Oxygen conditioning effect on an *in vitro* co-culture model of tendon-to-bone interface

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INTRODUCTION: Tendon-to-bone interface comprises a heterotypic cellular niche. The native interface is hypovascular, suggesting that the junction is physiologically hypoxic. As it bridges tendon and bone, which require different oxygen concentrations, a tight coordination of different oxygen concentrations along the junction must be considered when trying to mimic and understand biological events occurring within the tissue. Herein, an optimized *in vitro* co-culture model of tendon-derived cells (hTDCs) and pre-osteoblasts (pre-OBs) [1] was used to study the effect of a restricted oxygen environment on cell behavior.

METHODS: Single cultures of hTDCs or pre-OBs and direct contact co-cultures (1:1 cell ratio) were maintained for 14 days in a 5% oxygen (O_2) tension (hypoxia) using three medium conditions containing different osteogenic supplementation ratios (OM, 0%, 50%, 100%). Controls were performed under normoxia. Cell proliferation and protein synthesis, alkaline phosphatase (ALP) activity and mineral deposition (alizarin red, AZ) were quantified. Gene expression of tendon-, bone and interface-related was assessed by RT-PCR.

RESULTS: Hypoxia reduced cell proliferation, independently of OM supplementation, in comparison with normoxia for all cultures (p<0.0001). An overall increase in matrix mineralization (Fig. 1) and ALP activity was observed at 14 days in co-cultures independently of OM supplementation, compared to pre-OBs alone (p<0.0001). Interestingly, oppositely to co-cultures under normoxia, increasing OM concentration in 5% O_2 led to a reduction in matrix mineralization in co-cultures (50%OM, p<0.009; 100%OM, p<0.0001). In terms of total protein synthesis, hypoxia led to an overall reduction in synthesis, particularly in hTDCs. A synergistic effect between heterotypic cellular interactions, osteogenic medium and hypoxia was observed in the transcription levels of interface-related markers in co-cultures (*COMP*, *ACAN*, co-culture D14, $p<0.05$; versus single cultures D14, $p<0.0001$)

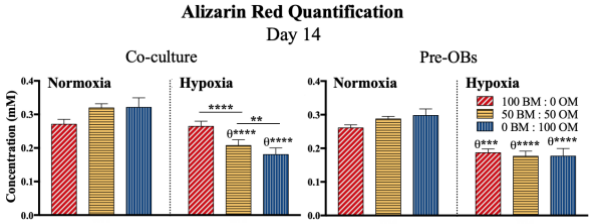

Figure 1: AZ quantification in co-culture and pre-OBs. Statistically significant differences: **, p<0.009; ***, p<0.0003; **** p<0.0001; θ is statistically significant in correspondence with the same condition in normoxia.

DISCUSSION & CONCLUSIONS: Overall, 5% O_2 diminished proliferation and protein synthesis. Combining osteogenic supplementation and hypoxia reduced matrix mineralization by cells in co-culture. Nevertheless, studying the expression of specific markers, such as HIF-1 alpha will allow a better assessment of the hypoxic response of cells in both single and co-cultures, toward identifying the role of cell-cell interactions and OM on the expression of bone, tendon and interface-related markers.

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