

EX-VIVO EXPANSION OF HUMAN MESENCHYMAL STEM CELLS UNDER HYPOXIA: EFFECTS ON PROLIFERATION KINETICS AND METABOLISM

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Due to the very low percentage of human mesenchymal stem cells (MSC) in their niches, an efficient *ex-vivo* expansion method is needed to meet the increasing MSC clinical applications. Attending to the low oxygen environment of bone marrow (BM) *in vivo*, it has been hypothesized that hypoxic conditions (under 5% O₂) might favour MSC expansion *in vitro*. Consequently, the effects of hypoxia on human BM MSC (from 5 donors) proliferation kinetics and metabolism were studied.

Under low oxygen tension (2% O₂), MSC reached a higher fold increase in total cells of 39±10 by day 12, compared to the normoxic condition (20% O₂) where a fold increase of 30±6 was obtained. Cell growth showed that hypoxia induced an earlier exponential growth phase, starting within the first 3 days of culture, when compared to normoxia (started between days 3 and 5). Cell division kinetics was also studied during the first week of expansion, by cell tracking with the membrane dye PKH67, allowing us to establish the cell divisional history of the cultures. It was possible to observe that a higher number of cells underwent mitosis under hypoxia, which reached later generations compared to cells expanded under normoxia after 6 days of culture.

The metabolite analysis showed similar average specific rates of glucose consumption and lactate production for cells expanded under hypoxic ($q_{\text{gluc}}=(1.5\pm0.3)\times10^{-8}$ mmol gluc/(cell.day), $q_{\text{lac}}=(3.0\pm0.3)\times10^{-8}$ mmol lac/(cell.day)) and normoxic conditions ($q_{\text{gluc}}=(1.4\pm0.1)\times10^{-8}$ mmol gluc/(cell.day), $q_{\text{lac}}=(3.2\pm0.5)\times10^{-8}$ mmol lac/(cell.day)).

It was possible to observe that glucose specific consumption rate maintained a stable value throughout the entire expansion period, whereas specific glutamine consumption rate was significantly higher in the beginning of the culture for both oxygen tensions tested - $(4.8\pm0.7)\times10^{-8}$ and $(3.8\pm0.1)\times10^{-8}$ mmol glut/(cell.day) for hypoxia and normoxia, respectively, however it decreased to considerably lower values upon exponential phase starting.

More interestingly, the MSC average specific ammonia production rate suffered a 34% reduction under low oxygen tension. Since ammonia is a known cell proliferation inhibitor, we hypothesized this hypoxia-induced metabolic adjustment might have a relevant role on cell yield.

For both conditions, the apparent glucose to lactate yield ($Y'_{\text{lac/gluc}}$) was higher early in culture (until day 5), which was consistent to a higher consumption of glutamine during this period of time. Nevertheless, overall, $Y'_{\text{lac/gluc}}$ values were not significantly different amongst hypoxia and normoxia cultures.

Cells expanded under both conditions maintained their phenotype (>95% CD73⁺, CD90⁺ and CD105⁺) and multilineage differentiative potential. Colony-forming unit fibroblasts (CFU-F) assays were also performed to assess multipotent stem/progenitor cell expansion and maintenance.

These results suggest that, under our culture conditions, a 2% oxygen tension might be advantageous towards the maximization of MSC yield for a potential clinical application.