Review

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Methods to study the tumor microenvironment under controlled oxygen conditions

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The tumor microenvironment (TME) is a complex heterogeneous assembly composed of a variety of cell types and physical features. One such feature, hypoxia, is associated with metabolic reprogramming, the epithelial–mesenchymal transition, and therapeutic resistance. Many questions remain regarding the effects of hypoxia on these outcomes; however, only a few experimental methods enable both precise control over oxygen concentration and real-time imaging of cell behavior. Recent efforts with microfluidic platforms offer a promising solution to these limitations. In this review, we discuss conventional methods and tools used to control oxygen concentration for cell studies, and then highlight recent advances in microfluidic-based approaches for controlling oxygen in engineered platforms.

Studying hypoxia in the tumor microenvironment

Molecular oxygen is used as a terminal electron acceptor in cellular respiration by most heterotrophic eukaryotes. Due to a variety of factors, including the transport of oxygen via diffusion, oxygen concentration gradients often exist within tissues [1]. When the transport of oxygen is unable to meet demand, hypoxia (sub-physiologic tissue oxygenation) occurs [2]. The partial pressure of oxygen in one such location where hypoxia is often found, the TME, ranges from 2 mm Hg (0.3% O_2) to 15 mm Hg (2.1% O_2) depending on tumor type (Table 1) [3,4]. Comparatively, arterial blood has a partial pressure of oxygen of 100 mm Hg (13.1% O_2), and the partial pressure introduced into traditional cell culture is 152 mm Hg (20% O_2) [5]. Therefore, to support the transition between *in vitro* knowledge and clinical successes, and to more accurately mimic oxygen conditions seen *in vivo*, advanced platforms of study are needed to more closely recapitulate hypoxia in the TME (Box 1) and tissue in general.

Controlling oxygen conditions in a laboratory setting is challenging due to sample/reagent handling and constant diffusion of oxygen from ambient air (~21%). For example, each step during an experiment must be performed under identical oxygen conditions to prevent bias and to ensure reproducibility. A standard laboratory practice, such as changing media for cells, requires the cell culture flask to be removed from a gas-controlled incubator and placed in a chamber with the same desired oxygen concentration. Further, fresh media must be pre-equilibrated to the same oxygen concentration. The dissolved oxygen concentration in the reagent, in this case media, should also be measured prior to addition. Hence, executing a series of relatively simple laboratory techniques quickly becomes arduous under controlled oxygen concentrations.

To control the gaseous environment for cell studies, a number of conventional methods are used. The two most common methods (Figure 1), enable *in vitro* cell assays under hypoxia by (i) having a chamber with an air-tight seal and introducing specific gas concentrations, or by (ii) biochemically inducing a state of pseudo-hypoxia within the cell. These two methods provide unique benefits, as well as limitations, for cell assays under hypoxia.

Perhaps the most prevalent method to control the oxygen concentration is modulation of the gas mixtures entering an incubator (Figure 1A). In this method, cells are grown and conditioned in an incubator with the desired oxygen concentration [6]. However, long oxygen equilibration periods and the burdensome measures taken to sustain hypoxia throughout all aspects of experimentation limit the effectiveness of this method. Furthermore, gas-controlled incubators require an additional system for manual handling of reagents such as a glove box. Similar tools, such as hypoxia chambers, have equivalent limitations when requiring real-time imaging or reagent manipulation [7]. To enable live imaging, perfusion chambers have been used in conjunction with microscopy to enable analysis of real-time data [8]. These perfusion chambers work by limiting diffusion of ambient air into the cell channel. The oxygen conditions are modulated by introducing liquids with a pre-equilibrated dissolved oxygen concentration into the chamber. However, handling and use of the reagents after equilibration of oxygen concentration is imprecise and challenging due to diffusion of oxygen from ambient air. Furthermore, current studies

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Keywords: hypoxia; microfluidics; tumor microenvironment.

0167-7799/

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Table 1. Partial oxygen pressure (pO_2) of solid tumors and the associated surrounding healthy tissue[a]

Tumor type	Median diseased pO_2	Median Health pO_2
Glioblastoma	4.9–5.6	–
Head and neck carcinoma	12–15	40–51
Lung cancer	7.5	38
Breast cancer	10	–
Pancreatic cancer	2.7	52
Cervical cancer	3.0–5.0	51
Prostate cancer	2.7	52
Soft-tissue sarcoma	6.2–18	–

[a]All pO_2 values are combined medians of individual values that were measured in mmHg using an oxygen electrode (adapted from [4]).

using this system have required cells to adhere to a microscope slide and, thus, the cells were not grown in a three-dimensional (3D) environment. While providing cells a 3D environment may be possible when using perfusion chambers, current studies have relied on 2D cultures. As such, this limitation may affect cell behavior, as 3D cell cultures are more physiologically relevant and have been found to differ significantly from 2D cultures in the proliferation, migration, and expression of cell-surface receptors [9–11].

A second approach used to study cellular responses to hypoxia is by biochemically inducing a pseudo-hypoxic state in cells (Figure 1B). This method is distinct from others as the aqueous solution remains oxygenated while investigators rely upon chemical treatments to induce signaling events associated with hypoxia. A number of chemicals such as prolyl hydroxylase inhibitors, nickel chloride, and, the most widely used chemical inducer of hypoxia, cobalt chloride, stabilize the transcription factor hypoxia inducible factor 1-α (HIF-1α) (Box 2) and thus mimic hypoxia [6,12–16]. The two proposed mechanisms by which cobalt chloride stabilizes HIF-1α include inactivating prolyl hydroxylases by chelating their iron core and replacing it with cobalt [17], or by taking up the VHL-binding domain of HIF-1α, thus rescuing it from degradation [18]. In either case, this state of pseudo-hypoxia has proven to be useful for many biochemical analyses, as oxygen can be present in samples without affecting the

Box 1. Hypoxia in the tumor microenvironment

The tumor microenvironment consists of populations of transformed cells, stromal cells (e.g., fibroblasts, chondrocytes), vascular cells (e.g., endothelial cells, pericytes), and inflammatory cells (e.g., neutrophils, macrophages), along with acidity (lactic acid from enhanced glycolysis), increased interstitial pressure, and low oxygen partial pressures [57,73,74]. As tumors grow outward away from the local vascular architecture, and become increasingly metabolically active, formation of variable hypoxic regions throughout the solid mass is inevitable [75]. Neoplastic angiogenesis occurs in an attempt to restore oxygenation, but this neo-vasculature is often insufficient and poorly arranged. This leads to the development of even further gradients of oxygen, nutrients, and waste materials [76,77]. This poses as a therapeutic challenge, as tumor hypoxia is associated with resistance to radiotherapy and chemotherapy and is a negative prognostic indicator for a more aggressive phenotype [78,79]. Furthermore, tumor cells associated with hypoxia are thought to be more malignant and capable of metastasis through avoidance of apoptosis, expression of invasion genes such as matrix metalloproteases, and downregulation of homeostatic genes such as E-cadherin [80–83].

Two aspects of the TME, lower pH and hypoxia, are functionally linked via aerobic glycolysis (Warburg effect), the production of lactate from glucose despite the presence of ample oxygen [84]. When compared to the complete oxidation of glucose to pyruvate through the citric acid cycle and electron transport chain, this metabolic pathway is markedly inefficient [85]. However, aerobic glycolysis likely represents an adaptation to the needs of hyper-proliferative and/or hypoxic cells [74]. Rapidly dividing cells have anabolic needs greater than ATP; glucose can be shuttled away from ATP production to form other macromolecular precursors such as acetyl-CoA, glycolytic intermediates for amino acids, and ribose for nucleotides [86]. Furthermore, other metabolic alterations in cancer cells, such as increased fatty acid synthesis and glutaminolysis, have been suggested as adaptive changes to the constraints of rapid growth in hypoxia. While the molecular switches governing these responses are still being unraveled, tumorigenesis and metabolism are clearly intertwined as all prominent oncogenic alterations have been experimentally linked to changes in metabolism [87]. Ultimately, however, the bioenergetic rearrangement towards aerobic glycolysis emerges from a number of factors including oncogene upregulation, tumor suppressor loss, mtDNA mutation, and stabilization of hypoxia inducible factor 1-α (HIF-1α) in the hypoxic microenvironment [88].

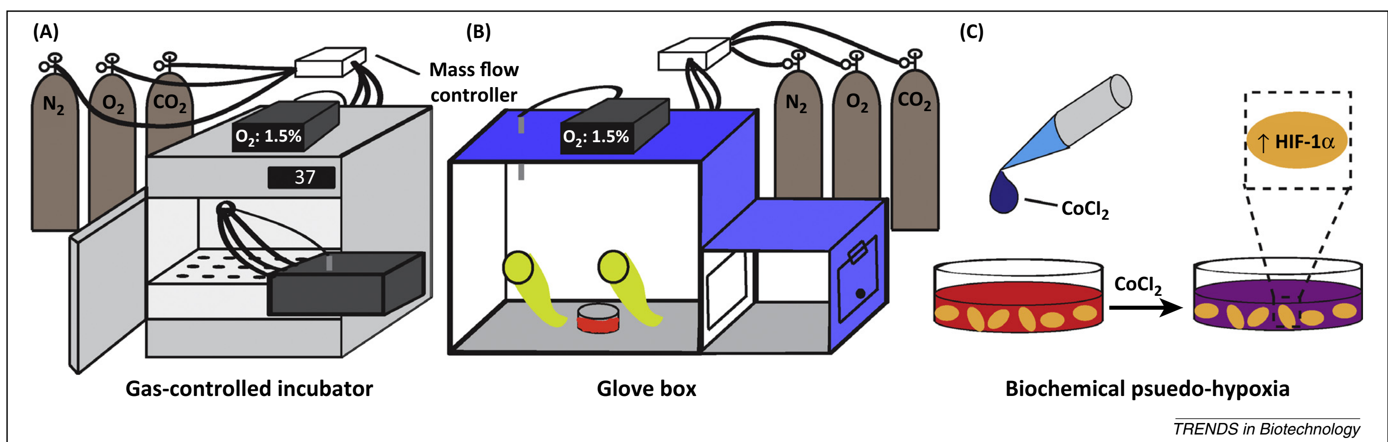

Figure 1. Illustrations of three conventional methods that enable control of oxygen concentration during cell studies: **(A)** a gas-controlled incubator; **(B)** a glove box; and **(C)** biochemical induction of a pseudo-hypoxic state. Abbreviation: HIF-1α, hypoxia inducible factor 1-α.

Box 2. The role of hypoxia inducible factor

Hypoxia inducible factor (HIF) is a master regulator of cellular responses to lowered oxygen concentrations. HIF-1α protein levels are the most frequently used marker of cellular hypoxia. As a DNA-binding transcription factor, HIF induces more than 100 genes, including those coordinating metabolism (especially glycolysis), cell signaling, erythropoiesis, angiogenesis, cell proliferation, and apoptosis [89]. Functional HIF is a heterodimer composed of an α and β subunit. HIF-α contains two oxygen-dependent degradation domains that are hydroxylated via prolyl hydroxylase domain-containing enzymes and subsequently degraded [90,91]. HIF-1α levels rise in hypoxia beginning at approximately 5% and peak at 1% oxygen by volume [92]. HIF-1α stabilization is of particular importance in carcinogenesis, where activity is induced or increased by numerous growth-promoting signals, tumor suppressor loss, oncogene signaling, and onco-metabolite stabilization [93,94]. Not unsurprisingly, tumors exhibiting higher levels of HIF-1α have correlated strongly to poorer survival times in many cancers including those of breast, head/neck, esophagus, stomach, and lung [78].

HIF-mediated effects are especially apparent in angiogenesis, in which the transcription factor regulates downstream events such as vascular permeability, extracellular matrix remodeling, endothelial cell migration/proliferation, tube formation, and pericyte interactions [93]. Angiogenesis is of such importance to neoplastic pathogenesis that much of the last half century's cancer research efforts were focused on angiogenesis' role in oxygen availability: understanding molecular underpinnings, revealing roles in tumor mass progression, and ultimately inhibition via receptor antagonism. However, these efforts have not directly led to clinical progress as strongly as predicted. In general, treatment failures seen with angiogenic inhibitors have been attributed to cell population selection towards those that can circumvent the specific angiogenic target (e.g., up/downregulation of angiogenic mediators, reliance on separate factors) and through a pre-existing immunity to the specific therapy (e.g., redundancy in signaling, pre-existing protection from stromal cells/inflammatory cells) [95].

experiment. However, this biochemical induction narrows the scope of the hypoxic study to those events downstream of the single HIF family of transcription factors. While HIFs are master regulators of many hypoxic cell responses, cobalt chloride fails to adequately reproduce mitochondrial reactive oxygen species (ROS) signaling; rather, generation of ROS from cobalt chloride physiologically differs from that of hypoxic ROS generation [19,20]. Furthermore, cobalt itself is cytotoxic [21], and cobalt chloride affects cell division and morphology [22], while in some cases, inducing mitochondrial DNA damage and apoptosis [23,24]. Overall, these conventional methods are limited in their ability to support the study of cell behavior under controlled oxygen conditions.

Microfluidic platforms for hypoxic studies

Microfluidic platforms enable precise control over the local microenvironment. This inherent characteristic allows spatial and temporal regulation of specific chemical and gaseous conditions. For example, microfluidic platforms capable of creating chemical concentration gradients have been used for cell migration studies [25,26]. Furthermore, spatiotemporal control over signaling molecules has enabled studies on cell–cell communication between multiple cell populations [27,28]. In addition to control over the microenvironment, a key aspect of microfluidic platforms is that equilibration times are significantly reduced compared to conventional methods due to differences in scale (micro versus macro) [7,29]. To control the oxygen concentration inside microfluidic platforms, several different mechanisms have been employed. The three most common methods (Figure 2) are: (i) introducing oxygen scavenging chemicals into the platform [30,31]; (ii) controlling the oxygen supply by implementing adjacent gas supply channels, for example, in multilayer platforms [29,32–39]; and (iii) reducing the oxygen supply by use of oxygen impermeable materials such as cyclic olefin copolymer (COC), polystyrene (PS), or poly(methyl methacrylate) (PMMA) [40–44]. A few other microfluidic approaches to control oxygen tension have been reported [42,45–47], but they are challenging to fabricate or more cumbersome in operation. The remainder of this review focuses mostly on the three aforementioned microfluidic methods of controlling oxygen concentration.

Oxygen scavenging chemicals

One method to control oxygen concentration in the microfluidic platform utilizes oxygen scavenging chemicals (Figure 2A). On a macroscale, oxygen scavenging chemicals have been used to control oxygen concentration in solutions [48]. However, temporal and spatial heterogeneity exist due to long diffusion times and a lack of control over mixing of the chemical solutions (convective mixing). In microfluidic platforms, mixing occurs only via diffusion due to fluid flow being in the laminar flow regime. Hence, the concentration and reaction rate of oxygen scavenging chemicals can be controlled well. Oxygen concentration gradients have been created by generating oxygen scavenging and oxygen generating reactions on either side of a cell channel [30]. Similarly, modulating channel dimensions and sodium sulfite flow rates enabled control over the oxygen concentration in a cell channel [31]. Utilizing the developed platforms, the anticancer drug Tirapazamine was tested under various oxygen concentrations on ardenocarcinomic human alveolar basal epithelial (A549) cells, demonstrating the utility of these platforms for therapeutic testing [30,31]. In addition, by introducing oxygen scavenging chemicals rather than gas streams, ancillaries such as gas cylinders and flow controllers are not required. However, the continuous flow associated with this method requires syringe pumps or passive pumping techniques such as gravity-driven flow and surface-tension driven flow to introduce the oxygen scavenging (or generating) chemicals into the platform.

Gas supply channels

The second method to control oxygen concentration is based on implementing gas supply channels adjacent to cell chambers (Figure 2B). An advantage of microfluidic platforms is their ability to be designed and rapidly fabricated to meet desired specifications. Hence, development of platforms with optimal positioning of gas channels enables control over oxygen concentration. The most common geometric arrangement of gas supply channels utilizes multilayer platforms, with the gas channel flowing over or under a monolayer of cells [29,35–38]. These platforms have been used to study intracellular calcium dynamics in neonatal rat cardiomyocytes under hypoxia [29]. Aqueous solutions with pre-equilibrated oxygen concentration have been introduced offering the additional advantage of reducing

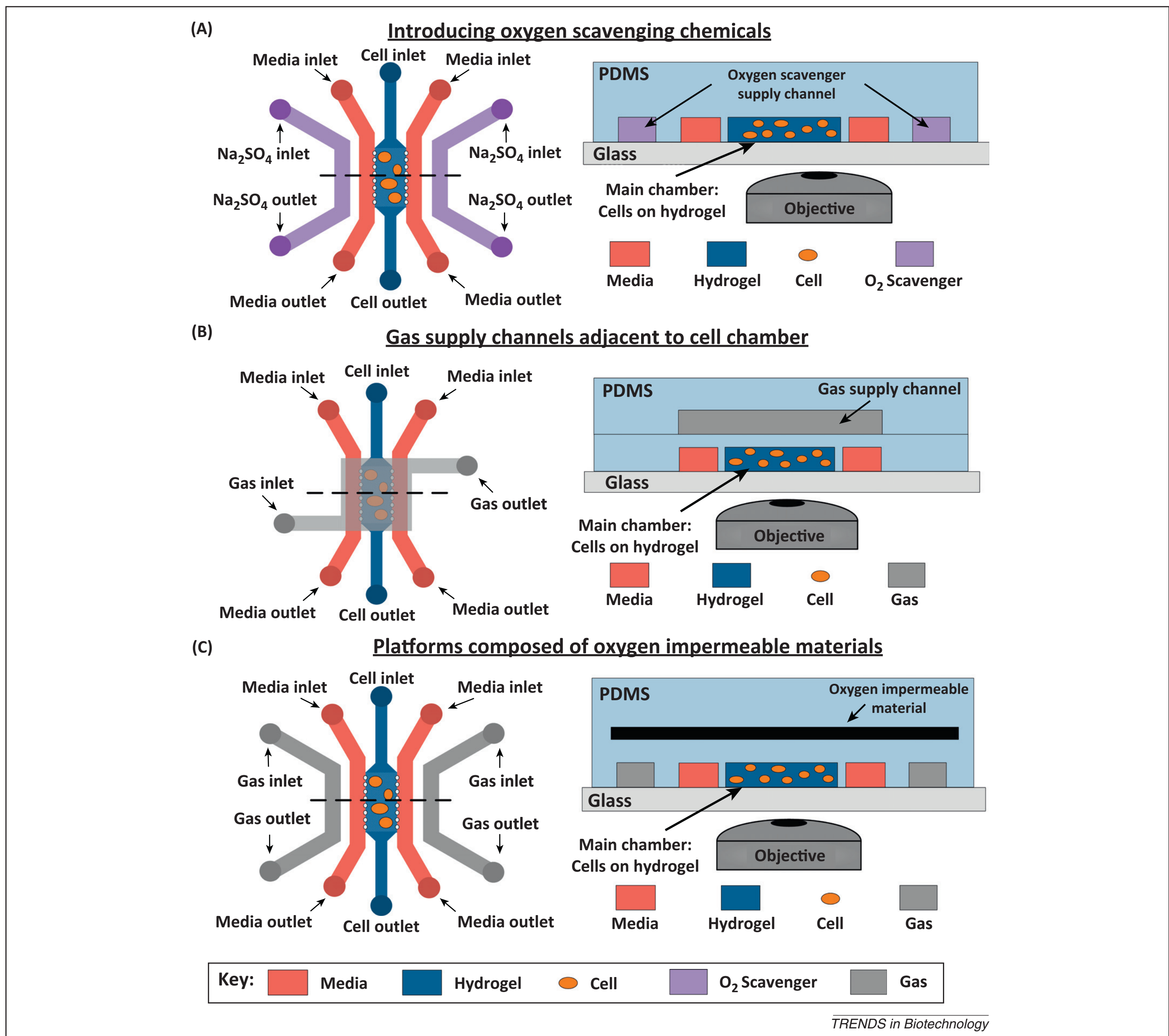

Figure 2. Microfluidic platforms that enable control over oxygen concentration, shown in top down view (left) and cross sectional view (right). Broken line indicates where the cross-section was made. The different platforms can control oxygen concentration by **(A)** introducing oxygen scavenging chemicals, **(B)** altering the diffusional distance of oxygen, or **(C)** incorporating relatively oxygen impermeable materials.

changes in solute concentration [34]. However, this method of pre-equilibrating aqueous solutions is imprecise and challenging due to diffusion of oxygen from ambient air during handling. In addition, these multilayer platforms can be more difficult to fabricate and use than single-layer platforms. For example, care must be taken that features in control layers do not obstruct imaging of cells.

Modifications to standard laboratory tools such as the Boyden chamber [49] and well plates [50] have been developed to control oxygen tension. These structures, commonly composed of poly(dimethylsiloxane) (PDMS) and created via replica molding, provide gas channels above the cell culture chamber. Similar to a well plate, cell chambers open to the atmosphere have been developed to control oxygen by flowing gas or oxygen scavenging chemicals underneath the cells [32,39]. This open well platform has been used to study intracellular ROS levels in Madin–Darby Canine Kidney (MDCK) cells under oxygen gradients [32]. These platforms enable easy addition of chemicals and are similar to a conventional tissue culture dish. The platforms require cells to adhere to PDMS in a monolayer to have a uniform oxygen concentration for all cells. To overcome the limitation of cells cultured on PDMS in 2D, a microfluidic platform capable of culturing cells in a 3D microenvironment while controlling the oxygen concentration was developed by positioning gas channels both above and below the cell chamber [33]. Overall, introducing gas supply channels adjacent to cell chambers has proven to be an effective method to control oxygen concentration. However, further advances in user operability are necessary for this method to gain widespread use.

Table 2. Oxygen diffusivity in materials commonly used for fabrication of microfluidic platforms

Material	Oxygen diffusivity (10^{-11} m^2/s)	Refs
Poly(dimethylsiloxane) (PDMS)	400	[96]
Polycarbonate (PC)	2.0	[97]
Polystyrene (PS)	4.0	[97]
Cyclic olefin copolymer (COC)	0.46	[43,98]
Poly(methy methacrylate) (PMMA)	0.25	[99]
Polypropylene (PP)	3.0	[100]

Oxygen impermeable materials

A third method to control oxygen concentration is by fabricating platforms out of oxygen impermeable materials (Figure 2B). Traditionally, microfluidic platforms have been fabricated using PDMS. One of the main benefits of using PDMS is ease and speed of replica molding, allowing for rapid iteration in design improvements. During cell culture, PDMS enables diffusion of oxygen from the atmosphere into cell channels. However, when desiring hypoxic conditions, this oxygen permeability limits the ability to control oxygen tension. To combat this issue, materials with low oxygen permeability (Table 2) can be selected and used to fabricate platforms. Oxygen diffusivity is orders of magnitude higher in PDMS than the other materials listed. Hence, microfluidic platforms comprised of alternative materials have the potential to control oxygen concentration more effectively by reducing diffusion from the atmosphere. For example, platforms comprised of PMMA prevented oxygen diffusion from the atmosphere [40,44]. Gas permeable membranes embedded within the platform enable oxygen diffusion between cells and gas channels. With this control over oxygen concentration, growth patterns of the bacterium *Pseudomonas aeruginosa* under varying oxygen concentrations was studied, demonstrating the capability to study microaerobic and anaerobic conditions [44].

Similarly, a hybrid platform consisting of PDMS and a polycarbonate (PC) film has been developed [41]. The relatively gas impermeable PC film, patterned above the cell channels, reduces diffusion of oxygen from the atmosphere above the device. Meanwhile, the PDMS enables oxygen diffusion from the gas channels to the cell chamber while physically separating the two. Using this platform, migration studies of MDA-MB-231 human breast cancer cells in 3D under hypoxia were performed [41]. To study oxygen uptake rate in hepatocytes and endothelial cells, an oxygen impermeable platform consisting solely of COC has been designed [43]. Due to the nature of the study, this platform did not require gas to be introduced into the system and, thus, did not require an oxygen permeable material. While constructing microfluidic platforms out of gas impermeable materials is promising, these materials are time consuming to pattern and can be difficult to bond and at times challenging to connect to ancillaries.

Microfluidic platforms (Table 3) enable real-time studies of cell behavior such as cell migration, ROS generation, and therapeutic sensitivity under controlled oxygen conditions. In addition, these platforms, reduce equilibration time, increase control of oxygen concentration, enable generation of oxygen gradients, and improve real-time measurements of cell behavior when compared to conventional methods [7]. Still, further work with microfluidic platforms is desired to increase their utility and ease of use in precise control of the oxygen concentration.

Future outlook of microfluidic platforms to control oxygen concentration

Many questions regarding cell behavior in the TME under controlled oxygen conditions remain. Further development of the tools used to control these conditions is needed to enable accurate analysis of cell behavior. Specifically, with respect to microfluidic platforms, a number of technical and pragmatic issues still exist. These challenges must be overcome to enable studies leading to a deeper understanding of the TME.

An issue that is beginning to plague microfluidic platforms is 'ease of use', especially pertaining to the

Table 3. Summary of reported microfluidic platforms that enable control of oxygen concentration in cell studies

Material used	Cells in 3D?	Device features	Refs
Introducing oxygen scavenging chemicals for oxygen control			
PDMS	No	Chemical reactions generate oxygen gradients	[30]
PDMS	No	Top of platform open to atmosphere and oxygen scavenging chemicals below cell chamber	[39]
PDMS	No	Oxygen scavenging chemicals in cell chamber	[31]
Gas supply channels placed adjacent to cell chambers for oxygen control			
PDMS	No	Gas channel above or below cell chamber	[29,35,37,38]
PDMS	No	Top of platform open to the atmosphere	[32]
PDMS	No	Valves actuated by Braille bin to circulate media	[45]
PDMS	No	Boyden chamber insert coated with Parylene-C	[49]
PDMS	Yes	Gas channels above and below cell chamber	[33]
PDMS	No	Insert for well plates	[50]
PDMS	No	Oxygen controlled with pre-equilibrated water	[34]
Platforms fabricated out of oxygen impermeable materials for oxygen control			
PMMA	No	PMMA exterior with gas permeable membrane	[40]
PC, PDMS	Yes	PC film patterned above PDMS channels	[41]
COC, PS	No	Patterned channels in COC and PS	[42]
COC, PMP	Yes	Completely composed of COC or PMP	[43]
PMMA	No	PDMS channels with PMMA exterior	[44]

requirement of external devices [51,52]. To this end, new techniques, procedures, and platforms have been developed to provide researchers who are not familiar with microfluidics the option to use these valuable tools. For example, surface tension based passive pumping enables addition of fluids and is compatible with micropipettes [53]. A technology that is already available, 'Kit-On-A-Lid-Assay', provides self-contained microfluidic cell-based assays, integrating all steps required to perform the assay [54]. Increased understanding of capillary flow in suspended microfluidic platforms offers increased accessibility, functionality, and simplicity of fabrication [55]. Still, the need for gas cylinders and mass flow controllers to introduce gas streams into microfluidic platforms still complicates experiment setup and execution. Hence, new and innovative designs and ideas are required to reduce the complexity of microfluidic platforms for oxygen dependent cell studies.

To move beyond validation of platform studies, scientists must be able to seamlessly integrate these microfluidic platforms into their laboratory toolkit. However, this integration requires familiarity and confidence with the particular microfluidic platform. Implementation of microfluidic platforms in current biology and biochemistry laboratory courses would provide the needed exposure to microfluidics for a broad range of potential users, which would lessen the barrier towards integrating this technology into many biological studies.

Technical issues, such as accurately recapitulating the TME on a microfluidic platform, also must be addressed. The TME directly affects tumor progression and therapeutic resistance, making accurate modeling critical [56,57]. At present, microfluidic platforms have been developed and applied to study interactions between the different cellular components of the TME [51]. However, to date these platforms have been used primarily to study proof-of-concept biology. To accurately model the TME, further development of imaging techniques that depict a tumor's cytotype are needed. The tumor cytotype, defined as the identity, quantity, and location of each of the different cell types that make up a tumor, influences how a tumor will evolve over time. Current imaging techniques such as optical coherence tomography (OCT) [58], positron emission tomography (PET) [59,60], magnetic resonance imaging (MRI) [59], as well as light sheet fluorescence microscopy (LSFM) [61], enable non-invasive imaging of the TME and associated characteristics such as hypoxia and pH. However, these techniques are limited by their chemistry, resolution, or inability to provide quantitative results and typically cannot fully depict the TME. Hence, more detailed analyses and improved imaging of the tumor cytotype is needed for engineers and scientists to more closely mimic these complex tissues, for example, within a microfluidic platform.

Another challenge associated with studying cell behavior under controlled oxygen concentrations in microfluidic platforms is the need to develop and integrate methods for endpoint measurements. Traditional endpoint measurements in cell studies performed with microfluidic platforms rely on image analysis for phenotypic behavior such as cell migration or proliferation. To provide more thorough analysis of cell behavior and function under hypoxia, further integration with current biochemical tools is necessary. For example, integrating a microfluidic platform with genetically encoded GFP-based redox sensors could enable measurement of the oxidative state of a cell under hypoxia [8,62,63]. Development of methods to quantify secreted molecules would provide insight into cell signaling under hypoxia, however, more research is necessary for current techniques to be adapted under controlled oxygen concentrations [64,65]. To measure levels of protein, RNA, or DNA, platforms enabling cell lysis for qRT-PCR and western blots off-chip have been developed and qRT-PCR completely on chip has even been performed [66,67]. Still, challenges with these techniques such as harvesting individual or small groups of cells cultured in complex 3D environments remain.

Beyond cancer and the TME, the stem cell microenvironment [68] as well as diseases such as Alzheimer's [69], rheumatoid arthritis [70], chronic kidney diseases [71], and non-alcoholic fatty liver disease [72] have all been associated with hypoxia. While the context of this article is specific to the TME, one should note that these tools could also be applied to these other hypoxia related microenvironments.

Concluding remarks and future perspectives

Hypoxia affects a number of cellular behaviors in the TME, however, studying these processes in a controlled manner has proven to be challenging. In general, conventional methods enable macroscale studies, but are limited with respect to real-time imaging. More recently, microfluidic platforms have been developed to study cell behavior under controlled oxygen concentrations. Although these platforms have been used primarily in validation studies, compatibility with microscopy provides an opportunity to further study cancer cell biology under hypoxia. However, further technical development of these tools is required for sophisticated biological experiments. Specifically, methods to increase ease of use will provide more facile addition and manipulation of reagents, while more accurate modeling of the TME is necessary to interpret the experimental results and thereby better understand tumor progression and therapeutic efficacy in a relevant system. In addition, to increase adoption of microfluidic platforms, increased training opportunities are necessary, for example, through implementation of experiments involving simple microfluidic tools in existing laboratory courses in molecular and cell biology programs.

Acknowledgments

Support was provided by the Midwest Cancer Nanotechnology Training Center (M-CNTC), National Institute of Health grants R33-CA137719 and R01-GM083601, and the University of Illinois at Urbana-Champaign.

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