Exploring the Efficacy of Additively Manufactured PLGA Implants for Fracture Repair at Early Time Points

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Introduction

Bone fractures can result in significant physical disabilities, chronic pain, increased healthcare costs, and an overall lower quality of life.[1] It has been established that micromotion at the fracture site can improve healing outcomes, so there is new interest in developing less rigid implants such as non-metallic plates.[2] Poly-lactic-co-glycolic acid (PLGA) is an attractive candidate material for bone plates due to its relatively high mechanical strength, biocompatibility, and controllable degradation kinetics, all of which make it suitable for fracture repair.[3] Additionally, its degradation products have been shown to promote osteogenesis and angiogenesis.[4] PLGA has been used in a variety of bone healing applications via additive manufacturing (AM).[5] However, we still do not know if AM PLGA can be used to create effective fracture implants. The purpose of this in vitro and in vivo study was to explore the potential for AM PLGA implants as devices for fracture repair at early healing time points. We hypothesized that AM PLGA implants would have decreased mechanical strength in comparison to non-degradable control implants, and that the bone healing response between groups would be similar.

Methods

In an IACUC-approved study, 19 male Sprague-Dawley rats underwent bilateral osteotomies of the femora (Figure 1). Each femur was fixed with either a PLGA (Lattice Medical) or BioMed Clear Resin (Formlabs) implant. PLGA implants were fabricated on a fused deposition 3-D printer with 85:15 PLGA filament (Prusa i3 MK3 3-D), and the resin implants were synthesized via photocuring (Formlabs Form 3). Because PLGA could not be sterilized in an autoclave, PLGA implants were soaked in 70% ethanol for 30 minutes. Resin implants were autoclaved. The polymer plates (193535 mm) were held in place with 4 non-locking screws (0-42 3 3/8"). The rats were allowed to weight-bear immediately after surgery. Rats were sacrificed at 3 and 6 weeks. Histology (n56) and micro-CT analyses (n56) were conducted at 3 and 6 weeks post-surgery. Torsional testing of healing femora was conducted at 6 weeks by performing a 90 ° internal rotation of the femur at 3°/sec (n57). Micro-CT outcome measures of the fracture callus included bone volume (BV) mean density, total volume (TV) mean density, and the BV/TV fraction. Histological analysis included Safrinin-O/FastGreen, hemotoxylin and eosin (H&E), and Picrosirius Red staining. Implants were harvested from all sacrificed animals and kept frozen at 220°C. To assess differences between in vitro and in vivo degradation of PLGA implants, additional PLGA and resin implants were manufactured (n510 per group) and incubated. Specimens were kept at 37°C on a rocker in a solution of 30% fetal bovine serum, 69% PBS, and 1% v/v Penicillin-Streptomycin-Fungizone.

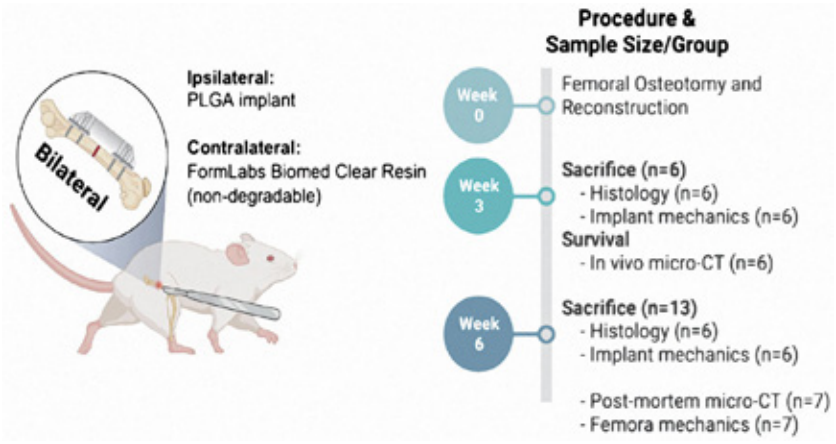

Figure 1. Left: Schematic of surgical procedure. Right: Relevant study timepoints including micro-CT, histology, and mechanical testing.

Serum changes were completed every 3-4 days. Harvested implants from the in vivo study and in vitro implants were subjected to torsional testing at 0, 3, and 6 weeks (90° rotation at 1°/sec). The primary mechanical testing outcome measure was virtual torsional rigidity (VTR). T-tests were used to make comparisons between groups at each time point. Paired t-tests were used to compare bones within each rat. A one-way ANOVA with a Holm-Sidak post-hoc test was conducted to compare outcomes from each implant type across all time points. Kruskal-Wallis tests with Dunn's post-hoc were used on nonparametric data sets. Significance was set to p,0.05.

Results

Micro-CT analysis revealed that PLGA significantly increased callus bone volume mean density from 3 to 6 weeks, but resin did not (Figure 2A). Significant increases in total volume mean density (Figure 2B) and BV/TV fraction (Figure 2C) existed for both implants between timepoints, but there were no differences between groups. Torsional testing of the femora at 6 weeks revealed no differences in VTR (Figure 2D). Histology results were still pending at the time of writing this abstract. In vitro degradation demonstrated significantly stiffer PLGA implants than resin at 0 and 3 weeks, but not 6 weeks (Figure 3A). PLGA implants retrieved from the in vivo study were different at all time points, and there were no significant differences between groups at 3 and 6 weeks (Figure 3B).

Discussion

To our knowledge, this is the first study to investigate

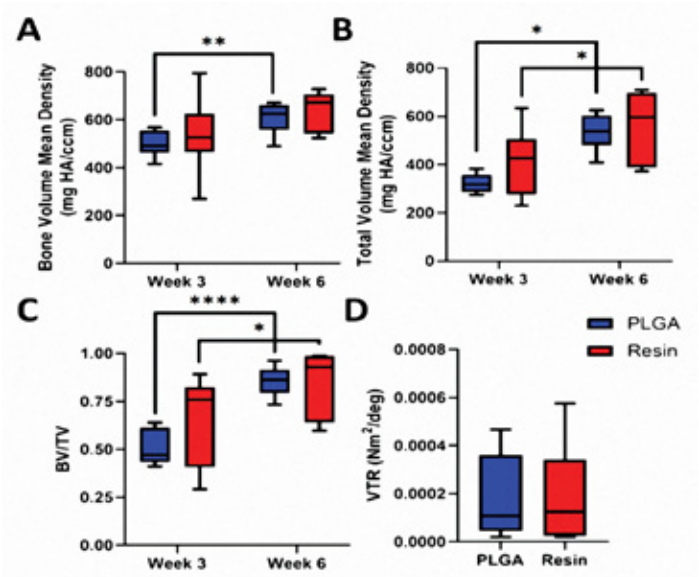

Figure 2. (A-C) Quantitative assessment of bone callus healing via micro-CT and **(D)** mechanical testing. *p<0.05, **p<0.01, ****p<0.0001.

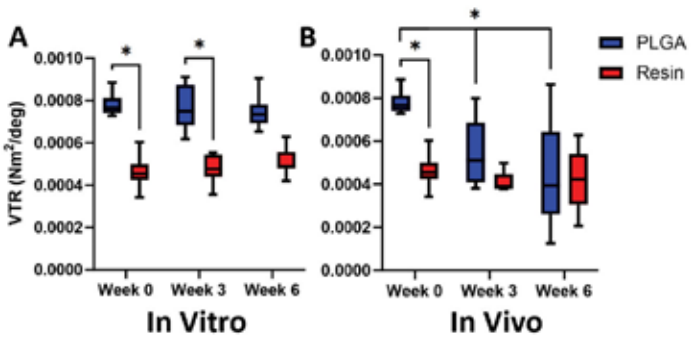

Figure 3. Mechanical testing results from in vitro **(A)** and in vivo **(B)** PLGA and resin implants. *p<0.05.

the effects of AM PLGA implants at early time points in fracture repair. At 3 and 6 weeks, we observed fracture healing, as indicated by the increase in BV mean density, TV mean density, and BV/TV. Notably, use of PLGA and resin implants led to similar bone healing responses. In vitro and in vivo analysis of the implant degradation demonstrates that mechanical loading in vivo significantly increased the degradation rate of the PLGA implants. These results reveal that unloaded in vitro degradation assays do not accurately reflect the degradation kinetics of AM PLGA, which is important for future experiments that will focus on PLGA implant form and function. Importantly, we found that PLGA implants did not have any detrimental effects on fracture healing progression at short time points (3-6 weeks). Further analyses at longer time points, when the strength of PLGA implants begins to go to zero, are necessary to determine the long-term relationships between AM PLGA implant degradation on mechanotransduction during bone healing.

Significance/Clinical Relevance

At early time points in the fracture healing process, the mechanical properties of biodegradable PLGA fracture implants were similar to matched non-degradable resin devices. Bone healing responses were similar between the two groups. We are encouraged by this finding, and we believe that the benefits of implant degradation at longer time points will lead to accelerated and improved bone repair.

References

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