Effects of Oxygen Tension on Terminal Differentiation of Chondrogenic Cells.
Sophie Portron1, Christophe Merceron1, Martial Masson1, Maïthé Gatius1, Pierre Weiss1, Olivier Gauthier2, Jérôme Guicheux1, Claire Vinatier1,3.
1 Inserm, U 791, LIOAD, STEP Group « Skeletal Tissue Engineering and Physiopathology» Nantes, France 2 Veterinary school of Nantes, Experimental Surgery Department, Nantes, France 3 Graftys SA, Aix en Provence, France

Introduction
Mesenchymal stem cells (MSC) have been recently considered as promising autologous cells for regenerative medicine of articular cartilage. MSC chondrogenic differentiation is similar to that observed in the growth plate leading to endochondral ossification. Therefore, MSC transplanted in vivo could give rise to the formation of a calcified and vascularized cartilage[1]. To prevent the formation of this mineralized and unfonctional cartilage, the hypertrophic differentiation needs to be controlled. Articular cartilage is avascular and consequently, the oxygen tension is between 7-13%O2. Hypoxia is considered as a key factor for the expression of early chondrogenic markers[2]. In this context, the aim of this study was to assess the influence of oxygen tension on chondrogenic hypertrophic differentiation.

Materials and Methods
The ATDC5 cell line differentiates into chondrogenic-like cells by mimicking the various steps of endochondral ossification including the hypertrophic stage. ATDC5 were differentiate until the hypertrophic stage (21 days) under normoxia (21% O2) and then cultured during 7 days either in normoxia or in hypoxia (5% O2). The hypertrophic differentiation of ATDC5 was evaluated by alcian blue and alizarin red stainings respectively for the presence of sulfated GAG and calcifications in the matrix. The expressions of hypertrophic differentiation markers (MMP13, type X Collagen, Runx2, ALP) were investigated by Real-Time PCR and TaqMan Low Density Array. Alkaline phosphatase activity was also evaluated.

Results
Our data indicate that hypoxia down-regulate the expression of terminal differentiation markers such as MMP13, type X Collagen, Runx2 and ALP, reduce the matrix mineralization and Alkaline phosphatase activity without influencing proteins production and cell number.

Discussion and Conclusions
Our data suggest that low oxygen tension inhibits the chondrogenic hypertrophic differentiation. Whether or not hypoxia could affect the hypertrophic differentiation of MSC remains to be investigated. These results make hypoxia a potential relevant tool to control the MSC chondrogenic differentiation for articular cartilage tissue engineering.

References

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Disclosures
The authors have no conflict of interest.