INTRODUCTION
The treatment of deep bone infections is generally difficult and time consuming. The current treatment protocols are based multiple-staged procedures. Aside with prolonged systemic antibiotic therapy, the protocols involve identification of the pathogen(s), radical surgical debridement, soft tissue coverage and treatment of the bone defect. Various local antibiotic carrier systems have also been developed, particularly antibiotic-impregnated polymethylacrylate (PMMA) beads with a need for subsequent removal. Recently, drug delivery systems (DDSs) using resorbable materials such as collagen, fibrogen and polyolactic acid have been developed. These materials do not require surgical removal, but they do not restore bone stock. An ideal resorbable osteoconductive DDS will have a dual-effect. First of all, it will provide controlled local release of the selected antibiotic for complete eradication of infection. In due course, the material would induce defect healing by new bone, thus minimizing the need of secondary bone grafting in contained defects following surgical debridement.

METHODS
The DDS tested in the current study consisted of three components. Resorbbale racemic poly(DL)-lactide (PDLLA 50:50) Resomer® R206 (Boehringer Ingelheim, Germany) acted for the controlled release of the antibiotic. The selected antibiotic (AB) was ciprofloxacin (Jinxing Kangle Pharmaceutical Factory, China), which was tested to be sensitive for most clinical pathogens of bone infections. The bioactive glass (BG) (ABM13 – 93, Abmin Technologies Ltd, Finland) was added to act as the osteoconductive component in order to promote new bone formation.

The local Ethical Committee approved the animal study design. The localized osteomyelitis model was modified from those of Mader’s rabbit (1) and Fitzgerald’s canine models (2). Surgery was performed on adult male New Zealand white rabbits (n=30, weight 3 kg). A cortical bone window (6 mm x 2.3 mm) was drilled in the medial cortex of the proximal metaphysis of the right tibia. Bone marrow was removed and the defect was filled with a block of bone cement, acting as foreign body. After the closure of deep soft tissue layers, 0.1 ml of a solution containing Staphylococcus aureus (1x10^7 /ml) (strain 52/52A/80, kindly provided by Dr. Jon T Mader) was injected into the defect behind the bone cement. Infection was allowed to develop for two weeks, when the bone cement was removed during debridement and the presence of osteomyelitis was confirmed by means of bacterial cultures. At this stage, the defects of the experimental animals (n=9) were filled with antibiotic containing composite (AB-PDLLA-BG). Three control groups were produced. The defects of the control animals received the same surgical debridement but no composite implant for treatment of infection (Osteomyelitis group, n=8); a PDLLA-BG composite without antibiotics (PDLLA-BG group, n=5). The negative control group (n=8), acting as a model of normal bone healing, underwent the same two-stage surgery but bacterial suspension was replaced by saline injection.

Peripheral QCT and positron emission tomography (PET) using F-18 fluorodeoxyglucose (FDG) were performed on time of debridement. FDG-PET measurements were performed on standardized region of interest (ROI) (circle of 3.8 mm radius) of the defect area of the right tibia and the corresponding region of the contralateral left tibia. Standard uptake values (SUV) were calculated as the radioactivity of the ROI divided by the relative injected dose expressed per animal body weight. Bone defect healing was evaluated with quantitative histomorphometry at 6 weeks. Concentration of ciprofloxacin in bone tissue was measured from the specimens taken from areas proximal and distal to defect. The statistical significance of the differences between the groups was calculated using one-way ANOVA with Tukey t-test.

RESULTS
Studied at the time of debridement, osteomyelitis was achieved in all rabbits that received S. aureus injection (Table 1). At sacrifice, the defects of all control animals (except the negative controls) still showed positive cultures. In contrast, the defect cultures of the AB-PDLLA-BG group had uniformly turned negative. Three rabbits of the AB-PDLLA-BG group, however, showed positive cultures of the soft tissues at sacrifice, as a sign of infection spread into soft tissues.

<table>
<thead>
<tr>
<th>Group</th>
<th>Debridement</th>
<th>Sacrifice</th>
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<tbody>
<tr>
<td>Healing bone</td>
<td>8</td>
<td>0/8</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>8</td>
<td>8/8</td>
</tr>
<tr>
<td>AB-PDLLA-BG</td>
<td>5/9</td>
<td>0/0</td>
</tr>
<tr>
<td>PDLLA-BG</td>
<td>5/5</td>
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Compared with the intact bone value, the negative control group (healing bone) showed a temporarily increased uptake of FDG at 3 weeks (p=0.019), but the uptake normalized by 6 weeks. In untreated osteomyelitis, the FDG uptake was many-fold increased compared with the healing bone values both at 3 and 6 weeks (Fig. 1). The treatment with AB-PDLLA-BG significantly decreased the FDG uptake and the difference was highly significant (p=0.013) at 6 weeks compared with the untreated osteomyelitis group. The FDG uptake of the AB-PDLLA-BG group was already approaching the level of normally healing bone at 6 weeks.

The local therapy with the composite (AB-PDLLA-BG) resulted in adequate bone concentration of ciprofloxacin in both proximal (median 2.1 µg/g, mean 12.6 µg/g, standard deviation 20.6 µg/g, n=9) and distal area (median 1.2 µg/g, mean 1.4 µg/g, standard deviation 0.7 µg/g, n=9). In pQCT, the cortical defects were open in all groups at 3 weeks. At 6 weeks the cortical defect was closed 89 % of its original length in AB-PDLLA-BG group and 77 % in healing bone group. Corresponding values for osteomyelitis and PDLLA-BG groups were 33 % and 5 %, respectively. For histomorphometry, the amount of new intramedullary bone in AB-PDLLA-BG was significantly higher than in healing bone group (p=0.045). There were no significant differences between other groups.

DISCUSSION
Local therapy with the composite resulted in high bone concentration of ciprofloxacin. The collaborative results of the bacteriologic and FDG-PET studies showed that the selected composite was successful in bone eradication of Staphylococcus aureus pathogen. Six weeks after the start of local antibiotic therapy, there was no significant difference in FDG-PET activity of AB-PDLLA-BG treated osteomyelitic bones and normal healing bones. In three cases, the local antibiotic therapy failed to treat the concomitant spread of infection into soft tissues, suggesting the need of simultaneous systemic antibiotics for the control of surrounding soft tissue infection under clinical conditions.

REFERENCES

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