Microfluidic dual gas control for the generation of oxygen gradients

New BioFlux dual gas controller allows users to have precise control over physiologically relevant gas gradients

The importance of establishing gas gradients for *in vitro* models

Under normal physiological conditions, oxygen plays an essential role in a host of cellular processes such as metabolism, cell growth, differentiation and DNA metabolism. The concentration of oxygen can vary significantly between different tissues and organs such as the lung parenchyma (4-14%), liver (4-14%), kidney (4-14%), heart (4-14%), brain (0.5-8%), eye (1-5%) and bone marrow (1-6%) (Haque et al, 2013). Moreover, once inspired air enters the lung and oxygen is transferred to the blood via the alveolar capillaries, the oxygen concentration gradually falls to hypoxic levels. This illustrates the importance of controlling the oxygen concentration, and establishing an oxygen concentration gradient that better reflects the *in vivo* environment. Despite these physiological differences in oxygen concentration, normoxic (20%) conditions are typically applied to *in vitro* cell culture models, causing environmental stress. This elicits physiological artifacts and biochemical/molecular changes, such as premature senescence, protracted population doubling times and an accumulation in DNA damage. These atypical cellular responses occur as a result of the aberrant accumulation of reactive oxygen species (ROS). Therefore, establishing *in vitro* culture models that correctly represent the spatio-

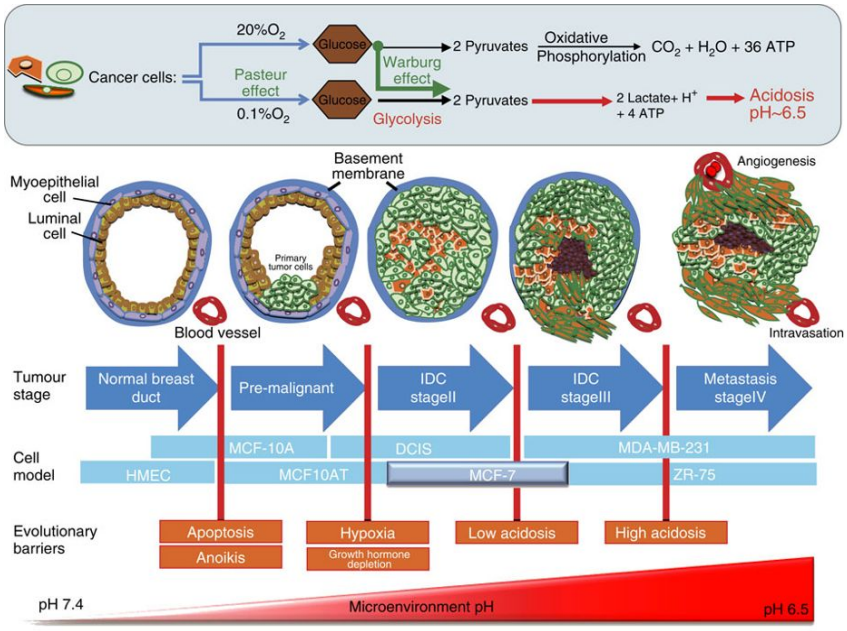

Figure 1. Tumor microenvironment showing the hypoxic region. Increased efflux of carbon through hypoxia, poor vascular system flow or fermentative degradation causes extracellular acidosis in solid tumors. Adaptation and development of tolerance to acidosis in the joints is one of the major issues of cancer development and evolution, leading to more aggressive phenotypes. This picture shows how the increase in acidosis during breast cancer progression from DCIS to stage IV metastasis, and how acid-adapted cells (tissue cells) become more aggressive and invasive. Reproduced from Damaghi et al., 2015

temporal variations in oxygen levels, and better reflect the normal physiological or pathophysiological states, is necessary to model the *in vivo* environment accurately. Under pathophysiological conditions, the importance of oxygen can be further exemplified in the abundance of disease states, including inflammation, wound healing, and cancer metastasis, where oxygen levels can have a profound effect. For example, the vast majority of solid tumors develop large hypoxic regions within the tumor microenvironment, resulting from insufficient vascularization, reduced blood flow, and low oxygen levels (Figure 1. Tumor microenvironment showing the hypoxic region). The presence of these hypoxic niches can have deleterious consequences, as the tumor undergoes a series of adaptations that promote a more refractory phenotype to chemotherapeutic agents. The importance of establishing oxygen concentration gradients to improve the biological relevance is further illustrated when modelling oral biofilms, where a plethora of over 700 bacterial species establish oxygen-dependent niches within the biofilm microenvironment on mucosal and dental surfaces (Darrene & Cecile, et al., 2016). (Figure 2. Oral biofilm model).

Dual gas flow and the BioFlux platform

In addition to oxygen concentration, the presence of physiological shear flow has been shown to have a profound impact on many physiological responses (Makwana et al, 2017), (Hayes et al, 2017). The BioFlux microfluidic system has allowed researchers to establish robust *in vitro* models that require a shear flow component such as that observed in blood flowing through the vasculature (Hayes et al, 2017), circulating tumor cells transmigrating through the endothelium (Gakher et al, 2013) and plaques forming on teeth (Kapila et al, 2015). The precise shear flow control (+/- 0.2% full scale) and the large range (up to 200 dyn/cm^2) has allowed researchers to instantly

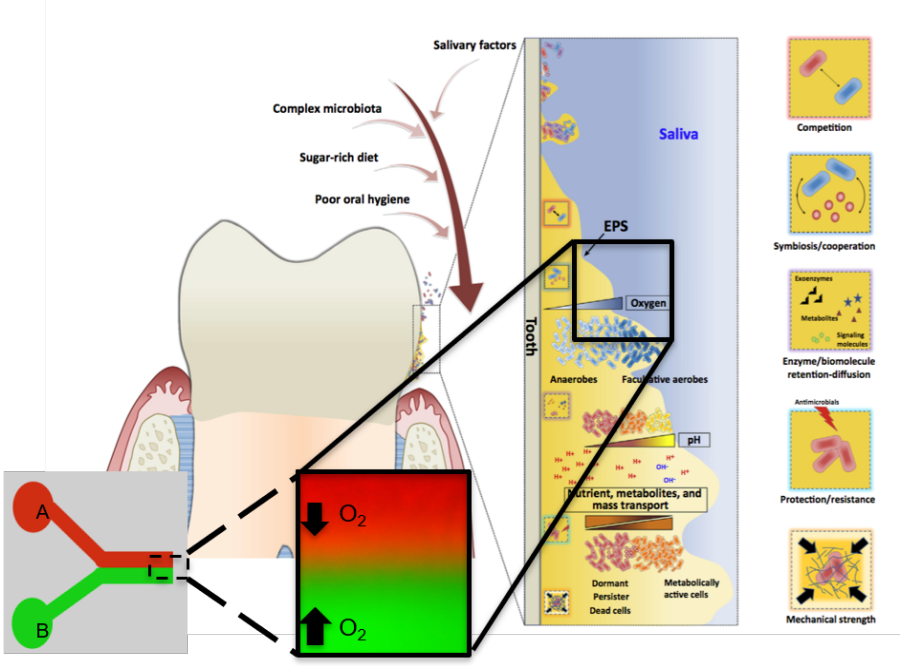

Figure 2. When modeling oral biofilm, it is important to set the oxygen concentration gradient to improve biological relevance. Oral biofilm establishes an oxygen-dependent gap in the biofilm microenvironment of mucous membranes and dental surfaces, with excess of more than 700 bacterial species. Adapted from Bowen, Burne, Wu & Koo, 2018

modulate the flow rate between a physiological and pathophysiological state, thus resulting in the establishment of robust *in vitro* models of disease including cystic fibrosis, thrombosis, atherosclerosis and metastatic disease.

Our aim was to further improve the biological relevance of complex *in vitro* models that not only necessitate a shear flow component, but require the formation of complex oxygen gradients. The recent introduction of the BioFlux dual gas controller provides the only commercially available shear flow system that gives the researcher the ability to immediately modify the oxygen concentration along a gradient, using our proprietary BioFlux Well Plate Microfluidic™ technology. The system uses two input wells held at differing gas concentration levels. They both feed into a viewing area where users can establish gradients in the viewing region or switch between the two solutions. In one example, CO_2 gas gradients drive pH gradients that are then detected via a pH-sensitive dye (Figure 3).

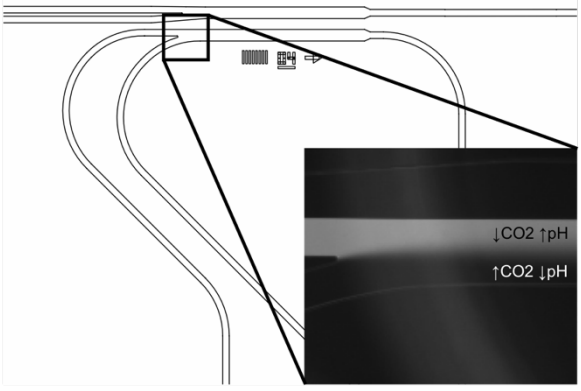

Figure 3. The system uses two input wells to maintain a different gas concentration. CO_2gas gradient induces a pH gradient that is detected via a pH-sensitive dye.

The importance of developing robust *in vitro* models can be illustrated by a recent paper published on the consequence of bacterial chemotaxis in biofilm formation. Here the authors utilized the BioFlux 24-well plate coupled to the BioFlux controller to demonstrate that single bacteria within a biofilm are incredibly motile and can move with sub-micron precision (Oliveira et al., 2016) in response to nutrient gradients**.**

Similar to the chemotaxis models described above, where the BioFlux system has been employed to rapidly modulate the chemical gradient, the ability to regulate the oxygen concentration across a single microfluidic channel adds a further important component when establishing an *in vitro* experimental dental biofilm model. Bacterial aerotaxis (Engelmann, 1881), an important active process that involves the movement of bacteria along an oxygen gradient seeking optimal oxygen concentration, has been demonstrated in many bacterial species (strictly aerobes, facultative anaerobes, microaerophile, and anaerobes). The process of aerotaxis has been shown to be important for optimal cell growth, as optimal oxygen levels are a prerequisite for efficient cell metabolism and growth. This has significant implications for the investigation of biofilm formation in the context of the evaluation of the efficacy of new antimicrobial agents. Thus, the availability of the new dual gas BioFlux controller allows researchers to model the impact of oxygen gradients on biofilm formation and therefore develop new strategies to eliminate biofilm-associated infections.

Perspectives

Despite the development of new assay technologies, the lack of robust *in vitro* models provides a significant bottleneck in the drug discovery process. For example, the increasing emergence of antibiotic resistant strains necessitates the establishment of improved *in vitro* experimental models that better recapitulate their *in vivo* counterparts. The Fluxion BioFlux dual gas controller, when coupled to a 24-well BioFlux plate, facilitates the establishment of robust *in vitro* models where tissue/organ oxygenation is severely disturbed in pathological states such cancer, coronary heart

disease and diabetes. It also allows control of oxygen concentration in processes where O_2 level plays a significant role in disease progression, such as that observed in infectious disease where bacterial species thrive. With the BioFlux system, up to 8 experiments can be conducted simultaneously while both shear rates and gas partial pressures are controlled.

The dual gas control part number is 950-0131 and is ordered as an option with a new BioFlux controller. Upgrading of existing controllers requires a return to the factory for retrofitting.

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