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A microfabricated platform for establishing oxygen gradients in 3-D constructs

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Abstract

Oxygen gradients are increasingly implicated in a number of biological processes, including stem cell differentiation and cancer metastasis. Unfortunately, the current *in vitro* tools designed to mimic conditions found *in vivo* lack application flexibility, simplicity in operation, and precise spatial control that most researchers require for widespread dissemination. The novel microfluidic-based device presented here addresses all the above concerns, offering a simple platform for enhanced control over the oxygen microenvironment exposed to three-dimensional cell-seeded constructs. The device utilizes an oxygen diffusion membrane approach to establish a gradient across a construct sandwiched between two continually perfused microfluidic networks. The device is capable of forming steady-state gradients at both the conditions tested - 0% to 5% O₂ and 0% to 21% O₂ - but a wide variety of profiles within the construct are possible. Cell viability with two model cell lines was also tested, with no adverse effects relative to the control.

Keywords

Oxygen; gradients; 3-D; cell culture; in vitro

INTRODUCTION

Oxygen is a key metabolic variable influencing many different biological phenomena, usually modulated by hypoxia-inducible factor-1 (HIF-1) (Wang et al., 1995). HIF-1 is a heterodimeric transcription factor that has been found to influence development, angiogenesis, cell proliferation, migration, metabolism, cancer cell biology, and apoptosis, among many other functions (Carmeliet et al., 1998, Semenza, 1999, Semenza, 2002, Semenza, 2000, Semenza, 2007, Wang et al., 1995). Gradients of oxygen are increasingly highlighted as crucial metabolic regulators when studying drug toxicity, stem cell differentiation, plant biology, liver zonation, and throughout developmental biology (Allen et al., 2003, Alvarez et al., 1998, Drew, 1997, Holzer et al., 1987, Parmar et al., 2007). Often, these gradients arise from cellular metabolic depletion of available oxygen diffusion from nearby capillaries, thus creating an oxygen gradient that approaches hypoxia the further the cell is from the vasculature. Replicating these *in vivo* gradients in an *in vitro* model accessible to any standard cell biology lab would have a huge impact across many fields.

Stem cells are defined by their ability for indefinite self-renewal given the appropriate culture media, and the ability to differentiate into a number of specialized cell types. Mesenchymal stem cells (MSCs) can differentiate into a number of cell types, including

chondrocytes, osteoblasts, adipocytes, and myocytes cells, among others (Chamberlain et al., 2007, Pittenger et al., 1999). MSCs are thought to have the potential to cure a number of diseases and allow *de novo* production of tissues from cell precursors. Recently, researchers have posited that oxygen plays a key role in stem cell maintenance and differentiation (Salim et al., 2004). Grayson *et al* demonstrated the increased osteoblastic and adipocytic differentiation potential of MSCs in 3-D ECM constructs exposed to constant hypoxia when induced by chemical means (Grayson et al., 2006). Murine-marrow derived MSCs undergo enhanced osteochondrogenic differentiation under chronic hypoxia (Lennon et al., 2001). Additionally, Kucia *et al* have demonstrated the commonality between cancer cell metastasis and injury-induced progenitor cell trafficking, as dependent upon SDF-1 and CXCR4 gradients, which are regulated by HIF-1 and presumably hypoxia (Kucia et al., 2005). Thus, oxygen plays a critical role in many stem cell behaviors, but it is unfortunately still poorly understood, perhaps in part due to the inadequacies of the tools available for research.

In addition to stem cells, oxygen gradients are also implicated in cancer cell biology. The process of cancer cell metastasis is quite complex, involving regulation of a number of genes that modulate migration, entry into and exit out of the vasculature, proliferation, survival, and angiogenesis (Baluk et al., 2003, Rofstad et al., 1999). Many of these genes are mediated by the HIF-1 α pathway, as hypoxia is one of the hallmarks of a metastatic phenotype (Chan et al., 2007, Rofstad, 2000, Sullivan et al., 2007). In tumors, exaggerated oxygen gradients typically exist due to the high metabolic rate of the cancer cells. In one *in vivo* study of tumor oxygenation, cells located only 200 μ m from the nearest capillary possessed a mean oxygen concentration of about 0.2%, whereas in normal tissues this distance can be upwards of 400 μ m (Helmlinger et al., 1997). These metastatic genes include those involved in cell and extracellular matrix (ECM) interactions such as the matrix metalloproteinases (MMPs), which break down the ECM to permit cell mobility and vascularization, and angiogenesis, such as vascular endothelial cell growth factor (VEGF) (Rofstad et al., 1998, Rofstad et al., 1999, Solorzano et al., 2001). Therefore, cancer metastasis could be further elucidated by a cell culture tool that could mimic the microenvironment found at initiation sites, which includes oxygen gradients.

One area of controversy involving cell culture is the relevance of 2-dimensional cell culture to modeling *in vivo* systems. In cell monolayers, which are typically employed in *in vitro* experiments, cells are forced to flatten down to a substrate, and only exhibit adhesion on one surface of the cell. However, *in vivo*, adherent cells are completely surrounded by ECM and these interactions are important in modulating cellular behavior (Wozniak et al., 2004). 3-D environments have been demonstrated to be important in a number of cellular behaviors, including stem cell differentiation (Hosseinkhani et al., 2006, Zhang et al., 2008, Zhang et al., 2009), drug metabolism (Kim et al., 1998, Nakamura et al., 2003), cell viability (Chen et al., 2003, Peyton et al., 2006), proliferation (Ceresa et al., 2009, Chen et al., 2003), morphology (Luhmann et al., 2009, Peyton et al., 2008), and gene expression (Fan et al., 2009, Li et al., 2007), among many others. With the growing interest in 3-D cell culture and the marked differences in cell behavior, the ability to modulate the oxygen conditions exposed to cells in a 3-D construct is attractive to a number of labs, and can be used to better model conditions found *in vivo*.

Unfortunately, the tools for *in vitro* oxygen exposure either lack the ability to mimic physiological oxygen regimes, as with the hypoxic chamber or lack the simplicity and control that is desirable to most labs for widespread dissemination. Other microfluidic approaches have been successful at generating oxygen gradients in two dimensions (Adler et al., 2010, Allen et al., 2003, Lo et al., Mehta et al., 2007). For example, Adler *et al* utilized the gas-permeable properties of PDMS by establishing an oxygen gradient across a cell-seeded liquid channel by infusing two different oxygen concentrations in gas channels above

the cell channel. The oxygen gradient device presented here however is applied to three-dimensional constructs, offering a tool that can more accurately mimic the *in vivo* environment. A diffusion-membrane approach to oxygen modulation as is utilized as with many other oxygen devices design for 2-D cell cultures (Adler et al., Mehta et al., 2007, Oppegard et al., 2010, Oppegard et al., 2009, Thomas et al., 2011, Vollmer et al., 2005). It is simple to use and can be disseminated to any lab with cell culture capabilities. The system functions by establishing an oxygen gradient between the infused gas microchannels and the cell cultures. Oxygen diffuses across a gas permeable membrane that separates the fluid and gas components of the system. The device possesses two microchannels with membranes sandwiching a cell-seeded construct that would be placed into the device. Two gases can be independently infused into the channels to establish a concentration gradient across the construct. For example, a gradient of 0% to 21% oxygen could be established by infusing 0% oxygen (balanced nitrogen) into one channel, and 21% oxygen into the other. The device was validated by measuring the oxygen concentration across the agarose surface for both 0%-5% and 0%-21% oxygen, using a PtOEPK oxygen sensor (Vollmer et al., 2005). Two model cell lines were used to assess the effect of the device on cell viability.

MATERIALS AND METHODS

Device Fabrication

A device was fabricated to permit the establishment of oxygen gradients across a 3-D cell-encapsulated construct, such as agarose. The main part of the device consisted of two microchannels covered with gas-permeable membranes sandwiching the construct, as shown in figure 1. A Petri dish houses the device and allows easy media exchanges for longer duration experiments. The membrane and microchannel components were fabricated as described previously (Oppegard et al., 2010, Oppegard et al., 2009). Briefly, a microchannel master was made using standard SU-8 photolithography and replica molding. SU-8 2150 (Microchem) was carefully poured on top of a silicon wafer and spun to a thickness of 400 μm using a Laurel spinner. The photoresist was selectively exposed to UV light through a photomask, and then the unexposed regions of the resist were removed during the development step. Polydimethylsiloxane (PDMS) was then cured on top of the master at 75°C for 2 h, delaminated from the mold, and diced. For the gas-permeable membrane, PDMS prepolymer was poured on top of a silicon wafer and spun to a thickness of 100 μm .

The lower microchannel was made to be slightly larger in diameter than the upper microchannel to permit inlet and outlet access around the periphery of the upper microchannel. PDMS membranes were bound to both the upper and lower microchannel. A spacer was then incorporated to support the upper microchannel over the agarose construct, and to contain the bored inlet and outlet holes. This spacer was bound only to the lower microchannel, and possessed notches so that media could enter the interior chamber of the device from the Petri dish containing the assembly. The upper microchannel thus remained a separate component and can be removed to access the chamber area where the agarose construct is housed. The entire assembly is contained within an autoclavable Petri dish, with the lid having built-in tubing adapters that insert into the inlets/outlets of the device.

Oxygen Sensor Validation of Device

A platinum-based fluorescent oxygen sensor (PtOEPK) was used to measure the oxygen concentration spanning across a 3 mm and 6 mm thick piece of agarose. The dye was prepared by dissolving PtOEPK powder in a polystyrene/toluene solution to yield 0.05% PtOEPK in a polystyrene (PS) matrix. The dye was then spin-coated on a glass slide at 2000 rpm for 30 s to evenly coat the substrate. The dye was then allowed to dry, causing the toluene solvent to evaporate and leave behind a PS layer with embedded PtOEPK. This

coating could then be carefully peeled from the coverslip, carefully laid atop a slab of PDMS, and then diced to 1.5 cm in length and 3 mm or 6 mm in width.

The approach used to measure the oxygen concentration across the gel in the z-axis involved rotating the entire sandwich assembly (microchannel-agarose-microchannel) on its side with the oxygen sensor facing up and in-between the two microchannels, with the membrane faces perpendicular to the oxygen sensor (figure 2). The piece of agarose used for validation was placed between the two microchannels and flush against the oxygen sensor as to prevent water-diffusion dominated effects that would primarily be measured if the agarose gel was not directly against the sensor. The sensor was placed low enough to allow focusing with the fluorescent microscope, but high enough to be at the same vertical plane as the microchannels. To bring the sensor to the appropriately height, a PDMS spacer was used. The custom-diced sensor was carefully laid atop this spacer, ensuring good contact between the two by expelling any air bubbles.

A fluorescence-equipped Olympus IX71 microscope, charged-coupled device camera (QImaging Retiga-SRV) and Metamorph image acquisition software (v7.6.3.0) were used for capturing images of the sensor. All images were acquired at room temperature using a PtOEPK-compatible fluorescent filter with an excitation/emission of 595/760 nm and a 620 nm dichroic mirror (Chroma, VT). Images were taken at 2× (PlanApo 2×/0.08). A 75W Xenon burner (Olympus U-LH75X) was used to illuminate the sensor. Image intensities were exported using Metamorph for later analysis.

For the calibration of the sensor, 0%, 10%, and 21% oxygen (in balanced nitrogen and 5% CO₂) were split and infused into both microchannels independently of one another at 50 mL/min. For example, calibration at 0% oxygen involved splitting the flow from the 5% CO₂ and balanced nitrogen tank and infusing into each of the microchannels simultaneously. This method of calibration yielded a good estimate of the actual oxygen concentrations spanning the agarose gel when a curve fit was generated relating fluorescent intensity with oxygen concentration. Heterogeneities in the PtOEPK film and accessibility of the dye in PS are normalized due to the calibration at each measurement position before each trial. The system was allowed to equilibrate for 1 h until steady-state was reached.

For the actual sample validation measurements, instead of splitting one oxygen concentration gas, two different gas tanks delivered gas to the system, one gas in each microchannel. For example, 0% oxygen was infused into one microchannel, whereas 21% was infused into the other. 50 mL/min flow rate was used for all experiments, but similar results were seen when lower flow rates were used, such as 20 mL/min. For the 3 mm agarose thickness, the entire width of the sensor and thus agarose could be imaged with one capture at 2×. Two images were captured and stitched together for the 6 mm width. Images were captured at 1 h, after the fluorescent intensity, and thus oxygen concentration, achieved steady-state. This experiment was run in triplicate with the mean oxygen concentration and standard deviation at each position calculated.

Using Metamorph imaging software, a linescan of 50 pixel width was drawn from one edge of the sensor to the other, as data from a single point across the linescan would suffer from stage drift issues. The data was then exported to Excel. Due to the heterogeneity of the coating, a calibration curve fit equation was required for each vertical position in the linescan. Data were taken for infusion of 0% and 5% oxygen into the two microchannels, as well as 0% and 21% oxygen. Both gradients were generated in 3 mm and 6 mm agarose thicknesses. The plots depict the average of three trials, with the error bars indicating the standard deviation.

Cell Viability Assessment

MDA-MB-231 cells (ATCC) and human mesenchymal stem cells (hMSC, Lonza) were used as cellular models to assess cell viability within the device. MDA-MB-231 cells were expanded in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were stored in a culture incubator (Fisher) maintained at 37°C and 95% humidity. Carbon dioxide was not required as Leibovitz's L-15 media is pH buffered for use at ambient CO₂ levels. hMSCs were expanded in a commercially-available growth medium (Lonza, PT-3001). hMSCs were stored in an incubator (NuAire) maintained at 37°C, 95% humidity, and 10% CO₂ for pH buffering. Both cell types were passaged at 90% confluency using Trypsin-EDTA (Lonza) and split 1:3 in T75 flasks (Corning).

For the device exposure, MDA-MB-231 cells and hMSCs (passage 3–6) were seeded within the agarose gel disc at 10⁶ cells/ml. Low melting temperature agarose (Sigma) was dissolved in PBS to 4% w/v and stored in the incubator to prevent gelling. Once cells were resuspended to an appropriate concentration after hemocytometer counting, 0.5 ml of the cell suspension was combined 1:1 with 4% agarose to yield a final concentration of 2% and a total volume of 1 ml. The agarose-cell mixture was gelled in tissue-culture Petri dishes (Corning), where 1 ml of the mixture yielded a thickness of 3 mm. The agarose discs were allowed to sit for 20 min at room temperature to properly gel. 1.5 cm diameter cores of the gel were then removed with a bore and placed in the oxygen gradient device. The device was assembled and then 5 ml of media was added through the media port. A separate cell-seeded agarose disc was cored and placed in a static petri dish as a control.

Device exposure experiments were conducted in a humidified incubator (95% relative humidity, 37°C) to prevent media evaporation and maintain proper physiological temperature. The same incubator CO₂ concentration was used as for the expansion depending on the media formulation. After placing the assembly in the incubator, tubing from the gas cylinders was connected to the device. Flow was regulated by a rotameter in-line with the tubing, and proper connections were confirmed by attached tubing downstream of the device and bubbling through a beaker filled with water. Cells were exposed to 21% oxygen infused into both microchannels (no gradient) for 48 h.

Cell viability was assessed with a Live-Dead stain (Invitrogen) for both agarose-seeded cell types cultured in the device, as well as the controls, using the manufacturer recommended concentrations. Constructs were sectioned and incubated in the stain for 30 min. Sections were then rinsed three times in PBS. Live cells were stained with calcein-AM whereas the dead cells took up the ethidium homodimer-1 dye. Images were captured with the same microscope used for oxygen validation, but with the manufacturer-specified filters and at 4×. Live and dead cells were imaged separately depending on which filter was used. After exposure of 21% oxygen for 48 h, the agarose discs were sectioned in halves and then incubated with the dyes in a phosphate-buffered saline solution. The cross-sections of the disc were viewed under the microscope, with images taken spanning the width of the agarose. ImageJ was used to threshold and count live and dead cells separately in each of the images. The images were stitched together for a position-dependent viability assessment, and then regions were specified with a box and analyzed. Cell viability experiments were conducted three times and error bars represent the standard deviation of those three trials.

RESULTS

Data demonstrate that a gradient can be formed in the device with a fairly linear profile as shown in figure 3 for both 3 mm and 6 mm thick matrices. For 0% to 21% infused oxygen, the oxygen concentration measured within the agarose ranged from about 2% to 20%. The

extent of hypoxia was improved for the trials infused with 0% and 5% infused oxygen, with values ranging from <1% to 5% oxygen.

Two model cell lines (MDA-MB-231 and hMSC) were used to demonstrate that the device has no adverse effects on cell viability relative to cell-seeded gels in a static Petri dish over 48 h. No significant differences were observed between the cultures after 48 h as seen in figure 4. Also, cell viability was not found to be dependent on the position within the agarose; in other words, the number of live cells in the interior of the gel was similar to near the edges adjacent to the device membranes. Figure 5 depicts hMSC viability as a function of agarose position, with three different positions represented (top, middle bottom).

DISCUSSION

The 3-D oxygen gradient device demonstrated the capability for establishing an oxygen gradient across a 3-D construct, such as agarose. Figure 3, which depicts oxygen concentration as a function of agarose position, demonstrates a fairly linear profile extending from nearly 0% to 5%, or 0% to 21%, depending on the gas infused into the microfluidic network. The device does not require complex oxygen control systems as in other microfluidic approaches (Adler et al., 2010) and the cell-seeded construct can be prepared in a culture dish of choice and transferred to the device.

The planar oxygen sensor used in these studies permitted high efficiency and resolution sampling of the entire width of the agarose. Fiber optic-based needle probes lack this spatial precision, and would involve disturbing the agarose which could affect oxygen diffusion through the construct. However, two notable disadvantages of the planar sensor system are apparent when inspecting the plot: 1) the oxygen concentration does not dip to the two extremes at each end of the agarose (ie not all the way to 0% and 21% oxygen), and 2) the plot appears noisy to a certain degree, lacking a smooth line when points are connected. Both characteristics are most likely an artifact of the validation setup rather than reflecting what is actually happening in the system. Although it is expected that the agarose adjacent to the membrane would be slightly higher than 0%, or lower than 5% or 21% oxygen as there is a 100 μ m diffusion gap across the PDMS membrane, the values measured are further from the extremes than expected. This deviation in oxygen concentration adjacent to the microchannels, along with the noisiness of the data, most likely arises from optical aberrations in the measurement system. The PDMS constituting the microchannels and spacer below the sensor, as well as the agarose slab itself, could affect the intensities measured across the gel. This is more of an issue near the microchannel wall, making measurements difficult in those regions. Thus, the oxygen validation data represent a worst-case scenario due to the limitations of the measurement system, as the actual gradient across the construct would be far less noisy and would approach the extremes of the infused oxygen gases into the device at the edges of the agarose disc.

The lack of a negative effect observed on the cell viability due to the device is not surprising as PDMS has been used as a cell culture material for over a decade (Whitesides et al., 2001). The single oxygen concentration condition of 21% represents the simplest experiment, as ambient air contains 21% oxygen and thus there is no equilibration involved. The experiment was aimed to assess the effect of infused gases into the microchannels below and above the construct, and indicated that the gases did not have an effect on cell viability.

Microfluidic devices aimed to modulate the oxygen microenvironment *in vitro* have typically been designed for 2-D cell culture as aforementioned. Few other devices for 3-D oxygen control exist, although there have been some successes. For example, Lovett *et al* designed and validated a device that establishes a gradient extending from a capillary

inserted through the middle of the agarose construct and infused with oxygen (Lovett et al., 2010), which offers an effective way to model oxygen diffusion from a vessel. However, the insertion of the capillary adds complexity to the experiment and makes assessment more difficult due to the radial profile of the oxygen gradient throughout the construct. Also, the oxygen concentration at the periphery of the construct is limited to the ambient oxygen conditions surrounding the device. Other notable microfluidic approaches include one device that was designed to generate a desired gradient based upon the metabolic requirements of the seeded cells and the dimensions of the scaffold (Verbridge et al., 2010). Although the approach was successful at generating oxygen gradients, the profile of the gradient is highly dependent on not only the cell seeding density and scaffold dimensions, but also on the cell type and media formulations that could affect cell metabolism. The same group also developed a method of incorporating microfluidic channels within an alginate construct (Choi et al., 2007). The microchannels can be infused with desired metabolites, such as media that has been equilibrated to a certain oxygen concentration, and a gradient can be formed across the cell-seeded construct. This approach was effective at generating fluorescent dye gradients as a model metabolite, but was not tested or designed for oxygen gradients. Perfusing equilibrated media may introduce problems associated with media bubbling and shear effects on the cells at adequate flow rates to maintain appropriate oxygen gradients.

The system presented here establishes an oxygen gradient equally across the 3-D construct and the gradient extremes can be controlled by infusion of a desired oxygen concentration. Also, the device is capable of generating steady-state oxygen gradients across constructs that are 6 mm thick. This was the largest thickness tested, but at suitable cell seeding densities and construct diameters, thicker constructs could also be explored. With 3 mm constructs, cell viability was uniform throughout the construct as seen in figure 5, supporting the idea that metabolite diffusion is adequate to reach the interior of the construct without perfusion. For very large or dense constructs, or with cell types possessing a high metabolism, slow perfusion could be incorporated into the device to provide adequate nutrients, without disturbing the oxygen gradients established by the device. Another advantage of the device is the ability to temporally modulate the oxygen concentration across the construct. Although equilibration times were on the order of a few hours for the 6 mm device, thinner constructs would equilibrate significantly faster and could permit modeling of physiological phenomenon. For example, due to the highly irregular vasculature and dynamic blood flow in tumors, oxygen concentrations can cycle dramatically over relatively short timescales (Bennewith et al., 2004, Brurberg et al., 2003, Brurberg et al., 2006, Lanzen et al., 2006). This cycling could be mimicked by the device.

There are a number of applications that would benefit from the 3-D oxygen gradient device. Many biological phenomena have been shown to be dependent on the oxygen microenvironment and would benefit from the device, such as cancer cell metastasis. Migration and invasion could be assessed within the construct, mimicking metastasis of cells from a primary tumor site *in vivo*. Another potent application includes stem cell maintenance, differentiation, and trafficking, which are critical processes in development and are relevant to tissue engineering. Oxygen could be investigated as a synergistic determinant or even as a sole stimulus for differentiation towards a particular phenotype. Instead of exposing a batch of cells to one concentration, the device permits exposure of a large population to a number of oxygen concentrations simultaneously. Although all these experiments could be done in traditional 2-D cell culture dishes, the device yields higher throughput and precise control over oxygen in more physiological 3-D constructs.

CONCLUSION

The 3-D oxygen gradient device presented here has been demonstrated to be effective at establishing oxygen concentration gradients across many thicknesses, permitting the researcher to investigate the effect of these gradients on a multitude of biological phenomena. The tool is simple in operation and can be disseminated to nearly any lab that has cell culture capabilities. The device has the potential to be utilized for novel experiments with 3-D *in vitro* cell cultures and will hopefully aid in biology and medical research.

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Figure 1.

The top is an illustration depicting the 3-D oxygen gradient device. Two microchannels with membranes sandwich an agarose construct seeded with cells. Each microchannel is infused with a different gas for establishment of an oxygen gradient. However, the device could also be used to expose the construct to a single oxygen concentration by infusing the same gas into both microchannels. The support ring stabilizes the upper microchannel over the construct. The whole assembly fits into a custom Petri dish that inhibits contamination and allows for easy media exchanges. The agarose is 1.5 cm in diameter and 3 mm or 6 mm in height. The bottom photographs show the device microchannel halves separated (left) and put together (right).

Figure 2.

Illustration depicting the setup used for validating the establishment of an oxygen gradient across an agarose gel using the 3-D oxygen gradient device. Two microchannels with membranes sandwich an agarose gel. The assembly is flipped on its side relative to the experimental setup. The oxygen sensor was placed atop a PDMS spacer to ensure proper alignment with the microchannel height.

A)

B)

Figure 3.

The 3-D oxygen gradient device was used to establish two different oxygen gradients; one from 0%–5% oxygen (A) and another from 0% to 21% oxygen (B). The device was validated with a PtOEPK sensor for easier incorporation into the calibration setup. Both gradients approached linearity in less than 1 h of equilibration. The error bars indicate the standard deviation across three trials. As both 3 and 6 mm gradient lengths were measured, the z-position was normalized to the overall length so that both plots could fit across the entire graph.

Figure 4.

Both MDA-MB-231 breast cancer cells (A) and human mesenchymal stem cells (B) were used for assessing cell viability after exposure in the device. A Live-Dead stain was used to die the cells for counting. Control scaffolds were placed in static Petri dish and sample scaffolds placed in device with 21% infused into both microchannels. The standard deviations for the MDA-MB-231 control and sample were 0.006 and 0.029, respectively. The standard deviations for the hMSC control and sample cells were 0.021 and 0.061, respectively.

Figure 5. hMSC viability as a function of agarose depth. The standard deviations for the sample cell counts at the top, middle, and bottom were 0.019, 0.012, and 0.014, respectively. The standard deviations for the control cell counts at the top, middle, and bottom were 0.019, 0.023, and 0.006, respectively. This plot demonstrates that there is no difference in cell viability throughout the thickness of the agarose.