Constrained intervertebral disc organ culture for simulation of cell therapy approaches

C. Neidlinger-Wilke\textsuperscript{1}  
A. Boldt\textsuperscript{1}  
G.Q. Teixeira\textsuperscript{2,3}  
C. Jahn\textsuperscript{1}  
J.A. Mollenhauer\textsuperscript{4}  
H.J. Wilke\textsuperscript{1}  
A. Ignatius\textsuperscript{1}  
M.A. Barbosa\textsuperscript{2,3}  
R. Goncalves\textsuperscript{2}

\textsuperscript{1}Institute of Orthopaedic Research and Biomechanics  
Center of Musculoskeletal Research (zmfu), University of Ulm  
\textsuperscript{2}Institute of Biomedical Engineering-INEB, Porto, Portugal  
\textsuperscript{3}Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Porto, Portugal  
\textsuperscript{4}NMI Natural and Medical Sciences Institute at the University of Tuebingen, Reutlingen, Germany
Introduction

Characterization of degenerated intervertebral disc model:
• lack of nutrition of disc cells and reduced oxygen supply (impaired endplate permeability)
• acidic microenvironment
• up-regulation of inflammatory mediators and metalloproteinases (MMPs).

Challenges of biological treatment strategies of disc degeneration:
• survival of injected cells in a degenerated environment
• maintenance of metabolic activity of injected cells

Difficulties for standardized studies:
• no availability of normal human disc explants
• no suitable animal models that simulate disc degeneration as it occurs in human beings
Main Aim

Establishment of a bovine disc organ culture model for in vitro simulation of cell therapy approaches:

• standardized “physiological” disc environment (disc size, disc height, culture conditions)

• simulation of degenerative conditions (proteoglycan composition, nutrient supply)

• simulation of pro-inflammatory conditions
Objectives

(1) Preparation of standardized bovine disc organ culture with a pro-inflammatory microenvironment for cell injection

(2) Characterization of the organ culture environment after induction of a pro-inflammatory response
   - morphological analysis of disc punches
   - matrix characterization
   - metabolic profile
   - quantification of Prostaglandin $E_2$ production
   - gene expression analysis for pro-inflammatory cytokines (IL-6, IL-8), MMPs (MMP1, MMP3), COL-2 and Aggrecan

(3) Identification of injected cells by fluorescence microscopy and immunohistochemistry
Methods: Organ culture of bovine disc punches

Disc isolation

Disc punch (13 mm)

Constrained culture

Injection of fluorescence labeled cells

0

2

4

6

8

Time (days)

**Preparation of IVD organ cultures**

**Pro-inflammatory stimulus:**
- needle puncture* plus LPS (10 µg/mL)
- needle puncture* plus IL-1β (10 ng/mL)
- needle puncture* plus IL-1β (100 ng/mL)
- control (without treatment)

*20G needle

**Output:**
- supernatant collection for PGE₂ quantification and metabolic activity analysis
- disc collection for gene expression

Medium renewal
Results: Organ culture characterization

➢ Static loading prevents swelling of disc punches
➢ disc explants loose proteoglycans with time in culture

Fig. 1. A) Morphology analysis of the constrained culture conditions. B) Disc height evaluation. C) Disc matrix analysis: A decreased alcian blue staining intensity of disc matrix with time in culture. D) GAG accumulation within the culture medium E) GAG content in the disc tissue.
Results: Metabolic profile and PGE$_2$ production

Fig. 2. A) Glucose and B) Lactic acid concentrations (g/L), calculated using the mean of three biological replicates. The dashed lines indicate medium renewal. C) PGE$_2$ concentration (ng/mL).

- Glucose production and lactic acid consumption are not influenced by different pro-inflammatory treatment strategies.
- PGE2 production does not differ from IL1β (10 ng/mL) to control after stimulation.
Results: Gene expression

**Fig. 3.** Gene expression of **A)** pro-inflammatory cytokines (IL-6 and IL-8) and of **B)** MMPs (MMP1 and MMP3). Results are shown as mean ± SD (n=3, p<0.05).
Results: Gene expression

Fig. 4. Gene expression of ECM proteins (COL-2 and Aggrecan). Results are shown as mean ± SD (n=3, p<0.05).

IL-6, IL-8, MMP1 and MMP3

Pro-inflammatory microenvironment

COL-2 and Aggrecan
Results: Identification of injected cells

GFP- and PKH- labeled cells could be detected at the different sampling time points.

After 14 days in culture injected cells are visible by fluorescence microscopy.

Fig. 5. Fluorescence microscopy of bovine NP cells that were stained with the fluorescent markers PKH26 or PKH67 in comparison to a SCP1-GFP cell-line after 14 days in culture. Cells were injected in a serum albumin hyaluronan hydrogel. The white dotted line marks the edge between nucleus pulposus tissue (NP) and the albumin-hyaluronan hydrogel scaffold (Gel). Scale bars: 500 µm.
Summary / Discussion

- GAG-release, glucose production and lactic acid consumption are not influenced by different pro-inflammatory treatment strategies.

- Treatments with needle puncture plus LPS or IL-1β induce a pro-inflammatory environment in disc organ cultures:
  - Increased PGE$_2$ levels
  - Up-regulated expression of MMP1, MMP3, IL-6, and IL-8
  - Down-regulated expression of COL-2 and Aggrecan

- Suitable approach for induction of a pro-inflammatory response: puncture plus IL-1β (stronger effect with 100 ng/mL)

- GFP- and PKH-labeled cells were able to be identified 14 days after injection.

Further strategies for anti-inflammatory approaches are subject of ongoing investigations!
Thank you for your attention!

Disclosure: The authors of this study have no conflict of interest to declare.