Glucocorticoid responses in a degenerative human meniscus ex vivo culture model



Sofia Pilão[1], Neila Ouldali[1], Luisa de Roy[1], Anita Ignatius[1], Jan Tuckermann[2], Andreas Seitz[1], Graciosa Quelhas Teixeira[1]

[1]Institute of Orthopedic Research and Biomechanics, Ulm University Hospital, Ulm, Germany
[2]Institute of Molecular Endocrinology and Physiology, University of Ulm, Ulm, Germany

sofia.pilao@uni-ulm.de

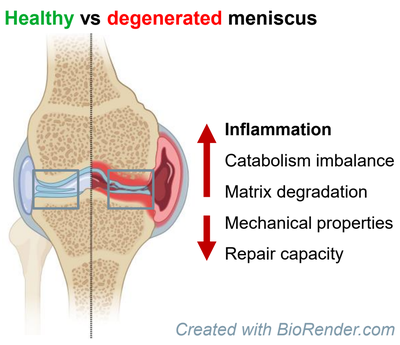
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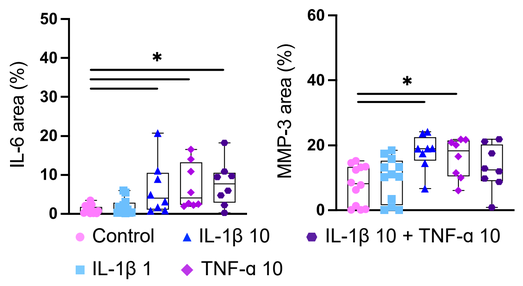
To investigate the effects of **glucocorticoids (GCs)** in **human menisci** using a **pro-inflammatory/degenerative ex vivo culture model**

BACKGROUND

- **Knee joint injuries** are associated with **inflammation**, matrix degradation, and functional impairment, contributing to **osteoarthritis (OA)** *(Riggs et al., 2023; Evers et al., 2022)*
- Glucocorticoids (GCs) are anti-inflammatory/analgesic drugs commonly used in OA treatment *(Savvidou et al., 2019)*
- **The direct biological effects of GCs on meniscus tissue remain poorly characterized**

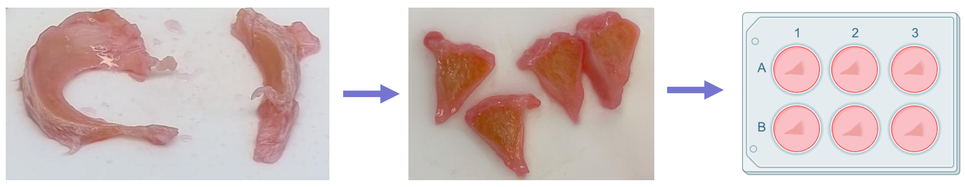


- Our group established an **ex vivo human meniscus culture model** that mimics **degeneration**
- Stimulation with **IL-1β (10 ng/mL)** maintains degenerative phenotype: increases pro-inflammatory and catabolic markers expression



METHODS

- **Mildly degenerated human meniscus samples** (n=25, 8 male/17 female, age=71±8, Pauli score 2-3) were cut into wedge-shaped explants for ex vivo culture



1 Time response to IL-1β stimulation and methylprednisolone (MP) treatment

d0 — d6 — 6h, 24h, 48h, 72h

CULTURE START

STIMULATION START
- IL-1β*
- IL-1β* + MP**

SAMPLE COLLECTION
- Gene expression

2 MP effects on human meniscus ex vivo cultures

d0 — d3 — d21

CULTURE START

STIMULATION START
- Control
- IL-1β*
- IL-1β* + MP**

SAMPLE COLLECTION
- Gene expression
- Histology/immunohistochemistry
- Biochemical quantifications
- Multiplex cytokine analysis
- Biomechanical testing

* 10 ng/mL
** 1 µM

CONCLUSIONS

- MP treatment repressed IL-1β-induced pro-inflammatory/catabolic phenotype in degenerated human meniscus explants
- **Potential immunomodulatory targets responsive to GCs** (e.g., IL-1β, MIP-1α, GM-CSF, IP-10) were identified in degenerated human meniscus explants
- **Ex vivo culture model was validated for studying meniscus inflammation/degeneration and testing novel therapies for OA**

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RESULTS & DISCUSSION

1 Time response to IL-1β stimulation and methylprednisolone (MP) treatment

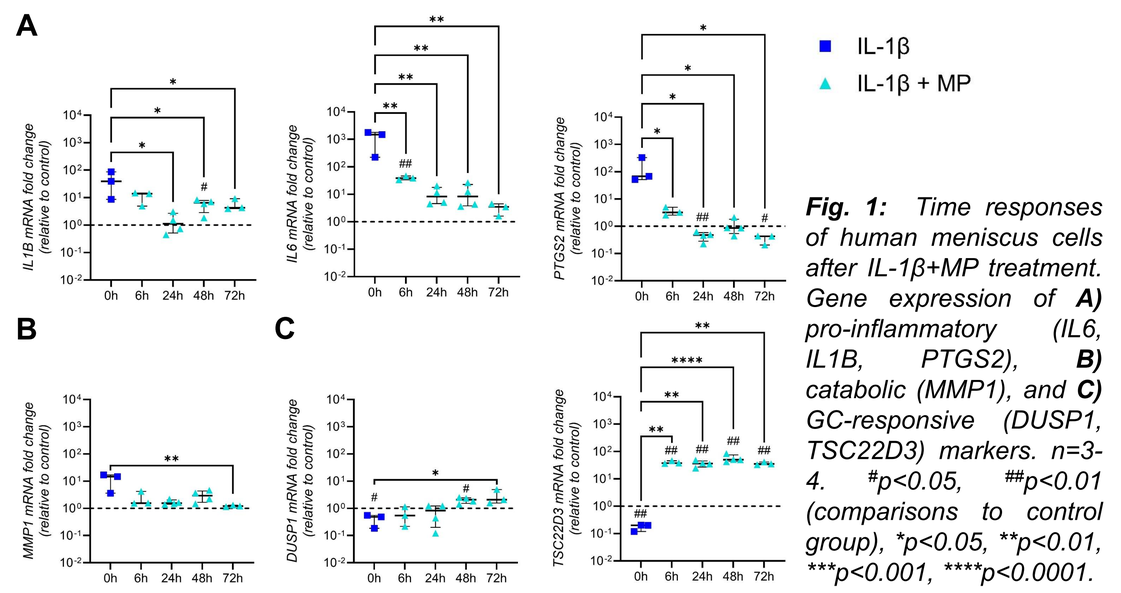


Fig. 1: *Time responses of human meniscus cells after IL-1β+MP treatment. Gene expression of **A)** pro-inflammatory (IL6, IL1B, PTGS2), **B)** catabolic (MMP1), and **C)** GC-responsive (DUSP1, TSC22D3) markers. n=3-4. #p<0.05, ##p<0.01 (comparisons to control group), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

- IL-1β induced upregulation of **pro-inflammatory and catabolic genes** immediately **6h** after stimulation
- MP reversed these effects and upregulated **GC-responsive genes** up to **72h** after treatment

2 MP effects on human meniscus ex vivo cultures

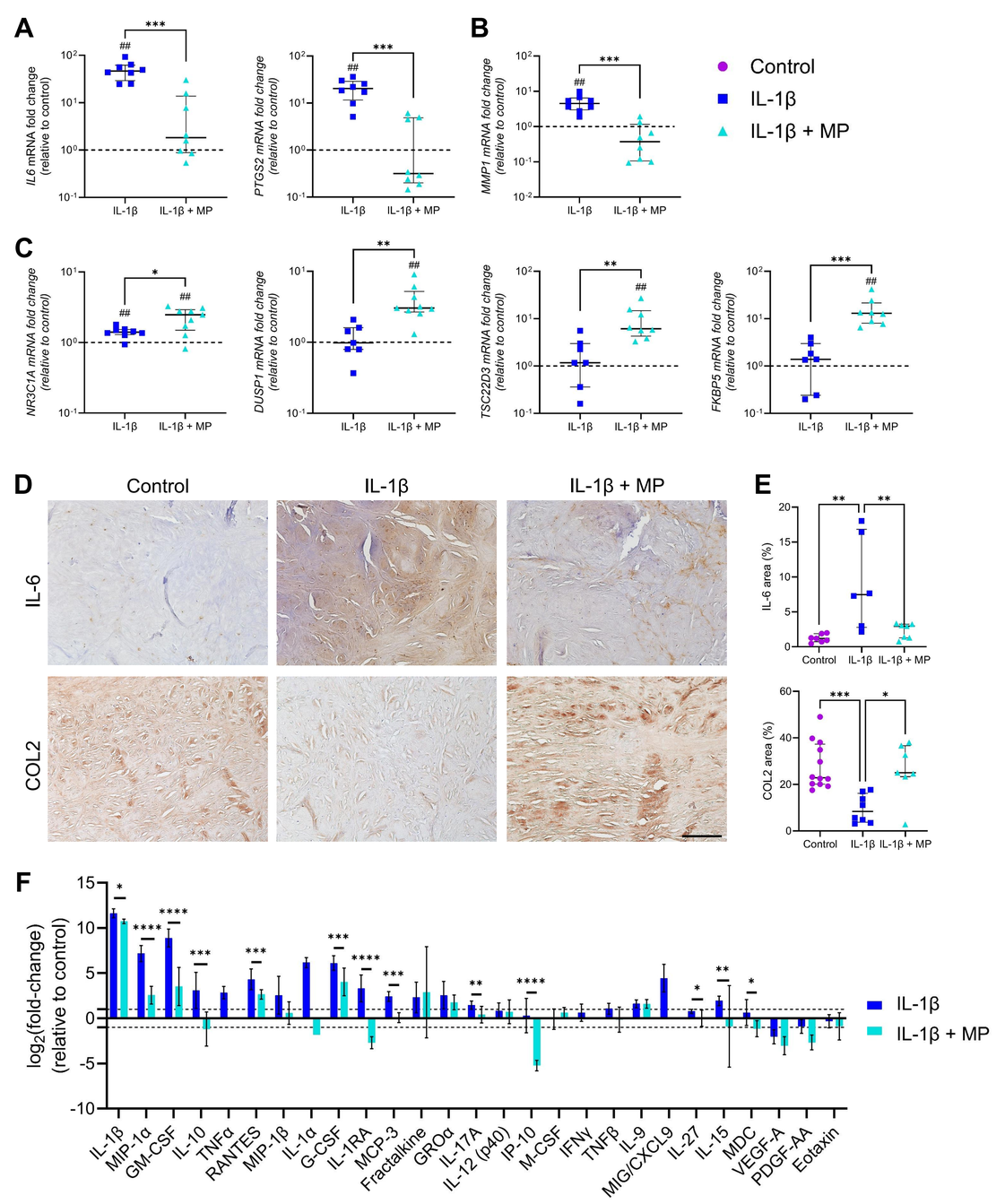


Fig. 2: *Characterization of human meniscus explants after 21 days in culture under control, IL-1β, or IL-1β+MP conditions. Gene expression of **A)** pro-inflammatory (IL6, PTGS2), **B)** catabolic (MMP1), and **C)** GC-responsive (NR3C1α, DUSP1, TSC22D3, FKBP5) markers. **D)** Representative images of interleukin (IL)-6 and collagen type II (COL2) immunostaining; scale bar=200 µm. **E)** IL-6- and COL2- positive area (%). **F)** Inflammation-related cytokine profile of meniscus explants. n=6-12. #p<0.05, ##p<0.01 (comparisons to control group), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

- MP upregulated the expression of GC receptor and downstream target genes up to 21 days in culture *(Reichardt et al., 2021)*
- MP exerted a **broad immunosuppressive effect** in degenerated human meniscus cultures, **reducing inflammatory mediators** (e.g., IL-1β, MIP-1α, GM-CSF, IP-10) **associated with immune cell recruitment and matrix degradation** *(Ehrchen et al, 2019; van Helvoort et al., 2020)*