Calcium Sulfate Void-Filling Does Not Prevent Fibrosis and Reduces Fixation Strength in a Mouse Model of Failed Osseointegration

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INTRODUCTION: Failed osseointegration is characterized by fibrotic encapsulation rather than bone apposition at the implant interface. We previously developed a murine tibial "overdrill" model that reliably induces peri-implant fibrosis[1]. In this study, we investigated whether transiently restoring physical continuity across the peri-implant void using a resorbable filler could modulate early healing away from fibrosis. Calcium sulfate ($CaSO_4$), a clinically established defect-filling and antibiotic carrier material, was selected for its rapid resorption profile over several weeks. We hypothesized that filling the peri-implant void with $CaSO_4$ paste would reduce fibrotic tissue formation at four weeks post-implantation.

METHODS: Skeletally mature 20-week-old male and female C57BL/6J mice (Jackson Laboratories) were used in equal numbers for all outcomes. Bilateral tibial implantation of custom 3D-printed titanium implants was performed using the overdrill protocol described by Thomson et al., 2024[1]. On one limb, the peri-implant void was filled intraoperatively with $CaSO_4$ hemihydrate powder (Sigma-Aldrich) mixed with sterile water (2.4g/ml ratio); the contralateral limb served as a paired control without paste. All procedures were approved by the Institutional Animal Care and Use Committee. At four weeks, mice were euthanized and tibiae were harvested for analysis. Dissected tibiae underwent microCT scanning to quantify trabecular bone volume (BV/TV) within a 500 µm height region surrounding the implant stem. Mechanical pull-out testing was performed to assess fixation strength. Calcein double-label fluorescence imaging (20 mg/kg injections, seven days apart prior to sacrifice) was used to determine mineral apposition rate (MAR). Samples were then methyl methacrylate (MMA) embedded and Pentachrome stained (Abcam) for histologic fibrosis quantification in QuPath within a peri-implant region of interest defined by the area within 200 µm of the implant surface. Paired two-tailed t-tests were used for statistical comparisons ($p < 0.05$).

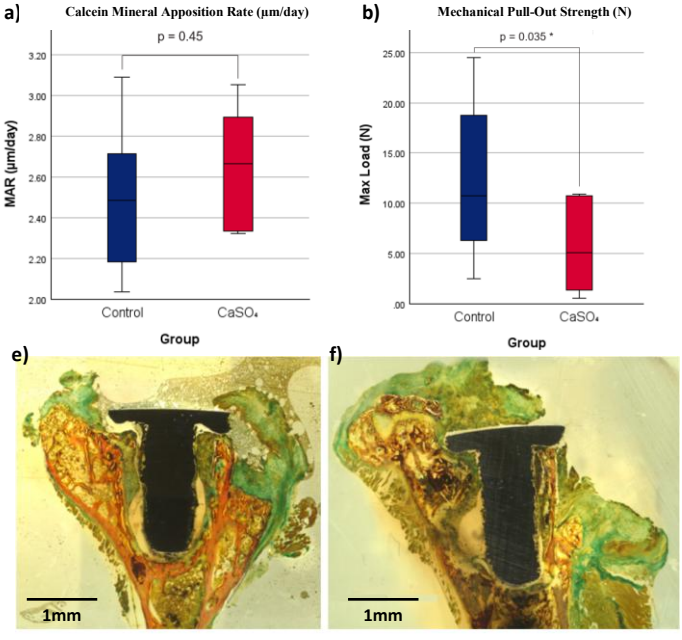
RESULTS SECTION: At four weeks, 10 mice were analyzed by microCT and mechanical testing, and 6 additional mice were used for dynamic histomorphometry and histologic quantification. Peri-implant BV/TV did not differ between groups ($CaSO_4$ 40.1 ± 13.3% vs Control 46.3 ± 17.0%, p = 0.34). MAR was similar (2.62 ± 0.34 µm/day vs 2.50 ± 0.38 µm/day, p = 0.45). Fibrosis area fraction was nearly identical (11.2 ± 5.0% vs 11.0 ± 6.4%, p = 0.97). However, $CaSO_4$-treated limbs demonstrated significantly lower pull-out strength (7.13 ± 7.76 N vs 12.50 ± 7.64 N, p = 0.035) [**Figure 1**].

DISCUSSION: Both the $CaSO_4$-treated and contralateral control limbs used the overdrill model, which predictably generates a fibrotic interface due to the initial gap and instability between implant and bone. Filling this gap with $CaSO_4$ was intended to temporarily restore physical continuity and intercellular stimulus while resorbing within weeks. However, the comparable fibrosis and bone metrics between groups indicate that the transient filler did not alter early healing dynamics or the fibrogenic trajectory of the model. The decreased mechanical fixation strength observed in the $CaSO_4$ group likely reflects an early period of decoupling as the material resorbed before stable bone bridging could form. In effect, the resorption may have transiently maintained and blocked the peri-implant space, leaving the implant poorly supported. Mechanical weakness may be seen in rapidly resorbing bone substitutes as the rate of resorption outpaces osseous remodeling. A limitation of this study is that only the early four-week timepoint was assessed out of interest in initial performance; longer-term outcomes may reveal compensatory bone infilling.

SIGNIFICANCE/CLINICAL RELEVANCE: $CaSO_4$ remains a widely used bone filler and antibiotic carrier, yet these findings suggest caution when applying it at load-bearing implant interfaces. In this murine model, transient void-filling with $CaSO_4$ did not prevent fibrotic healing and was associated with reduced early fixation strength, underscoring the importance of balancing resorption kinetics with early mechanical stability in implant design and surgical reconstruction.

REFERENCES:

1. Thomson AL, Suhardi VJ, Niu Y, Oktarina A, Döring K, Chao C, Greenblatt MB, Ivashkiv LB, Bostrom MPG, Yang X. A translational murine model of aseptic loosening with osseointegration failure. J Orthop Res. 2024;42(11):2525–2534. doi:10.1002/jor.25915



[Figure 1] Quantitative and histologic outcomes at 4 weeks following implantation in the overdrill model. **(a)** Mineral apposition rate (MAR) measured by calcein double labeling showed no difference between groups ($p = 0.45$). **(b)** Mechanical pull-out strength was significantly reduced in the $CaSO_4$ group ($p = 0.035$). **(c)** Peri-implant bone volume fraction (BV/TV) and **(d)** fibrosis area fraction were similar between groups ($p = 0.34$ and $p = 0.97$, respectively). **(e–f)** Representative Pentachrome-stained plastic-embedded sections show comparable peri-implant tissue morphology in control **(e)** and $CaSO_4$ **(f)** limbs (scale bar = 1 mm). Boxplots show median, interquartile range, and range.