

## DEVELOPMENT OF A NOVEL DRUG DELIVERY PLATFORM TO TARGET NLRP3 INFLAMMASOME AND ITS TREATMENT IN POST-TRAUMATIC OSTEOARTHRITIS

Hariitha Jacob<sup>1,2,3</sup>, James Early<sup>4</sup>, Sarinj Fattah<sup>5,6</sup>, Avril Roberston<sup>7</sup>, Oran Kennedy<sup>2,3,8</sup>, Annie Curtis<sup>2,3,4</sup>, Cathal Kearney<sup>1,2,3,9</sup>

<sup>1</sup> Kearney Lab, Dept of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin 2, Ireland; <sup>2</sup> Tissue Engineering Research Group, Dept. of Anatomy and Regenerative Medicine, RCSI, Dublin 2, Ireland; <sup>3</sup> Advanced Materials and Bioengineering Research Centre, RCSI & TCD, Dublin 2, Ireland; <sup>4</sup> Curtis clock laboratory, School of Pharmacy and Biomolecular Sciences, RCSI, Dublin 2, Ireland; <sup>5</sup> Drug delivery and Advanced Materials Team, School of Pharmacy & Biomolecular Sciences, RCSI, Dublin 2, Ireland; <sup>6</sup> Center Research in Medical devices, RCSI and NUI, Galway, Ireland; <sup>7</sup> School of Chemistry and Molecular Biosciences, University of Queensland, Australia. <sup>8</sup> Kennedy Lab, Royal College of Surgeons in Ireland (RCSI), Dublin 2, Ireland; <sup>9</sup> Department of Biomedical Engineering, University of Massachusetts Amherst, MA, USA.

**Background:** Post-traumatic Osteoarthritis (PTOA) occurs after a joint injury, such as a fracture, cartilage tear or ligament damage. Chondrocytes respond to joint injuries by releasing inflammatory mediators which drive PTOA. Activation of Nod-like receptor family protein 3 (NLRP3) inflammasome through NF- $\kappa$ B signalling induces Interleukin-1 $\beta$  (IL-1 $\beta$ ) secretion and mediates inflammatory cell death. MCC950 is a selective NLRP3 inflammasome inhibitor and prevents IL-1 $\beta$  secretion. Current pharmacological treatment options for PTOA primarily focus on symptomatic improvement of the pain, yet targeting immune mediators using MCC950 may enhance therapeutic outcomes or prevent/reverse injury. Here, we hypothesise that a local drug delivery device capable of delivering MCC950 to the joint space will demonstrate therapeutic efficacy in PTOA.

**Methods:** 3% Poly Lactic-co-Glycolic acid (PLGA) 75:25 (molecular weight 4000-15000) and 1mM MCC950 were spray-dried using dichloromethane. MCC950 release was measured in PBS (37°C) using HPLC. Macrophages were primed with lipopolysaccharide (LPS) for 3 hours. Cells were treated with 50mM MCC950 and MCC950 released from particles for 40 mins and then stimulated with ATP for further 45 minutes. Cells were tested for cytotoxicity and cultured supernatants were assayed for the levels of secreted IL-1 $\beta$  by ELISA. To explore the crosstalk between inflamed macrophages and chondrocytes, supernatants from inflamed macrophages treated with/out MCC950 were added on healthy chondrocytes for 24 hours. PCR on chondrocyte markers was used to analyse chondrocyte functionality (collagens, aggrecan, MMP13, ADAMTS4).

**Results:** Spray-dried PLGA microspheres provided sustained release of MCC950 with 30 $\mu$ g released by day 4. LPS is a potent activator of macrophages, and triggers the release of various cytokines including IL-1 $\beta$ . MCC950 inhibits LPS-induced IL-1 $\beta$  secretion in macrophages. Both 50 $\mu$ M MCC950 and MCC950 released from PLGA particles inhibited pyroptosis significantly and reduced cytotoxic effects on inflamed macrophages. Furthermore, IL-1 $\beta$  release from inflamed macrophages inhibited chondrocyte collagen formation and increased inflammatory protein level MMP-13. MCC950 (both pure drug and MCC950 released from PLGA particles) reversed these effects.

**Conclusions:** Spray-dried PLGA microspheres providing sustained release of MCC950 were successfully developed. MCC950 released from PLGA particles remain strongly bioactive and inhibited LPS-induced IL-1 $\beta$  release from inflamed macrophages. At the same time, MCC950 protected chondrocytes from the adverse effect of IL-1 $\beta$  released from inflamed macrophages. Our study highlights the potential of MCC950 as a potential drug delivery candidate for targeting IL-1 $\beta$ , which is believed to contribute to the pathogenesis of PTOA. Future work will test these particles in an in vivo PTOA model.