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Impact of Oxygen Levels on Human Hematopoietic Stem and Progenitor Cell Expansion

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Abstract

Oxygen levels are an important variable during the in vitro culture of stem cells. There has been increasing interest in the use of low oxygen to maximise proliferation and, in some cases, effect differentiation of stem cell populations. It is generally assumed that the defined pO$_2$ in the incubator reflects the pO$_2$ to which the stem cells are being exposed. However, we demonstrate that the pO$_2$ experienced by cells in static culture can change dramatically during the course of culture as cell numbers increase, and as the oxygen utilisation by cells exceeds the diffusion of oxygen through the media. Dynamic culture (whereby the cell culture plate is in constant motion) largely eliminates this effect, and a
combination of low ambient oxygen and dynamic culture results in a four-fold increase in reconstituting capacity of human hematopoietic stem cells compared to those cultured in static culture at ambient oxygen tension. Cells cultured dynamically at 5% oxygen exhibited the best expansion: 30 fold increase by flow cytometry, 120 fold increase by colony assay and 11% of human CD45 engraftment in the bone marrow of NOD/SCID mice. To our knowledge, this is the first study to compare individual and combined effects of oxygen and static or dynamic culture on hematopoietic ex vivo expansion. Understanding and controlling the effective oxygen tension experienced by cells may be important in clinical stem cell expansion systems, and these results may have relevance to the interpretation of low oxygen culture studies.

Introduction

Normal functioning of cells in vivo occurs under a wide range of oxygen concentrations, depending on the proximity to afferent arterioles and local oxygen consumption. Stem cells, in particular, appear to be highly sensitive to pO₂ levels and to hypoxia [1]. Hypoxia induced factor (HIF)-dependent mechanisms have been implicated in development [2] and function of the hematopoietic system [3], in the maintenance of embryonic stem cell self-renewal [4], and in the maintenance of stemness [5] and differentiation capacity [6] of mesenchymal stem cells (MSCs).

These findings have implications for the in vitro expansion of stem cells for clinical applications. We [7] and others [8-10] have demonstrated improved maintenance of self-renewal potential and in vitro expansion of hematopoietic stem/progenitor cells (HSPCs) when cultured at reduced oxygen. The cytokine cocktail used have hypoxia-mimicking effects and synergizes with low oxygen concentration in stem cell maintenance [11,12]. This
is perhaps unsurprising, given that the bone marrow, and in particular the HSC niche, is a low oxygen environment [8,13-15]. There is increasing evidence for a critical role of HIF-dependent mechanisms in control of HSPC self-renewal [8,16-18]. Hypoxia has also been shown to impact on human MSC expansion derived from bone marrow, adipose tissue, amniotic fluid, and umbilical cord blood and appeared to depend on the source of the cells [19].

A critical question in interpretation of in vitro studies is whether the cells were actually exposed to the effective pO$_2$ that was intended. More than 40 years ago Balin et al [20] showed the impact of stationary versus shaken cultures on the proliferative capacity of cells, while almost 20 years ago Metzen et al [21] demonstrated that the pericellular oxygen experienced by cells in monolayer culture may be substantially lower than the pO$_2$ in the air to which the culture was exposed. This effect is due to the limited diffusion of oxygen through media, such that the utilisation of oxygen by most confluent cell cultures exceeds the passive diffusion of oxygen through the medium [22], creating a gradient that can be directly measured [21] or visualised [23]. This effect has been modelled by Zhdanov et al [24], who showed that the critical factors in determining the effective oxygen experienced by cells includes atmospheric oxygen, sample geometry (eg. depth of media), cell density and the respiration rate of the cells. Given that stem cell expansion cultures can result in a many-fold increase in cell numbers over the course of the culture, we have measured the dissolved oxygen in HSPC cultures over 7 days. We have further investigated the functional impact of different atmospheric oxygen levels and of static and dynamic cultures on the repopulating capacity of HSPCs. These studies demonstrate the critical role of these variables on the outcome of HSPC cultures, and the importance of directly measuring
dissolved oxygen when performing studies on the effect of hypoxia on cell metabolism and function.

**Materials and Methods**

**Ethics Statement**

All research with human samples and mice was performed in compliance with the local ethical guidelines and with the approval of the Institutional Review Boards. NSG (NOD.Cg-Prkdcsid Il2rgtm1Wjl/SzJ) mice, which were used for xenografting human umbilical cord blood (UCB) cells were obtained from the Walter and Eliza Hall Institute and bred at the Monash Medical Centre Animal Facility under approval from the Office of the Gene Technology Regulator (IBC NLRD number: PC1-N30/09) and with animal ethics approval MMCA2013/28BC for breeding of the mice. NSG mice were bred and housed under specific pathogen free (SPF) conditions of automated 12 hours light and 12 hours dark cycle. Mice were fed ad libitum with standard mouse chow and autoclaved acidified water. After the irradiation and transplantation, the mice were given Bactrim antibiotic in their water. Approval to undertake this project was obtained from Monash Medical Centre Animal Ethics Committee A (AEC number: MMCA 2012/83). UCB was collected with informed consent under Monash Health human research ethics committee approval (HREC number: 12387B). Collection was carried out in accordance to the National Statement on Ethical Conduct in Human Research (2007) produced by the National Health and Medical Research Council of Australia.

**Cell culture**

**Isolation of CD34+ cells**
CD34<sup>+</sup> cells were isolated from freshly collected human umbilical cord blood (UCB) as described previously [25,26]. In short, diluted UCB was centrifuged using a Ficoll-Paque density gradient and mononuclear cells were obtained from the buffy coat. CD34<sup>+</sup> cells were then isolated from mononuclear cells via magnetic-assisted cell sorting using MACS CD34 Microbead kits (Miltenyi Biotec, NSW, Australia). The viability and purity of the isolated cells were assessed by flow cytometry and cells were cryopreserved in liquid nitrogen until required.

**HSPC expansion**

CD34<sup>+</sup> cells were cultured in serum-free medium, Stemspan ACF (Stem Cell Technologies, Victoria, Australia) supplemented with 100 U/mL penicillin-100 μg/mL streptomycin (Life Technologies, Victoria, Australia), 50 ng/mL stem cell factor (SCF) (BD Biosciences, NSW, Australia), 50 ng/mL thrombopoietin (TPO), 80 ng/mL Fms-related tyrosine kinase Ligand (Flt3-L) and interleukin 6 (IL-6; all three growth factors from Merck Millipore, Victoria, Australia). Cells were seeded in triplicates onto the middle wells of 24-well tissue culture plates at 10,000 cells/well. Prior to seeding, the tissue culture wells were coated with 10 μg/mL RetroNectin® (Takara Bio Inc., Shiga, Japan) for 1h at room temperature.

The cells were cultured in either 20% O<sub>2</sub> or 5% O<sub>2</sub> under 2 conditions – static or dynamic. Cells cultured under static condition were placed on a shelf in the incubator and those cultured under dynamic conditions were placed on an orbital shaker at 20 rpm within the incubator. Two identical plates were set up for each experimental group whereby one plate was used to quantify CD34<sup>+</sup> expansion while the other was for measuring oxygen concentration daily. All groups were cultured in a humidified environment at 37°C and 5% CO<sub>2</sub> for 7 days. Cells were harvested by gentle flushing, followed by two washes with PBS.
Viable cell counting was done with 1% trypan blue and the cells were further analysed by flow cytometry and colony-forming assay.

**Characterisation of expanded HSPCs**

After 7 days of culture, the HSPCs were harvested and counted using Trypan Blue exclusion. In addition, flow-cytometric analysis for surface markers was performed with the following four antibody combinations: CD45 FITC+CD34 PE+7AAD, CD34 FITC+CD38 PE+7AAD, CD33 FITC+CD34 PE+7AAD and CD34 FITC+CD133 PE+7AAD (all antibodies from BD Biosciences, NSW, Australia, except CD133 PE, which was from Miltenyi Biotec, Australia). PE- and FITC-labelled anti-mouse isotype IgG stains were used as compensation controls. Antibody-stained cells were analysed in a FACS Canto II (BD Biosciences, NSW, Australia) flow cytometer, using FACSDiva™ software. For determining the mean and standard error values, data from three experiments, with triplicate samples in each experiment, were used. Fold expansion of CD34+ and other lineages specific cells were calculated by comparing the proportion of cells after 7 days of culture to the initial seeding of 1 x 10⁴ cells.

Colony forming unit assay was carried out for all groups using Methocult media H4434 (Stem Cell Technologies) to assess the functionality of the expanded cells. The cells, resuspended in Methocult, were seeded in triplicates onto a 12-well plate at 1000 cells/mL and cultured at 37°C, 5% CO₂ and 20% O₂ for 14 days, according to the manufacturer’s instructions. The colonies were then manually counted to identify the Burst Forming Unit-Erythroid (BFU-E), Colony Forming Unit- Granulocyte Macrophage (CFU-GM) and Colony Forming Unit-Granulocyte, Erythrocyte, Macrophage, Megakaryocyte (CFU-GEMM).
**Measurement of \( pO_2 \)**

Dissolved oxygen concentrations were measured using OxyLite Pro (Oxford Optronix, Abingdon, UK). A “Bare-Fibre” fluorescent-containing oxygen sensor (Oxford Optronix) was placed as close as possible to the cells in a well of each experimental group (Figure 1), to measure the dissolved oxygen concentration that the cells were exposed to at specific time points over a 7-day culture. The oxygen sensor was permanently attached to the tissue culture well for the duration of the culture to enable real-time \( pO_2 \) to be measured.

The Oxylite Pro monitoring system was set on a 30s update mode and 8 measurements of \( pO_2 \) were obtained over 5 min at each time interval. Dissolved oxygen was measured in mmHg and converted to percentage by dividing the \( pO_2 \) measured by the atmospheric pressure and multiplying by 100.

**NSG mouse repopulation assay**

NSG mice were irradiated 6-8 hours before transplantation with 325 cGy. The progeny of 5000 CD34\(^+\) cells, post 7 day expansion, were injected into the tail vein of 8 to 12 week-old female and male mice (8 per group). The mice were killed 10 to 12 weeks after injection and the femurs and spleen were collected. Bones were flushed with 1 to 2 mL PBS/2% FBS, to remove bone marrow and the spleen was homogenized and filtered to obtain a single cell suspension. Flow cytometric analysis was performed to identify mouse CD45 and human CD45, CD33 and CD19 (all antibodies from BD Biosciences) positive cells after red blood cell lysis.
Statistical analysis

For flow cytometry analysis, 7000-10000 events were collected and analysed. Selected cell populations were shown as a percentage among the total gated live cells, based on the comparison with background staining shown when isotype controls of corresponding antibodies were used. Data of cell number, viability, cell surface markers, colony count and mice repopulation experiments were collected from three independent repeats (n=3), and all measurements were performed in at least triplicate to avoid sampling error. Results were reported as mean ± s.e. if not specified. Statistical relevance of the mean change in cell percentage and colony numbers during each experiment was determined via one-way ANOVA, using GraphPad Prism 5 software. Two-way ANOVA with 95% confidence was performed to compare the effect of oxygen, movement (static versus dynamic) and the combined effect of the 2 parameters on HSPC expansion. Significance level was set as (a: p ≤ 0.05; b: p ≤ 0.01; c: p ≤ 0.001).

Results

\( pO_2 \) in cultures under static and dynamic conditions

Dissolved oxygen was measured in static and dynamic HSPC cultures over 7 days at 5% ambient \( O_2 \) (Figure 2A) and 20% \( O_2 \) (Figure 2B). The \( pO_2 \) equilibrated with the ambient oxygen levels between 4 and 19 hours after initiating the culture, and levels remained stable until around 72 hours. Static cultures in 5% atmospheric oxygen exhibited a gradual decrease in \( pO_2 \) adjacent to the cells after 72h and, by day 7, a \( pO_2 \) of less than 1% was recorded. In the 5% Dynamic culture, \( pO_2 \) was maintained at approximately 4% adjacent to
the cells by day 7, even though the total cell number was 70% higher than in the 5% O₂ static culture (Figure 4A).

Cultures grown in atmospheric O₂ also showed a decrease in pO₂ after the third day of culture, though the dynamic cultures showed a greater fall (to 14%) by day 7 compared to static cultures (to 16%). There was no statistically significant difference in cell numbers between dynamic and static cultures grown in 20% O₂ (Figure 4A).

**Effect of pO₂ and Dynamic culture on HSPC expansion**

**In vitro analysis:**

The expansion of HSPC was assessed by phenotypic analysis (Figure 3, Figure 4) and clonogenic assay (Figure 5) after a 7 day expansion culture. FACS staining was performed in several combinations to mark different lineage specific cells. CD34⁺ CD45FITC⁺ cells mark multipotent and committed progenitor cells and CD34⁺ CD38FITC⁻ mark primitive HSPCs. CD133⁺ CD34FITC⁺ include HSPCs, multi-potential progenitors and some early committed progenitor cells, whereas CD33⁺ CD34FITC⁺ represent committed myeloid lineage stem cells.

As shown in Figure 4, cells cultured at 5% O₂ in a dynamic environment demonstrated the highest CD34 expansion, with almost 30 fold increase by FACS analysis and 120 fold by CFU assay. In general, cells cultured in 5% O₂ showed significantly greater expansion than those cultured in 20% O₂ (p <0.01). Within each of the oxygen concentration group, cells cultured dynamically expanded more than those cultured statically (p <0.001). Furthermore, an interaction between oxygen concentration and static versus dynamic culture was observed in that cells cultured dynamically in 20% O₂ exhibited comparable or better expansion than the 5% O₂ static group (p <0.05). Similar results were obtained with the clonogenic assay.
whereby there were more colonies when cultured at 5% O\(_2\) (p < 0.001) in a dynamic environment (p < 0.001) (Figure 5). Additionally, there was a significant interaction between oxygen and movement on HSPC expansion (p < 0.001).

**In vivo analysis:**

To examine the long-term repopulating ability of UCB-HSPCs, progeny of 5000 expanded cells were transplanted into NSG mice. After 10-12 weeks, there were significantly more human CD45\(^+\) cells detected in mice that were transplanted with HSPCs expanded at 5% O\(_2\) dynamically than statically, with approximately 11% human CD45\(^+\) cells found in the bone marrow in the dynamic culture group (p < 0.001) (Figure 6). The engraftment in the bone marrow for this group was 4 fold or greater compared to 20% O\(_2\) static culture. Engraftment of human CD45\(^+\) cells in the spleen demonstrated similar results. Mice transplanted with HSPC expanded at 20% O\(_2\) dynamically exhibited comparable levels of engraftment in the bone marrow to those expanded at 5% O\(_2\) statistically. The expression of lineage markers on the reconstituted human CD45\(^+\) cells was also analysed (Figure 6). All the recipients transplanted with the expanded cells possessed CD33\(^+\) myeloid cells and CD19\(^+\) lymphoid cells, confirming complete repopulation. While engraftment was significantly affected by both oxygen concentration and static versus dynamic culture, no significant effect was observed between the interactions of these 2 parameters.

**Discussion**

The small number of HSPCs present in an umbilical cord blood unit limits the use of this source of stem cells for transplantation, particularly in adults. The strong correlation between cell dose and outcome after cord blood transplantation has led to a progressively increased demand for large cord blood units from public banks [27-30]. Expansion is seen as
a critical to overcome this problem by enabling the use of the hundreds of thousands of smaller units currently stored in public cord blood banks around the world, for making cord blood transplantation safer and less costly.

The importance of oxygen levels in the proliferation and differentiation of stem cell populations, particularly HSPCs, is well established [31-33]. We have previously demonstrated the importance of oxygen in determining the characteristics of HSPC-supportive stromal cells [34], and a number of groups have identified the importance of oxygen in the efficiency of expansion of HSPCs [9,35]. Low oxygen has been reported to promote quiescence and regulate their differentiation, thereby maintaining stem cell phenotype. Even extremely low oxygen concentrations are permissive for slow self-renewing divisions of hematopoietic stem cells while higher (atmospheric) oxygen promotes terminal differentiation [36-38].

As part of the validation of a clinical-grade dynamic HSPC expansion system, we have sought to understand the in vitro environment, and, in particular, oxygenation of cells grown in our system compared to that in standard (static) culture conditions. In particular, we have examined the relationship between atmospheric oxygen, cell number during expansion, static/dynamic culture conditions and the effective pO\(_2\) experienced by cells in a culture system.

Oxygen is relatively poorly soluble in water, with a slow diffusion constant. Balin et al [20] in the mid 1970’s showed a relationship between oxygen levels, dynamic (“shaken”) cultures and proliferation rate. Metzen et al [21] demonstrated the impact of cell number on effective oxygenation in monolayer cultures, and derived an equation for the relationship between pO\(_2\) in the incubator (pO\(_2\)\(_{\text{gas}}\)), the tissue-specific rate of oxygen utilization (U\(_{O2}\), nmol O\(_2\) x min\(^{-1}\) x mg cellular protein\(^{-1}\)), the protein content of the cell layer (tProt), the
diffusion constant of $O_2$ ($D = 3.3 \times 10^{-5}$ cm$^2$ x sec$^{-1}$), the oxygen solubility constant ($\alpha$, 0.94 µmol $O_2$ x ml$^{-1}$ x atm$^{-1}$), the height of media in the culture (h cm) and the area of the culture vessel (A cm$^2$):

$$
\text{2(cell)} \quad \text{2(gas)}
$$

Metzen’s equation assumes that the tissue-specific rate of $O_2$ consumption, $U_{O2}$, is constant, whereas Balin’s study demonstrated that consumption is inversely related to available oxygen. Nevertheless, these studies form the basis for understanding the relationship between cell number (as represented by $t_{Prot}$), oxygen consumption, diffusion and available oxygen. Metzen showed that the measured peri-cellular oxygen was highly dependent on the cell type in culture, confirming that the tissue-specific rate of oxygen consumption can vary considerably. The application of these calculations to standard tissue culture is also complicated by the fact that the number of cells (and hence $t_{Prot}$) varies through the course of the culture. Pettersen et al [39] measured peri-cellular oxygen in subconfluent, confluent and superconfluent cultures of the breast cancer cell line T47D, and showed that the effective oxygen being experienced by the cells differed substantially from the gaseous oxygen as the cultures progressed.

We have measured the pericellular oxygen concentration in both static and dynamic HSPC cultures over 7 days, under conditions that result in 30-60 fold expansion of cell numbers. The probe for the measurements was placed immediately above the plastic surface (1-2mm), and so represented the conditions experienced by cells adhering to the tissue culture plastic. HSPCs are semi-adherent cells and will settle to the bottom of the plate under static conditions.
Our results demonstrate that the doubling time for HSPCs varied from 32.5 hours at 5% O$_2$ under dynamic conditions to 39.5 hours at 20% O$_2$ under static conditions. This difference resulted in a 2 fold difference in total cell numbers, and an almost 4 fold difference in CD34$^+$ cells over the seven days of culture used in these experiments. As shown in Sup Fig 1 in the Supplementary Document, no significant change in cell viability was observed between the different conditions. This result suggests that the combination of dynamic culture and lower O$_2$ levels may promote maintenance of a more primitive phenotype, which was supported by a similar fourfold difference in human CD45$^+$ engraftment in NSG mice. While total engraftment was significantly affected by oxygen concentration and movement, the ratio of myeloid vs lymphoid engraftment did not show any significant changes. The percentage of hCD45$^+$CD19$^+$ cells was 47-60% as compared to 22-35% of hCD45$^+$CD33$^+$ cells in the mice bone marrow and confirms the findings of Noort et al [40], where they demonstrated that after transfer of CD34$^+$ cells from UCB, the main cell lineage recovering in the NOD/SCID mice is of B cells.

This difference in expansion may be directly related to changes in effective O$_2$ experienced by the HSPCs during the culture. As shown in Figure 2, cells grown in 5% atmospheric oxygen under static conditions experienced extremely low (<1%) oxygen by day 7 of the culture. Such hypoxic conditions may promote relative quiescence of primitive HSCs, and so may inhibit expansion. Our results demonstrate that cells exposed to static cultures at 5% oxygen exhibited a pO$_2$ under 1% by day 7, supporting the concept those actual oxygen measurements in clinical cultures should be part of routine validation studies under GMP.

Jing et al [41] have reported that, in dynamic culture, an agitation speed of 30 rpm was better than that of 45, 60 and 80 rpm for the expansion of HSPCs. However, we found even lesser cell death at 20 rpm as compared to 30 rpm. According to Cabrita et al [42], cells in
static culture accumulate at the bottom of culture plates, generating oxygen and cytokine concentration gradients, which can negatively affect both cell proliferation and phenotype. However, in a dynamic culture system, the fluid shear stress can directly act as mechanical signal acting on cells. Under agitation an optimum degree of hypoxia may be maintained as the culture grows. This may operate alongside removal of negative regulators of growth form the cells and their surroundings, as well as mechanical stimulation. Yang et al [43], demonstrated that static cultures favoured the expansion of HSPCs and stirred cultures were more effective in preserving functional HSCs, so that static culture and stirred culture may be combined to guarantee both the quantity and quality of HSCs, providing helpful clues for further developing novel culture systems. Hosseinizand et al [44], who used a different model for dynamic cultures have shown that the agitation increases expansion of cord blood hematopoietic cells and promotes their differentiation into myeloid lineage.

In summary, although some studies have compared the effect of static versus dynamic cultures for HSPC expansion, while others have investigated the effect of O₂ levels on HSC expansion, the current study compared the combined effect of oxygen and static versus dynamic culture on UCB-HSPC ex vivo expansion. In this UCB expansion system, we have shown that there is a marked discrepancy between the oxygen concentration in the atmosphere of the incubator and the concentration to which the cells are directly exposed. Our results also indicate that HSPC expansion ex vivo and engraftment in vivo was significantly enhanced at 5% oxygen and dynamic culture system, suggesting that this culture condition can further improve transplantation outcomes.

Acknowledgments
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**Author Disclosure Statement**

MAK is Chief Scientific Officer of Cytomatrix Ltd, a for-profit biotechnology company. Other authors declare no conflict of interest.

**References**

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Figure Legends
Figure 1: Schematic diagram of the experimental set up showing placement of the oxygen sensor as close to the bottom of the culture well as possible to enable measurements of the oxygen concentration experienced by the cells.
Figure 2: Change in percentage of diffused oxygen provided to the cells in Static (S) and Dynamic (D) conditions for a culture period of 7 days.
Figure 3: Dot-plot analysis of HSPCs with lineage-specific surface markers (comparison of representative pre- and post-culture cells). (A–E) Preculture and (F–J) postculture CD34$^+$ cells. (A, F) Staining of the cells with isotype controls (B, G) viable CD34 PE$^+$ CD45 FITC$^+$ (which includes HSPCs, multipotent and committed progenitor cells), (C, H) viable CD34 PE$^+$ CD38 FITC$^-$ (primitive HSPCs), (D, I) viable CD34 PE$^+$ CD33 FITC$^+$ (committed myeloid progenitors) and (E, J) viable CD133 PE$^+$ CD34 FITC$^+$ (including HSPCs, multi-potential...
progenitors and some early committed progenitor cells, respectively). Cell target populations were gated according to size, granularity and fluorescence.
Figure 4: Fold expansion of the HSPC subpopulation based on phenotypic analysis: The effect of oxygen and movement on the fold expansion of target populations was determined by manual counts and detection of cell surface markers by flow cytometry. All analyses were gated for viable cells only (7AAD-). Fold expansion illustrates the increase in target population numbers over pre-culture values. Results are expressed as mean ±
standard error of the mean. N=3, each performed in triplicate. Statistical analysis was performed using one-way ANOVA (a: p ≤ 0.05; b: p ≤ 0.01; c: p ≤ 0.001).
Figure 5: Fold expansion of the HSPC subpopulation based on Colony forming unit (CFU) assay: The effect oxygen and movement on the fold expansion of target populations was determined by manual counts and colony counts. Functional assessment of multipotent cells was performed with colony forming unit assays for (A) BFU-E; (B) CFU-GM; and (C) CFU-GEMM; (D) total colony numbers, represented by the mean of all the colonies. Fold expansion illustrates the increase in target population numbers over pre-culture values. Results are expressed as mean ± standard error of the mean N=3, each performed in triplicate. Statistical analysis was performed using one-way ANOVA (a: p ≤ 0.05; b: p ≤ 0.01; c: p ≤ 0.001).
Figure 6: NOD/SCID Engraftment Assay: The effect of oxygen and movement on the engraftment of human CD34+ cells into NOD-SCID mice was determined by detection of human cell surface markers on mice bone marrow and spleen derived cells, by flow cytometry. Results are expressed as mean ± standard error of the mean. N=8. Statistical analysis was performed using one-way ANOVA (a: p ≤ 0.05; b: p ≤ 0.01; c: p ≤ 0.001). Results are based on following number of mice per condition: n=7/8 for 5% O2-S; n=7/8 for 5% O2-D; n=7/8 for 20% O2-S; n=6/8 for 20% O2-D.
Supplementary Data:

Sup Fig 1: The figure shows percentage of 7AAD- (live cells) in all the conditions as seen by flow cytometry and demonstrates no statistical difference.

Sup Table 1: The table shows the percentage of human and mouse CD45 detected in the bone marrow of all the living mice after 12 weeks.

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**Sup Table 2:** The table shows the average of dissolved oxygen pressure in mmHg in static and dynamic UCB CD34+ cultures over a period of 7 days. The Oxylite Pro monitoring system was set on a 30s update mode and 8 measurements of pO$_2$ were obtained over 5 min at each time interval. Dissolved oxygen was measured in mmHg and converted to percentage by dividing the pO$_2$ measured by the atmospheric pressure and multiplying by 100.

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