

## The effect of oxygen tension on the proliferation and differentiation of human mesenchymal stem cells

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**INTRODUCTION:** Mesenchymal stem cells (MSCs) reside *in vivo* under oxygen levels between 4-7% [1,2,3]. The present study investigated the effect of culture under 20%, 5% and 2% oxygen conditions on the proliferation and differentiation of human bone marrow derived MSCs.

**METHODS:** Fresh human bone marrow samples (Lonza, Wokingham UK) were aliquoted into culture flasks within a system permitting continuous and uninterrupted control of the oxygen environment (Biospherix, New Jersey USA). Flasks were fed with alpha-MEM + 8.5% FBS pre-equilibrated at either 20 %, 5 % or 2 % oxygen and placed into incubators set at the relevant oxygen conditions. The colonies were monitored until confluence with area and diameter assessed from photomicrographs using commercial software (SigmaScan Pro, Systat software, CA., US). Population growth curves were recorded for the cells over 5 passages. Senescence was assessed by staining for  $\beta$ -galactosidase activity (Sigma-Aldrich, Poole UK). Passage 2 MSCs were differentiated to osteogenic and chondrogenic lineages using defined medium under each of their cultured oxygen tension. Differentiation towards their specific lineages for each oxygen tension was assessed through biochemical assays (GAG and Alkaline phosphatase activity) and staining (alizarin red and toluidine blue).

**RESULTS:** The number of colonies formed at 5% oxygen was significantly reduced compared with 20% and 2% oxygen for two separate donors ( $p < 0.05$ ). The area and diameter of the colonies cultured at 2% were significantly smaller compared with oxygen in both donors ( $p < 0.05$ ). The number of cells recovered upon first trypsinisation was greatest at 20% oxygen suggesting a greater number of cells per colony compared with 5% and 2% oxygen cultures ( $p < 0.05$ ). Proliferation rate of cells cultured at 20% oxygen was initially greater than 5% and 2% oxygen, however, there was no significant difference in population doubling time between each oxygen condition after two passages (Fig. 1a). However, there was significantly greater

percentage senescence under 20% oxygen compared with 5% and 2% oxygen (Fig. 1b:  $p < 0.05$ ). MSCs showed osteogenic differentiation at 20% oxygen. However, chondrogenic differentiation occurred under each oxygen tension

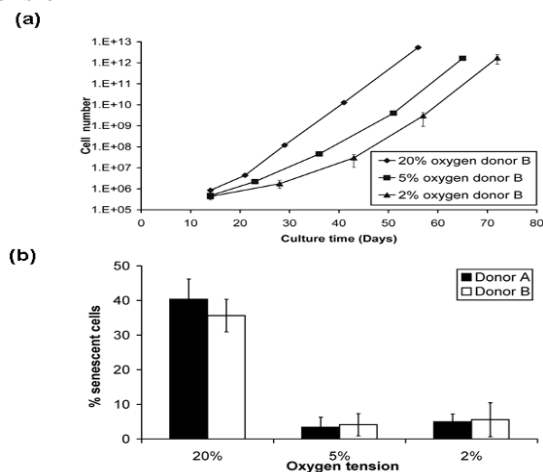


Fig. 1: (a) The proliferation growth curves for donor B and (b) the percentage of senescent MSCs grown under 20%, 5% and 2% oxygen conditions.

**DISCUSSION:** The results of the investigation show that the oxygen tension influences the colony-forming efficiency of MSCs and may enable the selection of MSC sub-populations that have been described in previous studies [2]. Greater cell numbers were generated under 20% oxygen from initial colony formation but there was greater cellular senescence for cells grown at 20% oxygen, which may be associated to the generation of reactive oxygen species [1]. Though under all oxygen conditions, chondrogenic differentiation occurred, inhibition of osteogenic differentiation upon culture under low oxygen conditions may also be associated with the metabolism of the cells during differentiation.

**REFERENCES:** <sup>1</sup> F. Mousavvi-Harami et al (2004), *Iowa Orthop. J.* **24**: 15-20. <sup>2</sup>G.D'Ippolito et al (2004) *J.Cell.Sci.*, **117**:2971-2981. <sup>3</sup> G. D'Ippolito et al (2006) *Bone*, **39**:513-522.