

# Vascular cell culture under physiological oxygen tensions – implications for cellular redox homeostasis

## Research Note

### Endothelial Cell Behavior under Physiological Hypoxia Conditions

Endothelial cells (ECs) line the inner surfaces of all blood and lymphatic vessels, forming a thin layer called the endothelium. Processes such as angiogenesis and vasculogenesis, hemostasis, vasomotor tone and immune and inflammatory responses depend on the capacities of ECs (Sumpio et al., 2002). In addition, ECs play a role in numerous pathologic states, e.g. human vascular diseases and chronic diseases associated with oxidative stress (Kanninen et al., 2015).

Oxidative stress emerges from exceeding the cells' antioxidant capacity to handle reactive oxygen species (ROS). ROS are formed by incomplete oxidation or reduction of O<sub>2</sub> during processes such as aerobic metabolism or electron transport and can damage proteins, lipids and DNA (Ishii et al., 2004, Tebay et al., 2015). Oxygen homeostasis is therefore a very important process in all nucleated cells, illustrating the delicate correlation between O<sub>2</sub> availability and its manifestation in cellular responses, e.g. angiogenesis, proliferation and differentiation

(Manalo et al., 2005, Rankin and Giaccia, 2016). Hypoxic microenvironments have been shown to promote angiogenesis in tumor tissues, immunosuppression and metastatic progression in cancer by hypoxia-inducible factor (HIF) activation (Bishop and Ratcliffe, 2015, Rankin and Giaccia, 2016). HIF induction is one of the mechanisms that is known to directly correlate with hypoxic states and prompt the transcription of angiogenic growth factors such as VEGF (vascular endothelial growth factor) (Manalo et al., 2005).

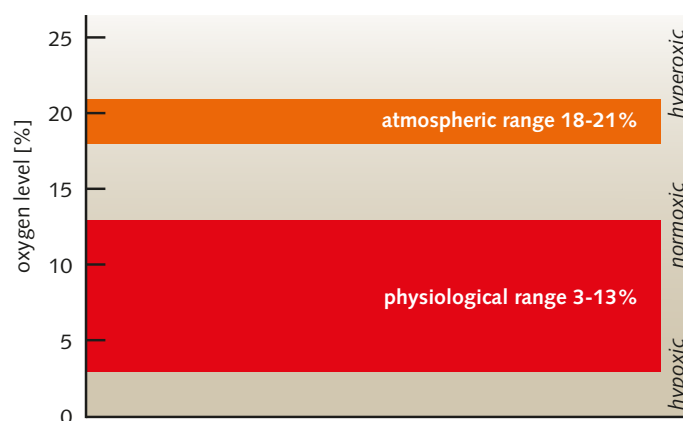
Transcription factor Nrf2 (NF-E2-related factor 2) is another factor that is known to regulate the expression of a very large number of genes involved in processes such as cytoprotection or lipid and carbohydrate metabolism, in both unstressed homeostatic and under stressed and perturbed conditions (Suzuki and Yamamoto, 2015, Tebay et al., 2015).

Whereas the physiological oxygen levels present in the vascular microenvironment *in vivo* range from 3 - 13% (Ward, 2008), common cell culture systems using traditional incubators maintain an atmospheric oxygen level of 18 - 21%. Endothelial cells cultured *in vitro* with

an oxygen level of 5% revealed altered regulation of Nrf2 downstream targets compared to ECs cultured at atmospheric oxygen levels (see Fig. 3) (Chapple et al., 2016). It is important to mention that most research results have been obtained in conventional cell culture systems with an atmospheric oxygen level used as "normoxia" control, which does not correspond to the "normal" oxygen levels *in vivo* (Chapple et al., 2016). Rather, the atmospheric oxygen level corresponds to a hyperoxic environment (see Fig. 1). Chapple et al. examined the differences between these culturing conditions and analyzed their impact on endothelial cell behavior (see Figs. 2 and 3).

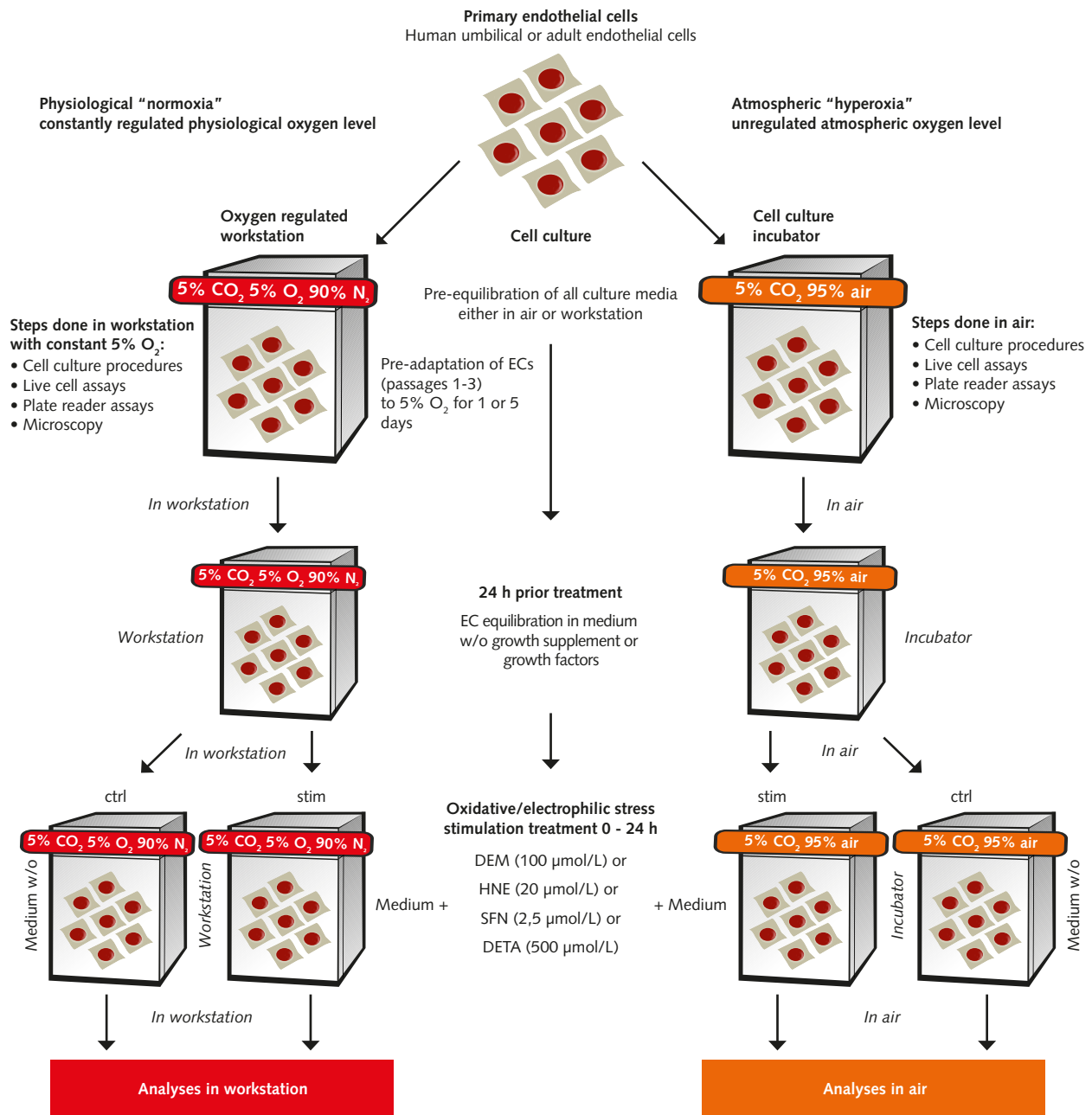
In general, it is advisable to consider how oxygen levels influence cell behavior and keep these implications in mind when working with redox-sensitive processes, to choose the appropriate culturing conditions.

However, the goal is to use a model that is as realistic and reliable as possible in order to mimic fundamental *in vivo* conditions present in process regulation and mechanisms and prevent from measuring artefacts (see Fig. 2 and Tab. 1).



**Figure 1: Oxygen level conditions:** Endothelial cells reside in a physiological O<sub>2</sub> environment *in vivo*, whereas under common cell culture conditions cells are normally exposed to atmospheric O<sub>2</sub> levels. For more detailed information, see Chapple et al. (2016).

## Endothelial cell culture under physiological (5% O<sub>2</sub>) vs. atmospheric oxygen conditions (18-21% O<sub>2</sub>)



DEM: diethylmaleate, DETA: NO donor, ECs: endothelial cells, ECGS: endothelial cell growth supplement, ECM: endothelial cell medium, FCS: fetal calf serum, HNE: 4-hydroxynonenal, NCS: newborn calf serum, SFN: sulforaphane, ctrl: control, stim: stimulated, w/o: without

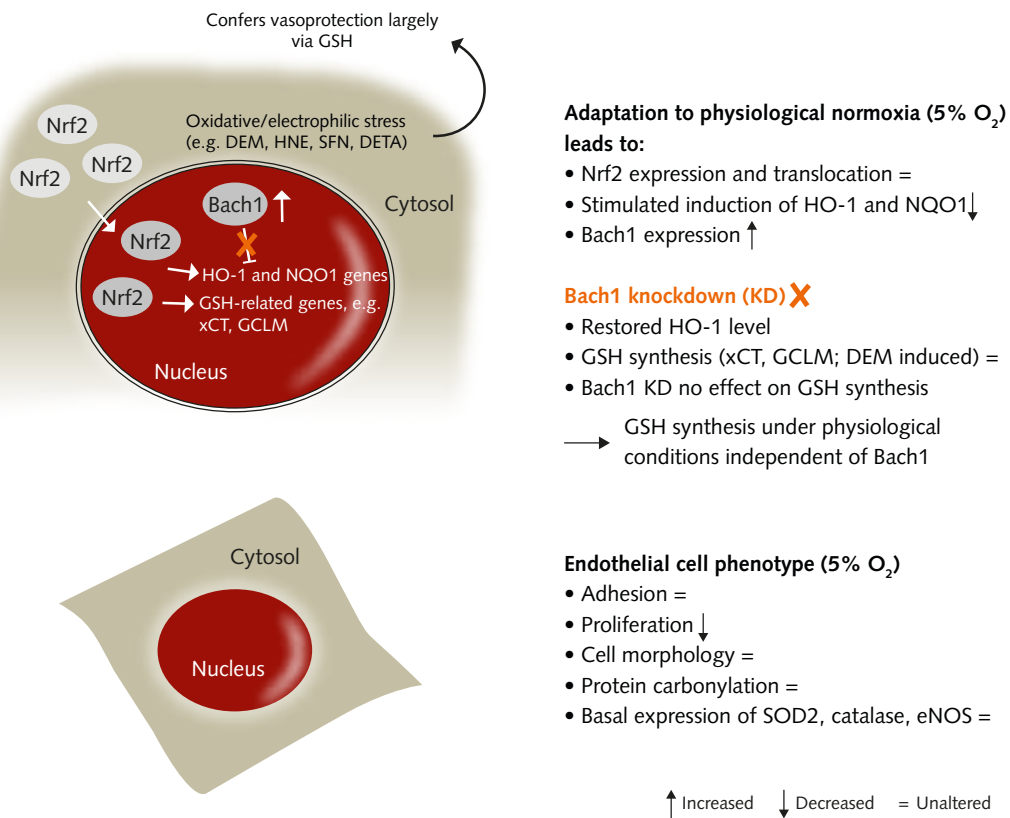
**Figure 2: Endothelial cell culture under physiological vs. atmospheric oxygen conditions:** Description and comparison of the respective culturing methods with emphasis on changes in the oxygen level. For more detailed information, see Chapple et al. (2016).

	Experimental control conditions	
	Physiological O <sub>2</sub> level (5%)	Atmospheric O <sub>2</sub> level (18 - 21%)
<b>Advantages</b>	<ul style="list-style-type: none"> <li>- No additional oxygen tensions during cell culture procedures</li> <li>- Closer to <i>in vivo</i> conditions</li> <li>- Generate more reliable data</li> <li>- Better insights into physiological processes</li> <li>- Better clinical translation</li> </ul>	<ul style="list-style-type: none"> <li>- Standard cell culture equipment</li> </ul>
<b>Disadvantages</b>	<ul style="list-style-type: none"> <li>- Oxygen regulated cell culture system required</li> </ul>	<ul style="list-style-type: none"> <li>- Artefactual observations possible</li> <li>- Additional oxygen tensions during cell culture procedures for ECs cultured under hypoxic conditions (passaging, live cell assays in air)</li> </ul>

**Table 1: Comparison of the advantages and the disadvantages of the two different methodological control conditions:** working with an O<sub>2</sub>-regulated workstation in contrast to unregulated atmospheric O<sub>2</sub> cell culture conditions

### Changes in Nrf2 pathway regulation in endothelial cell culture under physiological oxygen conditions (5% O<sub>2</sub>)

It has emerged, that the redox sensitive Nrf2/Keap1 (NF-E2-related factor 2/Kelch-like ECH-associated protein 1) pathway acts as a potent regulator of health and disease (Suzuki and Yamamoto, 2015). Experiments with human primary ECs (HUVEC, HCAEC, PromoCell) adapted to physiological O<sub>2</sub> conditions (5 %) showed a significantly altered Nrf2 regulation and corresponding downstream targets compared to atmospheric oxygen cell culture conditions (Chapple et al., 2016). Whereas cell ultrastructure, viability, basal redox status and HIF1- $\alpha$  were only minimally affected, the induced expression of Nrf2 downstream targets as HO-1 and NQO1 was attenuated due to Bach1 upregulation (Chapple et al., 2016) (see Fig. 3).



*Bach1: BTB and CNC homolog 1, DEM: diethylmaleate, DETA: NO donor, eNOS: endothelial NOS, GCLM: glutamate-cysteine ligase modifier subunit, GSH: glutathione, HNE: 4-hydroxynonenal, HO-1: heme oxygenase-1, NQO1: NAD(P)H quinone oxidoreductase 1, Nrf2: NF-E2-related factor 2, SFN: sulforaphane, SOD2: superoxide dismutase 2, xCT: cystine-glutamate transporter*

**Figure 3: Differential regulation of Nrf2-targeted genes in human endothelial cells adapted to physiological O<sub>2</sub> levels encountered *in vivo* compared to atmospheric O<sub>2</sub> levels. Illustrated and simplified according to Chapple et al. (2016).**

### Conclusion

Culturing endothelial cells at physiological O<sub>2</sub> levels provides a more accurate model by reducing artefactual observations *in vitro* as demonstrated here by the altered regulation of selected Nrf2 target genes by Bach1 (for details, see Chapple et al., 2016). This makes it possible to generate more reliable data from *in vitro* assays and translate them better into medical applications such as treatment of vascular diseases, wound healing, cancer or stroke (Manalo et al., 2005, Semenza, 2012).

## References

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## Products

Product	Size	Catalog Number
Human Umbilical Vein Endothelial Cells (HUVEC) pooled	500,000 cryopreserved cells	C-12203
	500,000 proliferating cells	C-12253
Human Coronary Artery Endothelial Cells (HCAEC)	500,000 cryopreserved cells	C-12221
	500,000 proliferating cells	C-12222
Endothelial Cell Growth Medium (Ready-to-use)	500 ml	C-22010
Endothelial Cell Growth Medium MV (Ready-to-use)	500ml	C-22020

## Related Products

Product	Size	Catalog Number
Human Umbilical Vein Endothelial Cells (HUVEC) single donor	500,000 cryopreserved cells	C-12200
	500,000 proliferating cells	C-12250
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, single donor	500,000 cryopreserved cells	C-12206
	500,000 proliferating cells	C-12207
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, pooled	500,000 cryopreserved cells	C-12208
	500,000 proliferating cells	C-12209
Human Umbilical Vein Endothelial Cells (HUVEC) pre-screened	500,000 cryopreserved cells	C-12205
	500,000 proliferating cells	C-12255
Human Umbilical Artery Endothelial Cells (HUAEC)	500,000 cryopreserved cells	C-12202
	500,000 proliferating cells	C-12252
Human Aortic Endothelial Cells (HAoEC)	500,000 cryopreserved cells	C-12271
	500,000 proliferating cells	C-12272
Human Pulmonary Artery Endothelial Cells (HPAEC)	500,000 cryopreserved cells	C-12241
	500,000 proliferating cells	C-12242



Product	Size	Catalog Number
Human Saphenous Vein Endothelial Cells (HSaVEC)	500,000 cryopreserved cells	C-12231
	500,000 proliferating cells	C-12232
Human Dermal Microvascular Endothelial Cells (HDMEC) juvenile foreskin	500,000 cryopreserved cells	C-12210
	500,000 proliferating cells	C-12260
Human Dermal Microvascular Endothelial Cells (HDMEC) adult donor	500,000 cryopreserved cells	C-12212
	500,000 proliferating cells	C-12262
Human Dermal Microvascular Endothelial Cells (HDMEC) pre-screened	500,000 cryopreserved cells	C-12215
	500,000 proliferating cells	C-12265
Human Dermal Blood Endothelial Cells (HDBEC) juvenile foreskin	500,000 cryopreserved cells	C-12211
	500,000 proliferating cells	C-12214
Human Dermal Blood Endothelial Cells (HDBEC) adult donor	500,000 cryopreserved cells	C-12225
	500,000 proliferating cells	C-12226
Human Dermal Lymphatic Endothelial Cells (HDLEC) juvenile foreskin	500,000 cryopreserved cells	C-12216
	500,000 proliferating cells	C-12218
Human Dermal Lymphatic Endothelial Cells (HDLEC) adult donor	500,000 cryopreserved cells	C-12217
	500,000 proliferating cells	C-12219
Human Cardiac Microvascular Endothelial Cells (HCMEC)	500,000 cryopreserved cells	C-12285
	500,000 proliferating cells	C-12286
Human Pulmonary Microvascular Endothelial Cells (HPMEC)	500,000 cryopreserved cells	C-12281
	500,000 proliferating cells	C-12282
Human Uterine Microvascular Endothelial Cells (HUtMEC)	500,000 cryopreserved cells	C-12295
	500,000 proliferating cells	C-12296
Endothelial Cell Growth Medium 2 (Ready-to-use)	500 ml	C-22011
Endothelial Cell Growth Medium MV 2 (Ready-to-use)	500ml	C-22022
Endothelial Cell Growth Medium Kit	500 ml	C-22110
Endothelial Cell Growth Medium 2 Kit	500 ml	C-22111
Endothelial Cell Growth Medium MV Kit	500ml	C-22120
Endothelial Cell Growth Medium MV 2 Kit	500ml	C-22121
DetachKit	30 ml	C-41200
	125 ml	C-41210
	250 ml	C-41220
Cryo-SFM	30 ml	C-29910
	125 ml	C-29912
PromoFectin-HUVEC	0.1 ml	PK-CT-2000-HUV-10
	0.5 ml	PK-CT-2000-HUV-50
VEGF-165, human, recombinant (E. coli)	10 µg	C-64420
VEGF-165, human, recombinant (HEK293)	10 µg	C-64423
Angiogenesis Assay Kit	50 assays	PK-CA577-K905
Cell Migration/Chemotaxis Assay Kit	please visit <a href="http://www.promocell.com/cell-biology">www.promocell.com/cell-biology</a> for more information	
Cell Invasion Assay Kit	please visit <a href="http://www.promocell.com/cell-biology">www.promocell.com/cell-biology</a> for more information	

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