INTRODUCTION
It is estimated that 20% of fractures sustained by children involve the growth plate [1]. In addition to damage during fracture, injury to the growth plate may also be caused by disuse, radiation, infection, and tumors [2]. Growth plate injury may result in formation of a bone bridge through the defect region that connects the epiphyseal and metaphyseal plates and can lead to limb length discrepancies and life-long musculoskeletal difficulties due to abnormal joint loading. Due to the limited success rate of existing clinical treatments, alternative therapeutic approaches are needed.

Small animal models may offer an effective test-bed for screening potential strategies for the treatment of growth plate injuries. Microcomputed tomography (μCT) has been previously used to visualize 3D changes in rat growth plate morphology and bony tether formation [3]. The goals of this study were to establish μCT-based methods to quantify alterations in the growth plate response to injury and test the ability of an in situ gelling hydrogel to restore growth function.

METHODS
All protocols were approved by the IACUC committee at Georgia Tech. A 2.0 mm central defect was created across the physis of the distal femur of male Sprague Dawley rats by drilling through the articular cartilage between the condyles, normal to the cross-sectional plane of the bone shaft. The depth of defect into the metaphyses was controlled to ~4 mm with a mechanical stop. The defect was then either left empty or filled with 1% agarose for 28, 56, or 112 days. Whole femurs and epiphyseal regions from the defect and contralateral control legs were scanned on a microCT system (Scanco Medical, Switzerland) at voxel sizes of 34 μm and 20 μm respectively. To determine the degree of bone bridge formation, the volume of bone infiltration into the original defect site was measured by isolating a 2 mm x 0.3 mm cylindrical region contained entirely within the growth plate. Whole bone lengths were measured from the images. Also, the bone volume fraction in the growth plate outside of the defect region was determined. The growth plate bone volume fraction (BVF) is a measure of mineralized tissue formation (tethers) between the epiphyseal and metaphyseal plates and can lead to limb length discrepancies. We have also shown that implantation of a conformal filling agarose with minimal cellular infiltration and some regions of mineralization, but no evidence of growth plate regeneration.

RESULTS
Characterization of the baseline healing response of growth plate defects
The volume fraction of bone infiltration into the original defect within the growth plate (bone bridge formation) was 26.1±5.3% and did not increase significantly with time (data not shown). At all time points, femoral length was significantly reduced in defect limbs compared to contralateral control limbs (Figure 1A). The growth plate became thinner over time and the presence of a defect through the tissue resulted in a further reduction in average thickness as compared to contralateral controls by day 56 (Figure 1B). Day 112 growth plates were too thin to reproducibly isolate from the surrounding bone tissue. The bone volume fraction within the entire growth plate (tether formation) was higher in the defect limb by day 56 (Figure 1C). Interestingly, the length of the defect legs expressed as the percent difference of controls was positively correlated in a non-linear fashion to both the percent difference in growth plate thickness (Figure 1D, R²=0.693) and tether formation (not shown, R²=0.508). Examples of the growth plate thickness maps and histological confirmation of tether formation are shown in Figure 2.

Effects of acellular agarose on the healing response of growth plate
Although implantation of agarose into the defect showed no improvement in growth plate thickness or tether volume fraction over empty defects, there was a significant improvement in percent of growth reduction (Figure 3). The limb length discrepancies between control and defect legs decreased from 9.7±4.06% in the empty defect to 5.02±1.19% in those defects filled with agarose after 56 days. Also shown in Figure 3 is a histological image of the defect region demonstrating the presence of agarose remnants with little cellular infiltration and some regions of mineralization, but no evidence of growth plate regeneration.

DISCUSSION
This small animal model replicates the key clinical features of growth plate injuries, including bone bridge formation and growth reduction. Compared to intact control limbs, transphyseal injury reduced growth rate by 63% between 28 and 56 days and 90% between 56 and 112 days. Based on the results of these studies, the mechanisms of limb shortening may include the observed cellular disorganization of the surrounding growth plate, an accelerated decrease in growth plate thickness, and an increase in bony tether formation. We have also shown that implantation of a conformal filling agarose within the defect decreased limb length discrepancy, but the fact that it did not restore normal growth plate function demonstrates the need for more sophisticated treatment strategies. Based on the results of this study, in situ gelling agarose is a viable scaffold for use in tissue-engineering approaches to growth plate defect repair. Future work in this area will include using agarose to deliver aggrecan, a major component of cartilaginous tissues, and cells that may promote growth plate regeneration and growth restoration.

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REFERENCES