Effects of an *in vitro* low oxygen tension preconditioning of MSC on their *in vivo* chondrogenic potential: application for cartilage tissue engineering

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Introduction

Adipose stromal cell (ASC)-based regenerative medicine is considered promising for cartilage repair. Cartilage is an avascular tissue in which cells experience hypoxia. Interestingly, hypoxia is known to promote the early chondrogenic differentiation of ASC. In this context, we aim at determining whether low oxygen tension may be an instrumental tool to exploit the regenerative potential of ASC for cartilage repair.

Chondrogenic potential of differentially preconditioned rabbit ASC

Methods

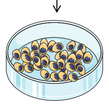
rASC

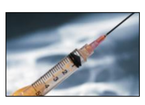
21% O_2 + Control medium (NCT)

21% O_2 + Chondrogenic medium (NCH)

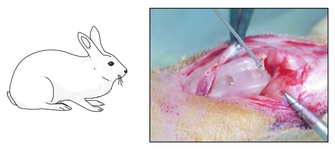
5% O_2 + Chondrogenic medium (HCH)

28 Days



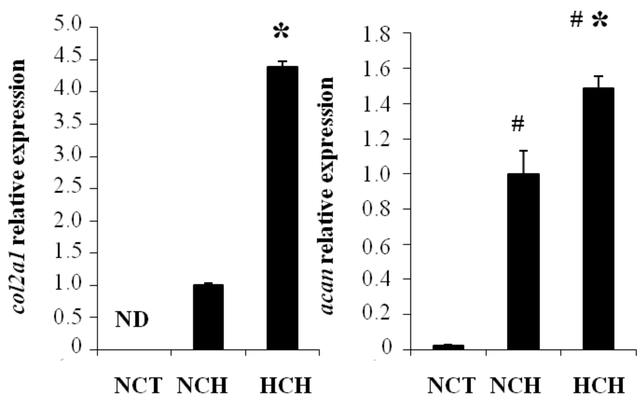


Si-HPMC hydrogel

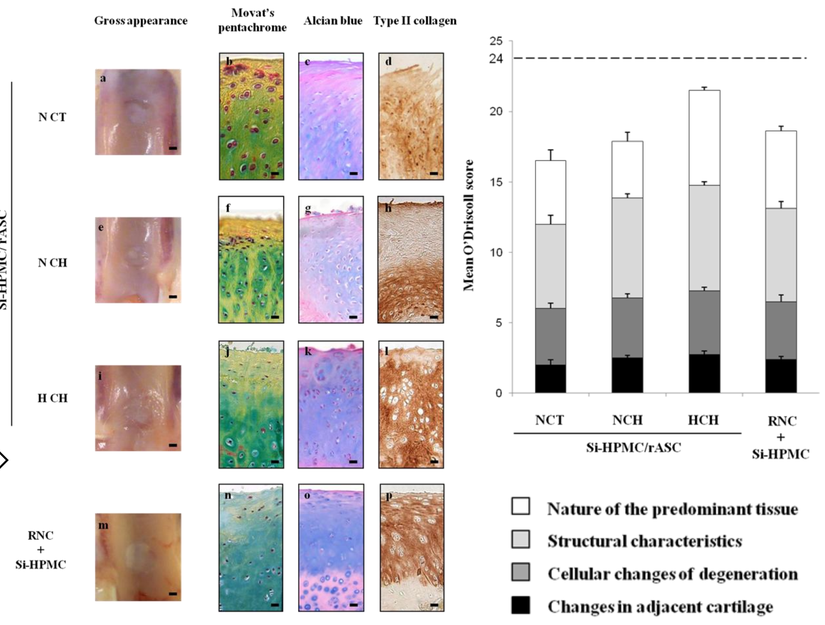


Articular implantation 18 weeks

Results



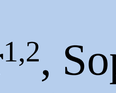
NCT : Normoxia Control medium
NCH: Normoxia Chondrogenic medium
HCH: Hypoxie Chondrogenic medium
# p<0.05 compared with the NCT condition
* p<0.05compared with the NCH condition



Whereas a 5% O_2 promotes the chondrogenic differentiation of rASC *in vitro*, it does not improve their *in vivo* chondrogenic potential.

Chondrogenic potential of differentially preconditioned human ASC

Methods



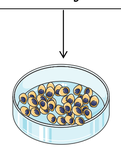
hASC

21% O_2 + Control medium (NCT)

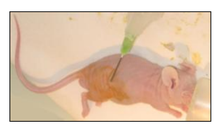
21% O_2 + Chondrogenic medium (NCH)

5% O_2 + Chondrogenic medium (HCH)

21 Days

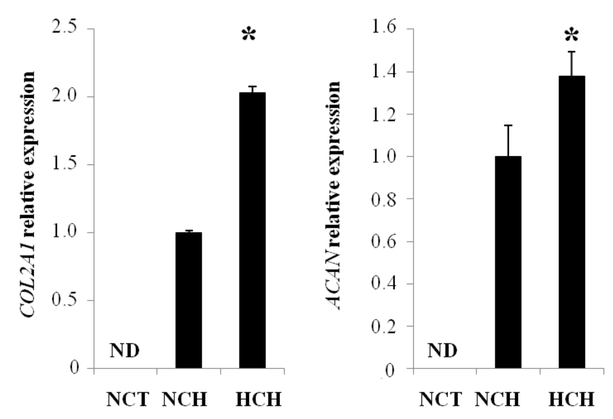


Si-HPMC hydrogel



Subcutaneous implantation 5weeks

Results



NCT : Normoxia Control medium
NCH: Normoxia Chondrogenic medium
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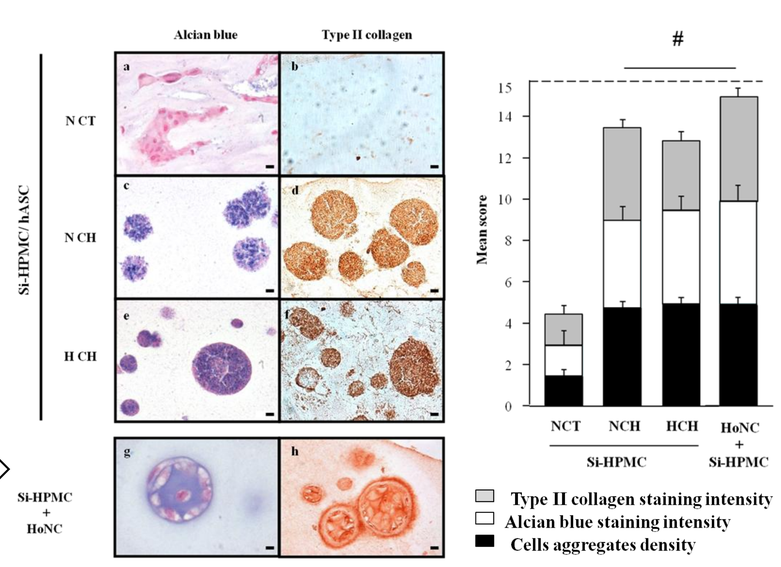
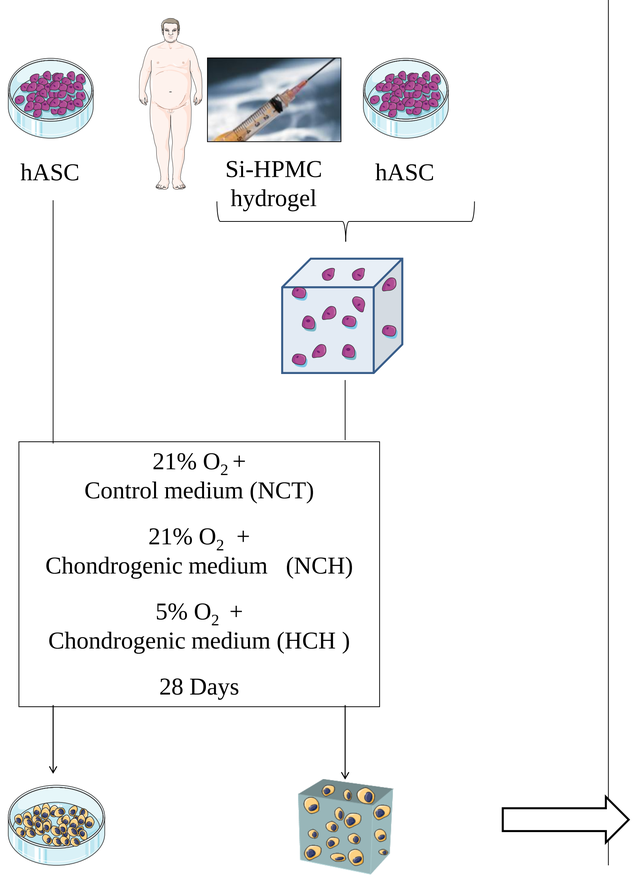
# p<0.05 compared with the NCT condition
* p<0.05compared with the NCH condition



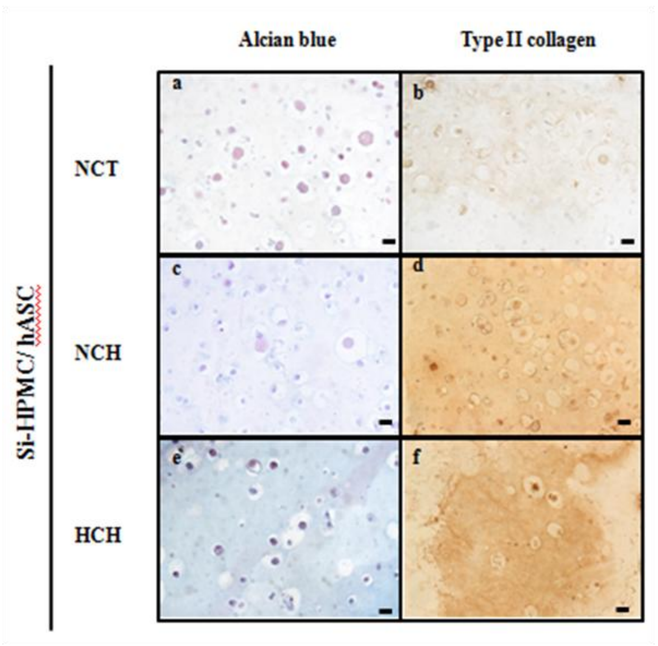
Whereas a 5% O_2 promotes the chondrogenic differentiation of hASC *in vitro*, it does not improve their *in vivo* chondrogenic potential.

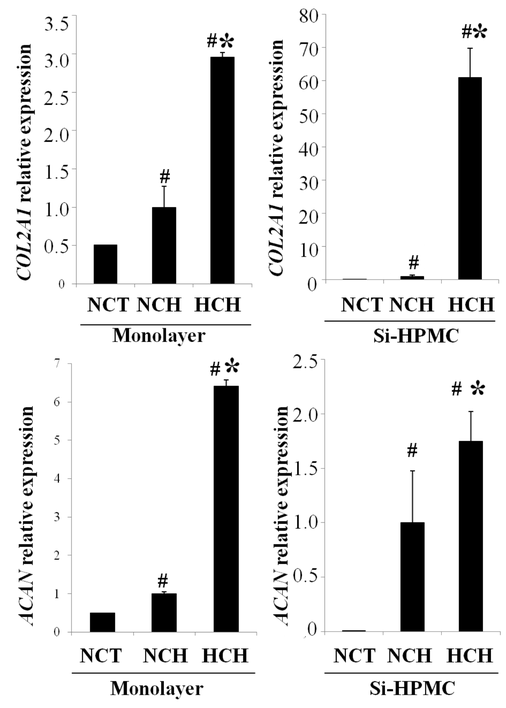
In vitro chondrogenic differentiation of tridimensionally cultured human ASC

Methods



Results





NCT : Normoxia Control medium
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HCH: Hypoxie Chondrogenic medium
# p<0.05 compared with the NCT condition
* p<0.05compared with the NCH condition

hASC cultured within HPMC-Si hydrogel are responsive to a pro-chondrogenic medium and to a 5% O_2 environment. In addition, these data suggest that a 3D culture within Si-HPMC hydrogel promotes the ability of an inductive medium and a 5% O_2 environment to up-regulate the chondrogenic differentiation of hASC.

Conclusions and perspectives

5% O_2 increased the *in vitro* expression levels of chondrogenic markers in rabbit and human ASC cultured in chondrogenic medium in 2D or within hydrogel. Interestingly, analyses of subcutaneous and articular implants revealed the formation of a cartilaginous tissue but only for cells cultured in chondrogenic medium. Surprisingly, no statistically significant difference could be observed between ASC cultured in 5 or 21% O_2. These data show that biomaterial-assisted ASC therapy could be a relevant strategy for the regenerative medicine of cartilage. Whereas a 5% O_2 up-regulates the *in vitro* chondrogenic differentiation of ASC, it does not stimulate the *in vivo* chondrogenic regenerating potential of ASC. With respect to the putative role of oxygen tension in the control of terminal hypertrophic differentiation, we questioned whether an *in vitro* hypoxic treatment may be instrumental to control the terminal differentiation of ASC after implantation.

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