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Letter

PO_2 Matters in Stem Cell Culture

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About a century ago, conditions were worked out for maintaining growing tissue and cells outside the body. From the beginning, care was taken to maintain cultures at a physiological temperature, and to include precise concentrations of salts and other compounds, but the oxygen concentration in the culture medium was simply the result of letting the medium equilibrate with air. This approach was a reasonable first approximation, given that values of partial pressure of oxygen (PO_2) in animal tissues were not measured until over a decade later, and all that mattered seemed to be to provide cells with "enough" oxygen. However, it is now firmly established that, in vivo, most stem cells are in environments with low, or very low, PO_2 (for review, see Simon and Keith 2008; Lin et al., 2008; bibliography in the Supplemental Data available online). Additionally, in vitro, it has been shown that oxygen concentration is sensed by stem cells and low PO_2 can radically modify their phenotypes. These facts suggest that we should think more carefully about PO_2 in cell culture.

Whereas ambient air has a PO_2 of ~150 mm Hg corresponding to a concentration of 21%, PO_2 in arterial blood is 95 mm Hg, and in normal tissues it is generally considerably lower, with the amount between 50 and 5 mm Hg (7%–0.7%). The PO_2s experienced in vivo by blastocysts in the uterine fluid, hematopoietic stem cells, and cancer cells in poorly vascularized tumors are at the lower end of this range (Supplemental Data, sections S3, S4, and S7). In vitro, low PO_2 is known to affect stem cell phenotype in an increasing number of ways (reviewed in Csete, 2005). For example, low PO_2 can reduce spontaneous differentiation and enhance clonality of human embryonic stem cells (hESCs). PO_2 can also influence the subsequent fate of stem cells. Amplification of neural stem cells in low oxygen increases the percentage of dopaminergic neurons obtained after differentiation, an effect partly mediated by an autocrine loop involving the expression and secretion of erythropoietin. In hypoxic regions of tumors, low PO_2 promotes dedifferentiation and could be a determinant of the cancer stem cell phenotype (see Keith and Simon, 2007). Now, in this issue of *Cell Stem Cell*, Yoshida et al. demonstrate that lowering cell culture PO_2 enhances the generation of induced pluripotent stem cells (Yoshida et al., 2009). Some of the mechanisms by which low PO_2 influences stem cell behavior have been elucidated. Activation of the Notch signaling pathway by the hypoxia-inducible transcription factor HIF-1α maintains the undifferentiated state, whereas expression of Oct-4, which controls stem cell renewal and pluripotency, is induced by HIF-2α. It is likely that many other pathways are affected by PO_2 given that when hESCs are cultured in 4% O_2, rather than 20%, 149 genes are either upregulated or downregulated (Westfall et al., 2008; see also Forsyth et al., 2008). In sum, PO_2 is important for stem cell function.

Unfortunately, accurately controlling pericellular PO_2 is technically challenging. Simply lowering the PO_2 in a standard laboratory incubator goes only a short way toward achieving a defined, low PO_2 at the cells themselves. The first problem is the time required for equilibrating a cell-culture medium previously exposed to atmospheric PO_2 with a gas phase at a lower PO_2. Routine laboratory practice is to change the medium and to passage the cells in a laminar flow hood under atmospheric PO_2. The gas trapped inside the culture vessel is initially at the atmospheric PO_2, and equilibration with the low PO_2 gas phase can take several hours (Westfall et al., 2008). Most of this problem can be overcome by equilibrating the culture medium at the desired PO_2 before adding it to the cells (and, ideally, by using a closed hypoxia workstation). If these precautions are not taken, not only is there an "oxygen shock" associated with changing the medium but also the time during which the cells were actually exposed to the low oxygen tension is shorter than the time they were in the incubator. The future value of current work might be increased if authors were to give precise details of the procedures they used.

There may also be an error in the other direction: when the oxygen tension in the bulk medium in the culture vessel finally falls to the value in the gas phase, the pericellular PO_2 will be even lower because oxygen consumption by the cells themselves reduces PO_2 (see Supplemental Data, section S9 for more complete references). Hence, when a PO_2 value is indicated in a publication, it is important to mention if this value corresponds to the incubator gas phase, to the bulk medium, or to the pericellular space. If the cells are grown as a uniform monolayer, then they will all experience roughly the same PO_2 (except at the edges of the vessel), so monolayer cultures are to be preferred when the aim is to subject all the cells to the same conditions. If the aim is to approximate in vivo conditions, spheroids are a useful model for embryonic or cancer stem cells. However, in a spheroid, oxygen consumption by peripheral cells reduces diffusion of oxygen into the center of the sphere and it is virtually impossible to impose a uniform PO_2 on all the cells. Little is known about the consequences of contiguous stem cells being in a PO_2 gradient, but new technologies offer hope that such questions might be investigated. For example, a system developed by Michel Maharbiz and colleagues is designed to allow the creation of gradients of PO_2 in cell monolayers (Park et al., 2006). Dissolved oxygen is locally generated at a controlled rate by electrolysis, and PO_2 can be imaged optically at the base of the culture chamber. Almost any desired 2D PO_2 microgradient can be produced with this apparatus.

An aim of much stem cell research is to produce stem cells for therapies, and existing evidence suggests that cells cultured in 20% oxygen will change their properties when injected into the low PO_2 environment in the patient. Equally, if the aim is to understand the behavior in vivo of stem cells, then experiments carried out at low, physiological PO_2s are likely to be necessary. In any case, it is desirable that reporting of how oxygen is provided to stem cells in culture should be more detailed.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Classified Bibliography and can be found with this article online at http://www.cell.com/cell-stem-cell/supplemental/S1934-5909(09)00394-4.

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