PTH Enhanced Osseointegration in a Physiologically Loaded Mouse Tibial Model

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Disclosures:

Introduction: Non-cemented joint replacements were developed primarily to improve the longevity of the bone implant interface. The success of non-cemented joint replacements depends on sufficient bone quantity/quality and rapid osseointegration. Clinically, the cancellous bone of the proximal tibia has not provided an optimal environment for the structural support and osseointegration of non-cemented implants. This issue has prevented the widespread adoption of non-cemented total knee replacements. Solving this problem requires an in vivo model of tibial cancellous osseointegration that captures the key clinical variables and allows optimization of the peri-implant environment. Currently, no clinically relevant small animal models exist to study the interaction between cancellous bone and metallic implants. Parathyroid hormone (PTH) is a potent anabolic agent that improves bone quantity and quality around an implant. Previous studies have shown that PTH enhances osseointegration of porous titanium implants in cortical and non-physiologic locations but data is limited on the effect that PTH has on tibial cancellous osseointegration [1-3].

The aim of this study was to develop a mouse model with an implant subjected to weight-bearing and physiological loading which is similar to that of the tibial component of human non-cemented total knee replacements. This novel model was used to test our hypothesis that PTH therapy enhances osseointegration.

Methods:
Animal Model: A titanium tibial implant was manufactured by a three-dimensional printer (Smith & Nephew, Inc.) The stem of the implant was press fit into the medullary canal of the mouse tibia. The proximal smooth surface of the tibial plateau of the implant articulated with the native femur. The roughened surface of the stem and undersurface of the tibial plateau provided an in-growth surface which facilitated initial stability and osseointegration.

Study Design: 10 week-old female C57BL/6 mice were subcutaneously injected with PTH (40µg/kg/day, N = 44) or vehicle (N = 44), 5 days/week throughout the experiment duration. After 6 weeks of injections, a baseline group was euthanized (n=6 mice/treatment group). The remaining mice received a right tibial implant and were euthanized at 1, 2 or 4 weeks after surgery.

Microcomputed Tomography (microCT): The right proximal tibia of each animal was scanned by microCT at a resolution of 6 µm. Baseline Group: (N=6/treatment group/time point): two volumes of interest (VOI) included epiphyseal cancellous bone and

Figure 1:
(a) Tibial implant
(b) Radiograph showing position of implant in mouse tibia.

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metaphyseal cancellous bone 1500 µm distal to the growth plate. The contours were drawn by tracing the endosteal border to encompass cancellous bone excluding cortical bone. **Experimental Group:** the VOIs were a 500 µm segment of cancellous bone distal to the tip of the implant and a separate 500 µm-long peri-stem VOI. The contours were drawn by tracing the endosteal border to encompass cancellous bone. For the peri-stem VOI, a 60µm region around the implant was excluded to avoid beam hardening artifact.

**Backscattered Scanning Electron Microscopy (BSE):** Samples (N = 7/treatment group/time point) from the 2 and 4 week time points were embedded in PMMA. Both axial and coronal sections were used. Axial sections were cut and scanned at four distances starting below the undersurface of the plateau. Coronal sections were taken from the proximal 0.75 mm of the implant and scanned. All scans were at 50X magnification. Osseointegration percentage (OI%), the proportion of the metal perimeter contacted by bone, was measured using ImageJ (NIH).

**Immunohistochemistry (IHC):** Samples (N = 7/treatment group/time point) from 1 and 2-week animals underwent decalcification, removal of the implant, and paraffin-embedding. Cross-sections located 1 mm distal to the surface of the tibial plateau trimmed during surgery were stained for osteoblasts and osteoclasts using pro-collagen I and cathepsin-K antibodies, respectively. Both cell types were quantified within 200 µm of the implant cavity and normalized per unit area.

**Statistical Analyses:** Baseline differences in microCT parameters between treatments were assessed with Mann Whitney U tests. Post-implantation differences in microCT and BSE parameters with treatment, region, and week were assessed with multi-factor ANOVAs with Turkey post-hoc tests. The level of significance was p < 0.05. Results presented in the abstract are significant unless stated otherwise.

**Results:**

**Animal Model:**

- The mice tolerated the implant well and started weight bearing immediately after surgery.
- At 4 weeks post-surgery, the range of motion of the right knee was preserved.

**Bone Mass:**

- In the epiphysis at baseline (Week 0), BV/TV was 31% greater in PTH-treated mice than in controls.
- In the metaphysis at baseline (Week 0), BV/TV was 15% greater in the control group than in PTH-treated animals.
- After implantation, metaphyseal BV/TV was greater in PTH-treated animals than in controls at weeks 2 (195%) and 4 (382%).
- BV/TV in control animals did not differ between post-implantation time points. BV/TV in PTH-treated animals was greater at weeks 2 (75%) and 4 (95%) vs. week 1, respectively.
- The metaphyseal region had a higher BV/TV in the PTH-treated animals than in controls at week 2 and week 4.

![Figure 2: Peri-implant bone volume fraction determined by MicroCT. ANOVA revealed significant differences between PTH and Vehicle when samples were pooled. *: p < 0.05 in post-hoc analyses.](image)

**Osseointegration:**

- PTH treated groups had significantly greater OI% vs. vehicle groups along the stem of the implant in axial sections.
- OI% was not different between the two treatment groups at any time points along the tibial plateau in the coronal
Bone Cell Density:

• PTH treated groups had 67% more peri-implant osteoblasts/mm² vs. vehicle groups.
• Week 2 animals had 27% fewer peri-implant osteoblasts/mm² vs. week 1.

Discussion: In this study, we demonstrated that PTH therapy enhanced peri-implant bone mass and osseointegration in a novel weight-bearing model of tibial cancellous osseointegration. The increase in osteoblast density in PTH treated animals indicates that PTH therapy had a net effect of enhancing bone formation. The combination of these data with the finding that bone volume fraction did not change significantly between weeks 2 and 4 suggests that the bone anabolic effect of PTH plateaus by week 2. While not significant, osteoclast density trended to being greater in PTH treated animals and increasing in week 4 relative to week 2, suggesting that bone turnover is overall increased and the remodeling phase initiates later than the anabolic phase.

Significance: This model is an effective platform to gain mechanistic insights regarding the process of osseointegration in non-cemented total knee arthroplasty and to test specific biological/mechanical mechanisms that inhibit and enhance osseointegration. Our data indicates that PTH therapy may benefit patients receiving non-cemented total knee replacements.

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References:

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