Precise control over the oxygen conditions within the Boyden chamber using a microfabricated insert†

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Cell migration is a hallmark of cancer cell metastasis and is highly correlated with hypoxia in tumors. The Boyden chamber is a porous membrane-based migration platform that has seen a great deal of use for both in vitro migration and invasion assays due to its adaptability to common culture vessels and relative ease of use. The hypoxic chamber is a current tool that can be implemented to investigate the cellular response to oxygen paradigms. Unfortunately, this method lacks the spatial and temporal precision to accurately model a number of physiological phenomena. In this article, we present a newly developed microfabricated polydimethylsiloxane (PDMS) device that easily adapts to the Boyden chamber, and provides more control over the oxygenation conditions exposed to cells. The device equilibrates to 1% oxygen in about 20 min, thus demonstrating the capabilities of a system for researchers to establish both short-term continuous and intermittent hypoxia regimes. A Parylene-C thin-film coating was used to prevent ambient air penetration through the bulk PDMS and was found to yield improved equilibration times and end-point concentrations. MDA-MD-231 cells, an invasive breast cancer line, were used as a model cell type to demonstrate the effect of oxygen concentration on cell migration through the Boyden chamber porous membrane. Continuous hypoxia downregulated migration of cells relative to the normoxic control, as did an intermittent hypoxia regime (IH) cycling between 0% and 21% oxygen (0–21% IH). However, cells exposed to 5–21% IH exhibited increased migration compared to the other conditions, as well as relative to the normoxic control. The results presented here show the device can be utilized for experiments implementing the Boyden chamber for in vitro hypoxic studies, allowing experiments to be conducted faster and with more precision than currently possible.

Introduction

Oxygen is critical in a wide variety of cellular signaling pathways, ranging from stem cell differentiation to cancer cell metastasis. Hypoxia, or low oxygen, has been strongly linked to a more invasive tumor cell phenotype4–8 and has been a good indicator of poor patient prognosis in a number of cancer types.4–8 More recently, researchers have discovered that the relationship between hypoxia and tumors may be due to the typically poorly developed tumor vasculature relative to normal tissues, often exhibiting blind ends, excessive branching, and leaky vessel walls that limit the supply of oxygen to the tumor.9–11 The mechanisms behind enhanced invasive potential of tumor cells exposed to hypoxia are not entirely understood, but are in part due to the attenuated degradation under hypoxia of hypoxia inducible factor-1α (HIF-1α), a subunit of the constitutively-expressed transcription factor HIF. The products of HIF have wide-ranging influences on development,12 angiogenesis,13 cell proliferation,14 migration,15 metabolism,16,17 cancer cell biology,12,16,17 and apoptosis.18 Tools allowing researchers to probe the effect of oxygen tension on cells are of great interest to the biological community due to the widespread impact of hypoxia.

The Boyden chamber (or Transwell insert) is one such tool that has been widely utilized for cell co-culture and migration/invasion studies due to its ease of use.19 The chamber is designed to insert into a single well of a multwell plate (ie 6, 24, 96-well). To perform migration studies, cells are seeded directly on the top of a porous polyethylene terephthalate (PET) membrane at the base of the insert, as shown in Fig. 1a. For experiments probing the effect of oxygen on cellular processes, a Boyden chamber is typically used in conjunction with the hypoxic chamber. The stimulus promoting chemotaxis, migration, or invasion depends entirely on the experiment and the phenomenon under investigation, ranging from signaling factors to mechanical cues.20–23

The hypoxic chamber is the gold-standard for exposing cells to different oxygen concentrations. The system is essentially an air-tight chamber into which cell cultures are placed, and is then sealed and flushed with the desired gas concentration. The chamber is simple and easy-to-use, which accounts for its widespread use. Unfortunately, it suffers from a relatively long oxygen equilibration time (>2 h),24,25 large size, inability to observe cells during the assay with microscopy, and absolutely no spatial control over the oxygen concentration. In the case of the Boyden migration assay, the experiment is normally run for 6 h, whereas it can take nearly 3 h for the cells to be exposed to
a steady-stage equilibration oxygen concentration within a hypoxic chamber. This greatly limits the hypoxic regimes that can be exposed to the cells, such as in oxygen gradients and intermittent hypoxia (IH), the latter of which involves cycling between two oxygen concentrations over time. The hypoxic chamber’s inadequacies could lead to misinterpretation of results due to improper experimental design rather than biological phenomena. Tumor cells show increased metastatic ability when exposed to IH over continuous hypoxia, and cardiomyocytes have been demonstrated to be protected against cardiac reperfusion injury-induced apoptosis by being pre-exposed to IH, yet performing IH experiments in the standard Boyden chamber is exceedingly difficult. Despite the physiological importance of precisely controlling the spatial and temporal oxygenation of *in vitro* cell cultures, the standard hypoxic chamber lacks the flexibility needed to investigate the role of oxygen in a number of physiological phenomena.

Microfluidic technology has been explored as a method for improving upon the hypoxic chamber, and many devices demonstrate impressive control over oxygen concentration, even permitting the generation of O₂ gradients at very rapid timescales (seconds). However, in many cases, the effectiveness of the oxygenation profile are highly dependent upon specific characteristics of the system, such as electrolysis-induced O₂ diffusion out of the device and microelectrode efficiency, or cell metabolic rate, seeding density, and perfusion flow rate in cell metabolism-induced gradients. Another approach has been to pre-oxygenate the media in perfusion systems. However, this perfusion exposes cells to shear stress, which needs to be taken into consideration. Many of these microfluidic devices
require specialized knowledge and equipment for their operation and do not adapt to established assays, making them successful in the labs they were developed in, but intimidating to researchers not already familiar with the technology.

In this paper, we present a novel device capable of controlling the oxygen concentration inside a standard off-the-shelf Boyden chamber to study migration. Essentially, the device is an insert within an insert that was tailored to fit inside the Boyden chamber as shown in Fig. 1b. The Boyden chambers nest into the six wells of a multiwell plate, and the hypoxic device has six pillars that nest into the Boyden chambers. At the base of each pillar is a microfluidic network continually infused with gas from a gas cylinder during an experiment. Oxygen and other gases like carbon dioxide can then freely diffuse across a thin 100 μm gas-permeable PDMS membrane underneath the channel. The device was coated with Parylene-C as a barrier layer to minimize oxygen contamination from ambient air, as demonstrated by Mehta et al. in their microfluidic oxygenator. The device effectively establishes a concentration gradient between the gas source (the microfluidic network), and the cell culture media, driving the system towards a desired steady-state equilibration concentration. The device is simple to use and can be easily implemented in any biomedical research lab currently utilizing hypoxic chambers.

To validate the device, the oxygen concentration was measured directly below the porous membrane of the Boyden chamber with planar fluorescent oxygen sensors. The device was later utilized to investigate the effect of different continuous oxygen concentrations on migration of an invasive breast cancer cell type. The goal of the device is to bridge the current gap between the physiologial precision of many of the microfluidic systems and ease-of-use of the hypoxic chamber so that researchers can probe novel oxygen-regulated biochemical pathways, such as those involved in cancer metastasis, and perhaps aid in the development of anticancer and other oxygen-dependent therapies.

### Experimental

**PDMS fabrication**

The Boyden chamber hypoxic insert device was fabricated with methods similar to those previously described and is shown nested into a Boyden chamber as a schematic in Fig. 1b and photograph in Fig. 1c. Briefly, the hypoxic device is fabricated in four main steps: 1) replica molding PDMS (Sylgard 184; Dow Corning) from a machined Delrin mold for the pillar array, 2) photolithography and soft lithography to create a PDMS microfluidic channel component, 3) defined spinning of PDMS on a silicon wafer to achieve a desired thickness of 100 μm for the gas-permeable membrane, and 4) bonding the components together using a handheld wand for atmospheric oxygen plasma treatment (BD-20, Electro-Technic Products). The overall height of each completely assembled pillar (excluding the scaffolding holding the pillars) is 16.2 mm in length and 22 mm in diameter. At the end of the pillar is a circular microchannel that has a diameter of 18.5 mm, a height of 300 μm, and has support pillars to minimize deformation of the attached PDMS membrane. The particular pillar length used yields a 1 mm gap distance between the Boyden chamber PET substrate and the hypoxic device gas-permeable membrane, which represents the oxygen diffusion path length and the cell culture media volume. The Boyden chambers (BD Falcon) themselves possess an 8 μm pore size that is suitable for mammalian cell migration. As these particular inserts are widely used in a 6-well plate configuration, the hypoxic device was designed and fabricated to fit within the specific dimensions of this platform. While no other manufacturer’s Boyden chambers were examined, the concept and design could be rapidly modified to extend to other chamber dimensions.

**Parylene deposition**

The hypoxic device was coated with a film of Parylene-C before being used in our oxygen equilibration and the cell migration experiments as this material is less permeable to air and prevents absorption or adsorption at the PDMS interface. Following sterilization of the device, the Parylene coating was deposited using a Labcoater 2 PDS 2010 chemical deposition system (Speciality Coating Systems, Indianapolis, IN). This vacuum vapor deposition polymerization process depositions Parylene-C films by initially loading a furnace chamber with a solid dimer, paracyclophane (di-para-xylene or DPX). In a first step the solid is converted to the dimer gas phase by sublimation (temperature in the range 120–160 °C, pressure 1 mbar). The vapors are pyrolyzed in a second step in a higher temperature zone (650–700 °C, 0.5 mbar), resulting in intermediate monomer di-radicals. Finally, the monomer gas enters the deposition zone (20–40 °C, 10⁻¹ mbar) where the polymer film is formed conformally onto all substrate surfaces. A reduction in the chamber temperature causes chloro-p-xylene to condense onto the PDMS surfaces to form a barrier membrane of Parylene-C. A cold trap is located between the deposition chamber and the pump in order to minimize the contamination of the pumping system with DPX. The initial loading weight of the DPX determines the thickness of the Parylene-C film as it is deposited at a continuous rate of 0.5 μm/g until no dimer remains. Using this protocol, we deposited a 2.5 μm thick Parylene-C film on the hypoxic device. Due to the reduced oxygen permeability characteristic of Parylene-C, the gas-permeable PDMS membrane is added at the end of the coating process so that the device functions properly. Thus, as depicted in Fig. 1b, the Parylene coating covers all surfaces of the device, with the exception of the membrane. During the coating process, the Parylene vapor penetrates inside the inlet and outlet channels and the top and sides of the microchannel itself.

**Device validation using fluorescent oxygen sensors**

Device validation was conducted with oxygen-sensitive fluorescent oxygen sensor slides (FOXY SGS; Ocean Optics), which are standard glass microscope slides coated with an oxygen-sensitive ruthenium complex. The sensor’s emitted fluorescent intensity is inversely proportional to the ambient oxygen concentration as oxygen quenches the fluorophore of the sensor. Fluorescent intensities of the slides were measured using a fluorescence-equipped Olympus IX71 microscope, charged-coupled device camera (QImaging Retiga-SRV), and Metamorph image acquisition software (v7.6.3.0). Images were taken at 2X (PlanApo 2x/0.08) using a FOXY-compatible fluorescent filter (Olympus 31020).
with an excitation wavelength of 475 nm and emission wavelength of 600 nm illuminated by a 75W Xenon burner (Olympus U-LH75X) to illuminate the slides. A motorized stage (Prior ProScan II) was used to store and return to saved image capture locations.

For the Boyden chamber hypoxic device equilibration studies, the FOXY slides were placed immediately below and adjacent to the Boyden chamber membrane. Thus, measurements reflect the oxygen concentration directly underneath the porous membrane of the Boyden chamber and effect the highest concentration the cells would be exposed to under hypoxia as they migrate further away from the device. Note that the Boyden chamber PET membrane itself is approximately 15 μm thick. The FOXY slides were placed at the bottom of the well. The insert device and water used for the equilibration studies were pre-warmed to 37 °C in an oven, and a heated multowell plate stage (Tokai Hit Thermo Plate) was used to maintain this temperature from below. A heating block was also used to warm the hypoxic insert device from above to prevent a temperature gradient that could affect equilibration. The effect of Parylene-C coating and heat on time and extent of oxygen equilibration was examined. The device equilibration conditions were as follows: 1) Parylene-C coated at 37 °C and 2) uncoated at room temperature. Devices were infused with 0% oxygen and the sensor consisted of only the membrane and not a volume connection of the inlet tubing leading from the gas tank. Coated and uncoated membranes were bonded to the microchannel component with oxygen plasma. The devices were placed directly atop the FOXY slide with the membrane adjacent to the sensor. Thus, the diffusion path of oxygen between the gas microchannel and the sensor consisted of only the membrane and not a volume of water, as in the device equilibration experiments. Images were captured at 4x every 2 s for a total duration of 5 min. The slide was calibrated and oxygen concentrations were calculated using the same method as for the device equilibration experiments.

Oxygen diffusion through Parylene-C

For the Parylene-C oxygen diffusion experiments, two uncoated PDMS microchannels were fabricated and bonded either to an uncoated or Parylene-C-coated 100 μm thick membrane. The Parylene-C coating procedure was the same as for the device, but now only for a membrane. The PDMS microchannel components were fabricated using the same SU-8 master as for the device, but with about twice the thickness to allow for easier connection of the inlet tubing leading from the gas tank. Coated and uncoated membranes were bonded to the microchannel component with oxygen plasma. The devices were placed directly atop the FOXY slide with the membrane adjacent to the sensor. Thus, the diffusion path of oxygen between the gas microchannel and the sensor consisted of only the membrane and not a volume of water, as in the device equilibration experiments. Images were captured at 4x every 2 s for a total duration of 5 min. The slide was calibrated and oxygen concentrations were calculated using the same method as for the device equilibration experiments.

Migration assay using the hypoxic insert device and Boyden chamber

MD-MBA-231 cells (ATCC), an invasive breast cancer cell line, were used to assess the ability of the device to modulate cell migration through Boyden chamber porous membranes, which were placed in the wells of a 6-well plate. Cells were cultured in Leibovitz’s L-15 media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (ATCC). Cells were seeded on top of the Boyden chamber membrane at a density of 5 × 10^5 cells/cm in complete media. Cells were allowed to settle and attach to the membrane for 3 h before starting the experiment to prevent cells from being pushed out to the periphery of the well once the pillar was inserted into the culture media. The hypoxic device was then inserted into the Boyden chambers and cells were exposed to 0%, 5%, 21%, 0–21% IH, and 5–21% IH oxygen for 6 h. Oxygen was cycled between concentrations every 30 min for the IH conditions. After exposure, the device was removed and cotton swabs were used to remove the unemigrated cells from the top of the Boyden chamber membrane. The top surface of the chamber was rinsed with serum-free media and then 2 mL of media was added. Hoechst DNA stain (bisBenzimide H 33342 trihydrochloride; Sigma) was used for high throughput cell nuclei counting. The dye was added to the underlying media to a final concentration of 3 μg/mL and cells incubated for 30 min at 37 °C. At least 7 images were taken per well at 2X using DAPI excitation, encompassing an overall area that covered a majority of the Boyden membrane surface under the gas microchannel. Images were then analyzed using ImageJ “Otsu” thresholding and “analyze particles” to count the number of cells per image. The average cell count per image was calculated per condition, and then was normalized to the 21% oxygen control for each trial.

Statistical analysis

All oxygen validation experiments were conducted in triplicate to establish a statistical experimental variance. Graphs depict the mean value with the error bars indicating standard deviation. For the migration assay results, data is presented as the mean normalized cell count for each condition over at least 5 trials, with error bars indicating the standard deviation. Error bars do not exist for the 21% oxygen condition as it was used for normalization. Single-factor ANOVA in Excel was used to calculate the p-values and thus significant difference between conditions. ANOVA could not be used to compare to the normoxic control, as there was no variance due to the normalization step.

Results

Oxygen equilibration and effect of Parylene-C coating

At 25 °C with a 1 mm diffusion gap, the device coated with Parylene-C equilibrated to a steady-state value of about 1% oxygen in about 20 min as shown in Fig. 2a. Zero percent oxygen equilibration time for the uncoated device was longer, taking about 30 min to reach 1% oxygen. A similar difference was observed for equilibration at 37 °C between the Parylene-C coated and uncoated device shown in Fig. 2b. Without the coating, equilibration was slower and had reached slightly below 1.5% oxygen in 30 min. However, coating the device with Parylene led to more rapid and effective equilibration, with the end-point concentration slightly over 0.5% at about 30 min.
Oxygen diffusion through Parylene-C

The Parylene-C coating significantly inhibited oxygen diffusion through the PDMS membrane for both the 0% and 100% oxygen equilibration experiments as shown in Fig. 3a and Fig. 3b, respectively. The uncoated PDMS membranes systems equilibrated in about 1 min for both oxygen extremes, whereas the Parylene coating yielded a change in oxygen concentration of only a few percent over the duration of the experiment. These experiments were done to validate the limited oxygen diffusion on Parylene-C coated devices, but it is important to note that the gas-permeable membrane of the actual hypoxic device remains uncoated. The Parylene-C layer blocks oxygen diffusion at other surfaces of the device to minimize oxygen contamination from ambient air.

Effect of oxygen concentration on cell migration

Five oxygen regimes (0%, 5%, 21%, 0–21% IH, and 5–21% IH) were infused into the device for 6 h to assess the effect of oxygen concentration on the migration of a metastatic breast cancer cell line (MDA-MB-231 cells) as shown in Fig. 4. The data reports the mean normalized cell count for each condition for 5+ trials, with the error bars representing the standard deviation, as described in the methods. Surprisingly, continuous hypoxia (both the 5% and 0% oxygen conditions) downregulated migration of cells relative to the normoxic control, as did the IH condition of 0–21% IH. However, the 5–21% IH oxygen condition yielded the most migration out of all the conditions, even slightly higher than the 21% control. Using a single-factor ANOVA, 5–21% IH was significantly different (p<0.05) from continuous 0% and 5% oxygen, as indicated by the asterisk. The p-value for the comparison between the two IH conditions was 0.067, indicating that there is a trend but are not significantly different.
p-values greater than 0.05. 5% oxygen, as indicated by the asterisks. All other comparisons yielded 5–21% IH was significantly different (p<0.05) from continuous 0% and even slightly higher than the 21% control. Using a single-factor ANOVA, control, as did the IH condition of 0–21% IH. However, the 5–21% IH hypoxia downregulated migration of cells relative to the normoxic conditions; 0%, 5%, 21%, 0–21% IH, and 5–21% IH. The IH conditions involved cycling between the two listed oxygen concentrations every 30 min for 6 h. Migration is expressed as the mean cell count for each condition normalized to the normoxic control. Surprisingly, continuous hypoxia downregulated migration of cells relative to the normoxic control, as did the IH condition of 0–21% IH. However, the 5–21% IH oxygen condition yielded the most migration out of all the conditions, even slightly higher than the 21% control. Using a single-factor ANOVA, 5–21% IH was significantly different (p<0.05) from continuous 0% and 5% oxygen, as indicated by the asterisks. All other comparisons yielded p-values greater than 0.05.

**Discussion**

The Boyden chamber hypoxic device presented here equilibrates on a much faster time scale than the hypoxic chamber (minutes rather than hours). The long equilibration time is a prominent disadvantage of the hypoxic chamber for shorter-term continuous hypoxia experiments like the migration assay, as equilibration can span a significant portion of the experiment itself. The temporal precision of the hypoxic device here not only allows for the establishment of steady-state conditions more rapidly, but also permits a number of physiological oxygenation regimes not possible before, such as intermittent hypoxia in cancer metastasis and cardiac ischemia reperfusion injury, among many others. Moving from a steady-state system like the hypoxic chamber to a temporally-dynamic one like the hypoxia insert device greatly expands the realm of experimental possibilities available to the hypoxia researcher. Coupled with the adaptability to a number of standard, high-throughput culture systems like the multwell plate and Boyden chamber, the device allows experiments to be conducted more rapidly and efficiently as well, greatly accelerating experimental throughput. With one device and plate, six different oxygenation regimes could be exposed to a cell population seeded simultaneously, negating the issues associated with exposing different batches of cells to different oxygen concentrations due to limited space or number of hypoxic chambers.

The device could also establish a wide variety of spatial oxygen profiles, limited by the media gap size and thus oxygen diffusion path similar to those previously shown in a device for standard 6-well plate culture. The device pillar could be made longer so the cell-to-microchannel network distance is closer to yield more precise spatial patterning, as lateral diffusion decreases with a decreasing vertical component. The number of simultaneously experiments then jumps from six to dozens as wells could be split up into multiple concentrations and temporal setups. Additionally, the pillar array format of the hypoxic device could be adapted to higher throughput multwell plates, such as 24 and 48-well plates.

When experiments were initially conducted with a heated stage warming the lower surface of the 6-well plate to 37 °C, equilibration was less efficient. Although the reasons for this inefficiency were difficult to quantify, it is thought to be a result of thermal gradients altering the diffusion profile. When the insert was heated from the top and bottom, the results were greatly improved over an unheated device. Therefore, for live microscopy experiments on a heated stage, it is important to heat the entire system, particularly the PDMS device, to avoid these temperature-gradient effects on equilibration. Experiments conducted in an incubator or warmed chamber would not be affected by these induced gradients and subsequent mixing. Alternatively, Parylene-C was employed on the device, except the gas permeable membrane, to prevent oxygen diffusion within the bulk of the insert as shown in Fig. 1b. Parylene-C has the added benefit of also blocking small hydrophobic compounds from adsorbing to or absorbing into the bulk of the PDMS, which has been previously demonstrated to alter experiments. As this data shows, the device with Parylene-C equilibrates slightly faster and reaches a lower level of hypoxia than the device without this coating. This is to be expected as Fig. 3 demonstrates how Parylene-C blocks oxygen diffusion. Despite the comparative oxygen impermeability of PET, which comprises the Boyden chamber membrane, oxygen concentrations were measured above and below the material and found to be similar, which is most likely due to the abundance of relatively large pores in this membrane. Thus, cells are exposed to the same oxygen concentration throughout their migration path in this assay.

The migration assay results were surprising in that they contradicted reports in the literature using invasion assays. For example, hypoxia has been shown to increase invasion of MDA-MB-231 cells through the Boyden chamber membrane, but in this study, the opposite was observed for continuous hypoxia and migration. This could be explained by a number of reasons. The first could be the absence of the ECM layer on the Boyden chamber membrane. Granted, the absence of ECM in this assay does differ greatly from the environment cells would be exposed to in vivo during migration, but many of the same processes like focal adhesion and actin cytoskeleton restructuring would be required even on an artificial substrate like the Boyden chamber membrane. Thus, it was hypothesized that migration would follow closely with invasion, as the former behavior is part of the latter process. However, many of the known proteins involved in metastasis are associated with ECM interactions. For example, lysyl oxidase and urokinase-type plasminogen activator receptor are both involved in metastasis, regulated by hypoxia, and play an important role in ECM remodeling. Factors involved in these ECM interactions like the ones mentioned could be upregulated, but are not observable using a migration assay. Thus, one future application of the device is to use it for an invasion study in the Boyden chamber.
Additionally, other Boyden chamber migration assays conducted involving hypoxia faced a few shortcomings, including utilization of the hypoxic chamber as the exposure system, which has been shown in other reports to be ineffective at hypoxia exposure both temporally and in the ability to establish concentrations below 2.5%. It also takes several hours to equilibrate and during this time, the cells are exposed to a gradually decreasing oxygen concentration. In shorter duration migration assays that could be run for less than 6 h, this equilibration time constitutes a majority of the overall experiment. Additionally, cell proliferation becomes an issue in longer versions of the assay, such as those conducted over 18 h as compared to 6 h. Unfortunately, the experimental duration for migration assays in the literature is highly variable, ranging from 3 h \(^{44}\) to 20 h \(^{42}\) which could lead to differential results due to the above reasons. Both extremes were tested during pilot experiments, but 6 h yielded the biggest differences in migration between conditions. Finally, the cell quantification methods of migrated cells often involve removal of cells from the substrate using a detachment solution, which with ineffective detachment could alter measured fluorescent intensities when cells or lysates are stained with a dye.

The migration results, although unexpected, did yield differences between continuous and intermittent hypoxia exposure, demonstrating the need for more temporal precision in \textit{in vitro} oxygenation. IH has been shown to induce a more metastatic phenotype in cancer tumors, which involves at least in part an upregulation of cell migration through tissues. In fact, due to the temporal precision of the hypoxic insert system and ability to generate severe hypoxia, the 5–21% IH condition could actually be more closely related to other studies reporting 0–21% IH while using the hypoxic chamber. As far as the downregulation of migration in continuous hypoxia versus normoxia, the result could again be due to the ability of the device to generate severe hypoxia, which could lead to cell apoptosis and necrosis in the 0% oxygen condition, whereas typically cells are exposed to higher concentrations of oxygen using the hypoxic chamber. This downregulation would be less likely using the hypoxia chamber due to its inadequacy at precisely controlling the oxygen concentration at the cell surface in a defined manner.

The Boyden chamber hypoxic insert device is not only capable of establishing a desired oxygen concentration much more rapidly than the hypoxic chamber, avoiding issues with gradually-changing oxygen environments exposed to cells, but also permits more complex spatial and temporal oxygen regimes. A similar technology developed by our lab for 6-well plates demonstrated extensive control over the spatial oxygen concentration in a well, as dependent on the microchannel design at the base of the pillar. \(^{40}\) The hypoxic insert device presented in this work could also modulate the spatial oxygen concentration using the same approaches as for the other device. Additionally, the device utilized in this study was used to establish intermittent hypoxia, which yielded different results than the continuous hypoxia condition. The migration assay results presented here serve as a validation of the device in terms of its ability to work in conjunction with common cell culture assays and to modulate cellular behavior. It is hoped that the technology will be disseminated to other labs that can conduct more innovative and intricate cell and molecular biology experiments.

**Conclusion**

The results described here demonstrate the ability of the Boyden chamber hypoxic device to permit more precise control over the oxygen concentration exposed to adherent cellular cultures over existing technologies. Additionally, use of the device with the Boyden chamber depicts the adaptability of the device to a number of cell culture systems, making it more easy-to-use and versatile. Multiwell plates permit much higher throughput experimentation, where numerous experiments can be conducted simultaneously with the same cell population. Therefore, experiments with hypoxia can be performed more quickly, accurately, and easily with the device. The Boyden chamber hypoxic insert device is a powerful tool that expands the experimental possibilities for chemotaxis, migration, invasion, IH, or reactive oxygen species signaling investigations that would be difficult to achieve with standard methods.

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**References**


