Effects of hypoxic culture conditions on umbilical cord-derived human mesenchymal stem cells

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Abstract
Following cultivation of distinct mesenchymal stem cell (MSC) populations derived from human umbilical cord under hypoxic conditions (between 1.5% to 5% oxygen (O₂)) revealed a 2- to 3-fold reduced oxygen consumption rate as compared to the same cultures at normoxic oxygen levels (21% O₂). A simultaneous measurement of dissolved oxygen within the culture media from 4 different MSC donors ranged from 15 μmol/L at 1.5% O₂ to 196 μmol/L at normoxic 21% O₂. The proliferative capacity of the different hypoxic MSC populations was elevated as compared to the normoxic culture. This effect was paralleled by a significantly reduced cell damage or cell death under hypoxic conditions as evaluated by the cellular release of LDH whereby the measurement of caspase 3/7 activity revealed little if any differences in apoptotic cell death between the various cultures. The MSC culture under hypoxic conditions was associated with the induction of hypoxia-inducing factor-alpha (HIF-1α) and an elevated expression of energy metabolism-associated genes including GLUT-1, LDH and PDK1. Concomitantly, a significantly enhanced glucose consumption and a corresponding lactate production could be observed in the hypoxic MSC cultures suggesting an altered metabolism of these human stem cells within the hypoxic environment.

Introduction
Tissue-derived stem cells including mesenchymal stem cells (MSC) provide enormous potential for appropriate tissue repair and renewal of damaged cells with cellular processes of retrodifferentiation and transdifferentiation extending this spectrum of developmental cellular flexibility and plasticity [1,2]. Thus, human MSC isolated from bone marrow (BM), adipose tissues or other sources are of great interest for tissue engineering and tissue replacement, since these cell populations are characterized by a high proliferative activity, self-renewal capacity, low immunogenicity and the potential to differentiate along the mesenchymal cell lineage to acquire phenotypes of osteoblasts, adipocytes and chondrocytes [3-5]. MSC are already used in clinical trials as cell suspensions [6,7]. For such clinical applications, a large number of cells are required which may still limit the implant preparation in the clinical applications. Another important property is the capability of MSC to survive after implantation when exposed to limited oxygen and nutrient supply due to a lack of vascularization.

Conventional in vitro cell cultivation is carried out under ambient oxygen concentration (21% of O₂) which is also defined as “normoxic”. In contrast, in vivo MSC usually are not exposed to such a high concentration of oxygen. Depending on the cell type MSC develop in certain environment niches with a low oxygen tension varying between 1% and 7% O₂ in BM and between 10% and 15% O₂ in adipose tissue (AT) [8-10]. Common consensus values of 30-50 μM (3% to 5%) of oxygen in tissues are generally accepted whereby the actual O₂ concentration in situ strongly depends on the vascularization of the tissue and its metabolic activity [11]. This suggests hypoxic in vitro conditions for these cells for a treatment under similar conditions resembling their natural physiological environment. Thus, previous work has demonstrated that high concentrations of oxygen can cause oxidative stress via production of reactive oxygen species (ROS) - free radicals that can damage lipids, proteins and DNA, altering cell metabolism [12]. Therefore, moderate hypoxia may lower the intracellular ROS generation and accumulation and may thereby increase the metabolic efficiency [13]. In this context, it is interesting to note that preculturing MSCs under hypoxic conditions before transplantation improves their tissue regenerative potential [14]. Thus, optimal oxygen
concentrations must be assigned in accordance with physiological niches and the type of cultivated stem cells, e.g. MSC derived either from bone marrow, peripheral blood, adipose tissue, placenta tissue or from umbilical cord. Under such circumstances, noninvasive optical on-line measurements of dissolved oxygen in the culture medium are very helpful in monitoring and control of cell incubation under conditions of variable oxygen concentration [15]. Birth-associated tissues including amnion, placenta, cord blood and the umbilical cord (UC) represent a very promising embryonic/fetal source of MSC populations and may provide a broader spectrum of cellular flexibility as compared to MSC obtained from adult tissues (bone marrow, adipose tissue). Thus, UC-derived MSC have a short doubling time, their harvest is not ethically restricted and there are no medico-legal limitations in their application [16,17]. Oxygen tension within the mammalian female reproductive tract was shown to be low, about 1.5% to 8% and lasts throughout the fetal development with dissolved oxygen in the fetal circulation rarely exceeding 5% [18,19]. Moreover, the UC with its blood vessels - two arteries and one vein - is lacking capillaries or lymphatic channels providing conditions that UC cells could also develop in situ in a hypoxic atmosphere. However, little is known about the effect of hypoxia on UC-derived MSC. Thus, the metabolic potential and the proliferative capacity of these UC-derived MSC at low oxygen tension has not been determined to date. In this study we therefore tested the effects of hypoxic conditions (1.5%, 2.5% and 5% O2) on a cultivation of 4 different individual UC-derived MSC populations to examine the proliferative and metabolic activities in these human stem cells.

Methods

Cell culture

Human MSCs were isolated from UCs of 4 different term-deliveries (38-40 weeks) by Cesarean section patients after obtaining informed written consent, respectively, as approved by the Institutional Review Board, project #3037 on 17th June, 2006 and in an extended permission #443 on 26th February, 2009. Recently, the isolated populations have been extensively characterized as mesenchymal stem cells by surface marker analysis and functional properties [20]. Moreover, the MSC were expanded and cryopreserved until the start of the experiment as described [20]. After thawing, the cells were expanded over two passages. At about 80% of confluence the MSCs were harvested by accutase treatment (PAA Laboratories GmbH, Pasching, Austria) and plated at a density of 3000 cells/cm² in 25 cm² cell culture flasks (Corning, CellBind Surface, Germany) and in 6-well plates (Sarstedt, Germany), respectively. Experiments were performed with cells of passages 3 to 7. Cells were cultivated in αMEM containing 1 g/l glucose (Biochrom, Germany), 2 mM L-glutamine (PAA Laboratories GmbH), 10% human serum (provided by the Division of Transfusion Medicine, Medical University Hannover, Germany) and 50 μg/ml Gentamicin (PAA Laboratories GmbH) in a humidified atmosphere containing 5% CO₂ and 21% O₂ at 37°C (Incubators: Thermo scientific, Germany). Twenty four hours after seeding, all non-adherent cells were removed by media changes and for the following 72 h the MSCs were incubated at various oxygen concentrations (1.5%, 2.5%, 5% or 21%), respectively.

Cell number, apoptosis and necrosis

At the end of cultivation, cells were washed with PBS, detached by accutase treatment (PAA Laboratories GmbH), sedimented by centrifugation for 5 min at 200 × g and counted using a haemocytometer following resuspension in 1 ml culture medium. Cell viability was determined by trypan blue exclusion (n = 4). Occurrence of apoptosis was measured with ApoOne® Homogeneous Caspase-3/7 Assay (Promega, UK) by the amount of the fluorescent product Rhodamine 110 (Ex355/Em460) cleaved by caspase-3/7 from the non-fluorescent substrate Z-DEVD-R110 after cell lysis following 6 h incubation at 37°C. Cell damage or cell necrosis was evaluated by measuring lactate dehydrogenase (LDH) activity in the cell culture supernatant (30 min incubation time, 25°C) using the CytoTox-ONE™ Assay (Promega, UK), by the amount of enzymatically reduced resorufin by its fluorescence intensity (Ex355/Em460) according to manufacturer’s instructions. All fluorescence measurements were performed using the Fluorescan Ascent microplate reader (Thermo Scientific, Germany).

Normoxic (21% O₂) cultures of the same passage were used as control cell population and the results of each oxygen concentration were presented as percentage of change to the normoxic controls.

O₂ and pH measurements

Dissolved oxygen and pH values in the cell culture supernatant were recorded online in 25 cm² cell culture flasks (Corning, CellBind Surface, Germany) every 10-20 minutes by using a SFR-Shake Flask Reader (Presens GmbH, Regensburg, Germany) with optical sensors, integrated and precalibrated by Presens GmbH. These measurements are based on the luminescence lifetime of the sensor dye, which depends on the oxygen partial pressure and the pH of the sample, respectively. The luminescence lifetime was detected non-invasively through the transparent flask bottom and represented equivalents of oxygen and pH values according to the
company’s software (Presens, Germany). Culture flasks with the same amount of medium (6 ml) without cells were used as a control. Oxygen consumption was calculated as difference of dissolved oxygen concentration in medium with and without cells divided by the number of living cells.

**Immunoblot analysis**

The isolated MSC cultures following incubation at different oxygen levels (1.5% to 21%) were washed three times in ice-cold PBS and immediately lysed in a buffer containing 10 mM Tris-HCl (pH 7.6), 140 mM NaCl, 10 mM EDTA, 1% (v/v) NP-40 with the addition of 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonylfluoride (PMSF) (all from Sigma). Protein concentration was adjusted using the colorimetric BCA-assay (Perbio Science Deutschland, Bonn, Germany), subjected to SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane (Millipore GmbH, Schweinfurt, Germany). The membranes were blocked with PBS containing 5% FCS and 0.05% Tween-20 (PBS/Tween). After washing four times with PBS/Tween, the membranes were incubated with the primary monoclonal antibodies (anti-HIF-1α (Biomol GmbH, Hamburg, Germany) and anti-ß-actin, clone AC-15 (Sigma, Saint Louis, Missouri, USA)) for 2 h at 37°C, washed four times with PBS/Tween and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (all from Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at 37°C. The membranes were washed with PBS/Tween and visualized by autoradiography using the ECL-detection kit (GE Healthcare, München, Germany).

**Real-Time RT-PCR (HIF Target genes Analysis)**

Total RNA from the cells incubated at different oxygen conditions was isolated by using RNeasy Mini Plus Kit (Qiagen, Germany) according to the manufacturer’s instructions and the RNA concentration was measured with a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Germany). Thereafter, 1 μg of RNA was transcribed into cDNA using Reverse Transcriptase (Promega, UK) and a mixture of oligo(dT) primers according to the manufacturer’s instructions. Primers for glucose transporter-1 (GLUT-1), lactate dehydrogenase A (LDHA), glucose-6-phosphate dehydrogenase (G6PD), pyruvate dehydrogenase kinase-1 (PDK-1) and hypoxanthine phosphoribosyltransferase-1 (HPRT1) genes were designed using OligoPerfect™ Designer Software (Invitrogen). Quantitative RT-PCR was performed using IQ™SYBR®Green Supermix and IQTM5 real-time PCR Detection System (Bio-Rad, USA). HPRT1 gene was used as internal control and non-template control was used as negative control. The dissociation curves were run for all completed SYBR Green reactions to rule out non-specific amplifications and primer-dimers. Data were analyzed using the comparative Ct (ΔΔCT) method. For each sample triplicate measurements were performed.

**Glucose and L-glutamine consumption, lactate and glutamate production (metabolic analysis)**

At the end of each cultivation concentrations of glucose and lactate were measured in the medium using an YSI 2700 SELECT analyzer (Yellow Springs, USA). L-glutamine and l-glutamate concentrations were determined using a gradient HPLC (column: Waters Resolve C18, 5 μm, 3.9 × 150 mm, 30°C, flow: 1 ml/min) with Fluorescence Detector RF-10AXL (Shimadzu, Japan).

Specific metabolite consumption rates (qmet) were calculated using the following equation:

\[ q_{\text{met}} = \frac{\mu C_x (0) - C_x(t) - \Delta C_x}{\Delta t} \]

whereby \( C_x(0) \) and \( C_x(t) \) represent the cell numbers and \( C_x(0) \) and \( C_x(t) \) the amount of metabolite at the start (0) and the end (t) of the exponential growth phase, respectively, \( t \) the time (h) and \( \mu \) the specific growth rate (h⁻¹)

Lactate to glucose yields were calculated as 

\[ Y_{\text{lacto-gluc}} = \frac{q_{\text{lact}}}{q_{\text{gluc}}} \]

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L-lactate to glucose yields were calculated as 

\[ Y_{\text{lacto-gluc}} = \frac{q_{\text{lact}}}{q_{\text{gluc}}} \]

where \( q_{\text{lact}} \) and \( q_{\text{gluc}} \) are specific lactate production and glucose consumption rates during the same time interval, respectively.

**Statistical analysis**

All data are represented as mean ± SD for triplicate measurements for each sample. Statistical significance was assessed with the one way ANOVA and t-test at P < 0.05.

**Results**

On-line measurements of dissolved oxygen and pH values of the medium

Twenty-four hours after seeding, the cell culture medium was changed and cells were placed on the SFR-Shake Flask Reader in an incubator with reduced oxygen concentration. For three days, dissolved oxygen concentrations in the medium and pH values were measured and recorded on-line every 10 to 20 minutes (Fig. 1). The measurements showed even at 1.5% O₂ only a marginal reduction of the available oxygen levels as compared to the appropriate cell-free medium indicating a faster gas diffusion into the medium than the rate of cellular consumption (Fig. 1). Thus, at the end of the cultivations (80% confluency) with 1.5% O₂, the concentration of oxygen in the cell culture supernatant was 15.03 μmol/l as compared to 15.7 μmol/l in the appropriate medium control (control) (Fig. 1A). Likewise, 2.5% O₂ incubation revealed 23.88 μmol/l of oxygen in
the culture supernatant versus 25.10 μmol/l in the control (Fig. 1B), 5% O2 resulted in 48.85 μmol/l versus 50.05 μmol/l in the control (Fig. 1C), and normoxic conditions at 21% O2 exhibited 196 μmol/l in the cell culture as compared to 198 μmol/l in the cell-free control medium (Fig. 1D). Accordingly, the calculated oxygen consumption at the end of cultivation (80% confluency) was 0.024 ± 0.002 pmol/h/cell in 1.5% O2, 0.035 ± 0.006 pmol/h/cell in 2.5% O2, 0.036 ± 0.006 pmol/h/cell in 5% O2, and 0.095 ± 0.005 pmol/h/cell in 21% O2 (Fig. 2).

Moreover, the pH values in the cell culture supernatant progressively decreased during cultivation time in parallel to an increasing cell number and reached a difference of about 0.15 pH values compared to control medium at the end of the cultivation (80% confluency) (Fig. 1A).

Cell proliferation, apoptosis and necrosis
Whereas other studies also use 6 h, 24 h and 48 h of hypoxic incubation, UC-derived MSC were exposed to various concentrations of oxygen for 72 h and the proliferation, apoptosis and cell damage/necrosis in the 4 different UC cell populations was investigated. Cell growth analysis revealed a marked increase in cell proliferation at 2.5% O2 as compared to normoxic 21% O2 (Fig. 3A). The hypoxic conditions were evaluated by HIF-1α expression and Western blot analysis revealed significant protein levels of HIF-1α in the hypoxic MSC cultures at 2.5% and 5% O2 in contrast to little if any detectable HIF-1α protein in the normoxic (21% O2) MSC population (Fig. 3B). The unaltered expression of β-actin was used as a control (Fig. 3B).

More detailed analysis of the hypoxic (1.5% O2) MSC cultures revealed little if any increase in apoptosis in cell
preparations from all four donors (Fig. 4A). In contrast, a markedly decreased cell damage or necrosis in all MSC populations became detectable under hypoxic conditions as evaluated by a significantly reduced LDH release in two of the four MSC donors (Fig. 4B).

Glucose metabolism-associated gene regulation

We also analysed the regulation of some energy metabolism pathway-associated factors including GLUT-1 (glucose transport into the cell), LDHA (glycolysis), G6PD (pentose phosphate pathway), PDK-1 (suppression of the oxidative phosphorylation) which also represent some targets of the transcription factor HIF-1α. Quantitative RT-PCT analysis revealed a significant upregulation of GLUT-1, LDHA and PDK-1 in 1.5% O₂, 2.5% O₂ and 5% O₂ as compared to normoxic cultivated (21% O₂) control cells (Fig. 5). In contrast, no upregulation of G6PD in hypoxic conditions was detectable (Fig. 5).

Metabolic analysis

MSC cultured at 1.5% O₂ consumed significantly more glucose (22.35 ± 1.56 pmol/day/cell) (Fig. 6A) and produced significantly more lactate (19.11 ± 3.58 pmol/day/cell) when compared to normoxic controls (12.00 ± 1.93 and 11.44 ± 2.93 respectively) (Fig. 6B). At 2.5% glucose consumption and lactate production rates were lower than at 1.5% O₂ (15.10 ± 1.39 and 12.48 ± 3.08 pmol/day/cell respectively), but still higher than in normoxic controls (Fig. 6A, B). At 5% O₂ there were no differences in glucose uptake and lactate production when compared to 21% O₂. The calculated lactate/glucose molar ratio was nearly the same at all oxygen concentrations (0.7 - 1.0 mol/mol) (Fig. 6C).

Consumption of glutamine was lower at 1.5% O₂ (1.94 ± 0.53 pmol/day/cell) and at 2.5% O₂ (2.65 ± 0.95 pmol/day/cell) with no detectable difference at 5% O₂ (2.79 ± 0.72 pmol/day/cell) when compared to 21% O₂ controls (2.82 ± 1.37 pmol/day/cell) (Fig. 7A). Glutamate production was lower at 1.5%, 2.5% and 5% O₂ when compared to 21% normoxic control (Fig. 7B).

Discussion

Cultivation of MSCs under hypoxic conditions mimic the natural microenvironment of these cells represents an important prerequisite to study cell proliferation,
differentiation, senescence, metabolic balance and other physiological processes [14]. Thus, a variety of studies for in vitro cell cultivation and subsequent clinical applications suggested the MSC culture at hypoxic (1% to 10% O₂) rather than normoxic (21% O₂) conditions [21,22]. Moreover, implanted MSCs in clinical applications without well-developed blood vessels would suffer from limited nutrient and oxygen supply which requires more knowledge about the ability of the cells to survive and adapt to the altered microenvironment.

The results from the present study reveal that UC-derived human MSC at 1.5% O₂ revealed little if any increase in apoptosis. Moreover, in 2.5% O₂ cells demonstrated an increased proliferative capacity. Similar data were obtained in bone marrow-derived MSC [14,27]. Moreover, the level of cell damage and/or necrosis under 1.5% hypoxia was significantly lower than in the normoxic control cell culture suggesting an adaptation to the energy requirements [21,22].

Figure 4 The effect of hypoxia (1.5% O₂) on apoptosis was tested by the measurement of caspase 3/7 activity (A) and cell damage or necrosis was tested by the measurement of LDH activity in the cell culture supernatant (B) of the 4 different donors of UC-derived stem cells. All measurements were normalized to 10,000 cells whereby the values of all measurements were calculated compared to the normoxic control conditions (21% O₂) at 100%. Data represent the mean ± SD for three independent measurements of each donor.

**Figure 5** The effect of hypoxia on glucose metabolism-associated gene expression which may also represent HIF-1a target genes. Gene expression was detected by Real-Time RT-PCR using primers for glucose-6-phosphate dehydrogenase (G6PD), glucose transporter-1 (GLUT-1), lactate dehydrogenase A (LDHA), pyruvate dehydrogenase kinase-1 (PDK-1) with hypoxanthine phosphoribosyltransferase-1 expression as an internal control. The data represent the expression levels in the UC-derived MSC populations at 1.5% O₂ (black bars), 2.5% O₂ (dark grey bars) and 5% O₂ (light grey bars) and compared to the steady state expression levels of normoxic control cultures at 21% O₂ (dashed line). Data represent the mean ± SD of three independent experiments.
Figure 6 The effect of hypoxia on the glucose consumption (A), lactate production (B) and yield lactate/glucose (Ylac/gl) (C) of the UC-derived stem cells in all donors. Data are the means ± SD for triplicate measurements for each donor, four donors per each oxygen concentration. (**p < 0.005, ***p < 0.001, *p < 0.05).

Figure 7 The effect of hypoxia on the glutamine consumption (A) and glutamate production (B) of the UC-derived stem cells. Data are the means ± SD for triplicate measurements for each donor, four donors per each oxygen concentration.
during hypoxia. This reduced concentration of oxygen in the hypoxic environment can contribute to a reduced production and availability of reactive oxygen species which are mainly responsible for the enhancement of cell damage [28,29]. The energy metabolism is mainly represented by glucose and glutamine, two important molecular carbon and nutrient sources. The analysis of metabolic activities of UC-derived MSC in our study were in agreement with previously described increases in glucose consumption and lactate production at low oxygen tension as a consequence of switching cell metabolism from oxidative phosphorylation to anaerobic glycolysis as well as an up-regulation of the glucose transport into the cells [27,30]. The yield of lactate production from glucose, however, was significantly lower in UC-derived MSC than it has been reported in bone marrow- and adipose tissue-derived MSC for both, hypoxic and normoxic conditions [30-33]. One possible explanation could be metabolic modifications within the mesenchymal stem cell populations originating from embryonic/fetal sources like umbilical cord as compared to adult bone marrow or adipose tissue. Indeed, recent work substantiates this hypothesis demonstrating alterations in the capacity of UC-derived MSC to differentiate along the adipogenic, chondrogenic and osteogenic pathway as compared to MSC obtained from adult adipose tissue [34]. This is furthermore supported by the suggestions of different MSC subpopulations exhibiting different levels of proliferative capacity and subsequent aging [20,35]. Likewise, hypoxia-induced MSC from different sources may also display different functional characteristics. Thus, a hypoxic environment can modulate the autocrine or paracrine activity of a variety of cytokines and growth factors in bone marrow-derived MSC [36], whereas in cord blood-derived MSC, two subpopulations were identified displaying low and high aldehyde dehydrogenase (ALDH) activity and significant differences in the proliferative capacity and the ability to differentiate [37].

Together, these findings demonstrated that UC-derived human MSC adapt the energy consumption and metabolism according to an appropriate hypoxic environment. Interestingly, distinct hypoxic conditions contribute to enhanced growth of UC-derived MSC in parallel to reduced cellular damage. Moreover, human mesenchymal stem cells obtained from the umbilical cord displayed metabolic differences during the adaptation to a hypoxic environment when compared to MSC derived from other tissues indicating tissue-originating variations in the functional properties of these different stem cell (sub)populations.

Acknowledgements
We thank Martina Weiss for technical assistance and Dr. A. Galkin (Queen’s University Belfast) for helpful discussions and critical reading of the manuscript. Part of this work supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH.

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Competing interests
The authors declare that they have no competing interests.

Received: 8 June 2010 Accepted: 16 July 2010 Published: 16 July 2010

References
1. Hass R. Retraddifferentiation–a mechanism for cellular regeneration? Biol Chem 2009, 390:409-416.
2. Hass R. Rejuvenation in distinct cell populations - What does it mean? Exp Gerontol 2009, 44:634-638.
3. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scandinavian Journal of Immunology 2003, 57:11-20.
4. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell 2002, 13:4279-4295.
5. Kern S, Eichler H, Stoeve J, Kloter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006, 24:294-1301.
6. Gordano A, Galertser U, Marino R. From the laboratory bench to the patients bedside: An update on clinical trials with mesenchymal stem cells. Journal of Cellular Physiology 2007, 211:27-35.
7. [http://clinicaltrials.gov/ct2/results?term=MSC].
8. Chow DC, Wenning LA, Miller WM, Papoutsakis ET. Modeling po(2) distributions in the bone marrow hematopoietic compartment. I. Krogh’s model. Biophysical Journal 2001, 81:675-684.
9. Bizzarri A, Koehler H, Cajlakovic M, Pasic A, Schaupp L, Klimant I, Ribitsch V. Continuous oxygen monitoring in subcutaneous adipose tissue using microdialysis. Analytica Chimica Acta 2006, 573:48-56.
10. Harrison JS, Rameshwar P, Chang V, Bandari P. Oxygen saturation in the bone marrow of healthy volunteers. Blood 2003, 99:394-394.
11. Ward JPT. Oxygen sensors in context. Biochimica Et Biophysica Acta-Bioenergetics 2008, 1777:1-14.
12. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. Biochemical Journal 1996, 313:17-29.
13. Miller WM, Wilke CR, Blanch HW. Effects of Dissolved-Oxygen Concentration on Hybridoma Growth and Metabolism in Continuous Culture. Journal of Cellular Physiology 1987, 132:524-530.
14. Rosova I, Diao M, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells 2008, 26:2173-2182.
15. Kohli O, Scheper T. Setup of a fiber optical oxygen multisensor-system and its applications in biotechnology. Sensors and Actuators B-Chemical 2000, 70:121-130.
16. Fong CY, Richards M, Manasi N, Biswas A, Bongo A: Comparative growth behaviour and characterization of stem cells from human Wharton's jelly. Reproductive Biomedicine Online 2007, 15:708-718.
17. Bongo A, Fong CY, Gauthaman K: Taking Stem Cells to the Clinic: Major Challenges. Journal of Cellular Biochemistry 2008, 105:1352-1360.
18. Fischer B, Bavister BD: Oxygen-Tension in the Oviduct and Uterus of Rhesus-Monkeys, Hamsters and Rabbits. Journal of Reproduction and Fertility 1993, 99:673-679.
19. Ma T, Grayson WL, Frohlich M, Vunjak-Novakovic G: Hypoxia and Stem Cell-Based Engineering of Mesenchymal Tissues. Biotechnology Progress 2009, 25:32-42.
20. Majore I, Moretti P, Hass R, Kasper C: Identification of subpopulations in mesenchymal stem cell-like cultures from human umbilical cord. Cell Commun Signal 2009, 7:6.
21. Eliasson P, Jonsson JI: The Hematopoietic Stem Cell Niche: Low in Oxygen but a Nice Place to be. Journal of Cellular Physiology 2010, 222:17-22.
22. Ivanovic Z: Hypoxia or In Situ Normoxia: The Stem Coll Paradigm. Journal of Cellular Physiology 2009, 219:271-275.
23. Brown MF, Gratton TP, Stuart JA: Metabolic rate does not scale with body mass in cultured mammalian cells. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 2007, 292:R2115-R2121.
24. Ivanovic Z, Caims RA, Fontana L, Lim AL, Denko NC: HIF-1 mediated adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metabolism 2006, 3:187-197.
25. James PE, Jackson SK, Grinberg OY, Swartz HM: The Effects of Endotoxin on Oxygen-Consumption of Various Cell-Types in-Vitro - an Epr Oximetry Study. Free Radical Biology and Medicine 1995, 18:641-647.
26. Brown MF, Gratton TP, Stuart JA: Metabolic rate does not scale with body mass in cultured mammalian cells. Am J Physiol Regul Integr Comp Physiol 2007, 292:R2115-R2121.
27. Grayson WL, Zhao F, Izadpanah R, Bunnell B, Ma T: Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs. Journal of Cellular Physiology 2006, 207:331-339.
28. Golstein P, Kroemer G: Cell death by necrosis: towards a molecular definition. Trends in Biochemical Sciences 2007, 32:37-43.
29. Bertram C, Hass R: Cellular responses to ROS-induced DNA damage and aging. Biol Chem 2008, 389:211-220.
30. Dos Santos F, Andrade PZ, Boura JS, Abecasis MM, da Silva CL, Cabral JM: Ex vivo expansion of human mesenchymal stem cells: a more effective cell proliferation kinetics and metabolism under hypoxia. J Cell Physiol 2009, 223:27-35.
31. Schop D, Janssen FW, van Rijn LDS, Fernandes H, Bloem RM, de Bruijn JD, van DiJKhuizen-Radersma R: Growth, Metabolism, and Growth Inhibitors of Mesenchymal Stem Cells. Tissue Engineering Part A 2009, 15:1877-1886.
32. Higuera S, Schop D, Janssen F, van DiJKhuizen-Radersma R, van Bokel T, van Bitterswijk CA: Quantifying In Vitro Growth and Metabolism Kinetics of Human Mesenchymal Stem Cells Using a Mathematical Model. Tissue Engineering Part A 2009, 15:2653-2663.
33. Fallmar KE, Decroos FC, Pinchard HL, Wang HT, Erdmann D, Olbrich KC: Effects of glutamine, glucose, and oxygen concentration on the metabolism and proliferation of rabbit adipose-derived stem cells. Tissue Eng 2006, 12:3525-3533.
34. Majore I, Moretti P, Stahle F, Hass R, Kasper C: Growth and differentiation properties of mesenchymal stromal cell populations derived from whole human umbilical cord. Stem Cell Rev & Rep 2010.
35. Wagner W, Ho AD, Zenke M: Different Facets of Aging in Human Mesenchymal Stem Cells. Tissue Eng Part B Rev 2010.
36. Dai R, Jahn H, van Osch GJ, Farrell E: The role of hypoxia in bone marrow-derived mesenchymal stem cells: considerations for regenerative medicine approaches. Tissue Eng Part B Rev 2010, 16:159-68.
37. Nagano M, Kimura K, Yamashita T, Ohneda K, Nozawa D, Hamada H, Yoshikawa H, Ochiai N, Ohneda O: Hypoxia responsive mesenchymal stem cells derived from human umbilical cord blood are effective for bone repair. Stem Cells Dev 2010.

Cite this article as: Lavrentieva et al: Effects of hypoxic culture conditions on umbilical cord-derived human mesenchymal stem cells. Cell Communication and Signaling 2010 8:18.