Inhibiting calcineurin activity under physiological tonicity: a win-win situation for cell-based chondral lesion repair?

INTRODUCTION:
Osteoarthritis (OA) is characterized by an imbalance between matrix synthesis and degradation. Increased synthesis of catabolic enzymes by hyaline chondrocytes causes collagen (mainly collagen type II, COL2) and proteoglycan (e.g., aggrecan) depletion in the extracellular matrix (ECM). As major structural ECM components, the COL2 network and the high fixed negative charge density of sulfated proteoglycans provide the tissue’s biomechanical properties. The physiological extracellular tonicity in healthy hyaline articular cartilage in vivo is 350 - 480 mOsm, but drops to 280 - 350 mOsm in OA.

We showed that calcineurin inhibitor FK506 improves chondrogenic marker expression [1] and that culturing osteoarthritic human articular chondrocytes (OA-HACs) under physiological tonicity (380 mOsm) is beneficial [2].

In the present study, we show that combining both treatments exerts synergistic effects: i.e. stimulating anabolic, but selectively suppressing catabolic/hypertrophic marker gene expression by OA-HACs.

METHODS:
Harvest: OA-HACs were harvested from knee cartilage (n=4, MEC2004-332) by overnight digestion in collagenase B in control (280 mOsm) or physiological (380 mOsm) medium [2].

Culture: OA-HACs were culture expanded in monolayer (7,500 cells/cm²) and kept in either control or physiological medium throughout all experiments. Passage 1 (P1) and P2 OA-HACs were seeded in high-density monolayers (20,000 cells/cm²) in triplicate. After 24 hrs 0, 50 or 500 ng/ml of FK506 was added to the culture medium and cells were harvested for mRNA (quantitative RT-PCR, qPCR) and protein expression (luminescent Western Blot) analyses six days later. The same experiments were performed with OA cartilage explants.

Statistical analysis was performed using SPSS 13.0 software and a Kruskall-Wallis H and post-hoc Mann–Whitney U test for data comparison between groups. Results represent means ± standard deviation.

RESULTS:
Culturing OA-HACs under physiological tonicity (380 mOsm) up-regulates anabolic markers such as aggrecan, SOX9 and COL2 compared to standard culture [2]. While FK506 has only a modest anabolic effect at 280 mOsm (Fig. 1), it significantly elevated the beneficial effects of physiological tonicity on COL2 (Fig.1A), aggrecan and SOX9 (Fig.2A): COL2 expression in OA-HACs increased up to 50-fold at 380 mOsm + FK506. Similar effects were found on protein levels (Fig.1B). Physiological tonicity reduced COL1 expression by 50% in P1 and addition of FK506 suppressed COL1 up-regulation in P2 (Fig.1A).


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DISCUSSION:
Physiological tonicity provides a simple, effective means to improve chondrogenic marker expression during cytokine-free isolation and in vitro expansion of human articular chondrocytes. Combining FK506 and physiological tonicity further improved chondrogenic marker expression, while suppressing toxicity-induced catabolic and hypertrophic markers.

Our findings may improve cell-based repair strategies for chondral lesions and could provide novel insights into the progression of OA.