# TissueGene-C (TG-C) Improves the Structural Recovery of Degenerated Discs in Rabbit Anular Puncture Model

Yujia Ge[1], Kiersten Red[1], Elisabeth Orozco[1], Madeline Brown[1], Mary Esparza[1], Yejin Do[1], Xi Wang[1], Jiyo Athertya[1], James Lo[1], Huan Tran[2], Moon Jong Noh[2], Yajun Ma[1], Koichi Masuda[1]

[1]University of California, San Diego, La Jolla, CA [2]Kolon TissueGene Inc, Rockville, Maryland

Email of Presenting Author; y8ge@health.ucsd.edu

**Disclosures**: Y Ge (N), K Red (N), E Orozco (N), M Brown (N), M Esparza (N), Y Do (N), J Athertya (N), J Lo (N), H Tran (3A-Kolon TissueGene, Inc), MJ Noh (3A-Kolon TissueGene, Inc), Y Ma (3B-Kolon TissueGene, Inc, Histogen Inc), K Masuda (3B-Anges Inc; 5-Anges Inc, Kolon TissueGene, Inc; 6-Histogen Inc, Darnatein; 8-JOR Spine, JOR)

**INTRODUCTION:** Degenerative disc disease (DDD) is a common disorder that causes low back pain and dysfunction. Recent studies revealed that growth factors, such as transforming growth factor-beta 1 (TGF-β1), have positive effects on extracellular matrix (ECM) metabolism and cell proliferation in vitro and induce structural repair of intervertebral discs (IVDs) in various animal models [1,2]. TissueGene-C (TG-C) is a novel cell-mediated therapy comprised of allogenic non-transduced human chondrocytes and irradiated/transduced GP2-293 cells expressing TGF-β1 that has been shown to improve pain and reduce cartilage degeneration in patients with knee osteoarthritis [3,4]. Previous studies have shown that using TG-C led to strong analgesic effects in the rat monoiodoacetate-induced model of osteoarthritic pain by downregulating pain mediators and inhibiting neuronal sensitization [5]. To show the clinical utility of this cell therapy product to treat DDD, the effect of the intradiscal injection of TG-C on structure modification of disc degeneration needs to be confirmed in larger species. Using the rabbit anular puncture model, we hypothesize that an intradiscal injection of TG-C can inhibit or reverse chronic disc degeneration. The objective of this study was to compare radiographic and magnetic resonance imaging (MRI) changes between groups that received TG-C treatment and the untreated group to determine the efficacy of TG-C as a potential therapeutic intervention.

**METHODS:** All experiments were performed with IACUC approval of our institution.
**Rabbit Anular Puncture Disc Degeneration Model [6]:** Surgeries and Injection of TG-C: Under general anesthesia, lumbar IVDs of female New Zealand white rabbits (n=80, five months old) were exposed, and an anular puncture (18-gauge, 5 mm depth) was performed at two non-continuous discs (L2/3 and L4/5), with the disc (L3/4) between the punctured discs left intact as a control. Four weeks after the initial puncture, either the control (CS10; 10 µL per disc) or TG-C (Three doses Low;$1.5x10^4$, Mid; $5x10^4$, High; $1.5x10^5$; 10 µL per disc) was injected into the center of the nucleus pulposus (NP) using a fine tip 26-gauge needle (XX*MS16, Ito Corporation, Shizuoka, Japan) attached to a MS*GFN25 syringe (Ito Corporation). After 12 and 24 weeks, rabbits (10 per group) were sacrificed and subjected to radiographic and MRI analysis.
Radiographic analysis of disc height index (DHI): Lateral radiographs of the lumbar spine were obtained at two-week intervals up to 12 or 24 weeks after the initial puncture. IVD height was expressed as DHI, as previously described [7]. The average percent change in DHI of injected discs (both L2/3 and L4/5) was calculated for each postoperative disc as a ratio to its preoperative DHI [%DHI = (postoperative DHI/ preoperative DHI) ×100] and further normalized to the DHI of the non-punctured disc (L3/4): [Normalized %DHI = (punctured %DHI/non-punctured %DHI) ×100]. All radiographs were assessed by an observer blinded to this experiment.
Magnetic resonance imaging (MRI) analyses: After sacrifice at 12 or 24 weeks, MRI examinations on isolated spine segments were performed using a Bruker BioSpec 3T scanner (BRUKER, Billerica, MA, USA). The average degeneration grade of injected discs (L2/3 and L4/5) was calculated according to Pfirrmann grade [8] using T2 weighted sagittal images; the evaluations were performed by two observers blinded to experimental groups.
Statistical analysis: The DHI was analyzed by two- or three-ways repeated ANOVA. The MRI grading data were assessed using Kruskal-Wallis Test with the Bonferroni correction.

**RESULTS:** Radiographic assessment of disc degeneration: Two-way ANOVA analysis for normalized %DHI, throughout the post-injection period, showed a significant improvement for all TG-C-injected groups over the CS10 control group ($p<0.001$). After 24 weeks of injections, all TG-C groups (Low, 84.6%, Mid, 86.6%, High 87.2%) showed significantly higher normalized % DHI than that of the CS10 group (69.6%, vs. all TG-C groups $p<0.001$) (Fig. 1).
MRI assessment of disc degeneration grade (16 weeks): The Pfirrmann scoring, including both time points, showed a significantly low grade in the Mid and High TG-C groups, compared to the CS10 group (Three-way comparison, $p<0.001$, $p<0.05$, respectively, not shown in figures). The comparison for the 12-week group showed the same trends (Fig. 2a).

**DISCUSSION:** In rabbit anular puncture model, a TG-C intradiscal injection showed a significant recovery of IVD height and improved MRI scoring with a single injection. These results suggest that TG-C has the potential to induce structural modifications in degenerated discs.

**SIGNIFICANCE/CLINICAL RELEVANCE:** We have shown that a combination of allogenic non-transduced human chondrocytes and irradiated/transduced GP2-293 cells expressing TGF-β1 (TG-C) attenuated intervertebral disc degeneration in a rabbit anular puncture model. Therefore, the intradiscal injection of TG-C has potential as an effective therapeutic strategy for degenerated IVDs in human.

**REFERENCES:** [1] Lee H+. Inflammopharmacology. 2020; 28: 1237-1252. [2] Masuda K+. In Härtl R, Bonassar LJ eds. Biological approaches to spinal disc repair and regeneration for clinicians. New York: Thieme, 2017: 101. [3] Cherian JJ+. Osteoarthr. Cartil. 2015; 23: 2109-2118. [4] Lee MC+. Bone Joint J. 2015; 97-B: 924-932. [5] Park E+. Osteoarthr. Cartil. 2023; in press. [6] Masuda K +. Spine. 2005; 30: 5-14. [7] Kato K+. Eur Cell Mater. 2021: 42, 90-109. [8] Pfirrmann CW+. Spine. 2001: 26, 1873-1878.

**IMAGES AND TABLES:**

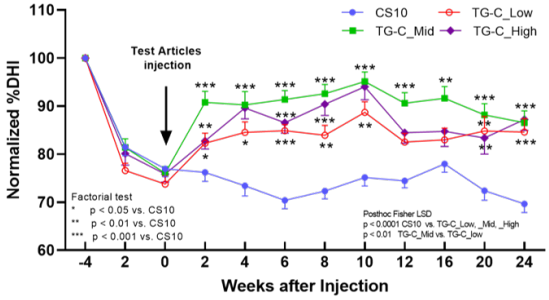


Figure 1. % Normalized DHI

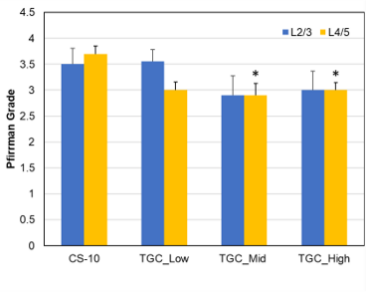


Figure 2a. Pfirrmann grade (12W)

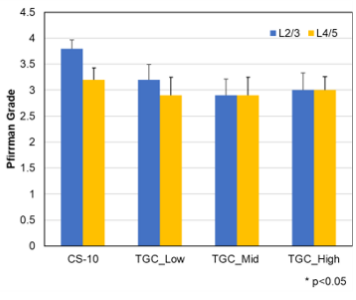


Figure 2b. Pfirrmann grade (24W)