ON-CHIP PERVAPORATION-FREE CONTROL OF GAS PARTIAL PRESSURES

Peter C. Thomas[1], Laurie E Locascio[1], Srinivasa R. Ragahavan[2] and Samuel P. Forry[1*]
[1]*National Institute of Standards and Technology, USA and*
[2]*University of Maryland, USA*

ABSTRACT
Pre-equilibrated aqueous solutions were used on-chip to simultaneously mitigate pervaporation and modulate the partial pressures of oxygen (P_{O2}) and carbon dioxide (P_{CO2}) in stagnant culture chambers. This approach enabled long-term (>1 week), stopped-flow microfluidic cell culture without requiring a cell culture incubator.

KEYWORDS: Cell Culture, Gas Partial Pressure, Pervaporation

INTRODUCTION
Gas partial pressures (e.g. O_2, CO_2) are critically important in biology. For cell-based assays, carbon dioxide is tightly maintained at 5% (0.05 atm) to mimic the *in vivo* environment, and differences in oxygen levels can lead to varying experimental outcomes [1,2]. For microfluidic systems, these levels are commonly controlled by placing devices inside bulky and expensive cell culture incubators. Alternately, some research groups have demonstrated on-chip modulation of gas partial pressures in PDMS devices using control channels filled with O_2 [3,4] or CO_2 [5,6] mixtures. However, this approach does not address (and may even exacerbate) pervaporation, which rapidly leads to changes in osmolarity that negatively impact cell cultures [7]. In this work, we pre-equilibrated aqueous solutions and used them on-chip to simultaneously modulate the gas partial pressure and mitigate pervaporation. This approach was characterized, and successfully enabled long-term, stopped-flow microfluidic cell culture.

EXPERIMENTAL
Aqueous solutions were pre-equilibrated off-chip with controlled gas compositions using gas-permeable Teflon tubing in a home-built gas exchanger (Fig 1). These solutions were then pumped through control channels on a microfluidic device that were routed adjacent to stagnant culture chambers. Equilibration through the PDMS enabled control over the gas partial pressure and minimized pervaporation by generating an on-chip water reservoir.

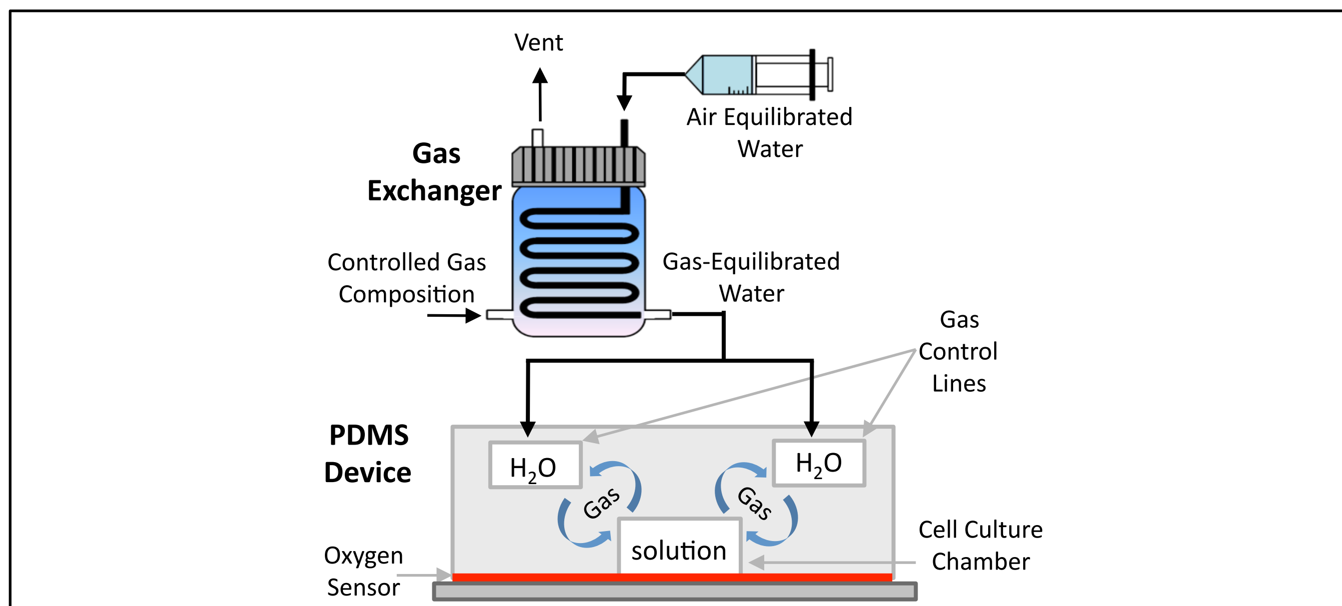

Figure 1: Schematic of the system used to pre-equilibrate and pump aqueous solutions through gas control channels. Equilibration through the PDMS allowed the gas partial pressure to be modulated on-chip. A thin-film oxygen sensor was incorporated into the floor of the device.

RESULTS AND DISCUSSION
Pervaporation was monitored by loading dextran-functionalized rhodamine and measuring fluorescence over time at 37 C (Fig 2). When humidified gas was pumped through control channels, fluorescence increased significantly, consistent with

pervaporation. As water volume was lost, fresh dye solution was pulled into the chamber. The dye (and salt) concentration doubling time was 2 hrs (≈0.25 nL/min water loss). Osmotic pressure changes of this magnitude would have negatively impact mammalian cells in culture.[7] In contrast, when water was pumped through the control lines, no change in fluorescence was observed, indicating significant mitigation of pervaporative losses.

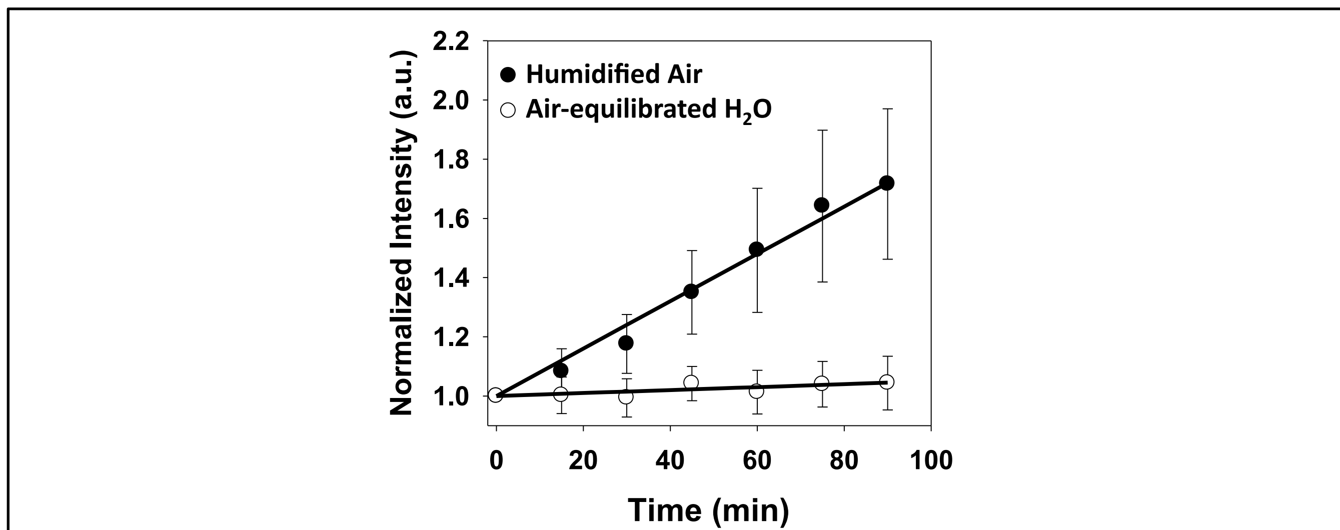

Figure 2: The fluorescence of dextran-congugated rhodamine in solution increased steadily when humidified gas was pumped through the gas control lines (black circles), consistent with rapid pervaporation and an increase in dye concentration. When the gas was replaced with gas-equilibrated water (open circles), no statistically significant change in fluorescence was observed. Devices were heated (37 C) to mimic cell culture conditions; fluorescence intensities were normalized (to t=0) to allow direct comparison of fluorescence increases.

Using pre-equilibrated aqueous solutions, the gas partial pressure was successfully modulated on-chip for O_2 and CO_2 (Fig 3a and 3b, respectively). Regions spatially close to the gas control channels more closely mimicked the partial pressure set points, as expected for a diffusive equilibrium through the PDMS (Fig 3a). The control over on-chip gas partial pressure exhibited sufficient long term stability to enable microfluidic cell culture experiments (Fig 4a).

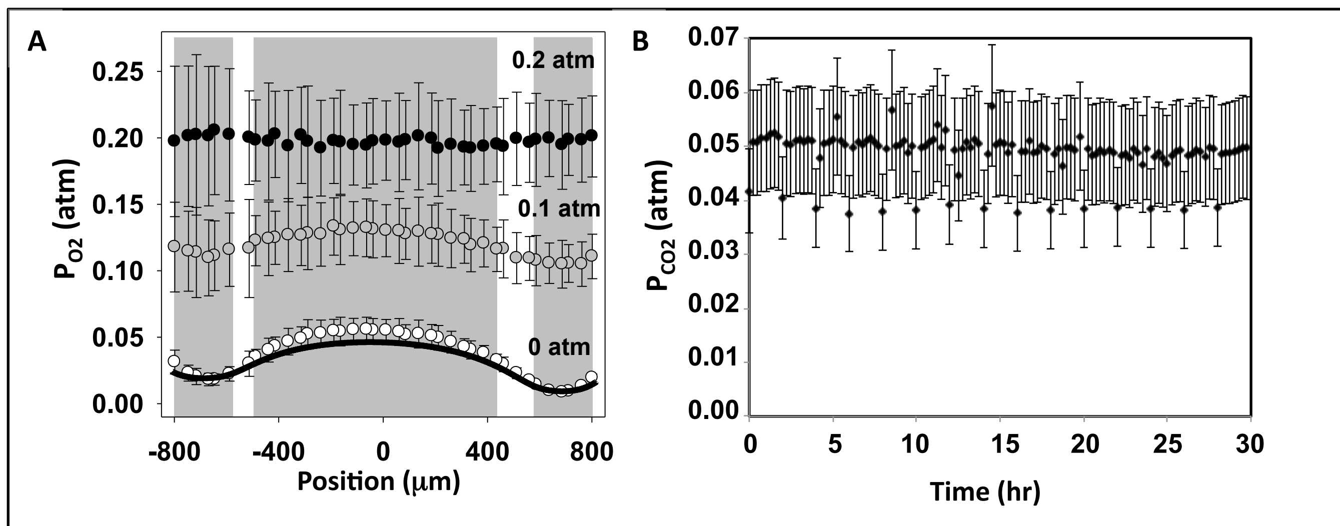

Figure 3: Pre-equilibrated H_2O was pumped through control lines to modulate on-chip the gas partial pressure for O_2 (a) and CO_2 (b). Spatial uniformity was monitored across the device from the control channels (outer grey regions of (a)) in to the culture chamber (central grey region in (a)). During periodic pumping (every 2 h), P_{CO2} exhibited long-term stability (>24 h shown in (b)). P_{O2} was measured with an integrated oxygen sensor; P_{CO2} was calculated from measured changes in solution pH.

Using fluid-filled gas control lines to maintain 5% CO_2, mammalian mouse fibroblasts (NIH 3T3) cells were cultured for more one week (Fig 4b) outside of a cell culture incubator. Cultures in the absence of on-chip CO_2 control (Fig 4a) or with gas-filled control lines deteriorated within 24 h.

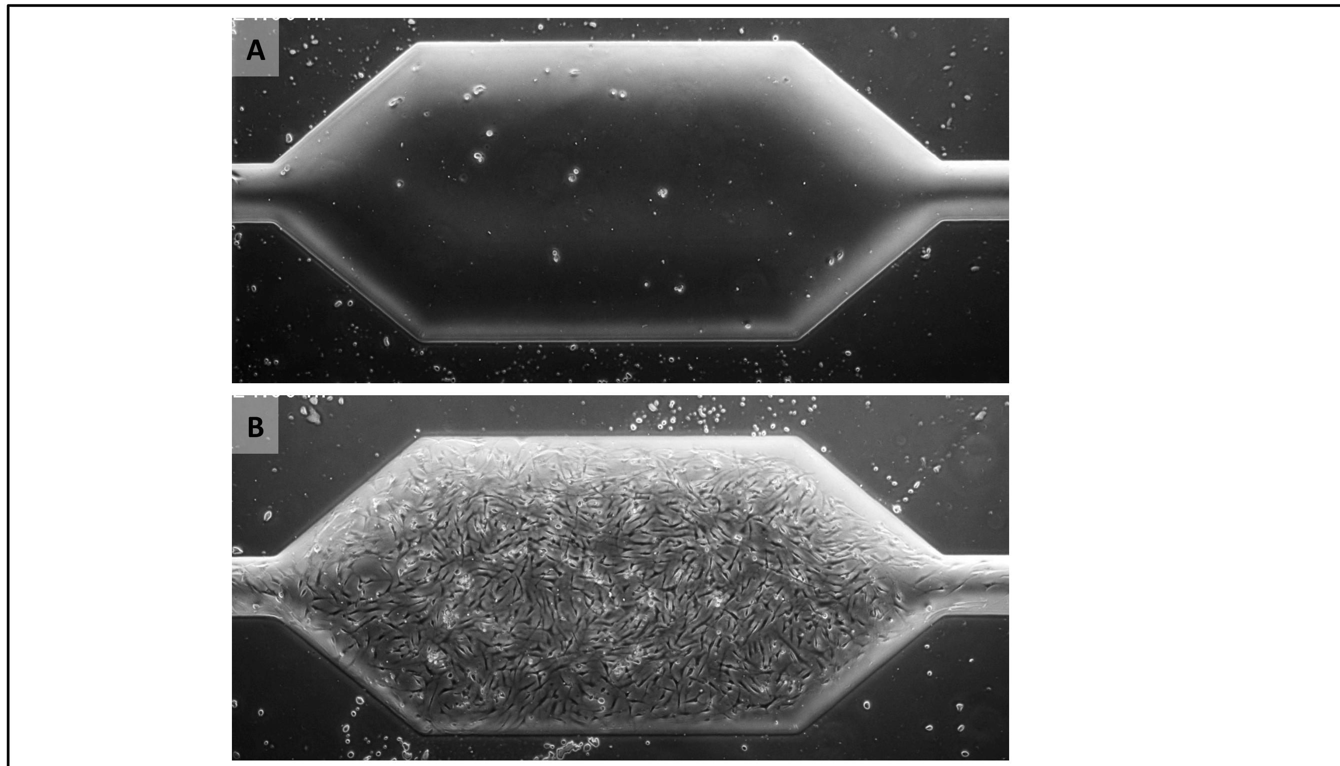

Figure 4: Mouse fibroblasts were cultured without (a) and with (b) on-chip control over P_{CO2} via pre-equilibrated aqueous solutions in gas-control lines. In both cases, cells were seeded in stagnant chambers for 2 h before initiating intermittent perfusion (one volume change over 90 s at the beginning of each hour). These phase-contrast images were acquired after 24 h; the culture in (b) remained viable for a week before being terminated.

CONCLUSION

This research demonstrates the use of pre-equilibrated solutions to modulate gas partial pressures on-chip while simultaneously mitigating pervaporation. This approach enabled, for the first time, long-term stopped-flow microfluidic cell culture without requiring a cell culture incubator or CO_2-independent media.

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CONTACT

*S.P. Forry, tel: +1-301-975-5246; sam.forry@nist.gov