Sulfated Glycosaminoglycan (GAG)-mimetics for the Repair of Osteochondral Defects

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INTRODUCTION: Over 500 million people are affected by osteoarthritis (OA) globally. OA is a chronic joint disease resulting in cartilage damage and if left untreated, these injuries will continue to deteriorate. Current surgical methods, such as allograft and autologous chondrocyte implantation have failed to restore functional, hyaline cartilage tissue. We have demonstrated in *in vitro* studies sulfated glycosaminoglycan (GAG)-mimetics derived from cellulose show promise for cartilage repair (1, 2) and in *in vivo* studies, fully-sulfated cellulose (NaCS) demonstrated the greatest cartilage repair when used to treat osteochondral defects in a rabbit trochlear groove model (3, 4). In this study, we investigated the NaCS-containing scaffold in an osteochondral defect in the medial condyle to more closely mimic the clinical-scenario where cartilage defects occur and is also a weight-bearing site. Comparisons were made with scaffolds containing partially sulfated cellulose (pSC) and native GAGs (chondroitin sulfate C-CS and heparan sulfate-HS). Since previous *in vitro* studies in dynamic loading conditions showed that complexing the GAG-mimetic with transforming growth factor-beta3 (TGF-β3) promoted chondrogenesis and resulted in a reduction in hypertrophic markers (5), we also investigated complexing the NaCS-containing scaffolds with TGF-β3 in this *in vivo* study.

METHODS: 5 wt.% GAG-mimetics (NaCS, pSC) and GAGs (CS and HS) were combined with gelatin to form fibrous scaffolds using the electrospinning technique, as previously described (5). Gelatin scaffolds alone and defect only served as controls. For studies complexing/loading with TGF-β3, TGF-β3 (10 ng/ml or 1 ng per scaffold, recombinant human, Prospec Bio) was incubated with NaCS-containing and gelatin scaffolds for six hours prior to implantation. Osteochondral defects (3 mm diameter by 3- 4 mm deep) were created bilaterally in the medial femoral condyle of New Zealand White rabbits (2-3 kg, male and female) using an IACUC approved protocol. An n of 6 was used per group, except for NaCS and pSC (n of 5 due to postoperative complications i.e. wound dehiscence). At 12 week post-implantation, harvested tissues were evaluated using a modified ICRS macroscopic scale (using a 12 points scale), processed for micro computed tomography (microCT) for bone volume fraction (BV/TV), fixed and processed for decalcified histology (H&E, safranin-O and fast green), immunostaining for types I and II collagen, and evaluated by modified ICRS histological assessment (using 12 point- modified ICRS scale). Mechanical testing was performed (n=4 per group) by determining the stiffness (N/mm) of the cartilage using a custom set-up. Stiffness of the treated groups were normalized to the stiffness of normal/healthy cartilage. Kruskall-Wallis non-parametric test was performed to test statistical differences between groups, $p<0.05$ for scoring data and a t-test was performed for mechanical testing data. Post-hoc planned comparisons were performed using Dunn's test for non-parametric test.

RESULTS SECTION: Cartilage repair and restoration of subchondral bone was evaluated at 12 weeks post-implantation. Initial studies using GAG-mimetic- and GAG-containing scaffolds alone (without TGF-β3) resulted in no statistical differences in microCT, macroscopic and histological scores where macroscopic and histological scores were appox. 7 and 6, respectively at 12 weeks. These findings were unlike previous results in the trochlear groove where NaCS-containing scaffolds repaired defects by 12 weeks as noted by a higher histological score (3, 4). Therefore, TGF-β3 was added to scaffolds by preconditioning in a TGF-β3 solution prior to implantation and evaluated in the medial condyle defect model. Histologically, the repair tissue had more columnar cellular distribution with proteoglycan staining for the NaCS+TGF-β3 group as compared to scaffolds without TGF-β3 and defect only controls (Fig. 1A). For the NaCS+TGFβ3 group, subchondral bone fill was 50-60% (Fig.1B) and showed improved integration with surrounding cartilage and a smoother surface in comparison to NaCS alone and the defect only groups, as noted by the statistically significant macroscopic score (Fig.1C). At 12 weeks, the stiffness of NaCS+TGFβ3 group was significantly higher than the Gelatin+TGFβ3 treated defects (Fig. 1D). Ongoing analyses include histological scoring, and immunostaining (types I and II collagen), and the analysis of the synovial fluid for inflammatory cytokines.

DISCUSSION: The present work evaluated GAG mimetic-containing scaffolds in osteochondral defects (in the medial condyle). The use of NaCS with bound TGF-β3 showed improved repair as noted by macroscopic scoring and mechanical testing at 12 weeks, which is considered early to mid-stage healing in this defect model. Statistical differences were not detected for gelatin loaded with TGF-β3 in comparison to the defect only group. Previous *in vitro* studies have demonstrated that TGF-β3 sequestration and retention was greatest for scaffolds containing NaCS as compared to other GAG-mimetics, native GAGs and gelatin alone (5). Therefore, the *in vivo* results in this study suggest that NaCS may provide a more effective delivery of TGF-β3. This study demonstrates the potential of the GAG-mimetic containing scaffold for cartilage repair.

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SIGNIFICANCE/CLINICAL RELEVANCE: These results demonstrate the potential of a GAG mimetic-containing scaffold for cartilage repair.

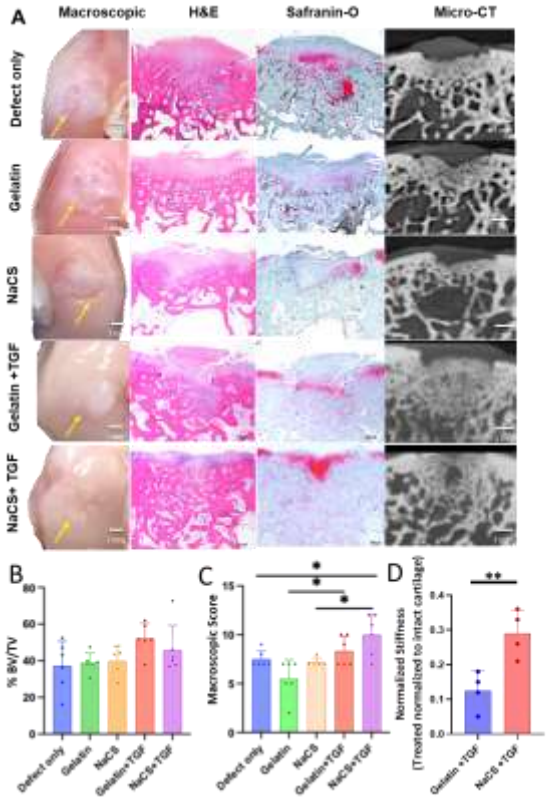

FIGURE 1. Osteochondral defects in the medial condyle treated with NaCS-containing scaffolds and Gelatin control scaffolds with or without TGF-β3 in comparison to defect only at 12 weeks post-implantation. A) Macroscopic appearance of the defect, histology (H&E,, Safranin O-fast green), and microCT B) % BV/TV. C) Macroscopic score. D). Normalized stiffness. *,** $p<0.05$.

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