doi:10.1017/S1431927610054693
Microsc. Microanal. 16 (Suppl 2), 2010

Microfluidic Systems for Microscopic Analysis of Islets and Hypoxia

D.T. Eddington

Department of Bioengineering, University of Illinois at Chicago, Chicago, IL 60607

Microfluidic polydimethylsiloxane (PDMS) systems are ideal for microscopic analysis due to their transparency and ability to deliver precise stimulants to tissues and cells. In order for non microfluidic labs to adopt these new technologies, they must be simple enough to seamlessly integrate into standard biomedical research labs. We have developed several systems that allow new experimental modalities not possible with current techniques and are simple and easy to use. The first is a hypoxic add-on for multiwell plates and the second is a microfluidic perfusion system for multimodal analysis of islet tissue.

The hypoxic add-on allows new possibilities in hypoxic research. Current devices permitting *in vitro* oxygen modulation are either greatly limiting in terms of the types of experiments they can conduct, or are complicated systems requiring specialized equipment and operational knowledge. In this study, a microfabricated insert for multiwell formats has been developed to control the gas concentration of each well independent of the global incubator's condition as shown in Figure 1. The platform consists of a polydimethylsiloxane (PDMS) insert that nests into a standard multiwell plate and serves as a passive network with a gas permeable membrane aimed to deliver gas to adherent cell cultures. Oxygen flows through microchannels embedded at the base of each pillar and is separated from the fluidic contents of the culture well by a 100 μm gas permeable PDMS membrane. The bottom of the membrane is fixed 170 μm from the base of the well by rigid glass posts. The oxygen microchannels connect to gas cylinders which provide the pressure to deliver the gas through the insert. Preliminary data demonstrate that the insert is effective in controlling the oxygen concentration at the cell surface inside a well. The three oxygen tensions, 0%, 10% and 21% O2, were achieved within 1.5 min and remained constant over the course of 5 days, as measured by a fluorescent oxygen probe, demonstrating the immersion of the device in media did not adversely alter the oxygen diffusivity. A wide variety of oxygen profiles can be attained based on the oxygen microchannel design. For example, a cyclic profile alternating between two concentration can be achieved. Even gradients in local oxygen concentration can also be generated using our device within each well to mimic those found *in vivo* for more biomimetic cellular models. These experiments serve as a demonstration that the platform can be used in conjunction with standard cell protocols and lines to achieve higher throughput and novel experimental possibilities within standard multiwell plates.

A microfluidic device to perfuse pancreatic islets while simultaneously characterizing their functionality through fluorescence imaging of the mitochondrial membrane potential and intracellular calcium ($[Ca^{2+}]_i$) in addition to enzyme linked immunosorbent assay (ELISA) quantification of secreted insulin was also developed and characterized. This multimodal characterization of islet function will facilitate rapid assessment of tissue quality immediately following isolation from donor pancreas and allow more informed transplantation decisions to be made which may improve transplantation outcomes. The microfluidic perfusion chamber allows

flow rates of up to 1 mL/min, without any noticeable perturbation of the islets, allowing for time lapse microscopic analysis under various flow stimulation profiles. This multimodal quantification was done on both mouse and human islets. The ability of this simple microfluidic device to detect subtle variations in islet responses in different functional assays performed in short time-periods demonstrates that the microfluidic perfusion chamber device can be used as a new gold standard to perform comprehensive islet analysis and obtain a more meaningful predictive value for islet functionality prior to transplantation into recipients, which is currently difficult to predict using a single functional assay.

Both these systems leverage microscale phenomena such as rapid diffusion and process integration to allow new experimental possibilities. By maintaining a simple device design, users not trained in microfabrication or microfluidics can easily operate and benefit from these systems.

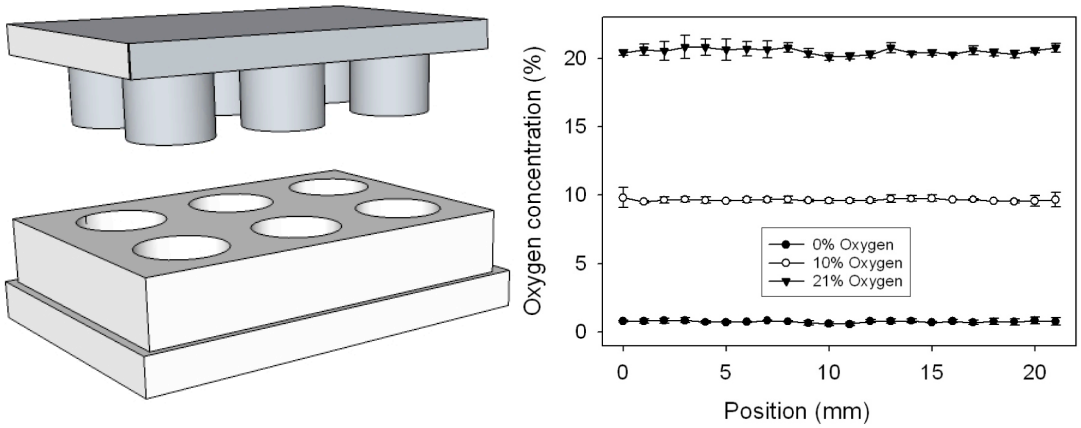

FIG. 1. Schematic of the hypoxic insert and calibration data across a single well with various gasses injected through the network.

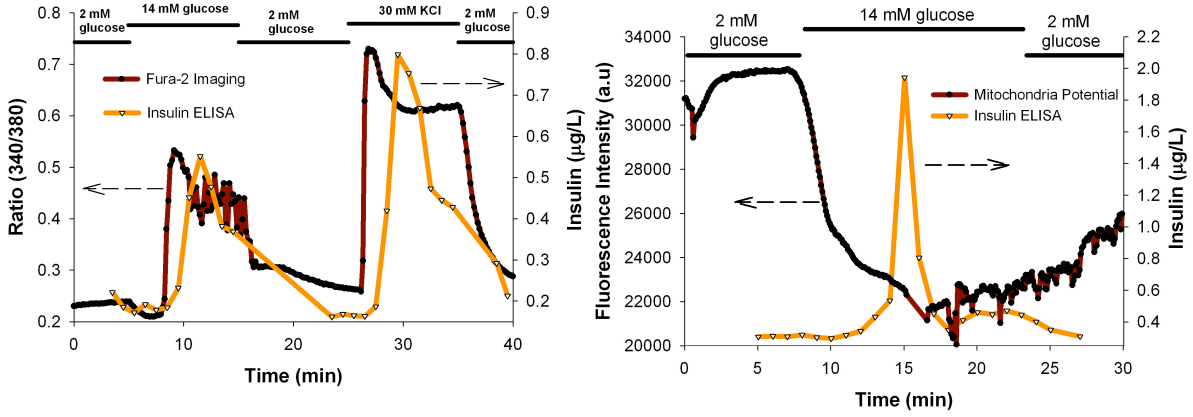

FIG. 2. Insulin secretion, mitochondria potential, and calcium flux simultaneously obtained with the same batch of islets within the device.

https://doi.org/10.1017/S1431927610054693 Published online by Cambridge University Press