GENERATION, DETECTION AND APPLICATIONS OF IN VITRO OXYGEN GRADIENTS

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Oxygen homeostasis is critical for the functioning of multicellular organisms. Deficiency of oxygen or hypoxia can lead to several pathological conditions such as ischemia, tumorigenesis and drug resistance. Most studies utilize specialized O_2 incubators to generate singular oxygen concentrations that vary significantly from the physiological conditions where hypoxic gradients exist within the tissue e.g. in solid tumors. Current microfluidic technology using polydimethylsiloxane-based (PDMS) devices enables generation of such oxygen concentration gradients, but yield low-to-moderate spatial resolution, involve tedious device assembly and are not feasible for practical research or pharmaceutical screening. We have developed a novel and simplistic approach of reproducibly and rapidly generating stable biomimetic oxygen gradients with high spatial resolution and integrated detection capability. The microfluidic split and recombine strategy utilizing O_2-rich and O_2-depleted media allows generation of prolonged dissolved oxygen (DO) gradients while an underlying platinum based sensor layer (PtOEPK) allows real-time detection of DO gradients generated. Deposition of an approximately 5-7µm thick three-sided glass coating prevents multi-directional diffusion of ambient oxygen through PDMS maintaining the gradient stability for hours or days. Two variations of the gradient devices have been developed, one offering the ability to generate continuous gradients within a single channel while another containing multiple outlet chambers each maintaining a specific concentration of DO (Figure 1).

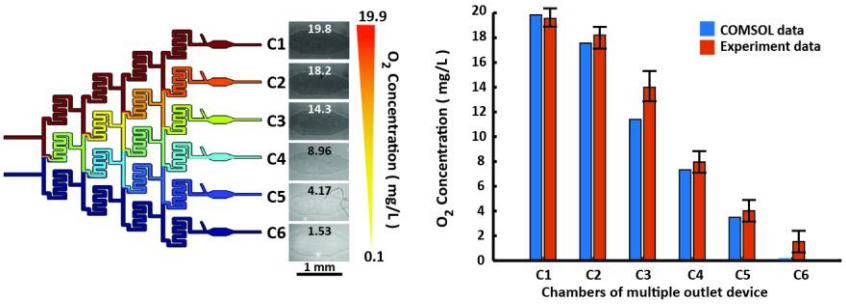
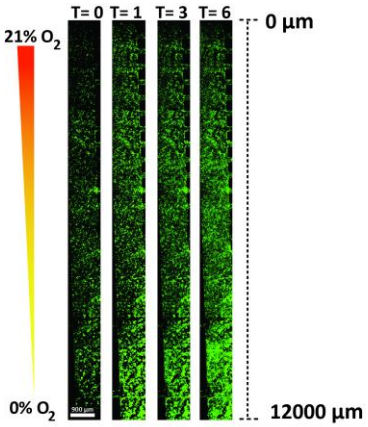


Figure 1. Left: Layout and modeling of the gradient geometry. Right: Simulation vs empirical O2 concentrations in each chamber.

Gradient generation was validated by introducing two aqueous solutions of known DO concentrations (0% and 21%) and observing the luminescence of the sensor layer. The gradient values obtained from experimental results closely matched when compared with those obtained through COMSOL simulations. Device functionality for cell based hypoxia studies was first demonstrated through viability analysis of immortalized mammary epithelial cells (MCF-12A) in hypoxic environments. We observed increased cell mortality with increasing hypoxic stress, with approximately 10% live cells being viable at 0% oxygen conditions. Since the chambers are physically separated, the cells are not able to migrate to oxygen rich areas providing a convenient way to monitor hypoxic effects on cells. To further evaluate the platform applicability for more complex cell functions, ER stress of breast epithelial cells (MDA-MB-468) was monitored under oxygen gradients from 0-21% O_2. As shown in Figure 2, cells demonstrated a 4-fold increase in ER stress levels after 6 hours and these levels also followed a gradient pattern within the channel.



Thus, our proposed technique enables convenient generation and simultaneous detection of biomimetic oxygen gradients in vitro for relatively long periods. The multi cell-outlet design mimics the functionality of several specialized O2 incubators at once. To further explore wide-scale applicability of this platform, the activity of hypoxia-activated prodrugs (HAP) in cancer cells under hypoxic gradient is currently being investigated.