Oxygen and mammalian cell culture: are we repeating the experiment of Dr. Ox?

Mammalian cell culture represents a cornerstone of modern biomedical research. There is growing appreciation that the media conditions in which cells are cultured can profoundly influence the observed biology and reproducibility. Here, we consider a key but often ignored variable, oxygen, and review why being mindful of this environmental parameter is so important in the design and interpretation of cell culture studies.

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In his 1872 short story 'Doctor Ox's Experiment', Jules Verne describes a sleepy Flemish town that is turned on its head by a rogue scientist, Dr. Ox, who is obsessed with oxygen. Bent on testing the effects of high oxygen on living beings, Dr. Ox proceeds to flood the town's atmosphere with pure oxygen under the pretence of modernizing the town's lighting system. The results of Dr. Ox's experiment are immediately apparent: oxygen 'animates' the previously tranquil townspeople, who become manic, excitable and ravenous; plants grow to monstrous proportions; and animals become aggressive. Although Dr. Ox would have let the experiment continue indefinitely, a fiery explosion at the oxygen factory brings everything to an end. The town returns to its serene existence, none the wiser. But what can we, as experimental biologists, learn from this fictional piece?

To a certain extent, Dr. Ox's experiment continues unfettered in modern mammalian cell culture. One of the most striking discrepancies between routine mammalian cell culture and the in vivo environment is the oxygen tensions to which cells are exposed. Room air at sea level consists of 160 mmHg O_2, and the air in a humidified CO_2 cell culture incubator contains 140 mmHg O_2 (20.9% and 18.5% O_2, respectively; conversion table in Fig. 1, inset). In contrast, the oxygen tensions to which human organs are typically exposed are far lower, ranging from ~5–100 mmHg (ref. [1] and Fig. 1). Organs at the lower end of the spectrum include the large intestine (3–11 mmHg) and uterus (15–19 mmHg), whereas the liver is on the higher end (30–55 mmHg). Within a given organ, substantial gradients can emerge, for example, within the kidney, O_2 tensions range from 15 mmHg in the medulla to 30–70 mmHg in the cortex[1]. O_2 tensions within a given tissue can undergo temporal fluctuations after increased metabolic demand; one study has found that during intense exercise, skeletal muscle oxygenation decreases from 30 mmHg O_2 to 7.5 mmHg O_2 (ref. [2]). Oxygen tensions also decrease in cancer and may be as low as 2 mmHg within the cores of untreated tumors[3], thus contributing to therapy resistance. Notably, standard tissue culture conditions (140 mm Hg) expose cells to a partial pressure of oxygen that is even higher than that experienced by the lung alveoli (~110 mm Hg), which are exposed to the highest partial pressure of oxygen of any site in the human body.

Although Dr. Ox had questionable motivations and poor ethical standards, our choice today to routinely culture mammalian cells at supra-physiological oxygen concentrations is one purely of convenience. Is it possible that we are inadvertently 'animating' cells when they ought not to be? Clearly, mammalian cell culture, even with supraphysiological O_2 tensions, has led to transformative discoveries, but investigators must bear in mind how certain aspects of biology can be either obscured or even unmasked by high O_2. To this end, we review six broad categories of cellular processes known to be affected by oxygen.

First, some of the most fundamental macroscopic properties of cells in culture show dependence on oxygen. Already by the late nineteenth century, Pasteur noted

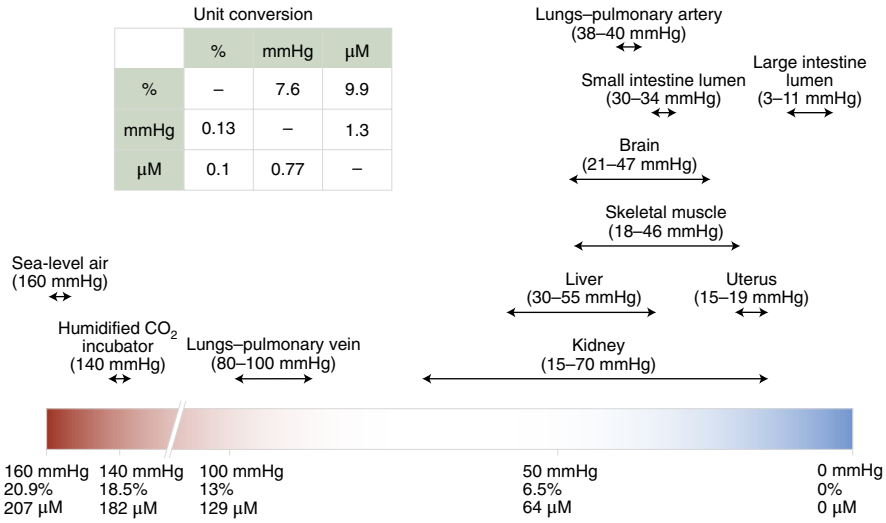

Fig. 1 | Oxygen tensions vary widely across tissues but are significantly lower than those in standard culturing conditions. Oxygen tensions reported across selected human tissues and comparison to standard cell culture conditions[1]. Oxygen tension is often expressed as a percentage representing the volume fraction of oxygen in air or as a partial pressure (mmHg) with respect to atmospheric pressure (760 mm Hg at sea level). In liquid medium, dissolved oxygen (µM) is proportional to the partial pressure of oxygen in air through Henry's law. Oxygen solubility in solution increases with atmospheric pressure and decreases with increasing temperature and ionic strength. Inset, conversion table for different units at which oxygen tensions are typically reported. These conversion factors are valid for measurements made at 37 °C at sea level, for liquids with an ionic strength of 175 mM.

in yeast that high oxygen tensions were capable of directly inhibiting glycolysis, and this 'Pasteur effect' was subsequently extended to mammalian cell culture systems. In his pioneering work, Leonard Hayflick discovered that diploid animal cells cultured at 160 mmHg O_2 cannot be passaged indefinitely. Subsequent studies have shown that the 'Hayflick limit' is a function of the oxygen tension at which cells are grown. In fact, diploid cells can be passaged far more times when cultured under low oxygen tensions (20–75 mmHg)[4]. This link between the Hayflick limit and oxygen has been attributed to several factors, including the lower amounts of DNA damage and transcriptional rewiring that take place under these conditions[5].

Second, numerous biochemical reactions use oxygen as a substrate, and in some instances, oxygen tensions can exert a large influence on chemical-reaction flux. Cytochrome *c* oxidase, the terminal reaction of the electron-transfer chain, and haem oxygenase, which catalyses the degradation of haem, have such low Michaelis constants (K_m) for oxygen (0.4–4 mmHg) that the choice of oxygen tensions in cell culture is not likely to affect their activity[6,7]. Other key O_2-consuming enzymes, however, such as stearoyl-CoA desaturase and certain NADPH oxidases, have activities that are expected to be sensitive to the choice of O_2 concentration in cell culture[8,9].

Third, several regulatory systems within human cells directly sense oxygen tensions and consequently influence physiology. Perhaps the best studied are the hypoxia-inducible factor (HIF) transcription factors[10]. In high oxygen conditions, levels of HIFα protein are kept low, because HIFα is hydroxylated by the PHD1–3 prolyl hydroxylase enzymes and is subsequently tagged for proteasomal degradation. The prolyl hydroxylase enzymes use molecular oxygen as a substrate, thus endowing the system with the ability to directly sense oxygen. HIFα protein levels increase exponentially as a function of decreasing oxygen tensions, starting at 45 mmHg O_2 (ref. [10]). Although the HIF system has received much attention, a similar paradigm is likely to extend to a very large number of enzymes mechanistically associated with the prolyl hydroxylases. For example, the activity of Jumonji-C (JmjC) domain–containing histone lysine demethylase is oxygen dependent (K_m = 10–100 mmHg), directly linking lower oxygen tensions to epigenomic modifications and transcriptional activity[11,12]. In addition, the E3 ubiquitin ligase FBXL5 contains a haemerythrin domain that has been posited to sense both iron and molecular oxygen[13] and subsequently activate the iron-starvation response in low-oxygen conditions.

Fourth, importantly, although oxygen is kinetically extremely stable, it can readily react with metals and cofactors. In the presence of oxygen, iron is oxidized from Fe^{2+} to the non-bioavailable Fe^{3+}, thus catalysing the formation of reactive oxygen species (ROS). Moreover, oxidation can directly damage essential cofactors such as iron–sulfur clusters[14], which function in DNA polymerization, ribosome homeostasis and respiration. Thus, cellular oxidative damage via ROS is affected by the oxygen tensions at which cells are grown, although this correlation does not appear to be linear and instead is bimodal. Although cells grown at physiological oxygen tensions (40–60 mmHg) appear to experience less oxidative stress[15], there is evidence of a paradoxical increase in ROS in low oxygen tensions (<7.5 mmHg) linked to the respiratory chain[16].

Fifth, there is growing appreciation that developmental and differentiation programs are sensitive to oxygen. In the field of stem cell biology, human embryonic stem cells are often cultured at 15–40 mmHg O_2. These conditions support enhanced proliferation, inhibit spontaneous differentiation and lead to significantly less accumulation of DNA damage[17]. Other cell types have been reported to benefit from culturing at physiological O_2 tensions[1]: T cells cultured in lower oxygen tensions display enhanced viability and lytic capacity; preosteoblasts exhibit earlier and greater Ca^{2+} mineralization; and adipocytes differentiated under physiological oxygen tensions secrete higher levels of adiponectin and show elevated hormone-induced lipolysis.

Finally, some cellular models of human disease exhibit pathology only at certain oxygen tensions. We have recently shown that several cellular models of mitochondrial respiratory-chain disease and Friedreich's ataxia exhibit proliferative defects in traditional culturing conditions that can be buffered by growing cells at very low oxygen tensions[18,19]. Other disease phenotypes could conceivably be masked or unmasked as a function of the oxygen tension used.

Confronted with the manifold effects of oxygen in cultured cells, what are researchers to do? Being mindful of the possible influence of oxygen on the given experimental system offers an opportunity to improve reproducibility. The seeding density, surface area-to-volume ratio of the cell culture vessels and time in culture influence the oxygen tension at the start of an experiment and the rate at which oxygen is depleted. Although we have focused on the potentially adverse effects of supraphysiological oxygen tensions, it is also important to bear in mind that culturing cells under anoxic conditions can trigger cell death[20]. Some of these considerations may be particularly important in cells that naturally respire at a higher rate, such as cardiomyocytes or hepatocytes[1].

In the event of discrepancies between cell-culture findings and in vivo results, researchers should consider performing cell culture experiments under oxygen tensions that are more physiological. Several options are available that can be readily scaled to fit researchers' requirements. On the smaller end are airtight chambers, which can be housed in standard CO_2 incubators that have been flushed with a gas mixture of choice, and can accommodate several standard tissue culture flasks. On the larger end, tri-gas incubators, dedicated hypoxia workstations or bioreactors are available to dial the oxygen tension down to the range of interest. However, of note, incubator chambers and tri-gas incubators both have the drawback of requiring all cell manipulation (such as monitoring and subculturing) to be carried out in ambient room air. This acute re-exposure to supraphysiological oxygen tensions, especially during long-term culturing, may not fully recapitulate the effects of chronic hypoxic culturing.

Cell culture is one of the mainstays of modern biomedical research, and, without a doubt, routine cell culture in room air will continue to drive fundamental new discoveries. By considering how oxygen can influence cell culture findings, we have the potential to discover new facets of biology, make our experiments more robust and increase the likelihood that these findings have in vivo relevance. In this era of cellular therapy, being mindful of the importance of culturing cells under more physiological oxygen conditions may be necessary, with the goal of maintaining their health and functional integrity for clinical applications, so that we do not inadvertently repeat the experiment of Dr. Ox. ❐

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Competing interests

V.K.M. is on the scientific advisory boards of Raze Therapeutics, Janssen Pharmaceuticals and 5AM Ventures, and is listed as an inventor on patents filed by Massachusetts General Hospital on the use of hypoxia as a therapy.