Time Course of Peri-Implant Bone Regeneration Around Loaded and Unloaded Implants in a Rat Model: A Pilot Study

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Introduction: Implant osseointegration is a complex process that involves recruitment of stem cells to traumatized tissue, tissue differentiation, mineralization, and bone remodeling[1]. Methods to increase peri-implant cancellous bone mass especially in osteoporotic patients are highly desirable clinically. Many orthopedic implants undergo mechanical loading, which can affect osseointegration, and post-surgical rehabilitation protocols affect this loading. In this study, we have developed a novel rat model that allowed us to observe the time course of peri-implant bone regeneration and remodeling around loaded versus unloaded implants implanted in the contralateral limb. We hypothesized that mechanical loading alters the time course of peri-implant bone healing.

Methods: Six cylindrical polyether-ether ketone (PEEK) implants (dia. 0.80 mm) were fabricated using computer-controlled machining (Fig. 1(a)). The implant had a grooved topography that was sputter coated with titanium (thickness of 200 nm). The implant was held in place with a housing, which remained percutaneous for external loading[2]. Implants were implanted into proximal tibia metaphyses of both limbs of three female Sprague-Dawley rats aged 12-14 weeks (Charles River Laboratories, Wilmington MA). Controlled external cyclic loading (60 μm displacement, 1 Hz, 60 sec) was applied every other day for 14 days (Fig. 1(b)), to one implant in each rat, via an electronically controlled mechanical test machine (LM1 TestBench, Bose, Eden Prairie MN). The contralateral limb with the implants served as the unloaded controls. Rat hind limbs were imaged in vivo with high resolution μCT (voxel size 12.5 μm) (VivaCT 40, Scanco, Switzerland) at 2, 4, 7, 9, 11, and 14 days post-surgery. The μCT scans from various time points from a single limb were registered together in space using 3 radiopaque microspheres (100 μm dia.) that had been included within the implant (Avizo software, VSG US, Burlington MA). A peri-implant cylindrical volume twice the diameter of the implant was selected and thresholded to compute bone volume (BV)/total volume (TV). After 14 days, animals were euthanized and pull-out tests were performed by stabilizing the bone and displacing the implant head at 5 μm/sec. Statistical analyses were performed in SAS. All animal experiments were conducted in accordance with Penn State Hershey IACUC.

Results: At day 9, the mean %BV/TV for loaded and unloaded limbs were 43.2 ± 8.5 % and 39.9 ± 3.9 %, respectively. By day 9 both the loaded and unloaded peri-implant regions demonstrated statistically significant increases in bone volume, averaging 15% (p < 0.05) and 22% (p < 0.05) greater than at day 2, respectively. Bone volumes did not continue to increase during the second week, and in some animals decreased. There was no statistical difference in the mean pull-out force between the loaded (5.5 ± 5.7
N) and unloaded (7.2 ± 1.6 N) implants. By day 14, the mean %BV/TV for loaded and unloaded limbs were 23.4 ± 12.7 % and 41.1 ± 18.0 %, respectively.

**Discussion:** In this study we report initial data on the time course of bone regeneration surrounding implants, in both mechanically loaded and unloaded implants in the rat. Following surgery, a regional inflammatory response is expected to be present and dominates the early tissue response. In the rat tibia, bone volumes were seen elevated at earlier time points and then decreased by day 11 after continued loading. This decrease in bone volume could correspond to the bone-remodeling process that occurs after initial tissue differentiation and formation of woven bone. Van der Meulen MC et al.[3] used a rabbit model to study cancellous functional adaptation to mechanical loads and observed a transient increase in bone mass from 2 to 4 weeks and constant response from 4 to 8 weeks. Slaets E et al.[4] studied histomorphometrically the bone regeneration with titanium implants in tibial epiphysis of rabbits over 42 days and observed simultaneous osteoclast and osteoblast activity Day 7 onwards. Limitations of the study include a shorter observation period (2 weeks) and simplified loading environment. Future work includes a histological evaluation and longer observation period, and more number of animals in order to better understand the changes in bone regeneration between different implant materials and loading regimes.

**Significance:** This study is the first to the best of our knowledge to report on the change in mineralized peri-implant tissue longitudinally across a series of time points in response to mechanically loaded implants.

![Figure 1](image_url)

**Figure 1.** (a) Layout and assembly of the cylindrically shaped PEEK implant and its housing components (b) Controlled cyclic mechanical loading applied in vivo to percutaneous implant.
Figure 2. Micro-CT images of the peri-implant region of the same mechanically loaded rat tibia at 2, 4, 7, 9, 11, and 14 days post implantation. Scans are 3D registered based on fixed implant microspheres to reveal local changes in mineralized tissue.

Figure 3. Peri-implant bone volume (%BV/TV) as a function of time (days). The bone volumes for both loaded and unloaded implants were observed to increase up to day 11.