Effect of Confined Bioreactor Culture on Human degenerated Nucleus Pulposus Explants

Y. Dilek[1], J. Du[1], J. P. Garcia[1], L. B. Creemers[1]
[1]Orthopedics, University Medical Center Utrecht, Utrecht, Netherlands
Y.Dilek@umcutrecht.nl

Disclosures: Yeter Dilek(N), Jie Du(N), Joao Pedro Garcia(N), Laura Creemers(N)

INTRODUCTION: Chronic low back pain (LBP) is predominantly attributed to intervertebral disc degeneration (IVDD), a complex process that remains a subject of ongoing debate in terms of its pathogenesis. Therefore, adequate models are needed to understand the mechanisms behind IVDD-associated LBP, thereby facilitating the development of innovative therapeutic strategies. Specifically, bioreactor-based systems, capable of restraining swelling and applying physiological loading, offer a unique avenue for investigating IVD degeneration. By utilizing living, degenerated disc tissue from human donors, these systems hold the promise of faithfully replicating the biological intricacies of the in vivo cellular microenvironment. The main objective of our investigation was to establish the validity of employing a bioreactor culture system, previously used with bovine NP tissue (Arkesteijn et al), for human nucleus pulposus (NP) tissue culture, focusing on the effect of culturing in confined conditions in comparison to unconstrained free-swelling.

METHODS: Human NP explants obtained from Thompson grade II/III donors (8 mm diameter) were cultured free swelling condition or in bioreactor culture chambers developed and described previously (Arkesteijn et al). T0 explants were utilized as reference controls. Medium was renewed twice weekly and conditioned medium collection. After 28 days, explant wet weight was measured and divided samples. Half of the tissues were paraffin-embedded for subsequent histological evaluations, while the other half were freeze-dried, their dry weight was measured and subjected to papain digestion for biochemical assays. Paraffin histology and immunohistochemistry employing Alcian blue and picrosirius red staining were employed to assess collagen type I&II and aggrecan expression. Biochemical assessments encompassed the DMMB assay for GAG content, hydroxyproline assay for collagen quantification, and PicoGreen for DNA content. Additionally, GAG content was measured in conditioned media, while enzyme-linked immunosorbent assays were employed to quantify interleukin-6 (IL-6), interleukin-8 (IL-8), and MMP activity.

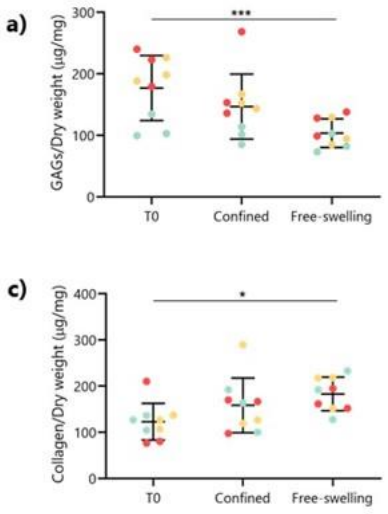
RESULTS SECTION: Free-swelling culture of NP tissue led to a diminished GAG content of the tissue, while collagen content was increased (Fig. 1). GAG release over time was higher in bioreactor culture. In bioreactor culture, also IL-6 and IL-8 production was increased (Fig.2). MMP activity was not detected.

DISCUSSION: NP tissue cultured in confined conditions seemed to maintain the initial matrix composition more effectively than under free swelling conditions. It remains unclear whether the higher release of GAGs in bioreactor culture stems from degradation or synthesis. Moreover, the implications of the increase in cytokine production remains to be investigated, although induction of degenerative enzyme activity was absent.

SIGNIFICANCE/CLINICAL RELEVANCE: This study advances our tools in understanding intervertebral disc degeneration, a major contributor to chronic low back pain, by utilizing innovative bioreactor-based models to replicate complex disc behavior. The use of human-derived tissue adds biological accuracy, offering insights into degenerative mechanisms that could guide the development of targeted interventions. In conclusion, the findings from this research have the potential to improve therapeutic approaches and patient care.

REFERENCES: Arkesteijn I., et al. A Well-Controlled Nucleus Pulposus Tissue Culture System with Injection Port for Evaluating Regenerative Therapies. Ann Biomed Eng. 2016; 44: 1798–1807.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No:825925 and from the Turkish government under Republic of Türkiye Ministry of National Education scholarship programme.



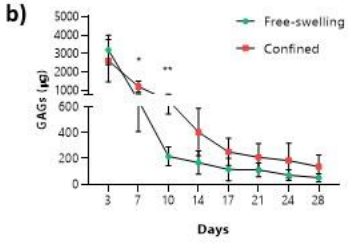


Figure 1: ECM content and release in NP explants after culture in free swelling or confined condition a) glycosaminoglycan (sGAG) content normalized to dry weight, b) GAG release in bioreactor and free swelling culture for 28 days, c) collagen content normalized to dry weight. *P<0.05

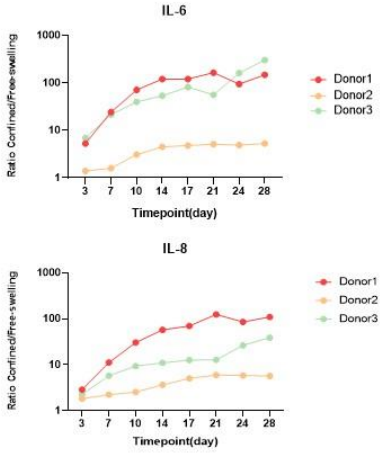


Figure 2: Secretion of IL-6 and IL-8 in NP explants after culture in free swelling or confined condition.