Validation of anti-inflammatory and anti-degenerative drug therapy using a bioreactor guided intervertebral disc organ culture model

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Introduction and Objective

- Degenerative disc disease (DDD) = discogenic pain associated with disc height loss, biomechanical alterations, neovascularization and disability, pathogenesis poorly understood
- DDD is correlated with an proinflammatory microenvironment due to upregulation of proinflammatory cytokines, such as Tumor Necrosis Factor-α (TNF-α)
- Etanercept, a TNF-α inhibitor, has already shown an analgesic effect in human patients [1]

Study aims

1) To investigate the interplay between degeneration and inflammation within a loaded bovine intervertebral disc (IVD) organ culture system

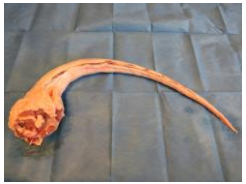
2) To investigate the effects of Etanercept on degeneration and inflammation in a bovine intervertebral disc organ culture model

[1] Sainoh et. al, 2016, Single Intradiscal Administration of the Tumor Necrosis Factor-Alpha Inhibitor, Etanercept, for Patients with Discogenic Low Back Pain, Pain Med., 17(1): 40-5

Part 1 - Methods - Degenerative and Inflammatory Intervertebral Disc Organ Culture Model [1]

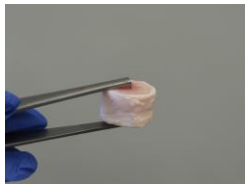
Dissection

Intervertebral discs with intact endplates (IVD) from 10 month old calf tails were dissected within three hours after slaughter



Tail obtained from slaughter

Tail after soft tissue removal

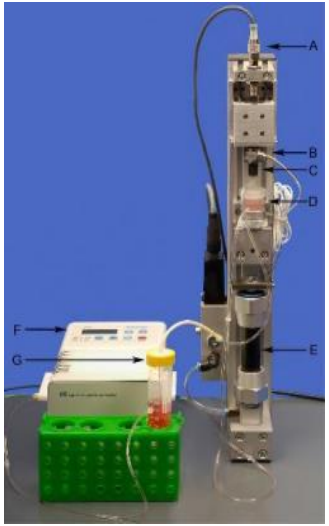


IVD Organ

Synergistical stimulation of proinflammatory and degenerative condition within DDD

Degeneration

Dynamic loading



(1) Deg

degenerative loading: (0.32-0.5 MPa; 5 Hz; 2h/day, 3 days) + Low Glucose medium

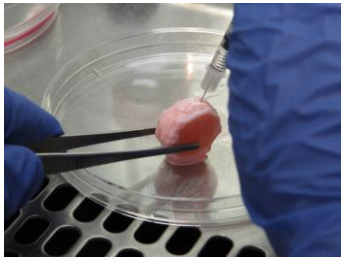
(2) **Phy**

physiological loading (control): (0.02-0.2 MPa; 0.2 Hz; 2h/day, 3 days) + High lucose medium

Entire system maintained in an incubator at 37° C, 5% CO_2 and ambient O_2.

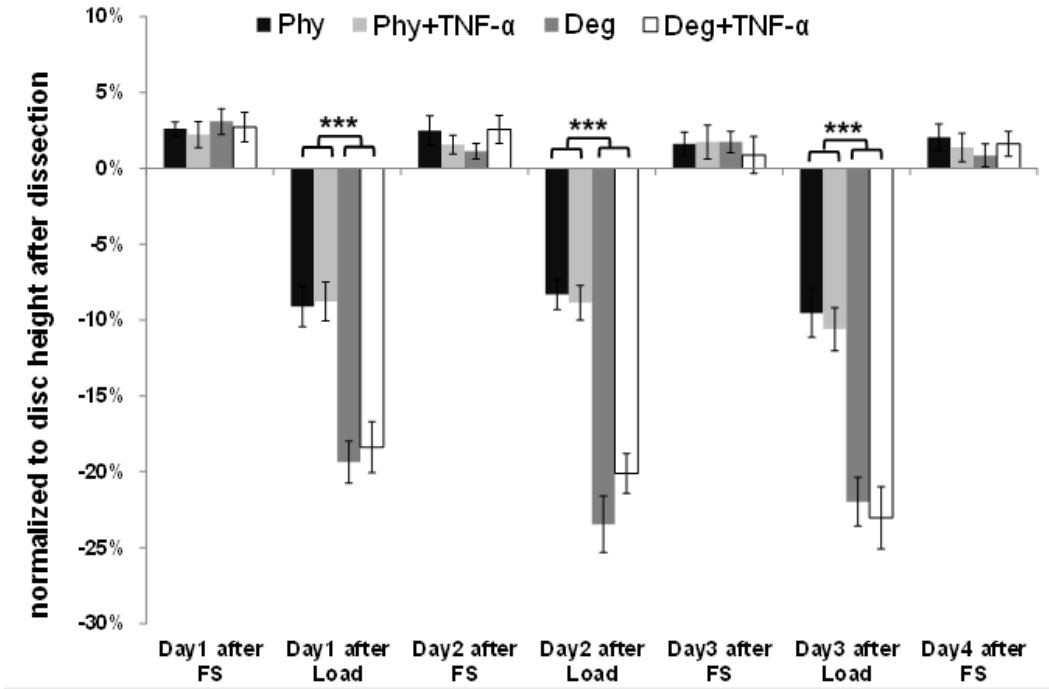
Inflammation

TNF- α injection (100ng)



[1]Lang et al., 2018, An intervertebral disc whole organ culture system to investigate proinflammatory and degenerative disc disease condition, J Tissue Eng Regen Med. 2018 Apr;12(4):e2051-e2061

Results – Disc Height Change [1]



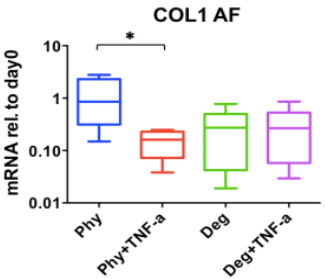
- **Degenerative** loading caused significantly higher disc height loss (~ -20%) compared to the **physiological** loading (~ -10%, $p < 0.001$)
- **TNF-α** intradiscal injection did not further induce disc height loss after loading
- After free swelling recovery, all the IVDs recovered to the initial disc height before load

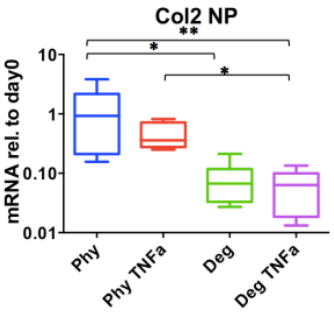
n=6, ***p<0.001

[1]Lang et al., 2018, An intervertebral disc whole organ culture system to investigate proinflammatory and degenerative disc disease condition, J Tissue Eng Regen Med. 2018 Apr;12(4):e2051-e2061

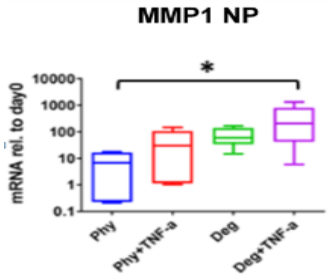
Results – Gene Expression Analysis

Anabolic genes

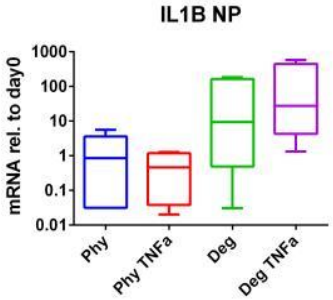


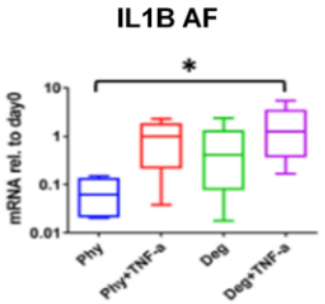


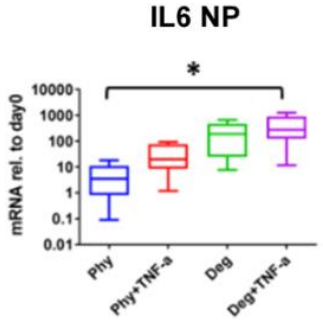
Catabolic genes



Inflammatory-related genes

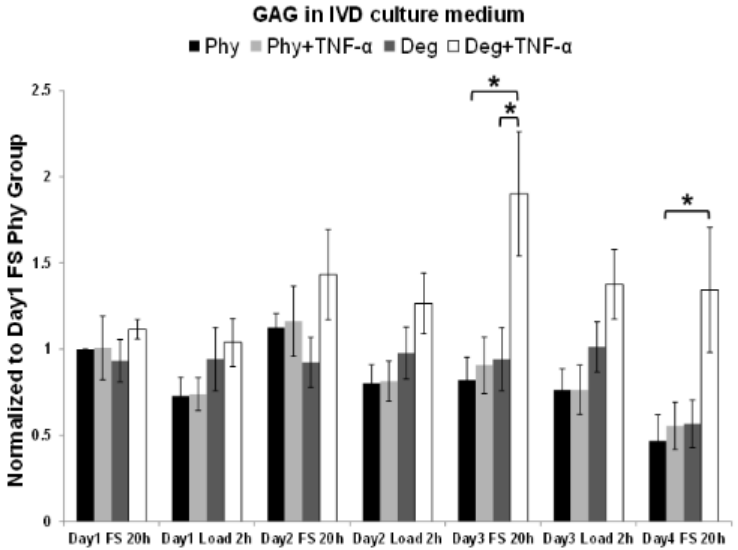


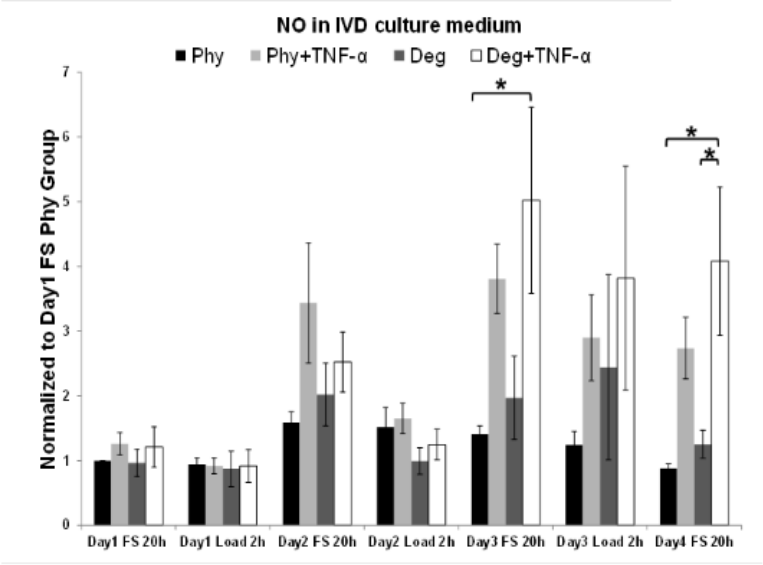




Relative mRNA expression in disc tissue of organ cultured IVD after 4 days of culture.n=6, *p<0.05, **p<0.01,Tissue of Nucleus pulposus (NP) and Anulus fibrosus (AF)

Results – Biochemical Analysis[1]

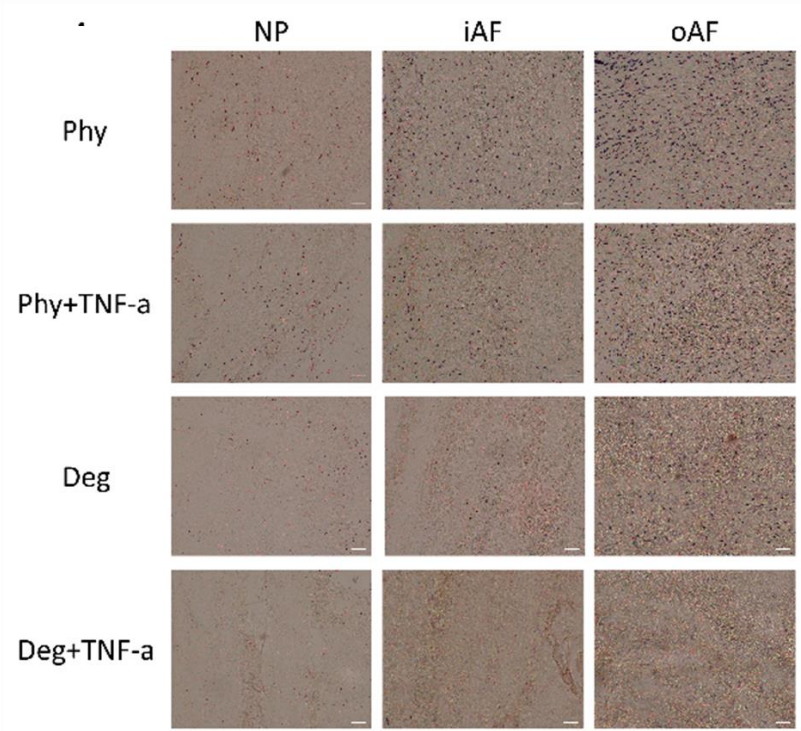


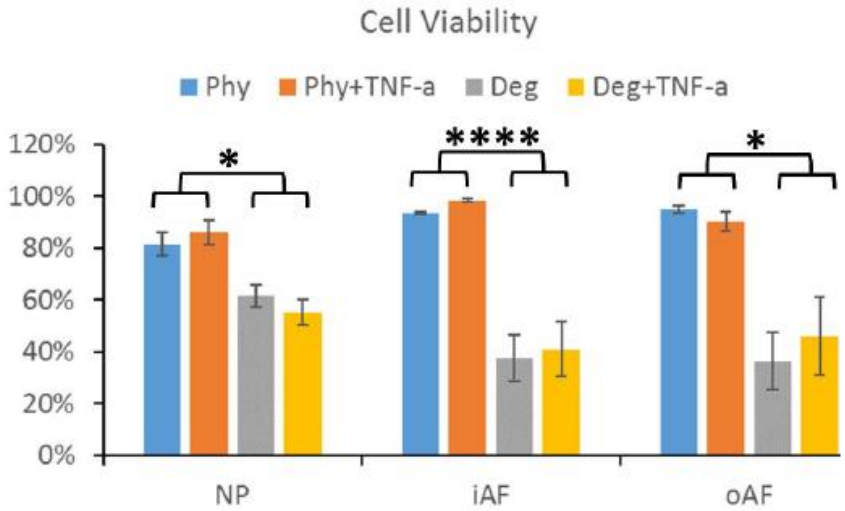


Intradiscal TNF-α injection combined with Deg culture condition enhanced Glycosaminoglykan (GAG) as well as nitric oxide (NO) release of IVD compared to Phy culture condition in the conditioned media during the free swelling period from day 3 on, n=6, *p<0.05

[1]Lang et al., 2018, An intervertebral disc whole organ culture system to investigate proinflammatory and degenerative disc disease condition, J Tissue Eng Regen Med. 2018 Apr;12(4):e2051-e2061

Results - Cell viability - Lactate Dehydrogenase (LDH) / Ethidium homodimer [1]

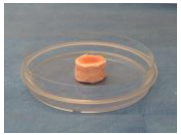


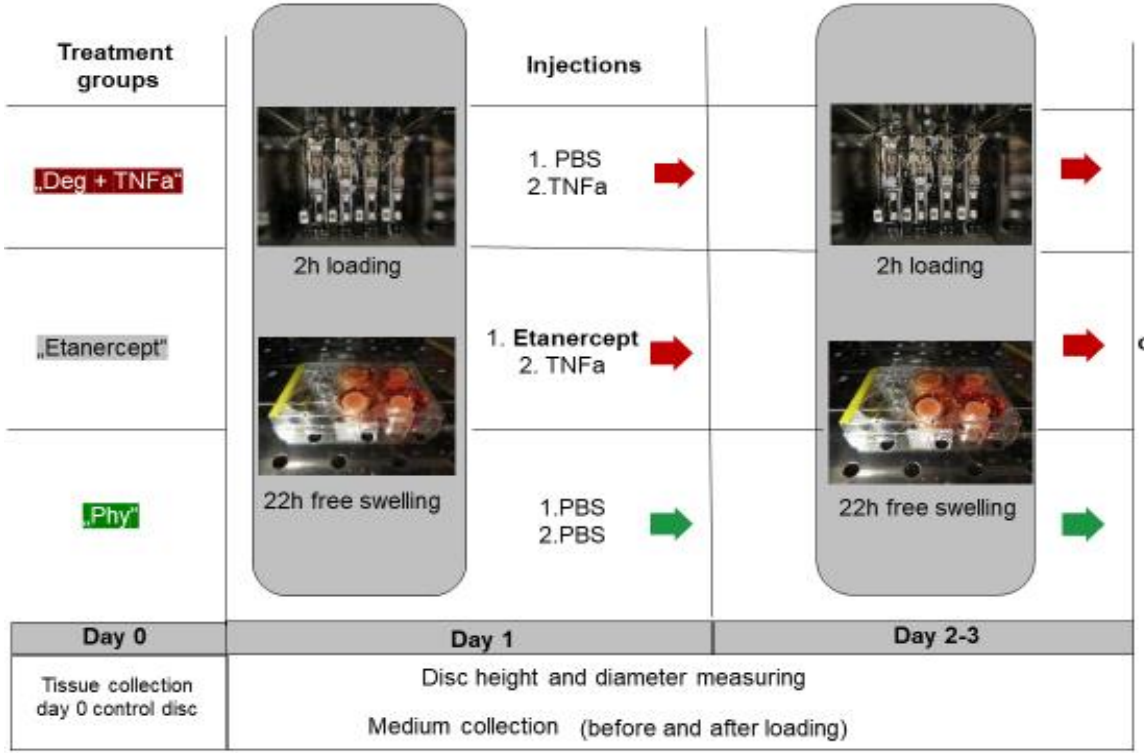


n=8, means ± sem, *p<0.05, ****p<0.0001

[1]Lang et al., 2018, An intervertebral disc whole organ culture system to investigate proinflammatory and degenerative disc disease condition, J Tissue Eng Regen Med. 2018 Apr;12(4):e2051-e2061

Part 2 - Methods – Intradiscal Etanercept Application





Degenerative loading protocol + Low glucose DMEM culture medium

Physiological loading protocol + High glucose DMEM culture medium

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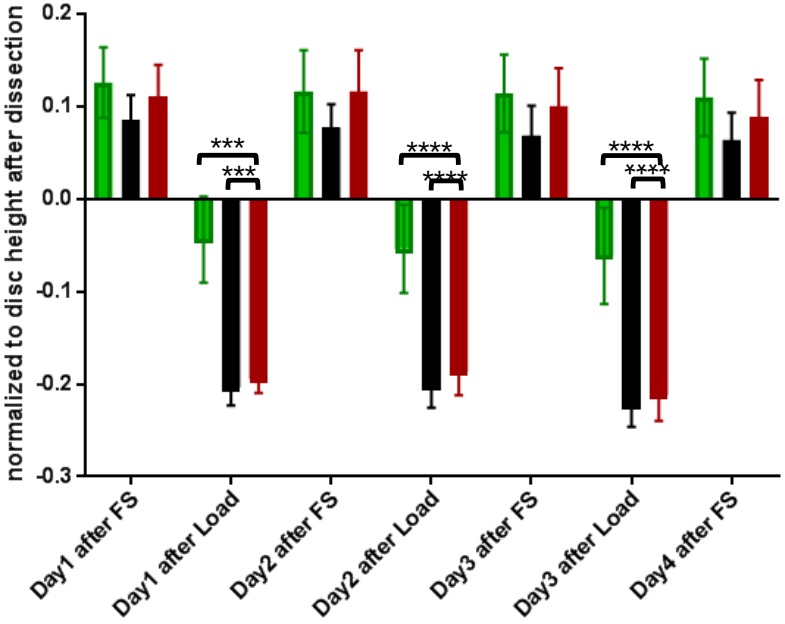
Results – Disc Height Change

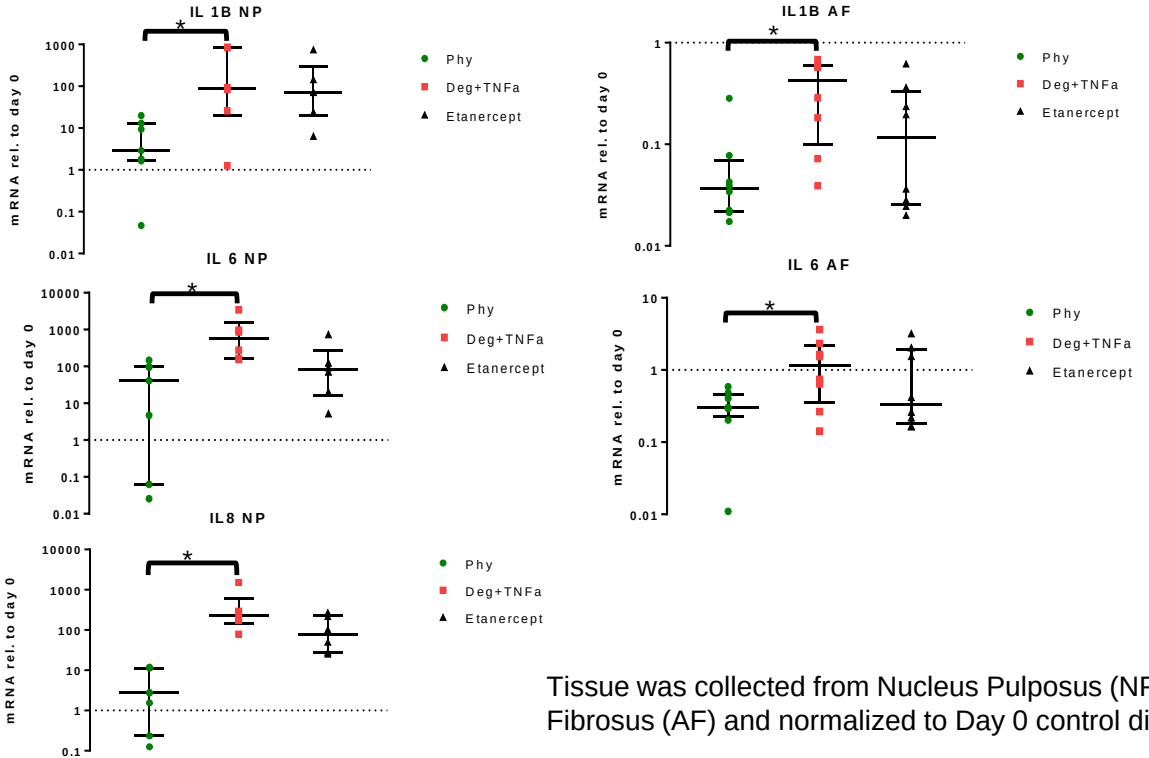


N= 9-11, ***p < 0.001, ****p < 0.0001

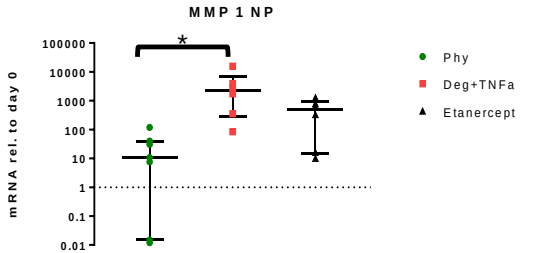
- **Degenerative** loading caused significantly higher disc height loss, compared to **physiological** loading
- No impact of drug treatment on biomechanical behaviour

Results – Gene Expression Analysis

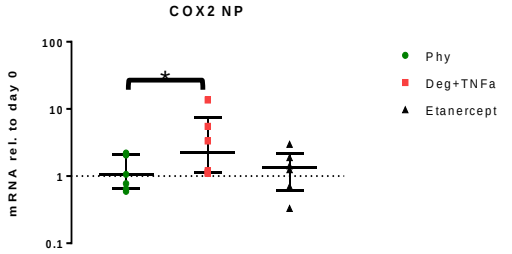
Inflammatory-related genes



Catabolic genes

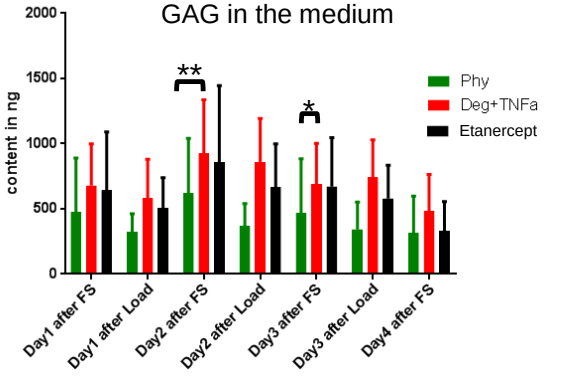


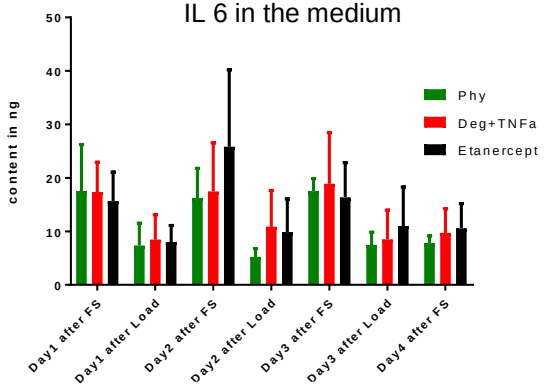
Pain-related genes

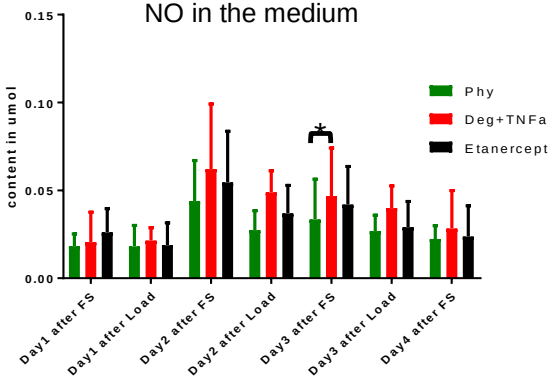


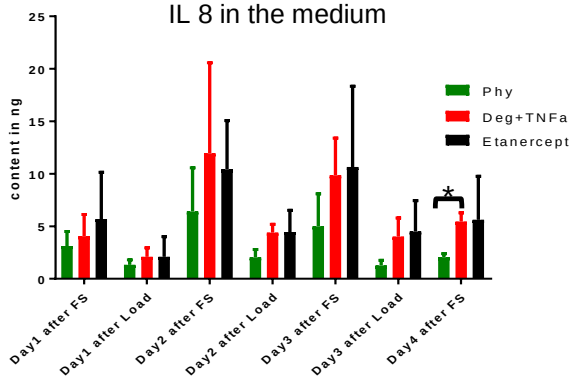
Tissue was collected from Nucleus Pulposus (NP) and Annulus Fibrosus (AF) and normalized to Day 0 control disc, n= 6-9, *p < 0.05

Results – Biochemical analysis









- Etanercept treatment partially reduced GAG and NO release into the medium

- Proinflammatory interleukin determination shows no drug effect on protein level, but IL-8 is significantly upregulated under degenerative and inflammatory culture condition

n= 4-9, *p < 0.05, **p < 0.01

Conclusions

- The combination of detrimental dynamic loading, nutrient deficiency and intradiscal TNF-α injection could synergistically simulate the proinflammatory and degenerative condition within a degenerative IVD, **demonstrating the potential of our whole IVD organ culture model for testing of anti-inflammatory or regenerative therapies.**
- **Etanercept** shows the ability to develop cellular effects on disc tissue and is capable of **reducing degenerative and inflammatory effects**
- Augmented treatment is required to completely counter the induced pathological processes

Disclosure

Acknowledgements

set Foundation for the promotion of alternate and complementary methods to reduce animal experiments

Competing interests

No benefits in any form have been or will be received from a commercially party directly or indirectly related to the subject of this article. The authors declare that there are no conflicts of interest