysis model we previously showed that bone resorption can be markedly decreased by a single s.c. dose of zoledronic acid (9). Using the same model, we aimed to investigate the in vivo effects of zoledronic acid on osteoblastic bone formation under conditions of polyethylene particle-induced osteolysis.

Materials and Methods
A recently established murine calvarial model of ultra-high molecular weight polyethylene (UHMWPE) particle-induced osteolysis (10) was applied in twenty-eight C57Bl/J6 mice in accordance with the official guidelines and following approval by the University and the local government. Animals were equally randomized to four groups. In group 1 animals underwent sham surgery only, in group 2 animals were treated with UHMWPE particles (about 6 x 10⁶ particles), in group 3 animals were treated with particles and received additional Zoledronic acid (Zometa, Novartis Pharma AG, Basle, Switzerland) at a dose of 25 mg/kg of body weight directly after surgery, seven animals received zoledronic acid 4 days after surgery (group 4). This dose regime was in the range of the dosage recommended for application in humans. The group size of seven was determined in a power analysis. Statistical analysis was performed using a one-way ANOVA and a post-hoc Student’s two tailed t-test. All p-values were compared to an a-value of 0.05 to determine significance.

Particles
Commercially pure UHMWPE polyethylene particles were obtained from the manufacturer (Ceridust VP 3610, Clariant, Gersthofen, Germany). The particle size was described by the manufacturer as 50 % of the particles being smaller than 5 µm and 90 % smaller than 9 µm. Specimen retrieval and histotechnical processing 14 days postoperatively, the animals were sacrificed in a CO₂ chamber. Retrieved calvaria were processed utilizing undecalcified hard tissue techniques. Four µm sections were taken in the frontal plane centered over the area of particle deposition. These sections were stained with Giemsa.

Histomorphometric analysis
Bone formation was assessed by quantifying osteoid formation in Giemsa stained sections. Osteoid formation was determined by tracing the perimeter of osteoid. In detail, using one microscopic field at a magnification of x10, the osteoid tissue area was encircled and the area was calculated automatically. Bone thickness was measured as an indicator of net bone growth. Then specimens were divided in four 0.5 mm measured steps to the left side and four measured steps to the right side of the midline suture. Bone thickness was measured successively at these measured steps.

Results
UHMWPE particle burden without any further intervention (group 2) induced bone resorption to sham control levels (Figs. 1, 2). Additional treatment with Zoledronic acid significantly increased osteoblasts’s activity and the osteoid formation (Figs. 3,4) as quantitatively assessed by histomorphometry. Osteoid formation was 0.00 ± 0.00 mm² in sham group (group 1), 0.02 ± 0.03 mm² in animals with particle implantation (group 2), 0.25 ± 0.08 mm² in animals with particle implantation and Zoledronic acid treatment directly after surgery (group 3) (p=0.0018), and 0.21 ± 0.11 mm² in animals with particle implantation and Zoledronic acid treatment on the fourth postoperative day (group 4) (p=0.0042) (Fig. 5). The mean bone thickness was 0.2 ± 0.04 mm (range 0.17 to 0.31) in group 1 and 0.16 ± 0.02 mm (range 0.14 to 0.19) in group 2, 0.31 mm ± 0.04 mm (range 0.28 to 0.39) in group 3, and 0.29 mm ± 0.02 mm (range 0.28 to 0.34) in group 4. Student’s t-test revealed a statistical significant difference in mean calvarial bone thickness between group 2 and 3 (p=0.00042), and group 2 and 4 (p=0.0019). There was no statistical significant difference in bone thickness between group 3 and 4 (p=0.07).

Conclusions
In our previous experiment a single s.c. dose Zoledronic acid was given and shown to be effective in preventing particle-related osteolysis (9). Here we report that zoledronic acid further enhanced osteoblastic bone formation under conditions of particle-induced osteolysis. Consistent with previous studies (6-8), our results suggest that osteoblasts exposed to a single dose of zoledronic acid s.c. are able to increase their bone forming potential. The mechanisms by which they mediate their actions remain as yet unclear. The bisphosphonate-mediated effects of locally increased bone formation in a situation where bone may be excessively resorbed, e.g. due to wear debris, could be of significant benefit in order to ultimately improve the durability of total joint replacements.

References

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