

# Investigations of the mechanical, biological and biochemical effects of asymmetric dynamic loading on the intervertebral disc

Straumann, Lukas

Master thesis, Master of Science in Life Sciences, Therapeutic Technologies

Principal: Prof. Dr. Michael de Wild, School of Life Science  
Expert: Dipl. Phys. Daniel Behr, Graviton GmbH  
Supervisor: Dr. Sibylle Grad, AO Foundation

## INTRODUCTION

Adolescent idiopathic scoliosis (AIS) is a severe deformation of the spine in young people, who were previously healthy children. The cause of this disease is still unknown, but it is assumed to be multifactorial. Genetics may play a major role; however, one of these suspected influences is a non-physiological loading of the intervertebral disc<sup>1</sup>. This thesis is a part of a larger project with the same goal of finding biological traces of this disease. The metabolism of healthy, traumatic and scoliotic human intervertebral tissue is compared with another on the gene and protein level. In addition to that study, the effect of asymmetrical loading on intervertebral discs is sought. Due to lack of human material, it is the aim of this study to establish an intervertebral disc culture model with bovine tail discs loaded with an asymmetrical loading regime.

Further observation in advanced stage scoliosis, showed that intervertebral discs become wedged along with degenerative signs. Inappropriate mechanical loading has been associated with disc degeneration too. It is hypothesised that asymmetric loading, as found in scoliosis, is inducing disc matrix remodelling. Disc tissue was analysed with the aim of determining loading dependent effects and signs of degeneration.

## CONCEPT

Bovine tail discs (4-10 month old) with endplates were obtained from local abatement and dissected. Discs were loaded parallel or asymmetrically with an angle of 10°. Sinusoidal axial compressive stress of 0.02 – 0.4 MPa was applied for 1 h per day. Tissue was collected after 3 and 6 days of loading. Analysis methods included cell viability assessment, cellular gene expression (n=6 per group), matrix synthesis, biomechanical stiffness determination and histology with safranin-O/Fast green.

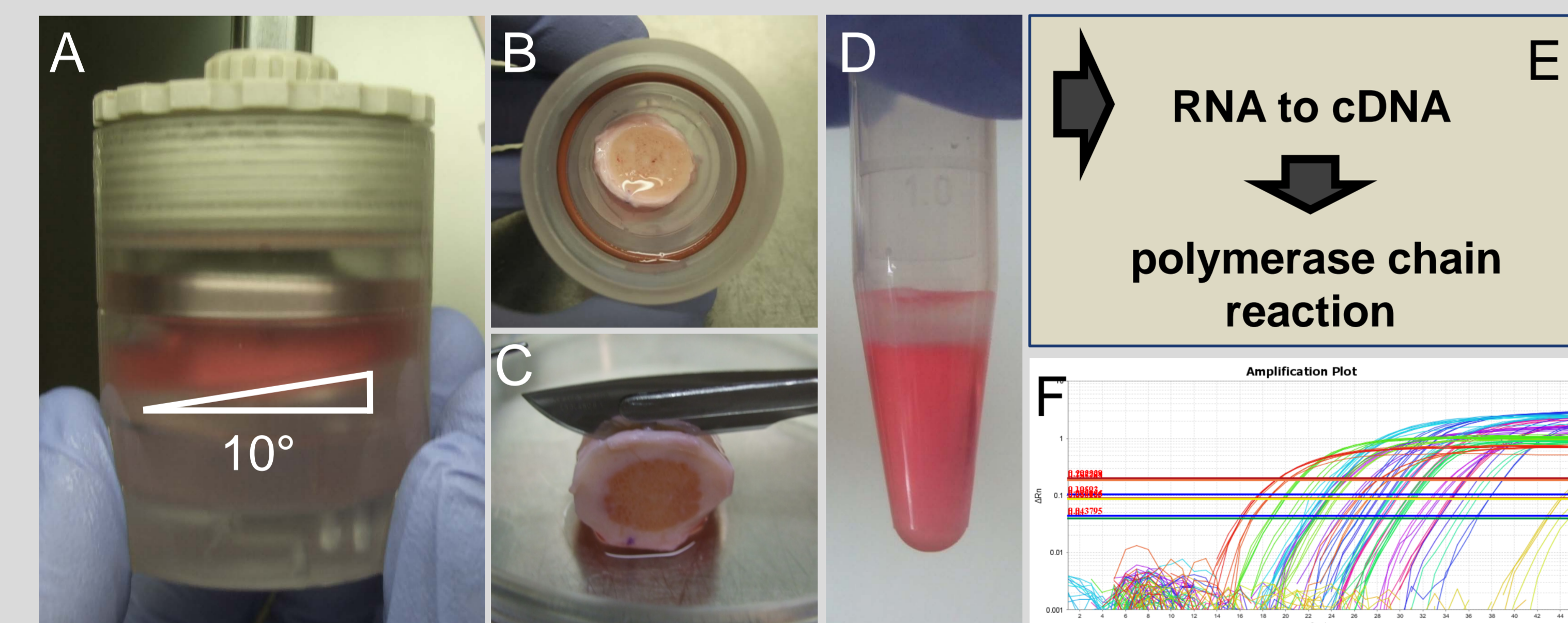


Fig. 1: Methods  
A: Loading chamber with wedge inlet, B: Bovine tail disc with endplate, C: Tissue collection, D: Total RNA extraction. E: Reverse transcriptase process F: Amplification plot of polymerase chain reaction analysis.

## RESULTS

During the 1 h of axial loading the discs lost approximately 10% of its height (~10 min after loading). This is due to dehydration of nucleus pulposus tissue (inner disc part) resulting from the externally applied pressure. The loading duration dependent height loss is shown in Fig 2 A. A temporary tilt of the wedged loaded disc was measured (Fig 2 B). However, after an overnight swelling in medium, discs fully recovered to its original shape during the experimental duration.

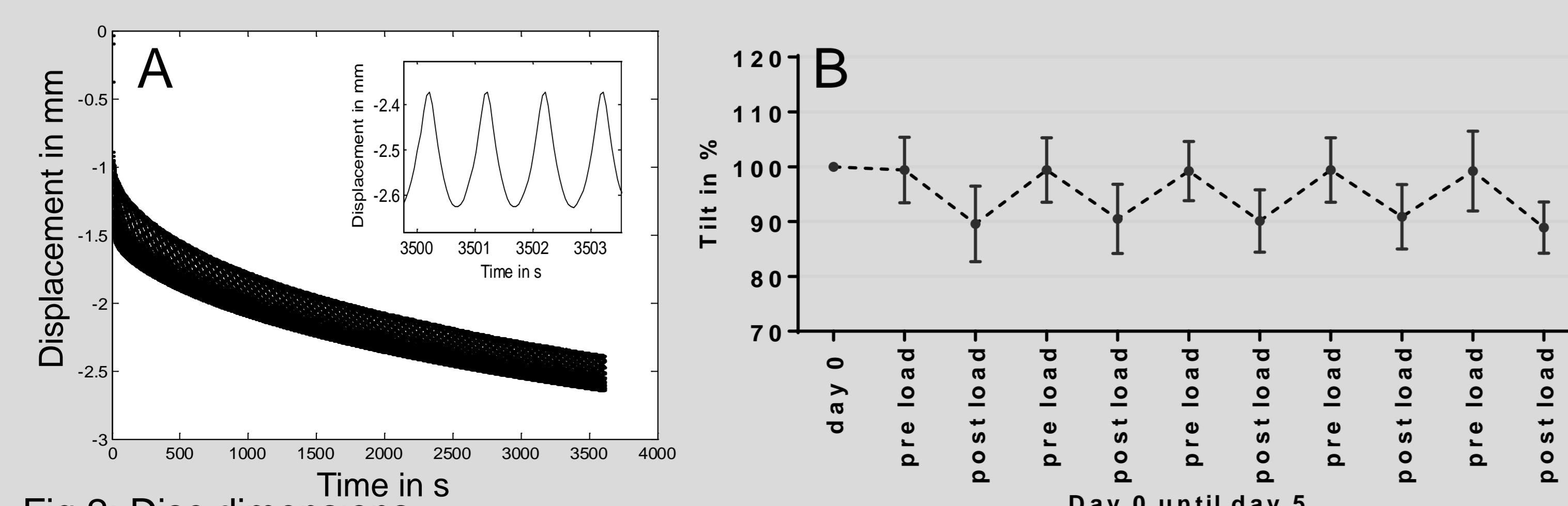


Fig 2: Disc dimensions

A major focus of this relatively short experimental duration of 7 days was to gain an actual picture about metabolic activity of AF cells in the highly mechanical stressed area of the asymmetrically loaded discs. This was determined with gene expression changes. Two time points (day 4 and day 7) were selected in order to note time dependent changes. In the Fig. 2 shows the gene expression changes of day 4. Expression levels were normalized to a day 0 untreated control disc.

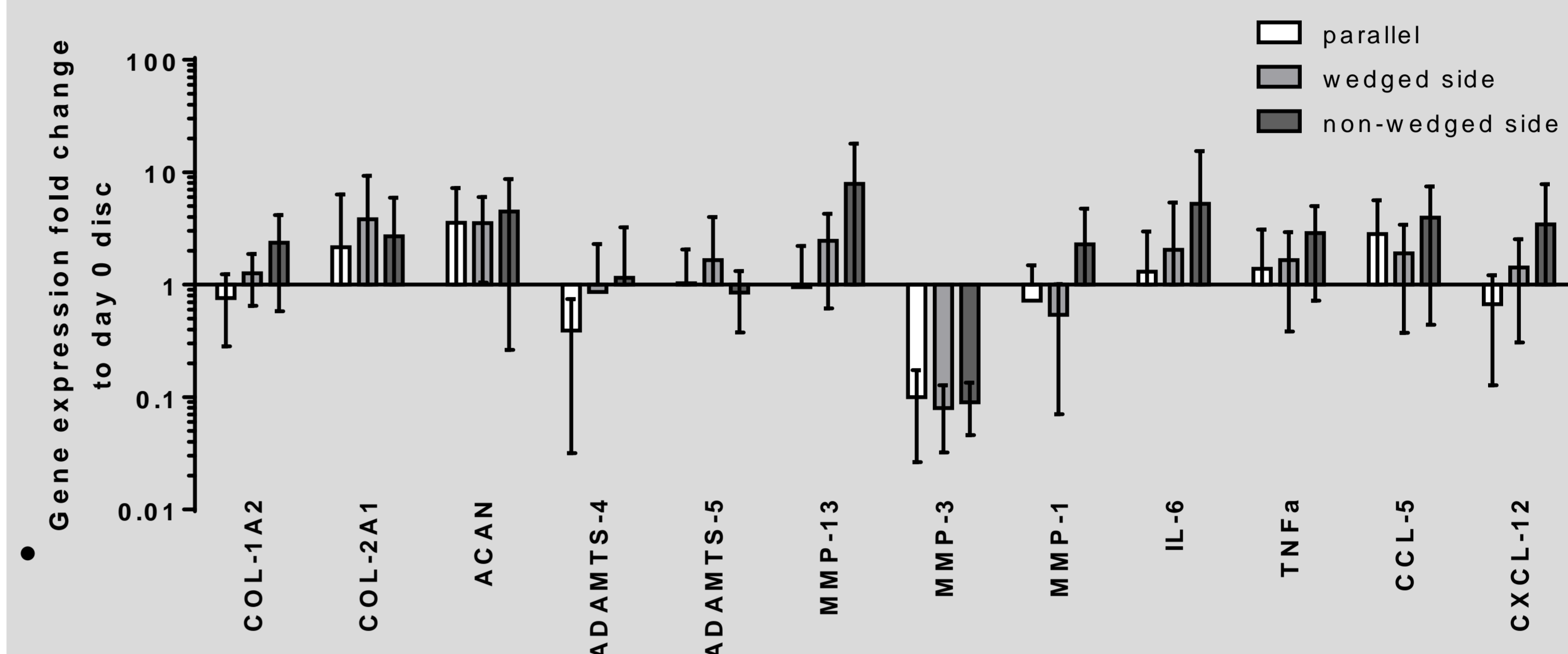


Fig. 2: Gene expression results after 3 days of loading (n=5).

Genes to analyse the disc's metabolism were selected according to their relevance in studies of non-physiological loading, degenerated or scoliotic discs. The applied loading on bovine tail discs did not induce a firm metabolic changes (Fig. 2).

Histological sections were stained with Safranin-O/Fast green:

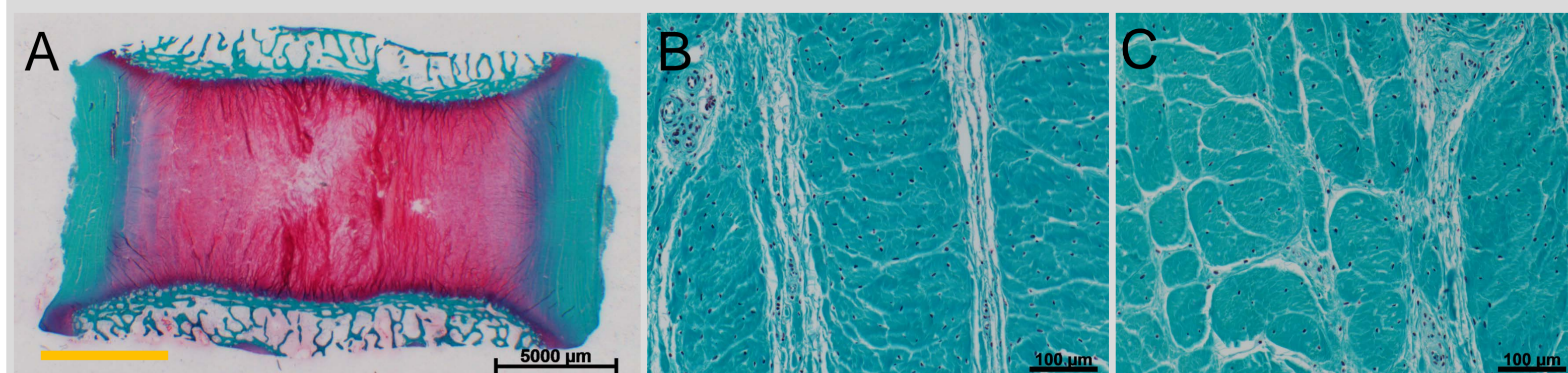


Fig. 3 Safranin-O stained sagittal sections. A: Overview, wedged side marked yellow. B: wedged side. C: non-wedged side. Background/collagen appears green, proteoglycans red and cell nuclei blue.

Tears and clefts in structural collagen fibres as well as red safranin-O stain between the lamellae (sign of degeneration in annulus fibrosus tissue in fig. 3 B, C) was looked for<sup>2</sup>. No differences between the wedged and non-wedged side was observed. No differences were found in histological sections for a cell viability study. Asymmetric loaded discs were always compared to the parallel loaded disc as well.

An examination of the dissection process indicated metabolic changes towards a strongly decreased collagen-1 gene expression. This suggests the use of a day 0 control disc processed before the dissection process as a more relevant physiological control.

## CONCLUSION

Neither side of the asymmetrically loaded discs showed any catabolic or anabolic metabolism shift towards matrix remodelling, as assessed by gene expression. Cells remained viable for 7 days of culture in both parallel and asymmetrically loaded discs. Neither transversal nor sagittal histological sections did show any degenerative changes

## REFERENCES

- [1] Kouwenhoven, J. W., & Castelein, R. M. (2008). The pathogenesis of adolescent idiopathic scoliosis: review of the literature. *Spine (Phila Pa 1976)*, 33(26), 2898-2908. doi: 10.1097/BRS.0b013e3181891751
- [2] Hsieh, A. H., & Twomey, J. D. (2010). Cellular mechanobiology of the intervertebral disc: new directions and approaches. *J Biomech*, 43(1), 137-145. doi: 10.1016/j.jbiomech.2009.09.019