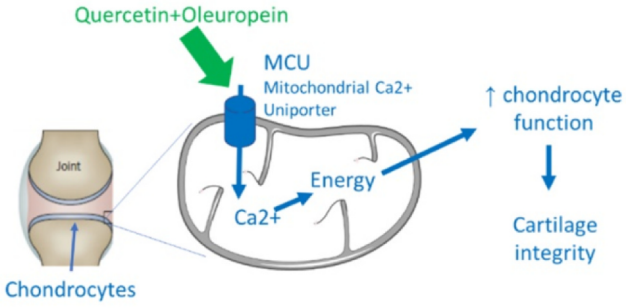
To rescue mitochondrial Ca^{2+}-linked defects with micronutrients, we used the natural polyphenolic MCU activators Quercetin, which partially rescued both mitochondrial Ca^{2+}-uptake and chondrocyte function in OA chondrocytes, but not in MCU-ablated cells, demonstrating the efficacy and MCU-dependency of this intervention. Similar effects were obtained with Oleuropein, another natural bioactive MCU activator. Acute treatment with the combination of these compounds, which synergistically activated MCU, completely rescued chondrocyte function in OA condition.

Conclusions: We identify causality between mitochondrial activation and chondrocyte function via MCU, demonstrating that this mitochondrial transporter is a novel molecular target for cartilage health in OA conditions. We establish Quercetin and Oleuropein as nutrients that specifically target MCU to rescue chondrocyte function in OA condition.



Model of chondrocyte protection via mitochondrial activation, by targeting MCU with the specific two natural bioactives activators Quercetin and Oleuropein

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ULTRASOUND COUNTERACTS INFLAMMATION BY PROMOTING THE CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STROMAL CELLS IN 3D PIEZOELECTRIC HYDROGEL

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Purpose (the aim of the study): Articular cartilage injuries have a limited potential to heal and, over time, may lead to osteoarthritis, an inflammatory and degenerative joint disease. Developing hydrogels capable of providing a biomimetic environment to drive mesenchymal stromal cells differentiation and counteract inflammation is a major challenge in cartilage tissue engineering. Low-intensity pulsed ultrasound (LIPUS) and piezoelectric nanomaterials represent an interesting tool for inducing chondrogenesis.

The aim of this study was to evaluate the effects of LIPUS in counteracting inflammation and positively committing the chondrogenesis of encapsulated human adipose stromal cells (hASCs) in 3D piezoelectric hydrogels.

Methods: hASCs at $2*10^6$ cells/mL were embedded in a 3D VitroGel RGD® hydrogel without nanoparticles or doped with 25 µg/ml of GO nanoflakes and 50 µg/ml BTNPs piezoelectric nanoparticles. The hydrogels were exposed to basal or inflammatory condition (+IL1β 10ng/mL), stimulated with LIPUS (frequency: 1 MHz, intensity: 250 mW/cm^2, duty cycle: 20%, pulse repetition frequency: 1 kHz, stimulation time: 5 min) every 2 days until day 10 of culture and chondrogenic differentiated for 28 days.

At day 2, 10 and 28 cell viability, cytotoxicity, gene expression (*COL2A1, ACAN, SOX9, COL1A1, COL10A1, MKI67, MMP13, TIMP1*), immunohistochemistry (COL2, aggrecan and COL1) and electron microscopy were evaluated. Inflammatory markers (*NFKB1* and *NFKBIA*) and cytokines release (IL6, CXCL8, TNF-α, CCL2, CCL4 and CCL5) were also analysed at each time point.

Results: In all 3D hydrogels we evidenced that LIPUS treatment did not affect negatively the viability and citotoxicity of the embedded cells. In basal condition, LIPUS considerably pushed the chondrogenic differentiation of hASCs embedded in 3D piezoelectric hydrogel, in particular chondrogenic markers COL2, aggrecan and anabolic extracellular matrix remodeling factor TIMP1 were overexpressed, while the fibrotic marker COL1 and proliferative marker MKI67 decreased compared to control samples without nanoparticles. Interestingly LIPUS also had effective anti-inflammatory effects by reducing NFKB1 and its inhibitor NFKBIA, as well as IL6, CXCL8, TNF-α, CCL2, CCL4, and CCL5 cytokines, so boosting the chondrogenic differentiation.

Conclusions: Overall, these data confirmed that LIPUS stimulation of 3D piezoelectric hydrogels promoted the chondrogenic differentiation of hASCs and it also was efficient in counteracting inflammation. However, only when the inflammatory cytokines were switched-off at day 10, the chondrogenic commitment was induced. This represents a promising tool in osteoarthritic cartilage regeneration field.

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THERAPEUTIC POTENTIAL OF PERINATAL MESENCHYMAL STROMAL CELLS DERIVED EXTRACELLULAR VESICLES ON INFLAMED CHONDROCYTES *IN VITRO*

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Purpose (the aim of the study): Introduction: Osteoarthritis (OA) is a degenerative joint disease for which no disease-modifying treatment is available. Mesenchymal stromal cells (MSCs) show promising therapeutic effects based on their inherent immunomodulatory and pro-regenerative properties. However, the therapeutic potential of MSCs decrease with ageing. Compared to adult derived stem cells, MSCs of perinatal origins exhibit superior proliferation ability and lower immunogenicity. In addition, perinatal annexes such as umbilical cord blood, Wharton's jelly (WJ), placental tissue, and amniotic membrane are rich sources of MSCs that can be obtained non-invasively from medical waste material at birth. The therapeutic effect of MSCs is mainly mediated by paracrine signals and there is accumulating evidence that extracellular vesicles (EVs) derived from MSCs preserve the therapeutic action of the parent MSCs. **Aim:** Therefore, this study aims to compare the therapeutic efficacy of WJ and Amnion-derived EVs on inflamed (tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β)) adult ovine chondrocytes *in vitro*.

Methods: To obtain the EVs, MSCs from a human immortalized perinatal MSCs cell lines from WJ (WJ-MSC/TERT273) and amniotic membrane (P-MSC/TERT308) were seeded and maintained in serum-free medium in the extracellular space of a hollow fiber bioreactor of different sizes (450 cm^2/4000 cm^2) following the manufacturer's instructions. EVs, isolated from the collected supernatant by tangential flow filtration (300kDa cut off), were characterized for particle size and number, as well as presence of marker proteins (Tetraspanins CD9, CD63, CD81) and typical EVs morphology using ELMI. The therapeutic effects of WJ-MSC/TERT273 and P-MSC/TERT308 derived EVs ($1x10^9$ particles/mL) on inflamed chondrocytes (1ng/mL IL-1β+TNF-α) were assessed in 2D and 3D culture and compared to three control groups: healthy, inflamed non-treated and inflamed Dexamethasone-treated chondrocytes. Wound healing (scratch) and proliferation assays, a multi-omic approach combining RNA-Seq, miRNA-Seq and Mass Spectrometry-based proteomics were employed to gain insights into the molecular mechanisms underlying the observed effects.

Results: Treatment with EVs from WJ-MSC/TERT273 promoted a higher proliferation rate of chondrocytes than P-MSC/TERT308-derived EVs, but a similar wound closure rate. Both EVs preparations, showed superior immunomodulatory effects compared to Dexamethasone by significantly ($p = < 0.05$, false discovery rate (FDR) < 0.05) downregulating several pro inflammatory mRNAs ((chemokine (C-C motif) ligand 20 (CCL20), C-X-C motif chemokine ligand 8 (CXCL8), interleukin 6 (IL6) and cyclin dependent kinase inhibitor 1A (CDKN1A)) and miRNAs (miR-146a-5p, miR-146b-5p and miR-34). However, P-MSC/TERT308-derived EVs significantly increase matrix metalloproteinase-1 (MMP1) and showed a non-significant effect on influencing matrix metalloproteinase-3 (MMP3)