IN VITRO MODELLING OF OSTEOARTHRITIS AS PLATFORM FOR TESTING MIRNA-BASED THERAPEUTIC POLYPLEXES

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Introduction: OA is the main chronic form of joint disease. Pro-inflammatory cytokines play a key role in OA pathogenesis by affecting the anabolic activity of chondrocytes in favour of catabolic activity, leading to protease-mediated Articular Cartilage (AC) degradation[1].
Objectives: To design and manufacture an AC *in vitro* scaffold-free organoids model in Healthy and OA conditions, with the aim of studying the disease evolution at cellular level and testing therapeutic polyplexes.
Materials and Methods: Spheroids of human articular chondrocytes (HC) and immortalised mesenchymal cells differentiated to chondrocytes (Y201-C) were formed in round-bottom 96-well plate and cultured in three culture conditions: Healthy (DMEM/F12), Low concentration OA (LC-OA) (DMEM/F12 loaded with: IL-1ß:1ng/mL, TNF-α:1ng/mL, IL-6:10ng/mL) and High Concentration OA (HC-OA) (DMEM/F12 loaded with: IL-1ß:5ng/mL, TNF-α:5ng/mL, IL-6:50ng/mL). Spheroid growth kinetics and metabolic activity were evaluated over 10 days and then spheroids were assembled and cultured up to 21 days on gelatin-coated poly(lactic-co-glycolic acid) electrospun membranes (10 spheroids/cm^2), using a protocol inspired by the clinically approved Chondrosphere® (CO.DON AG) technique[2]. Gene expression analysis (SOX9, COL2A1, ACAN,COL1A2, MMP13 and ADAMTS-5), Histology (H&E, Alcian Blue, Picrosirius red) and Immunohistochemistry (IHC)(Collagen II , Aggrecan and Ki-67) were performed to assess OA markers in all conditions. The therapeutic effect of miRNA-140-5p-based (20 µM) chitosan polyplexes (250 µg/mL) was evaluated using the model.
Results and Discussion: HC and Y201-C spheroids showed a diameter decrease over culture in Healthy state compared to OA conditions, where the diameter remained stable. Cell metabolic activity decreased over culture for HC and increased for Y201-Cs in all conditions, but both cell types showed a higher metabolic activity in HC-OA condition at day 10. At day 21, cells in OA environment showed significant decreased expression of anabolic markers (COL2A1, SOX9 and ACAN) and an upregulation of catabolic markers (MMP13 and ADAMTS5), as well as a significantly lower production of collagen and glycosaminoglycans. Collagen II and Aggrecan (Fig. 1A) IHC confirmed this trend. The uptake of polyplexes into the model was successful (Fig.1B) and demonstrated their potential as therapeutic nanoparticles, being able to decrease the expression of MMP13 catabolic marker and increase the expression of COL2A1 in OA-induced models.
Conclusions: We have developed a reliable *in vitro* OA model where the presence of pro-inflammatory cytokines led to: *(i)* an increased cells metabolism, possibly due to the biosynthesis of OA-related inflammatory and degradative enzymes at day 10; *(ii)* decreased expression of anabolic genes in favor of catabolic genes by cells after 21 days culture ; *(iii)* lower production of collagen II and aggrecan as effect of the inflammation; (iv) demonstration of the therapeutic effect of miRNA-based polyplexes as a potential OA treatment strategy.

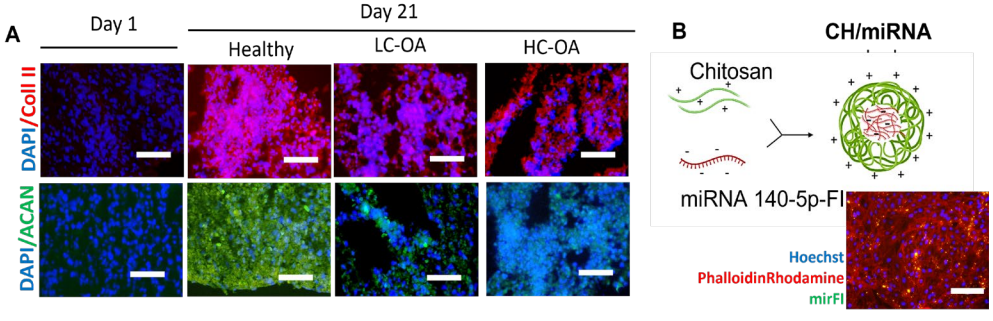

Figure 1: IHC at day 1 and day 21 of Collagen II (Col II) and Aggrecan (ACAN) in healthy LC-OA and HC-OA (A). miRNA/CH polyplexes uptake by HC cells after 48h. Bars: 150µm

*References:*1. Kloppenburg et al, *Osteoarhtr Cartil* 28;3,2020; 2.Libera et al. US7887843B2 Patent