Delayed Treatment of Critically-sized Femoral Defects in a Rat Model of Chronic Non-union

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Introduction: Large bone defects are one of the most challenging problems faced by orthopedic surgeons today. Over 600,000 bone grafting procedures are performed in the US every year with an associated cost of over 5 billion dollars [1,2]. Although there have been improvements to these procedures in recent years, delayed union and non-union are not uncommon, and often result in multiple revision surgeries involving more grafts and/or BMP-2 delivery. Despite the magnitude of this problem, the pathophysiology of non-union remains poorly understood and the critical events that determine whether bones do or do not heal are not well known. Consequently, therapeutic interventions for these delayed union and non-union cases are unclear and may require considerations different from treatment of primary injuries. Our goal was to understand the healing of segmental bone defects originally fated for non-union, but subsequently subjected to a second revision surgery and delayed treatment with BMP-2 - a clinically relevant therapeutic route. Previous work in a critically-sized femoral segmental defect rat model has established a range of BMP-2 doses delivered in an alginate-based hybrid delivery system that induce varying levels of healing, or mineralized bridging of the defect [3]. We hypothesized that the previously determined minimum dose required for healing (2.5 μg BMP-2) would not be adequate to heal an established non-union but a higher dose (10 μg BMP-2) would.

Methods: All procedures were reviewed and approved by the Georgia Tech IACUC. Eight 13-week-old female Sprague-Dawley rats received unilateral 8 mm critically-sized mid-femoral bone defects. A perforated polycaprolactone (PCL) nanofiber mesh tube was implanted around each bone defect, and then treated with 150 μl of RGD alginate hydrogel, 150 μl of RGD alginate hydrogel plus 1 μg BMP-2, or left empty. All of these treatments have been shown previously by our group to result in non-union. After 3 weeks, a second operation was performed where the nanofiber mesh and enclosed tissue were removed, a new mesh was implanted, and a healing dose of 2.5 or 10 μg BMP-2 was delivered in RGD alginate. Bone regeneration was assessed via in vivo radiographs and quantitative microcomputed tomography (μCT) scans at 2, 4, and 8 weeks.

Results: Radiographs and μCT analysis revealed extensive bridging of all except one defect (alginate, 2.5 μg BMP-2) by 8 weeks after the second operation (Fig. 1). Quantitative μCT analysis did not suggest differences in bone volume or bone mineral density between treatment groups (Fig. 2 and 3). However, there were large increases in both parameters from 4 to 8 weeks for all treatment groups. Interestingly, we observed a “ballooning” effect of mineralization with the high 10 μg BMP-2 treated defects, reminiscent of heterotopic bone formation with high doses of BMP-2 (Fig. 1). Further μCT quantification is ongoing and may reveal differences in the spatial patterns of bone formation.

Discussion: Despite this being a pilot study with only preliminary data available, interesting trends are already apparent. Even with the low sample numbers, we can infer that delayed delivery of BMP-2 to an established non-union, at least in the hybrid delivery system, does not impede regeneration. Furthermore, adequate healing with delayed BMP-2 treatment may not require higher BMP-2 doses after all, which could in turn lead to better spatial definition of the mineralization response.
Biomechanical testing and histological characterization of these defects are planned at 12 weeks of healing for this ongoing study. An improved version of this experimental model is proposed for future studies by allowing non-union to progress longer than 3 weeks, to when the bone ends have capped. This will provide the most challenging scenario in which to overcome non-union and will better recapitulate what is observed clinically with delayed union and non-union injuries.

**Significance:** Currently, most large bone defect animal models involve creation of the defect followed immediately by a treatment. This fails to account for cases where tissue ingrowth has already occurred in the defect and/or non-union has been established. Therefore, developing a chronic non-union animal model addresses a clear clinical need. Once fully characterized, this model will be a useful tool for investigating non-union and aid in the development of better treatment strategies.

![Image](image.png)

**Figure 1:** Representative 8 week radiographs of each treatment group.
**Figure 2:** μCT analysis of bone volume (BV) at 4 and 8 weeks (n=1-2 per group)

**Figure 3:** μCT analysis of bone mineral density (BMD) at 4 and 8 weeks (n=1-2 per group)