A Potential Role for Osteocyte Derived NLRP3 in Post-Traumatic Osteoarthritis (PTOA)

Antonio Franchi[1], Bridget Twombly[1], Steffi Tiburcius[1], Anugerah Yaumil Ramadhani Aziz[1], Michela Uberti[1], Meric Goker[1], Chenjun Su[1, 2], Ingmar Schoen[1], Annie Curtis[1], Tom Hodgkinson[1], Oran D Kennedy[1, 2]

[1] Tissue Engineering Research Group (TERG), Dept. of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland;
[2] Trinity Centre for Biomedical Engineering, Trinity College Dublin, Ireland.

Introduction

- Osteoarthritis is a degenerative joint condition and the most common type of arthritis[1] (Figure 1)
- No treatment to prevent or reverse OA as normally detected at a late stage
- Post-traumatic osteoarthritis (PTOA) accounts for 12% of all OA cases and develops after acute knee injury (ACL rupture)[2]
- Around 80% of ACL ruptures result in Bone Marrow Lesions (BMLs - possibly linked to bone microdamage)[3,4]

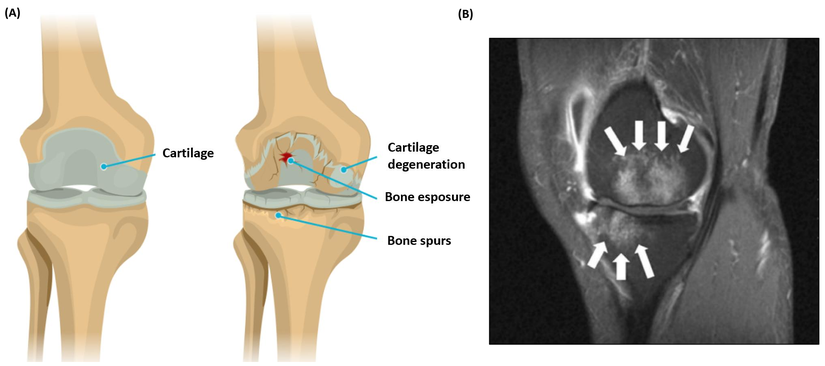


Figure 1: (A) Comparison between a healthy (left) and osteoarthritic (right) knee joint. (B) MRI scan of distal femur and proximal tibia showing the presence of bone marrow lesions[5].

- BMLs likely contain regions of bone microdamage, which causes osteocytes to undergo cell death and subsequently modulate RANKL and OPG to recruit osteoclasts, with the potential release of inflammatory factors through the NLRP3 inflammasome [3,4,6]

Aims and Objectives

To characterize the specific nature of inflammasomes in osteocytes and to establish osteocytes as a potential therapeutic target for PTOA

Aim 1: To characterize inflammasome activation and inhibition in osteocytes

Aim 2: To characterize the osteocyte response to different extracellular matrix substrates, in terms of inflammation, cell viability, gene and protein expression

Aim 3: To develop a targeted delivery system for inflammasome inhibition

Methods

- Comparison between primary osteocytes (murine) and the MLO-Y4 osteocyte cell line
- Determine the optimal components for the ECM substrate (collagen I, collagen I-hydroxyapatite) to recapitulate the *in vivo* environment (Figure 2)
- Treat cells with inflammatory agents (LPS, TNF-α) and ATP to induce endogenous inflammation through inflammasome activation (Figure 2)
- Use NLRP3 inflammasome inhibitor MCC950 to determine the specificity of inflammation[7]

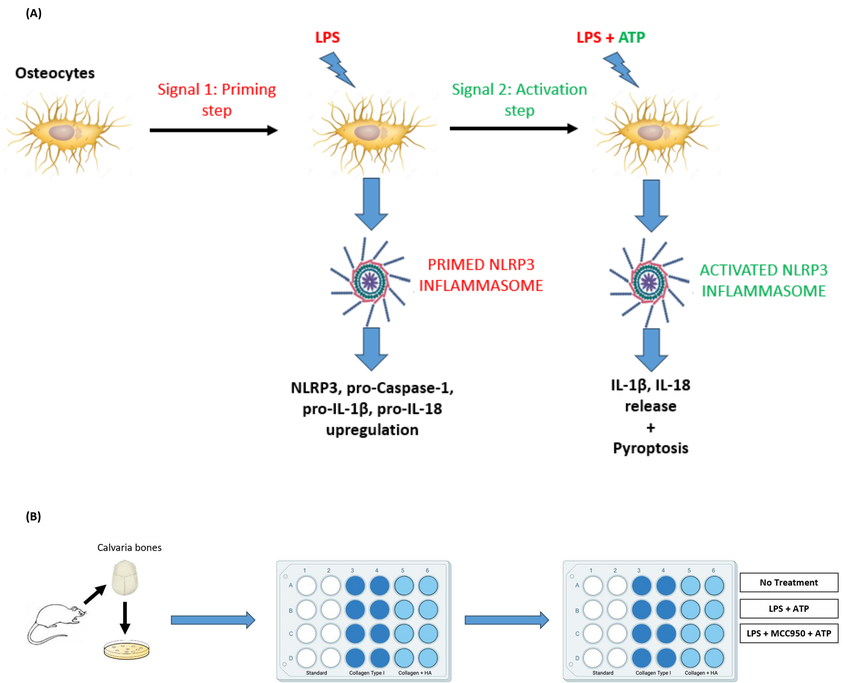


Figure 2: (A) Priming and activation steps during NLRP3 inflammasome activation in osteocytes using LPS and ATP as first and second stimuli respectively. (B) Experimental set-up for NLRP3 inflammasome activation and inhibition on different 2D substrates.

Results

✓ **Collagen I and collagen I/hydroxyapatite coatings promote the development of a dendritic shape, resembling osteocytes *in vivo***

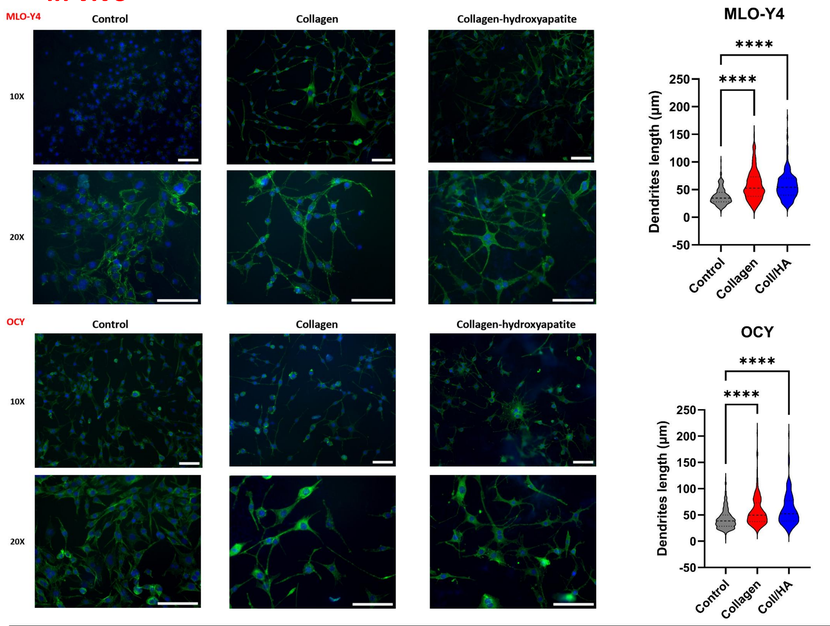


Figure 3: Fluorescence images of DAPI (blue) and Phalloidin (Alexa 488, green) stained MLO-Y4s and OCYs seeded on ECM coatings. Images were taken 3 days post-seeding. Scalebar: 100 µm. Data are presented as means ± SD. n=4. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

✓ **Collagen I and collagen I/hydroxyapatite coatings modulate metabolic activity, proliferation and gene expression of MLO-Y4 and OCY**

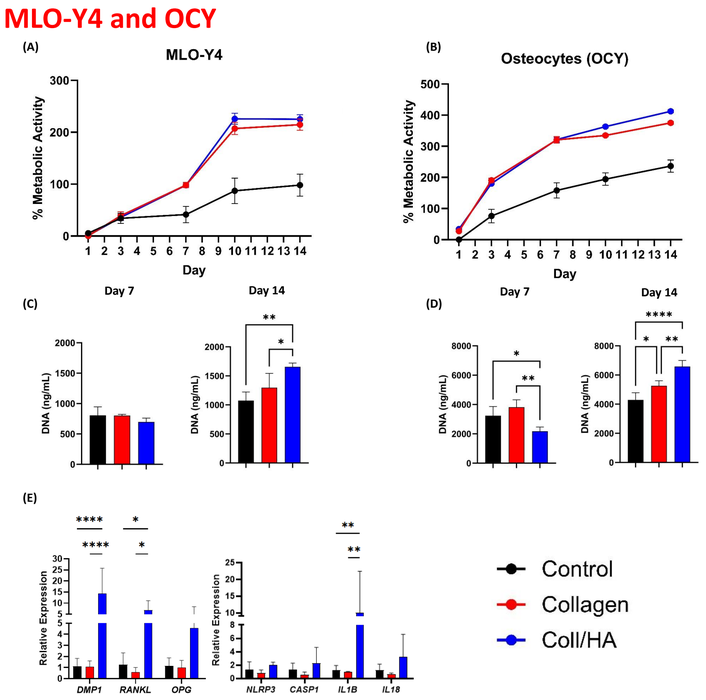


Figure 4: (A, B) Metabolic activity of MLO-Y4 and OCY over time. n=8. Data are presented as a percentage of the control group with means ± SEM. (C, D) DNA content of MLO-Y4 and OCY. n=4. (E) Gene expression of MLO-Y4. Data are presented as means ± SD. n=3. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

✓ **LPS exposure induces pyroptosis in both MLO-Y4 and OCY, while the simultaneous addition of MCC950 reduces inflammation**

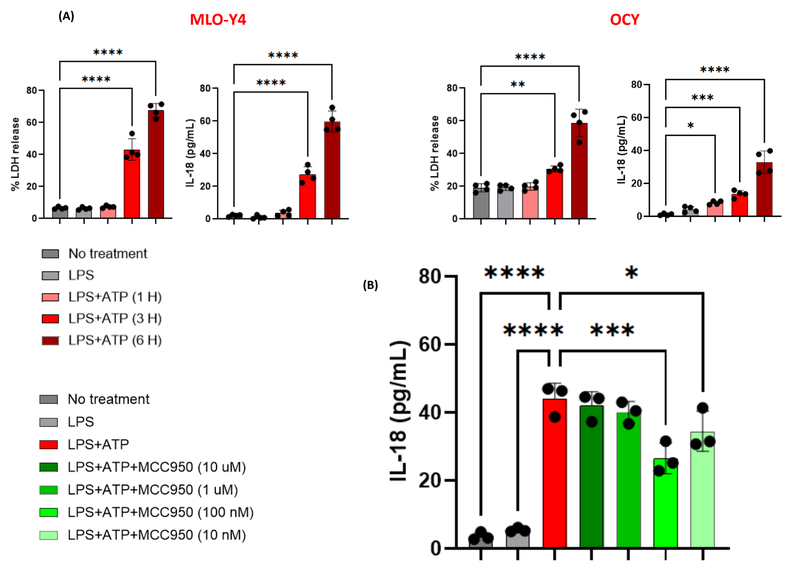


Figure 5: (A) Cytotoxicity and IL-18 release upon LPS+ATP exposure of MLO-Y4 and OCY. Data are presented as a percentage of the positive control group or concentrations (pg/mL) with means ± SD. n=4. (B) IL-18 release of OCY after inflammasome activation (24 hours ATP) and inhibition with MCC950. Data are presented as concentration (pg/mL) with means ± SD. n=3. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

✓ **Presence of ASC in response to inflammasome stimulation**

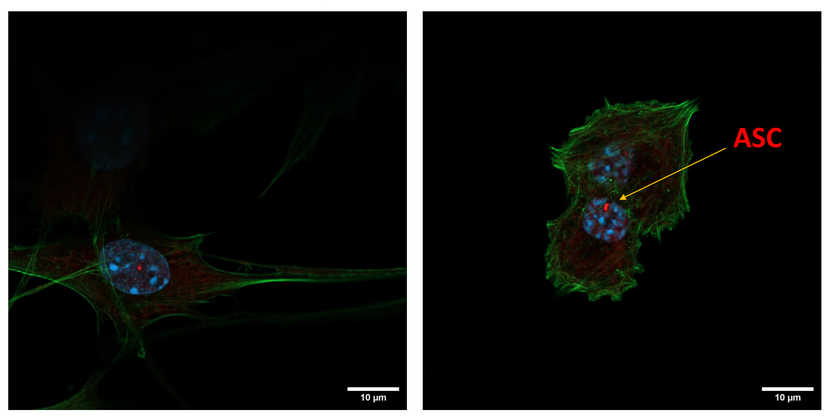


Figure 6: Super Resolution Microscopy (SRM) image showing the presence of ASC in MLO-Y4 cells. Cells were treated with LPS (10 ng/mL) for 24 hours and ATP (5 mM) for 1 hour to allow ASC-speck formation (right) and compared to the control (left). Stain was performed with Phalloidin 488 (green), DAPI (blue) and ASC/TMS1 rabbit monoclonal antibody + Alexa fluor 594 Donkey anti-rabbit.

Results

✓ **Activators of osteocyte inflammasome (TNF-α) induce upregulation of inflammatory and pro-bone remodelling genes**

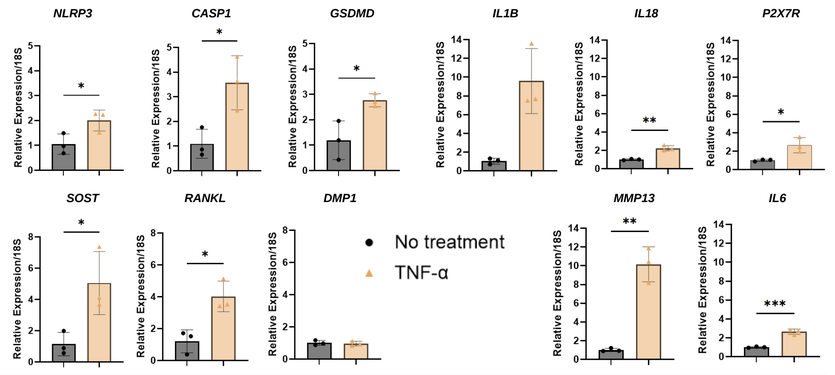


Figure 7: Gene expression of OCY exposed to TNF-α (10 ng/mL) for 24 hours. Cells were seeded onto collagen I functionalized wells. Data are presented as fold-change of the No treatment group with means and individual values ± SD. n=3. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

✓ **NLRP3 priming and activation promote osteocyte pyroptosis**

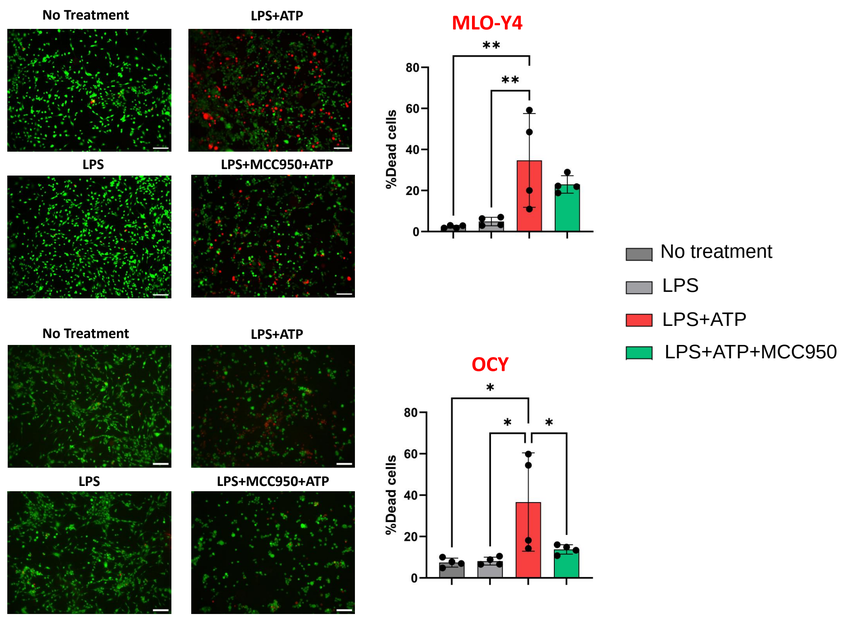


Figure 8: Live/dead cells stain of MLO-Y4 and OCY. Cells were seeded on collagen I 2D coatings for 3 days and treated with LPS (10 ng/mL) for 24 hours. Cells were then exposed to +/- MCC950 (1 mM) and +/- ATP (5 mM) for 1 hour. (Left) Fluorescence images. Scalebar: 200 µm. (Right) % of dead cells over total number of cells detected. Data are presented as means and individual values ± SD. n=4. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0. 0001.

Conclusion and Clinical Significance

- Collagen and collagen-hydroxyapatite matrices increase metabolic activity, proliferation and promote the development of osteocytes phenotype
- Inflammasome activators promote the upregulation of inflammatory and pro-bone remodelling genes in osteocytes
- Osteocytes undergo cell death in response to NLRP3 inflammasome activation, with the release of IL-18

IMPACT

This study highlights the novel role of osteocytes as an endogenous source of inflammation and suggests their possible involvement during the pathogenesis of PTOA

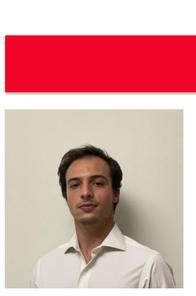
Future works

- Studies on 3D collagen-based scaffolds to resemble osteocytes microenvironment
- Use of PLGA and nHA nanoparticles to optimize MCC950 delivery
- Application of shear stress to enhance inflammasome activation in osteocytes

References

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Contacts



linkedin.com/in/antonio-franchi-700150195

antoniofranchi23@rcsi.com

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