Oxygen tension-mediated regulation of chondrogenic differentiation: application to stem cell-based osteochondral repair

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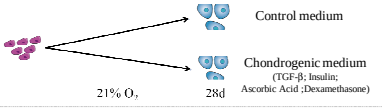
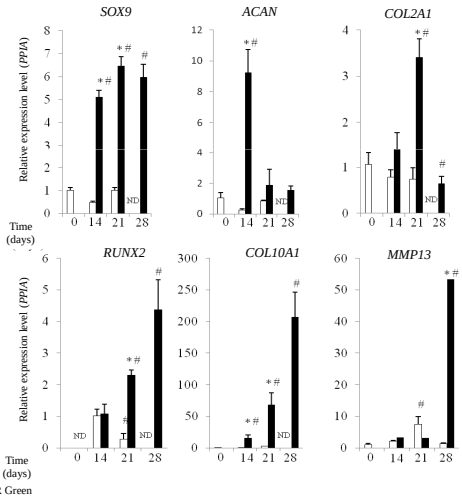
Introduction

Multipotent stromal cells (MSC) have been considered promising for the regenerative strategies of articular cartilage. However, the MSC chondrogenic differentiation can ultimately lead to the formation of hypertrophic chondrocytes responsible for the calcification of cartilage. To prevent this MSC-dependent production of a calcified matrix in articular site, MSC hypertrophic differentiation has to be carefully controlled. Given that articular cartilage is avascular, we questioned whether in addition to its stimulatory role in the early differentiation of chondrogenic cells, hypoxia may prevent their late hypertrophic conversion. To address this issue, we used two different chondrogenic cell types: human adipose stem/stromal cells (ASC) and chondrogenic ATDC5 cell line.

Chondrogenic differentiation of ASC





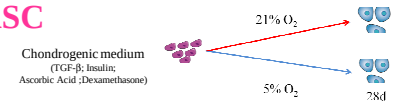
RT-PCR SYBR Green

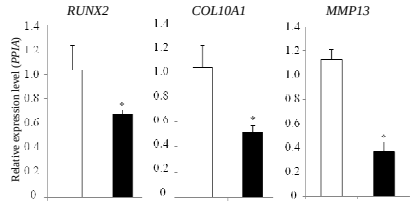
ASC exhibits chondrogenic and hypertrophic phenotypes

Control medium ■ Chondrogenic medium

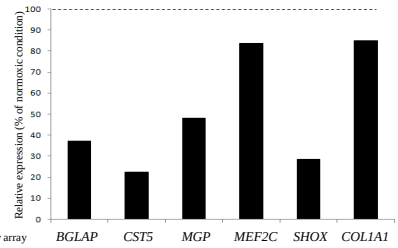
# $p<0.05$ compare to NCT conditions at day 0
* $p<0.05$ compare to NCT conditions at the same day

Effects of 5%O_2 on the terminal differentiation of ASC





RT-PCR SYBR Green



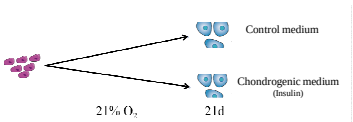
Taqman low density array

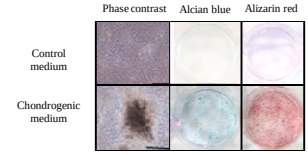
5% O_2 down-regulates the expression of terminal differentiation markers and alkaline phosphatase activity

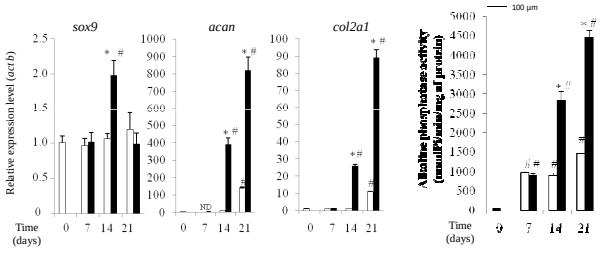
Chondrogenic medium; 21% O_2 ■ Chondrogenic medium ; 5% O_2

* $p<0.05$ compare to NCH conditions

Chondrogenic differentiation of ATDC5 cells







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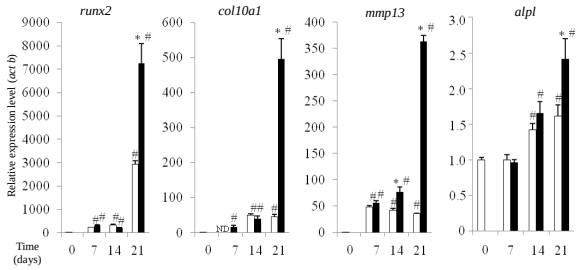


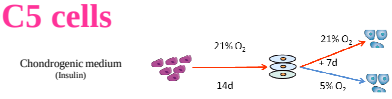
RT-PCR SYBR Green

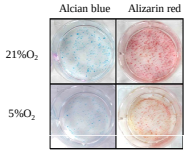
ATDC5 cells exhibit chondrogenic and hypertrophic phenotypes

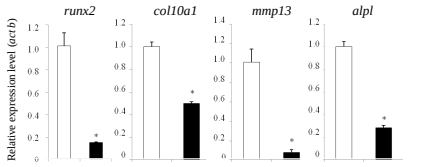
Control medium ■ Chondrogenic medium

# $p<0.05$ compare to NCT conditions at day 0
* $p<0.05$ compare to NCT conditions at the same day

Effects of 5%O_2 on the terminal differentiation of ATDC5 cells







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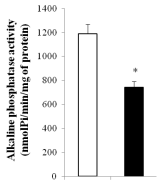
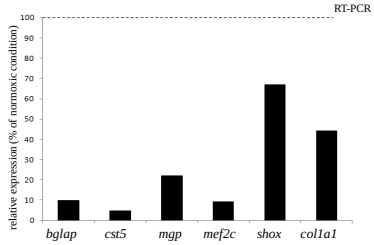
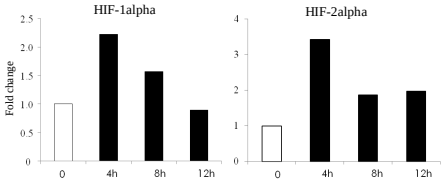




* $p<0.05$ compare to NCH conditions

Taqman low density array

5% O_2 down-regulates the expression of terminal differentiation markers and reduces matrix mineralization



ELISA-based EMSA (No shift assay)

5% O_2 promotes the DNA binding activity of HIF-1alpha and HIF-2alpha

Chondrogenic medium; 21% O_2 ■ Chondrogenic medium ; 5% O_2

Conclusions and perspectives

Our data suggest that a 5%O_2, in addition of being able to chondrogenically commit ASC, inhibits the hypertrophic differentiation of chondrogenic cells. These results make hypoxia an instrumental tool to prevent the formation of a calcified matrix in ASC-based cartilage tissue engineering. On the contrary, 21%O_2 was found to up regulate the terminal differentiation of chondrogenic cells. These data make normoxia a potent factor useful for bone repair through endochondral strategy.

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