INTRODUCTION:
The objective of this study is to evaluate by means of imaging and biomechanical analysis, the effect of local Bisphosphonate application on screw fixation in cancellous bone. An overdrilling procedure enabled consistent modeling of reduced screw stability comparable to compromised cancellous bone. The biomechanical evaluation of local drug delivery allowed determining the grade of in vivo stability at various time points after surgery (Aspenberg, 2009; Pioletti, 2008).

METHODS:
Coating: Titanium bone screws (4.0mmΩ) were coated with Fibrinogen (Aspenberg, 2008) and loaded with 150ng/cm² of zoledronic acid (Axxora, Switzerland). After single-packaging in peel-pouches, test articles as well as uncoated controls were sterilized by γ-irradiation.

Surgical Procedure: 48 adult NZW rabbits were randomly divided into 5 groups, representing investigational time points of 1day, 5 days, 10 days, 6 weeks and 11 weeks. All rabbits underwent bilateral femur implantation. Drilling was initiated by introducing a 1.4mmΩ guide wire allowing appropiate allocation of the defect. To the final drill size was performed using a cannulated Ø3.7mm drill. In a paired approach, each medial distal femoral condyle received one bone screw coated with fibronogen loaded with Zoledronate (drug) or coated with fibrinogen but no Zoledronate (no drug). Surgical as well as implantation procedure were referenced by Hoshikawa et al., 2003.

Micro-CT imaging: The screw head was clamped in a specially designed screw holding sleeve to ensure the alignment of the screw with the scanning axis. The holding sleeve was then placed in the polystyrene support of the in-vivo µCT Scanner 1076 (SkyScan, Belgium). Samples were scanned at 100kV/100mA source voltage/current, with a 1mm aluminium filter. The pixel size (resolution) was 18 µm, rotation step was 0.6° over 360°, exposure time was 400ms. The dataset was reconstructed with NRecon software (SkyScan, Belgium). Moderate beam hardening was applied in the reconstruction process. The µCT analysis was done with dedicated software CTAN (SkyScan, Belgium). The HU and BMD calibrations were first applied to the dataset. The region of interest (ROI) was chosen as a sleeve around the screw with 0.5 mm of thickness (Figure 1b). The cortical bone at the entrance point of screw was excluded. Afterwards, the bone volume (BV), tissue volume (TV), and BMD of bone tissue was measured for each dataset.

Pull-out test: The specimens were embedded in Neukadur cement (Troller, Switzerland) while making sure that the screw axis is perpendicular. The screw head was then gripped by a special apparatus and subjected to pull-out (Instron, USA) until bone failure.

Statistical analysis: All statistical analysis was performed in MATLAB (Mathworks). Group comparisons were done using two-way ANOVA. As the no drug screws were paired with the drug screws in each rabbit, we employed paired t-test for pair-wise comparison. Biomechanical differences were considered significant if p<0.05.

RESULTS:
Bone density (BV/TV) was measured as the percentage of bone tissue in a defined ROI around the screw. During the pull-out tests we could observe that the amount of bone tissue taken out with the screw increases over time. This observation is confirmed by measuring bone density over time which is significantly increasing (p<0.01) (Figure 2a). The effect of drug on bone density is significant at 6 and 11 weeks (p<0.01 and p=0.015, respectively), but not at the early time points. BMD of bone tissue in ROI was significantly increasing in time, but there was no difference between the two groups. The effect of drug was significant at 11 weeks (P=0.5) and not at prior time points (Figure 2b). The correlation between BD and maximum pull-out force was 0.95.

DISCUSSION:
Mechanical testing and micro-CT imaging was used to assess the effect of local drug delivery of Zoledronate on screws fixation. In the early time points (1, 5, and 10 days), no significant difference could be seen between the no drug and the drug groups. At 6 weeks, the bone density was significantly higher in the trabecular region of the drug group. However, this increase did not have a significant effect on the pull-out force. At the last time point, 11 weeks, both the bone density and the pull-out force were significantly higher in the local delivery drug group.

The results of this study suggest that local delivery of bisphosphonate enhances the stability of bone screws in long run.

REFERENCES:

Figure 2. Box plot of the paired difference between local drug and no drug groups (a) bone density (BV/TV), (b) pull-out force, at different time points.