A prevalent neglect of environmental control in mammalian cell culture calls for best practices

In biomedical studies, the environmental conditions used in mammalian cell culture are often underreported, and are seldom monitored or controlled. Best-practice standards are urgently needed.

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Human cell lines, first cultured in the 1950s[1], are indispensable in biomedical research. Today, a wide range of cell types are available, and sophisticated advanced 'omics' and visualization techniques allow for the routine assessment of cell identity and cellular responses[2]. However, the culture methods have remained relatively unchanged. Major advances in culture systems were made over three decades ago[3,4], yet the old standard approach of batch cell culture — the culture of cells either in suspension or as adherent monolayers of cells in standard media[5–7] — remains the predominant method in biomedical research.

Culture media provide crucial nutrients, signalling molecules (such as growth factors) and suitable osmotic conditions. The gaseous and thermal environments of cell cultures are typically controlled by the incubator. The initial media conditions are generally stabilized by adjusting them to 18.6% O_2 and a standard pH of 7.4, and this adjustment is achieved by adding a given amount of HCO_3^- salt (a base), and by enriching the media with CO_2 to a given percentage in the air (usually 5% or 10%). However, cell metabolism involves the exchange of gases — specifically, the release of CO_2 and the consumption of O_2 — and this can affect cellular growth via the alteration of, for example, the pH and the level of dissolved O_2 (dO_2) in the cellular microenvironment[8]. In theory, the equilibration of the medium with the gaseous and thermal environments of the incubator provides a way to reliably mimic O_2, CO_2 and HCO_3^- homeostasis in metazoan bodily fluids. Yet this doesn't take into account the fact that homeostasis in a living mammal is supported by the active exchange of gases with the atmosphere. The absence of such active gas exchange in cell cultures suggests that, over time, cellular metabolic activities may acidify and deoxygenate the cellular microenvironment[8–10], if intermittent monitoring and (when necessary) corrective action are not carried out.

To mimic a physiological environment when using cell cultures, careful control over environmental factors (such as pH, CO_2 and O_2) is typically needed, in particular, because even small deviations of environmental parameters from physiological levels may impair cellular function. For instance, in human blood, pH values below 7.2 (acidaemic conditions) and above 7.44 (alkalaemic conditions) can be fatal[11–13]. In cell cultures, the optimal growth of normal cells (that is, non-cancerous cells and non-transformed cells) occurs within a specific alkaline pH range, whereas cancer cells grow in a broader pH range that is shifted towards acidic values[14–18]. Cells have evolved mechanisms, including the use of Na^+/H^+ antiporters or histone deacetylation, that restore the alkaline pH of the cytoplasm when the extracellular pH deviates from physiological levels[19–25]. However, such regulatory mechanisms require cellular energy, and changes in the acetylation state of chromatin can alter gene transcription and reduce cellular growth[26,27]. O_2 and CO_2 are similarly crucial for optimal cellular growth and physiology. The discovery of how O_2 affects cellular performance — for which William Kaelin, Peter Ratcliffe and Gregg Semenza were awarded the Nobel Prize in Physiology or Medicine 2019 (ref. [28]) — underscored the fact that dissolved gases can substantially affect cellular physiology. Deviations in the levels of dO_2 and dissolved CO_2 (dCO_2) from the physiological ranges can cause abnormalities, such as hypocapnia (defined in different reports as 2.7% CO_2 or 1.5% CO_2, and a partial pressure of CO_2 (p_{CO_2}) less than 40 mm Hg)[29–31], hypercapnia (7.5–10% CO_2 or 15% CO_2, and a p_{CO_2} of 60–120 mm Hg)[32–34], hypoxia (variously defined as 1%, 2%, 2–10%, 3% or 8% O_2)[35–39] and hyperoxia (95% or 40%)[40,41]. All of these abnormalities can result in altered cellular function. In fact, even small deviations from physiological levels can result in substantial cellular perturbations, potentially affecting the function of biomolecules and the proteome[42]. Therefore, monitoring, reporting and intervening (more than is currently the norm) to control culture parameters may be required to ensure that experiments closely mimic organismal physiology. Such efforts would aid the interpretability, utility and reproducibility of biomedical research involving cell cultures.

Reproducibility concerns

In basic and preclinical biomedical research, reproducibility shortcomings are pervasive and persistent. A primary contributor to irreproducibility in the biomedical sciences is the inability of scientists to replicate experiments that involve in vitro cell cultures[43]. Growing concerns spearheaded the Reproducibility Project: Cancer Biology (RP:CB), which led to research efforts, published in 2014, to directly replicate influential cancer biology experiments initially reported between 2010 and 2012 (ref. [44]). After ten replication studies were completed, an analysis from this project concluded that five of the original studies were mainly repeatable, that it was unclear whether three of them were replicable and that two could not be replicated (still, for most of these studies, the original findings have been confirmed by other laboratories)[45]. Out of the 16 original studies assessed by the RP:CB, 13 used mammalian cell cultures. We found that 7 out of 13 of the original studies failed to report the nominal set points for CO_2 and temperature, and that none of the studies reported the measured environmental conditions within the cell cultures (Supplementary Table 1), thus limiting the ability of the replicating studies to adopt the same environmental conditions. The replicating studies indicated the nominal settings used, but they did not report measuring the environmental conditions of the cell cultures.

In 2018, an analysis of 200 papers regarding the practice of reporting O_2 conditions in cell cultures revealed that only 6% of the papers provided information about the factors necessary to replicate the O_2 conditions[46]. There is, in fact, some evidence suggesting that variable conditions in cell culture can substantially affect the reproducibility of cell-culture experiments. In particular, barcoding experiments involving cancer cell lines revealed that cell-line evolution occurred because of positive clonal selection that was highly sensitive to culture conditions[47]. These observations suggest that the neglect of environmental conditions, alongside other sources of variations in culture conditions (for example, batch variations in the chemicals used, the presence or absence of the pH indicator phenol red, and type of serum used) could contribute to culture instabilities that lead to cell-line heterogeneity[47,48]. Additional investigations are urgently needed to assess whether unstable environmental parameters promote genetic instability in cultures using cell lines, and whether this compromises reproducibility.

The fact that even deliberate reproducibility efforts such as that of the RP:CB did not consider the importance of reporting data of the cellular environments in mammalian cell cultures, together with evidence of underreporting of such environmental conditions[46], prompted us to assess current reporting practices. In this Comment, we report the results of an evaluation of experimental practices in research involving mammalian cell cultures and published between 2014 and 2019. We assessed 810 randomly selected papers out of 29,192 papers that reported research on mammalian cell lines. Out of the 810 studies, 688 contained relevant data from 1,749 individual cell-culture experiments (Supplementary Fig. 1 and Supplementary Methods). Our assessment reveals a surprising level of neglect regarding the monitoring, reporting and control of pH, CO_2 and O_2 in mammalian cell cultures. We postulate that better reporting, measurement and control of the environmental conditions of cell culture ought to improve reproducibility, drive new discoveries and increase the likelihood that preclinical research has higher in vivo relevance. We also provide practical recommendations of best practices for the monitoring, reporting and control of the environmental conditions of mammalian cell cultures.

Prevalence of underreporting

Our literature assessment revealed inconsistent and insufficient reporting of the environmental conditions of cultured mammalian cells (Fig. 1). The type of culture medium used was typically declared, but over one-third of the cell-culture experiments (hereafter, referred to as just 'experiments') did not report the culture system used (Fig. 1a). In our assessment, nominal incubation temperatures and CO_2 percentages were declared for less than half of the experiments, less than 10% of the experiments reported the atmospheric O_2 levels, and less than 0.01% reported the pH of the medium. Culturing procedures, such as cell density, rates of sub-culturing and cell-passage numbers, were seldom reported. For the studies that did report such data, cell density was often reported at the time of seeding but was infrequently reported at later stages. The rates of sub-culturing and passage numbers varied hugely between experiments, from a few days and a few passages up to nearly one month and over 200 passages, with mean ($\pm$ standard error) values of 4.97 ($\pm$ 0.27) days and 12.19 ($\pm$ 1.2) passages.

Differences in reporting prevalence were small yet substantial for cell types, animal models, and for aspects of the environmental factors and culture method (Fig. 1a–e and Supplementary Table 2). Factors relevant to experiments conducted on stem cells were more frequently reported than factors relevant to experiments with other cell types, yet the majority of experiments did not describe any factor or reported only one factor (Fig. 1b). The number of reported environmental factors was higher for human cells than for other mammalian cells, but less than 50% of the experiments with human and mammalian cells reported two or more factors for either the environmental conditions or culture methods (Fig. 1c).

Sixty-four per cent of the experiments were conducted using batch-culture systems, and 35% of all experiments failed to report the culture system used. The remaining 1% of experiments were conducted in bioreactors or chemostats, where environmental conditions could be accurately monitored and controlled (Fig. 1d). Nominal temperature and CO_2 settings were reported more often than atmospheric O_2 or the media pH (Fig. 1e). However, less than 0.5% of the experiments measured the pH of the medium, and none of the studies reported p_{CO_2} or p_{O_2} in the medium (Fig. 1e). Hence, most of the environmental parameters were defined nominally, but do not seem to have been verified during the experiments.

To assess the robustness of our findings, we conducted a complementary assessment of the prevalence of parameter reporting at the paper level (687 papers; see Supplementary Methods) to ensure that differences in the number of experiments reported for each study assessed did not skew the results. Overall, this robustness analysis (Supplementary Fig. 2) resulted in findings broadly similar to those of the analysis conducted at the level of individual experiments (Fig. 1). For the paper-level analysis, we designated a paper as reporting growth conditions if at least one of the experiments in the paper did so (Supplementary Methods). Despite the use of such an inclusive criterion, we observed only slight differences in reporting percentages, and the outcomes of all statistical analyses remained the same (Supplementary Table 4). In particular, consistent with our analysis at the level of individual experiments, approximately half of the papers failed to report nominal settings for temperature and CO_2, less than 10% of the papers reported the nominal atmospheric O_2 level, and less than 0.01% reported the nominal pH of the medium.

Influence of the environmental conditions

A solid body of experimental evidence implicates pH as a driver of various aspects of cell physiology, and the impact of changes in O_2 on cellular performance has also been amply investigated. However, despite the discovery of cellular sensing of O_2 and of cellular adaptability to hypoxia[49–51], the potential effect of simultaneous changes in pH, CO_2 and O_2 during cell-culture experiments has been largely overlooked. Only one of the 1,749 individual cell-culture experiments that we analysed assessed pH conditions in the culture medium, and not a single experiment reported measurements of dO_2 or dCO_2.

We conducted a thorough and temporally unrestricted search (Supplementary Methods) using PubMed and Google Scholar to ascertain how many peer-reviewed papers have reported environmental conditions for the media of standard batch cultures of mammalian cells. Our search retrieved only six studies that measured pH, dO_2 or dCO_2 conditions during standard batch cultures. Hence, although the sample size of our literature assessment represents only a fraction of the total number of papers published between 2014 and 2019, our conclusion that less than 0.5% of the studies measured environmental conditions in mammalian cell cultures seems robust. The six studies reported substantial changes in the pH of the culture medium during experimentation, with a median shift from the initial pH conditions by 0.425 pH units, and deviations approaching 1 pH unit. Only one of the six

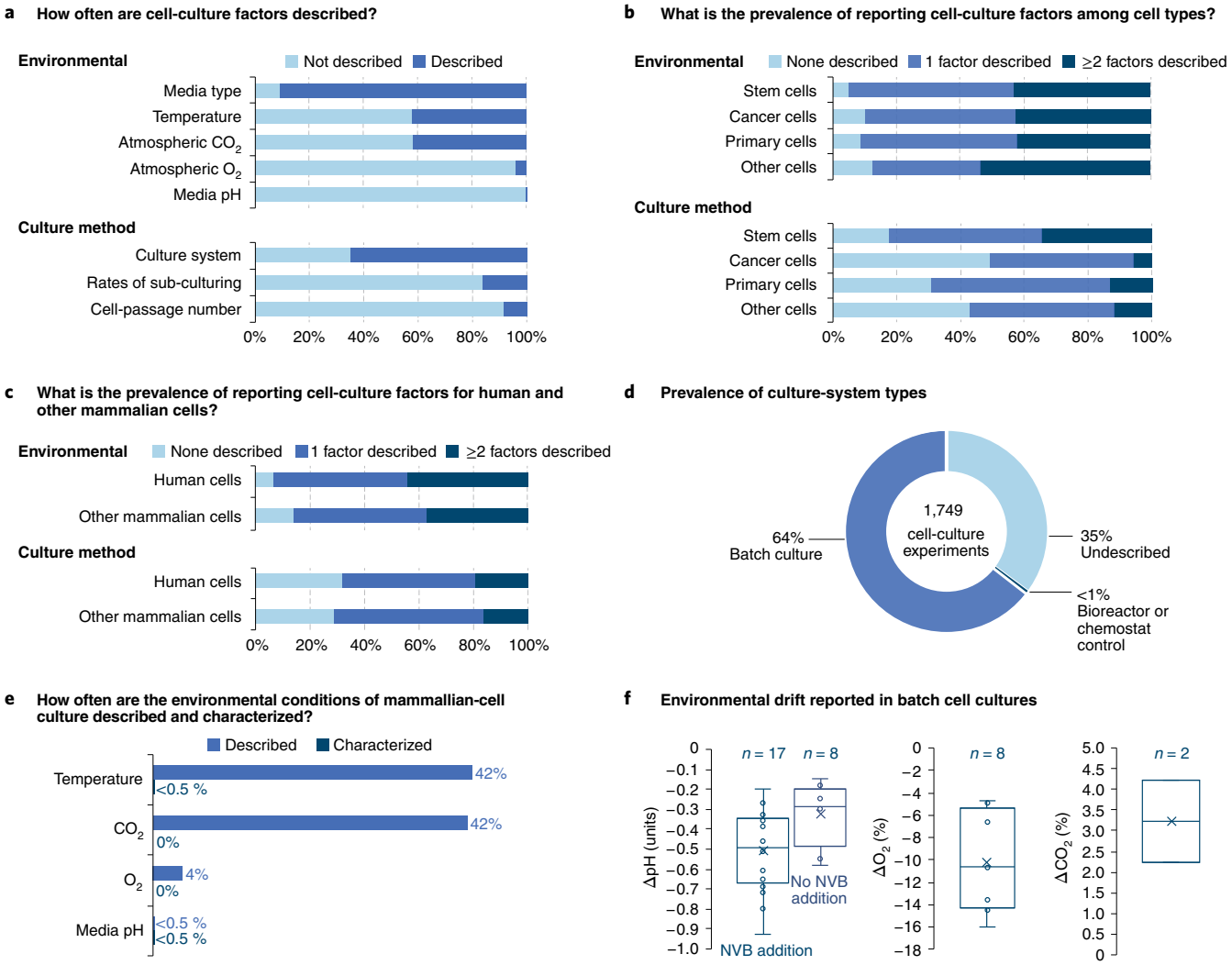

Fig. 1 | Prevalence of reporting of the environmental conditions of mammalian cell culture in 810 randomly selected biomedical studies published in 2014–2019. a, Environmental and culture-method factors for 1,749 cell-culture experiments (detailed in Supplementary Table 2). **b**, Environmental and culture-method factors for common cell types. **c**, Environmental and culture-method factors for human cells and other 'model' mammalian cells (such as cells from rodents). **d**, Type of cell-culture system for the 1,749 experiments. **e**, Physiochemical factors described (that is, a nominal set point was reported) and characterized (that is, the factor was measured during cell culture). **f**, Reported changes in pH, dissolved O_2 and dissolved CO_2 (Supplementary Table 3). Circles represent individual data points, and crosses represent the mean. NVB, non-volatile buffer.

published studies included daily medium exchanges in the culture protocol[52]; and this paper reported that, despite this practice, pH deviated by 0.4 pH units after 72 hours in culture. Indeed, cell densities increase exponentially over time, thereby dramatically increasing metabolic demand and reducing the ability of culture medium to buffer pH changes, even within a 24-hour period[52]. Although the available evidence is limited, it nevertheless shows that daily media exchanges may not necessarily buffer the increasing metabolic demands that arise from exponential cell growth, raising the possibility that such demands would acidify and deoxygenate the medium to reach levels beyond the physiological range.

In addition, we documented the major buffering components of the media to investigate how non-volatile buffers (NVBs), such as HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), affect acid–base regulation. NVBs are traditionally used to enhance the buffering capacity of the medium because the physiological HCO_3^-/CO_2 buffering system can exhibit high volatility and a weak buffering capacity. However, the addition of NVBs can bring about unpredictability (owing to chemical reactions involving CO_2/HCO_3^- and CO_2/NVB), and there is evidence showing that they may not fully prevent cell-induced pH changes[9] (Supplementary Table 3). Indeed, our data also suggest that the addition of NVBs does not fully prevent drifts in the pH (Fig. 1f). Exogenous buffers such as NVBs introduce active molecules and drive acid–base reactions that are absent in mammalian fluids and that can cause long-term toxicity[53,54]. These buffers also stimulate the production of lactic acid via glycolysis[10], potentially introducing artefacts that could compromise the experimental results. Reductions in the pH of the media occurred together with median decreases in dO_2 by 10.6% (in absolute concentration; a 63.6% relative change). Only one published study measured dCO_2 in the culture medium: the dCO_2 levels in two different experiments

increased by 2.24% and 4.21% (an average relative change of 61.3%; Fig. 1f and Supplementary Table 3).

It is important to note that a lack of control, monitoring or reporting of the environmental parameters of cell culture does not necessarily imply that attempts to reproduce the experimental conditions will fail. It is in fact reasonable to hypothesize that if a replicating study uses the same nominal culture conditions (for example, cell density, medium formulation and incubator settings), then the pH, dO_2 and dCO_2 conditions during the experiments would be similar and therefore have little influence on reproducibility.

We did not find reported data that allowed us to assess whether environmental conditions are consistent between experiments that use identical cell lines and nominal culture settings. However, we investigated the degree of variation reported for pH and dO_2 drifts among replicates that were reported in the six studies that measured conditions during standard batch cultures (Supplementary Table 3). Although not all of the six studies examined variations among replicate cultures, the variations reported for pH and dO_2 were substantial in most of these studies. For instance, reductions in pH varied by a range of 0.48 units, and O_2 varied by a range of 5.64% among replicate cultures of hamster ovary cells. After 96 hours of culture, despite similar pH and dO_2 levels at the time of inoculation, replicate cultures exhibited pH levels that varied between 6.77 and 6.47 pH units and dO_2 levels that varied between 10.6% and 3.74%. These observations indicate that, despite using the same nominal culture conditions, the influence of cell metabolism on pH and dO_2 in the culture environment may be difficult to predict, potentially resulting in poor experimental control, non-intuitive outcomes and erroneous inferences that could affect reproducibility[10]. Importantly, even in cases where replicate cultures exhibit similar environmental deviations, such drifts will almost certainly deviate from physiological conditions, limiting the relevance of the experimental results to in vivo physiology.

Among the studies that have investigated the role of extracellular pH on cellular responses, few studies manipulated pH via the addition of CO_2 in media containing physiologically relevant levels of HCO_3^-. In particular, hypercapnia accelerated the differentiation of human primary preadipocytes in culture[33]. In this study, the researchers manipulated the pH of the medium by using different levels of CO_2 enrichment in the atmosphere of the incubator to produce various initial pH values. However, the pH levels as a result of the treatments tested were verified only at the beginning of the experiment and, in some of the treatments, the researchers used HCl to adjust the pH of the medium, potentially introducing artefacts associated with exogenous reactions[32]. Indeed, most studies on the effect of pH on cell physiology have manipulated the pH of the medium by using acids that are not present in the primary buffering system (HCO_3^-/CO_2)[55–57]. However, most published pH manipulations do not depend on modifications to the physiological buffering system (HCO_3^-/CO_2).

It has been shown that a reduced pH in cell culture can unleash a pro-inflammatory signalling response (for instance, in cells of the human nucleus pulposus[56]; and in human aortic smooth muscle cells, it can drive prostaglandin I2 expression and the accumulation of cyclic adenosine monophosphate[57]). Also, human cells can change their metabolic state in response to reduced pH by reconfiguring mitochondrial physiology in order to prevent mitochondrial fragmentation[58]. Indeed, mitochondria increase fission or fusion events when experiencing cellular stress to promote cellular survival[59]. Nevertheless, it is difficult to disentangle the role of reduced pH from effects that are driven by the exogenous acids used to manipulate pH conditions in these studies. However, one study that manipulated pH using the CO_2/HCO_3^- buffering system showed that a reduced pH promoted global transcriptional alterations, and human fibroblasts undergoing CO_2-driven reductions in pH (to pH 6.7) had altered expression of 2,068 genes, including genes encoding for signalling components of apoptosis[60].

Stem cells, in particular the embryonic-pluripotent and adult-multipotent varieties, are widely used as model systems for biomedical research. Their ability to self-renew, to maintain a stable genome and to differentiate into different functional cell types enables the development of two-dimensional (2D) and 3D cellular models that are a better representation of in vivo physiology than models based on cell lines[61]. There is growing evidence that the stem-cell niche can, through metabolic pathways, program stem cell fate[62,63]. For example, pluripotent stem cells and many adult stem cells (including haematopoietic stem cells, neural stem cells and mesenchymal stem cells) reside in hypoxic (1–5% dO_2) niches, and prefer to use glycolysis over oxidative phosphorylation as their energy-providing pathway[63]. A body of experimental evidence has shown that elevated p_{O_2} promotes exit from quiescence for haematopoietic stem cells and exit from senescence for mesenchymal stem cells[63]. By contrast, if stem cells grow to produce structures such as 3D organoids, these depend on having ample oxygenation to grow reproducibly in culture and to faithfully recapitulate tissue physiology[64]. Despite this difference, the vast majority of studies that we analysed and that used stem cells failed to report nominal p_{O_2}, let alone provide any indication that any efforts were made to monitor and control the p_{O_2} experienced by stem cells (Fig. 1b). Moreover, studies that manipulate p_{O_2} in stem cell cultures typically do so by adjusting the percentage of O_2 in the atmosphere of the incubator, with the implicit assumption that the O_2 concentration surrounding the cells is always in equilibrium with the gas phase. However, evidence from this study (Fig. 1f) and other studies[8,46] shows that this assumption does not hold true: the actual p_{O2} depends on many factors, such as cell type, cell density, culture vessel, temperature, O_2 solubility and the diffusion properties of the medium[46].

The impact of variabilities in pH and p_{CO_2} on stem cells has scarcely been explored. It is generally assumed that between changes of medium, the pH is maintained via buffering capacity, as the media are formulated to maintain physiological pH under 5% atmospheric CO_2. This assumption should be reconsidered in light of published evidence to the contrary (Fig. 1f and Supplementary Table 3). Whether stem cells cultured using the standard approach of batch cell culture experience similar pH changes merits further investigation. Interestingly, a study showed that the pH of the culture medium (6.6–7.8), adjusted by changing the concentration of $NaHCO_3^-$, affects the reprogramming of somatic cells and the differentiation of pluripotent stem cells[65]. However, in this study, the presence of two buffering systems (CO_2/HCO_3^- and NVB) in the medium could have caused unstable pH changes in the cultures[10]. The effect of p_{CO_2} on stem cells is difficult to investigate because of difficulties associated with disentangling the effects of CO_2 and pH on cell physiology[33]. It is known, however, that hypercapnia can affect different cellular processes in diverse cell types[34]. Thus, monitoring and potentially controlling pH and dCO_2 in stem cell cultures may improve stem-cell-based models of human physiology.

Best practices

The acknowledged importance of controlling the environment (temperature, pH, dO_2 and dCO_2) in mammalian cell

cultures[10,32–34,42,46,66,67] is in stark contrast with the findings of our assessment of current practices. Our results show a general neglect of the monitoring and control of environmental variables other than temperature. Specifically, published studies typically report nominal temperature and atmospheric CO_2 levels, but most fail to provide the measured environmental conditions and other culture factors such as cell density, passage number and rates of sub-culturing. These are required to ensure acceptable degrees of environmental variation (that is, within the ranges seen in vivo) as well as to aid reproducibility.

Little effort goes into attempting to control dO_2, possibly because of the belief that O_2 is abundant in the atmosphere of the chambers where cultures are maintained. However, passive diffusion may not suffice to supply enough O_2 to support the high O_2 demands of cells undergoing exponential growth[8,46] (Fig. 1f). The importance of carefully controlling the environmental conditions is, however, not a new realization: recommendations made over three decades ago included the use of chemostats[3] and bioreactors[4] for improving the monitoring and control of culture conditions.

There are commercially available systems — including ready-to-use cell-culture flasks[68,69] with integrated and autoclavable sensors — for measuring temperature, pH, dO_2 and dCO_2 with the precision and accuracy required in cell cultures. Commercial culture systems for controlling the environmental parameters range in cost, scalability, maintenance requirements and functionality. Chemostats or perfusion systems[70–72] can maintain environmental conditions via continuous dilution with fresh medium, and advanced bioreactor systems can automatically control the temperature and the addition of gases (O_2 and CO_2), acids and bases to maintain set targets for dissolved gases and pH[73,74]. Although such advanced bioreactor systems can be costly, they provide the best capacity for environmental control, and are ideal for the maintenance of long-term culture and for applications that require the precise management of environmental conditions. To determine the frequency of measurements required to capture real conditions, a basic understanding of the expected variability of the environmental parameters in specific experimental setups is necessary. In the simplest case, initial and final values may be sufficient for experiments where linear declines in pH and dO_2 (and parallel increases in dCO_2) are expected, such as in batch cell cultures (Supplementary Table 3). In other instances, frequent recordings (for example, at one-minute intervals) are required for automated bioreactor systems (involving, for example, the addition of gases), where abrupt changes in environmental parameters are probable. Fortunately, such systems are typically equipped with sophisticated sensors. For initial assessments, pH sensors and meters are typically available in most biomedical laboratories, and measuring systems for dissolved gases are commercially available. And O_2 conditions in standard batch cultures can be predicted via Fick's law from basic culture parameters (such as atmospheric incubator settings, depth of the medium and cell density)[46].

Although all the cell-culture experiments that we assessed consistently exhibited environmental variations (Supplementary Table 3), improving existing protocols could help to constrain environmental drift within acceptable ranges or physiological ranges. Such improvements could involve increases in the frequency of passages or dilutions, reductions in cell density, and the adaptation of culture vessels to increase 'headspace' (to allow for surface-area equilibration). All of these types of experimental features would reduce the impact of cellular metabolism on the conditions of the medium. Importantly, the monitoring and control of cell-culture conditions must be accompanied by the reporting of the environmental measurements. For each of the monitored environmental parameters, the minimum reporting requirements must include the mean and a metric of dispersion (typically, the standard deviation, the standard error of the mean, or the range). Detailed reporting of the data and methodology associated with the environmental conditions will help to identify possible environmental artefacts that may affect the reproducibility of the experimental findings, to ensure the validity of any data comparisons and to assess the in vivo relevance of the conditions.

Improving the standards for environmental control, monitoring and reporting requires a systematic approach. The first step will be to develop standard reporting, control and measuring procedures that do not represent an inordinate burden (in time and human resources). This will require the design and commercial availability of instruments that are purpose-built to control the culture environment for various cell types and applications. The next step will be to determine the impacts of environmental instability on cellular responses (such as proliferation, metabolism, changes in gene transcription or epigenetic regulation), and the reproducibility of the experimental findings. Funding agencies could consider supporting research initiatives aimed at investigating the effects that environmental factors have on commonly studied biological responses (for example, gene expression, histone modifications or metabolic pathways) in model cell lines. Postgraduate university curriculums for biomedical programmes may need to be revised to better impart conceptual, theoretical and experimental understanding of how environmental conditions affect cellular responses and key experimental outcomes. And scientific journals should consider establishing reporting standards and requiring adequate monitoring and control of pH, dO_2 and dCO_2. For relevant research papers, some journals (including the Nature-branded research journals) publish a reporting summary — a standardized declaration of reporting practices — that the authors are asked to fill in; when best-practice standards for the environmental control of cell cultures become available, such reporting forms could be amended to include these.

Outlook

The urgent need for best-practice standards for the control, monitoring and reporting of cell-culture environments is underscored by the rising demand for stem-cell-based cellular models that faithfully and reproducibly recapitulate human physiology. The benefits of developing and updating such standards are exemplified by the success of the Encyclopaedia of DNA Elements (ENCODE) Project, in which a large number of collaborating groups followed stringent experimental and reporting standards to ensure reproducibility and data harmonization[75]. ENCODE was launched in 2003, with the aim of identifying all of the functional elements in the human genome[76].

Much of the cost of conducting biomedical experiments is currently related to the tracking of cell identity and function via sequencing and data analysis. Achieving acceptable environmental conditions for cell growth would require the investment of a small fraction of the costs usually devoted to analysing cellular data. We also argue that the return for such relatively minor investment could increase the relevance and reproducibility of the experimental findings and that the absence of environmental control can jeopardize the value and relevance of cellular data. Addressing this need will contribute towards safeguarding scientific data from any experimental artefacts, to modelling human physiology with higher precision, and to removing a likely contributor to the reproducibility problems undermining biomedical research. ❐

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Author contributions

S.G.K., S.M.A., A.S., S.A., A.J.P., K.A. and M.L. extracted metadata. S.G.K. and S.M.A. performed data analyses. S.G.K., S.M.A., M.L. and C.M.D. interpreted the results. M.L. and C.M.D. wrote the initial draft, with major contributions from S.G.K. and S.M.A. S.G.K. and S.M.A. revised the text during the review process and consolidated edits from all authors. C.M.D., J.C.I.B. and M.L. conceived and supervised the study. All authors approved the final manuscript.

Competing interests

The authors declare no competing interests.

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