Effects of Scaffold Chemistry and Fixation of 3D-Printed Growth Plate Mimetic Composite for the Treatment of Growth Plate Injuries

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INTRODUCTION: The growth plate is a cartilage layer near the ends of long bones in children and responsible for longitudinal bone growth. Growth plate injuries (GPI) can result in bony bar formation and subsequent skeletal deformities. Our group has developed a 3D-printed growth plate mimetic composite that improves limb lengthening and reduces angular deformity after GPI [1]. The composite consists of a 3D-printed scaffold that provides mechanical support that is then infilled with a soft cartilage-mimetic hydrogel that provides chondrogenic cues. While promising, the composite often gets embedded into newly forming bone and does not remain with the growth plate over time. Maintaining the scaffold within the growth plate area during bone lengthening may improve outcomes. A composite that degrades once skeletal maturity is reached would also be beneficial. The objectives of this study were to: (1) evaluate a composite containing a degradable scaffold and (2) tether the composite to the epiphyseal bone during implantation. Outcomes such as composite location, limb length and angular deformity were compared to our original 3D-printed growth plate mimetic composite [1].

METHODS: Twenty-four male NZW Rabbits (6 weeks old) were subjected to a GPI [1] in the right tibia (left-uninjured control). Animals were immediately treated with a composite consisting of a scaffold 3D printed with either poly(ethylene glycol) diacrylate resin (PEGDA) [1] or degradable poly(β-amino ester) diacrylate (PBAE B6) resin and infilled with degradable poly(ethylene glycol) cartilage-mimetic hydrogel [2]. Within each scaffold chemistry, animals were further divided into: composites anchored to the epiphysis (via suture; Fig. 1A) or unanchored (n=6/group). Rabbits underwent weekly X-ray imaging (60kV, 0.4mA) and were euthanized at 2 weeks post-treatment to evaluate limb length and tibial angle. Micro-computed tomography (microCT) (voxel size of 17.2 µm) was performed at euthanasia to assess bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th.), and trabecular spacing (Tb.Sp.) and normalized scaffold location relative to the growth plate. Repair tissue was evaluated with alcian blue hematoxylin (ABH) staining. Paired Student's t-test with control limbs and mixed model analysis with post-hoc Tukey's HSD determined differences between scaffold chemistry and anchoring ($p<0.05$).

RESULTS SECTION: Limb length and tibial angle were assessed as percent difference (with contralateral control; 0% = no difference); neither measure indicated a significant effect due to chemistry (F=0.98, 0.25) or anchoring (F=0.57, 0.23) (Fig 1B and 1C). Similarly, no significant differences were seen when comparing across treatment groups for any bone morphology measures either with uninjured controls or between treatment groups (BV/TV F=0.09, Tb.Th. F=0.25, Tb.Sp. F=0.52) (Fig 2). Histological imaging suggests that the anchored scaffolds remained more aligned with the growth plate (Fig. 3A). Positional measurements from microCT showed that when normalized to the growth plate (ideal location=0) the PEGDA composite without an anchor had a mean location of 0.48 ± 0.30 while the anchored composite had a position of 0.26 ± 0.10 (Fig. 3B). While statistically non-significant these results suggest that the PEGDA anchored composite maintained a position nearer the growth plate compared to the unanchored PEGDA (a similar trend was not observed with the B6 chemistry) (Fig 3B).

DISCUSSION: No differences were seen between the injured and control limbs (low % difference values) suggesting, as previously shown [1], that the composite helps to prevent clinical deformities. Modifying the 3D printed scaffold to a degradable resin had no statistical impact on any outcome measure. Anchoring the composite tended to maintain the scaffold position closer to the growth plate, but no statistical differences were seen in any outcomes regardless of anchoring. Additional samples may be needed to increase power, and longer timepoints might be required to observe impacts of anchoring. Likewise, a more comprehensive evaluation of the rate of degradation of the 3D-printed B6 scaffold is needed.

SIGNIFICANCE/CLINICAL RELEVANCE: Modifying the 3D printing resin to a degradable PBAE chemistry did not have any significant impact on any outcome measure assessed compared to our original PEGDA scaffold, and PBAE scaffolds maintained the ability of the PEGDA scaffolds to reduce angular deformities after 2 weeks post treatment . As well, anchoring the 3D-printed composite tended to better maintain the scaffold position with the growing limb. These two modifications will continue to be used in future studies to assess the therapeutic potential of our 3D-printed cartilage mimetic composite for growth plate injury.

REFERENCES: [1] Yu et al.,2022, *NPJ Regen. Med.* 7(1):60 [2]: Schoonraad et al.,2021., *Biofabrication*. 13(4)

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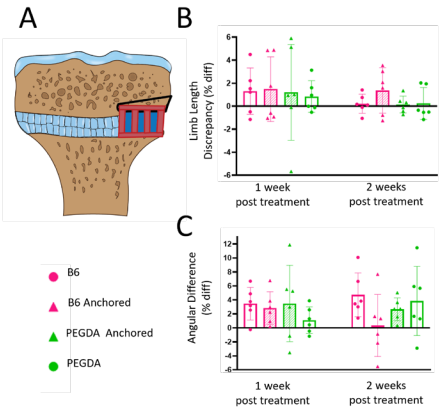

Fig 1: A) Limb length difference B) Tibial angle difference

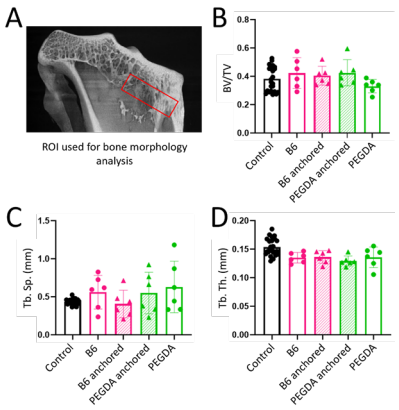

Fig 2: A) microCT region assessed for morphology (red outline) B) BV/TV C) Tb.Sp. D) Tb.Th.

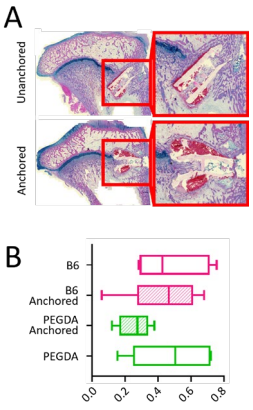

Fig 3: A) ABH B) Scaffold position normalized (0=growth plate)