Cerclage Wire for Affordable Plate Fixation in Murine Critical Sized Defect Models

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INTRODUCTION: Bone can naturally repair itself relatively easily when affected by a small, well-stabilized defect. However, defects caused by trauma, osteomyelitis, or cancer resection that are greater than ~2.5cm or 2x the injured bone's diameter usually cannot heal spontaneously despite adequate stabilization. These critical-sized defects (CSDs) require robust reconstruction interventions to preserve limb function and avoid amputation. Orthopedic research has established that rodents largely parallel the bone repair mechanisms of humans, making rodents valuable models to investigate bone repair and CSD treatment. Replicating CSDs in mice is technically challenging due to the exceptionally small dimensions and costly since precision implants, tools, and surgical guides are needed. Ideally, internal plate fixation can be used to better replicate clinical implementation and to limit secondary, transcutaneous infection. Unfortunately, commercially available microscrews for integrating bone and the limb-stabilizing plate can be cost-prohibitive as they can easily cost well over $60 per animal plus additional premium costs for the accompanying precision drill/saw guides, drill bits, and screwdrivers. **The objective of this study was to create a more affordable alternative for murine CSD research using cerclage wire.** To thoroughly challenge this method, we created internal fixation environments of varying anticipated stability by altering the plate material and the defect size. **We hypothesized that cerclage wires could be used to achieve satisfactory fixation at a much lower cost, even with high motion implants.**

METHODS: Material properties of Tough 1500 and polyether ether ketone (PEEK) plates were characterized *in vitro* and then their efficacy was evaluated *in vivo*. Tough 1500 is a relatively weak 3D resin from FormLabs, while PEEK is a radiolucent polymer with strong mechanical properties and the current standard for *in vivo* plate implantation. To estimate *in vivo* performance, plate specimens were exposed *in vitro* to different conditions: 1XPBS vs air, room (25°C) vs body (40°C) temperature, 24 vs 48 hours. All plates (n=3-4/group/material, N=31-32/material) were subjected to 3-point bend testing (preload=3N, 0.1mm/s displacement to failure). Results were analyzed via staged MANOVA leading to pooling of time and temperature (final pooled factors: material & medium, final factors when blocked by material: medium). IACUC-approved CSD survival surgeries (Fig1A-B) were performed on the right femora of C57BL/6 mice to implant a plate and remove a 3mm or 4mm bone segment from the mid-diaphysis. Four conditions were tested: 1) Tough 1500 plate + 3 mm defect (N = 13 mice, 8M/5F, 26.9±7.1 weeks old); 2) PEEK plates + 4 mm defect (N=6 mice, 5M/1F, 16.4±0.29 weeks old), 3) PEEK plate + 3 mm defect (N=10 mice, 4M/6F, 16.2±0.62 weeks old), 4) PEEK plate + 3mm defect + morselized bone graft from sex-matched littermate (N=10, 4M/6F, 16.5±0.52 weeks old). To assess plate stability and wire, three unbiased assessors were given a standard grading guide (Fig 1C-D) to blindly grade randomized x-ray pairs for all non-grafted groups (1, 2, & 3) taken up to 20 weeks post-operation (≤10 timepoints/animal, N=133). Their independent scores were averaged and tested via Kruskal-Wallis non-parametric test. The grafted group (4) was taken out 8 weeks but was not included in the x-ray grading. To verify wire integration and non-union even in the presence of a graft, microCT scans were taken of all PEEK-plate groups (2, 3, & 4) to determine union status as well as quantify bone volume fraction and mineral densities within the defect (MicroCT 50, ScanCo Medical; 70kVp, 114µA,8W, 15µm, Dragonfly software, Version 2022.2, Object Research Systems, Inc.). Data collection is on-going as 9 out of 26 samples have been analyzed. Results collected to date were analyzed with one-way ANOVAs between groups 2 & 3 and 3 & 4.

RESULTS: Initial MANOVA showed neither plate materials were significantly affected by time or temperature. After pooling, PEEK significantly outperformed ($p<0.0001$) Tough 1500 in every metric analyzed (i.e. stiffness, elasticity, rigidity, toughness, yield strain and stress, ultimate strain and stress, and break strain and stress). There were significant covariations in toughness, yield strain, ultimate stress, and break stress, so these metrics were analyzed after blocking for material. Tough 1500 was significantly different ($p<0.0001$) between mediums in all 4 metrics while PEEK was significantly different ($p<0.05$) in only ultimate stress and break stress (Fig2 A-B). X-ray grading confirmed an unstable environment was achieved *in vivo* with Tough 1500 plate as evidenced by increased incidence in plate breaks requiring early removal (Fig 2C). Plates greatly deformed after 2 weeks causing severe bone misalignment. Regardless, the wires were relatively undamaged and secured the plate without bone lysis or overgrowth (Fig 2D). Both PEEK groups kept bones well aligned and demonstrated good wire integration with the 3mm defect group being more consistently stable. MicroCT analysis is currently ongoing, but preliminary data has shown no differences in BV/TV, BMD, or TMD between the 3 and 4 mm groups without bone graft. BV/TV increased between the 3mm grafted and 3mm non-grafted groups while there is no difference in BMD or TMD; x-rays indicate that 3/10 grafted samples achieved union while none of the non-grafted samples united (Fig3A). While not yet quantified, all bones showed excellent wire integration.

DISCUSSION: In vitro mechanical testing of Tough 1500 and PEEK plates indicated the former is much weaker, especially when exposed to internal body conditions. In contrast, PEEK plates were either unaffected or strengthened by exposure to PBS, confirming their superiority for internal fixation. These results support that both unstable and ideal environments were achieved. X-ray grading of Tough 1500 plates showed that while plates often failed 4-weeks post-implantation, the cerclage wires were relatively undamaged and continued plate fixation without compromising the bone via lysis or new bone growth. PEEK plates generally had much better stability and the cerclage wires in these animals performed as well as or better than those in the Tough 1500 group. Even at 10 weeks post-op, none of the PEEK implanted animals needed to be removed. This was expected given the superior mechanical properties of PEEK. Preliminary microCT results showed non-grafted animals were unable to achieve union, even 20 weeks post-op, indicating a true CSD model was achieved. Additionally, bone growth directly upon the cerclage wires was observed, further indicating satisfactory fixation. Most importantly, the low union rate (30%) of the grafted group mimics clinical findings using grafts alone in large CSD repair. This demonstrates that interventions tested with our model would reflect treatment effectiveness not confounding innate repair capacity. A limitation of this study is that a 4 mm group with Tough 1500 plates was not included. As this would have been an extremely unstable environment, we did not include this group since it would be inconducive to the animals' well-being. X-ray grading is currently being reperformed by orthopedic surgeons and microCT analysis is ongoing.

SIGNIFICANCE: Cerclage wires were able to maintain fixation without causing extreme damage to the bone material despite plate instability, indicating that cerclage wires are a viable option for plate fixation in murine CSD models. Additionally, the low union rate of the grafted mice indicates a need for advancement in CSD repair. **Developing a fixation method that is both affordable and durable could make mouse CSD repair research more accessible to labs with limited resources and thereby expedite the improvement of CSD treatments.**

IMAGES AND TABLES: