INTRODUCTION
In orthopaedic trauma surgery, osteoporosis carries an increased risk of surgical complications. Bone fragility, the tendency for fracture comminution, and the presence of large fracture defects produce challenges in operative stabilization of osteoporotic fractures. To overcome these problems, the osteosynthesis devices have been re-designed and internal fixation is frequently supplemented by use of synthetic bone graft substitutes.

Bisphosphonates are antiresorptive agents extensively used in treatment of osteoporosis. Many osteoporotic fracture patients are candidates for concurrent treatment with bisphosphonates and synthetic bone graft substitutes. Recently, we found that bioactive glass microspheres induce high local bone turnover. Thus, adjunct bisphosphonate therapy may affect the process. The current study examined the effect of adjunct zoledronic acid therapy on bioactive glass incorporation.

MATERIALS AND METHODS
Harlan Dawley female rats (n=80) (age 15-19 weeks, weight 208-287 g) were used. The study protocol was approved by the Institutional Animal Care Committee. A standardized region of the proximal tibia was subjected to ablation of local bone marrow through two cortical windows and filled with bioactive glass (BG) microspheres (Ø 250-315 µm). The BG composition (glass 13-93) was SiO₂ 53 %, Na₂O 6 %, CaO 20 %, K₂O 12 %, MgO 5 %, P₂O₅ 4 % by weight. In controls, the rinsed medullary space was left to heal without filling. Experimental animals received zoledronic acid (1.5 µg/kg sc, once a week, started one week before surgery) or doxycycline (a metalloproteinase inhibitor) (33 mg/kg, daily gavage) as a control agent. According to the systemic drug therapy and local defect filling, the animals were divided into six groups:
- zoledronic acid therapy with BG filling (BG+ZOL, n=14)
- doxycycline therapy with BG filling (BG+DOXY, n=12).
- BG filling without systemic adjunct therapy (BG, n=12)
- zoledronic acid therapy without defect filling (ZOL, n=13)
- doxycycline therapy without defect filling (DOXY, n=13)
- unfilled defect without adjunct therapy (empty control, n=12).

Sequential in vivo peripheral quantitative computed tomography (pQCT) was performed and strength strain index (SSI) was measured. The final outcome was analyzed at 8 weeks. Six animals from each group were used for Northern analyses. The mRNA levels of markers for bone resorption (cathepsin K, TRACP, MMP-9, MMP-13) and synthesis (type I, II, III collagens, osteocalcin, osteonectin, osteopontin) were measured for determination of local bone turnover. The remaining animals of each group (n=6-8) were used for digital X-ray imaging, µCT, hard-tissue histology and backscattered electron imaging of scanning electron microscopy. The histologic specimens were measured for intramedullary bone area. The bone affinity index, defined as the fraction of the outer perimeter of bioactive glass microspheres in contact with bone, was also measured. One-way analysis of variance with Tukey’s posthoc test was applied with Bonferroni correction. In the analysis of mRNA levels, non-parametric Mann-Whitney test with Bonferroni correction was applied.

RESULTS
Effect of zoledronic acid on bone healing
In response to zoledronic acid treatment, the healing bones demonstrated massive intramedullary new bone formation. Histology, BEI-SEM imaging and µCT imaging revealed complete or near complete healing of the cortical windows. In histomorphometry, the amount of intramedullary bone was the highest in the zoledronic acid treated group and accounted 33.9 ± 5.5 % of the intramedullary space (p=0.001 compared with empty controls)(Fig. 1). The intramedullary pQCT density increased approximately 3.5-folds during the first four postoperative weeks (p=0.001 compared with empty control), but did not change thereafter reflecting the high retention of intramedullary new bone. At the 9th week of zoledronic acid treatment (eight weeks after surgical procedure), Northern analysis of mRNA levels showed a pattern typical for the resorption phase secondary to accumulation of new bone. The healing bones showed significantly decreased mRNA levels for bone synthesis markers, type I collagen (p=0.009) and osteocalcin (p=0.035), and increased mRNA levels for all osteoclastic resorption markers, cathepsin K (p=0.009), TRACP (p=0.009) and MMP-9 (p=0.017).

DISCUSSION
In the previous study, bioactive glass microspheres administrated into intramedullary space of rat tibia were found to accelerate local bone turnover and enhance intramedullary new bone formation. Based on this observation, we hypothesized that high local bone turnover is crucial for the osteopromotive action of bioactive glass. Therefore, adjunct treatment with zoledronic acid, known to decelerate high bone turnover, was proposed to have effects on the molecular mechanism of action of bioactive glass. Based on the current results, the process of bioactive glass incorporation seems to benefit from adjunct antiresorptive zoledronic acid therapy. Adjunct treatment with doxycycline had no adverse or additive effect on bioactive glass function suggesting a minor role of matrix metalloproteinase in the process.

The current study supports the concept that bisphosphonate could promote the biologic action of bioceramics in bone. The high mRNA levels of osteoclastic markers suggest that the therapy is safe in maintaining a physiological regulation of remodeling in healing bones.

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