Understanding physiologic oxygen and red blood cells function using microfluidics

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Problem: Physiologic oxygen and carbon dioxide, two gases that are the essence of what defines the cell function. Innovation in laboratory equipment to control oxygen (O_2) and carbon dioxide (CO_2) has lagged behind other areas in research. Current solutions are cumbersome, expensive, and do not replicate the rapid changes that occur in the body. Cells in the blood cycle about every 3 minutes from oxygen repletion (~ 10% O_2) to oxygen depletion (~ 1% O_2). It is reasonable to ask why virtually all studies to date require hours for equilibration or simply default to room air (~ 20% O_2). We believe this default is increasingly indefensible. It is interesting to see that most of the published research use cell culture plates with wells as a reaction chamber, where the blood cells settle down in time. Under the oxygen depletion condition, the diffusion limits the interaction of the oxygen with the blood cells and the interaction is also non-homogeneous. Another disadvantage of the classical technique is that the cells remain in a static condition during the experimental period. In contrast, the blood cells are always in motion in the physiological condition. We believe that we can achieve conditions that are closer to the physiological conditions using microfluidics.

Design: Our polydimethylsiloxane (PDMS) based microfluidic device contains two inlet serpentine channels that merge to a zigzag channel where the reagents and the cells mix.[1] The PDMS is an elastomer that is **semi-permeable to gases** and non-permeable to liquids, so the cavities inside the microchannels equilibrate the environmental condition in a few minutes. The height and the width of the channels are 150 µm and 350 µm respectively. The total length of each of the serpentine channels is 800 mm. The buffer and the blood cells are simultaneously introduced from the inlet channels, at 2 µl/min, and get mixed when they pass through the zigzag channel. This zigzag channel is used as the **reaction chamber** in the experiments. A complete experiment is performed inside an oxygen controlled multigas incubator at 1% O_2 where excess of O2 is displaced by nitrogen. The blood cells in the microfluidic channel remain in flow during O_2 depletion. Fig. 1A shows the microfluidic device with red blood cells infused from the serpentine inlets, and the oxygen depleted blood cells exiting from the zigzag outlet. This image was taken just after the device was taken out from the incubator at 1% O_2. The inter-channel gradient in color darkness is due to variation in exposure length of the cells to the physoxia condition. Intra-channel difference is due to the flowrate variation of the blood flow, with a slower the flow resulting in darker blood due to longer exposure. Fig. 1B shows the blood collected at normal oxygen condition and at oxygen depletion condition (1% O_2) from the microfluidic device.

Application: The trick that biology uses is to coordinate stepwise changes from 1% O_2 followed by 3-10% O_2. And the "trick" is normally done by using exercising muscle to extract O_2, release hypoxanthine, and "charge" the red blood cells. If the O_2 conditions are reversed, biology sends the purine to the waste bin as uric acid. Because of this a simple delivery of purine bases into the blood will fail because most exposure will be in blood that is at greater than 3% O_2.

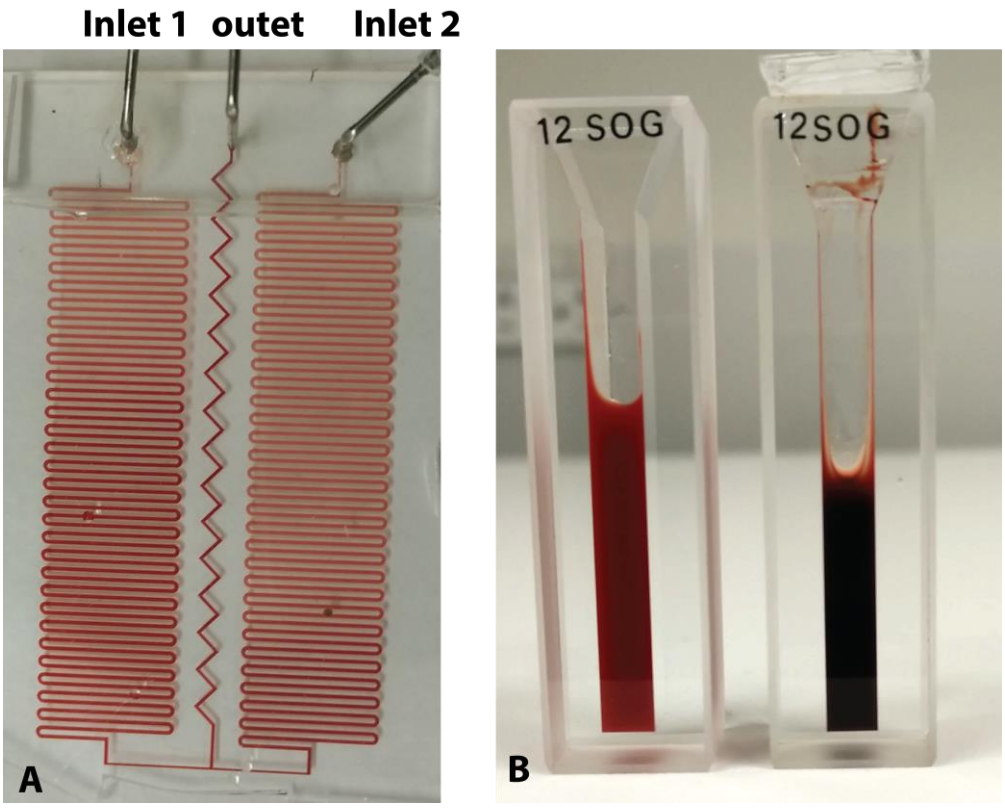

Figure 1: A) Microfluidic device consisting inlets and outlet channels. In the figure, blood cells on both of the inlet channels. The downstream is darker because cells are exposed to hypoxia condition (1% O_2) longer than at the upstream. The flowrate of inlet 1 is slower compared to inlet 2, therefore darkness is observed more at the upstream of channel 1 (inlet 1). B) Blood collected from microfluidic device at atmospheric condition (left) and at 1% O_2 (right).

We have shown that the O_2 content of red blood cells can be changed within 15 minutes with the microfluidics approach, but requires hours in the traditional cell culture dish. We are now at the stage of scaling up the device and proving the value by application to real patient red blood cell function.

Implementation: Our first demonstration of utility will be to demonstrate salvage of purine bases. The purine structure is the building block for energy – ATP. Making ATP from "scratch" is expensive – it takes 7 ATP to make 1 ATP. Biology – as the ultimate recycler – has evolved a purine salvage system. Tissues, particularly the brain, rely on the salvage pathway. Basic science studies have shown that if you feed ischemic brain the ATP building block inosine, the damage due to ischemia can be reduced or prevented. Red blood cells are an important component of the purine salvage system. These cells take up hypoxanthine or xanthine in tissues with 1% O_2 and then make inosine. The inosine is then released in the brain to allow the brain to maintain its ATP, i.e. energy, essential to cell functions. Hence, there is a need to develop an *ex vivo* method that takes advantage of the physiological functions of the red blood cell and apply this to the patient at risk for ischemia.

[1] Mengeaud, V., Josserand, J., and Girault, H.H., "Mixing processes in a zigzag microchannel: Finite element simulations and optical study," Analytical Chemistry 74(16), 4279–4286 (2002).