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IN-SILICO CHARACTERISATION OF THE KIRKSTALL QV900 IN-VITRO SYSTEM FOR ADVANCED CELL CULTURE

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SUMMARY

We have developed an in-silico model of the Kirkstall Quasi-Vivo QV900 cell culture system. The QV900 consists of a series of connected sealed chambers, within which cells can be cultured in a low shear stress flow environment. By modelling fluid flow and oxygen transport within the chambers, we have been able to simulate flow patterns and shear stress/oxygen levels experienced by the cells, and we have demonstrated that physiologically relevant oxygen concentration gradients can be achieved by configuring connected chambers in a particular way.

Key words: *Cell culture systems, bioreactors, fluid dynamics, drug toxicity testing*

1 INTRODUCTION

Understanding the behaviour of cells, especially when subjected to stimuli, is one of the key goals in toxicology research. Current experimental investigations focus on either in-vivo experiments, which involve animals, or in-vitro experiments, where cells are grown outside of the body in conditions that poorly mimic reality. Recent developments in 3D bioreactor technology have provided a way of better representing the in-vivo situation [1]. Kirkstall Ltd have developed a family of cell culture systems called Quasi Vivo which consist of interconnected cell culture chambers, each used to culture cells or tissues under dynamic nutrient flow, providing more physiologically accurate conditions [2]. This technology provides a functional median between traditional whole-organism animal and human studies and the microfluidic human-on-a-chip systems still under development.

The QV900 (Fig.1) system consists of an optical tray suitable for holding six chambers which can be connected together in any combination, providing a high degree of flexibility and the potential to culture cells over a more defined set of conditions. Additionally, three dimensional structures of cells can be incorporated within this system: cells can be placed in the chamber as multi-layer structures, such as scaffolds or spheroids, leading to an environment which more closely mimicks in-vivo conditions.

The primary aim of this work was to simulate flow patterns and shear stress/oxygen levels experienced by the cells and to establish whether or not physiologically relevant oxygen gradients could be obtained using this system.

2 METHODOLOGY

In this study, we focus on HepG2 liver cells. These cells typically suffer from a lack of expression of (phase 1 and 2) metabolising enzymes in-vitro, despite being highly expressed in-vivo. It is believed that a flow environment may improve the expression of these metabolising enzymes. An additional complication is that HepG2 liver cells are very sensitive to shear stress, with high levels resulting in decreased cell viability, so we must ensure that low levels of shear stress are maintained at the cell surface.

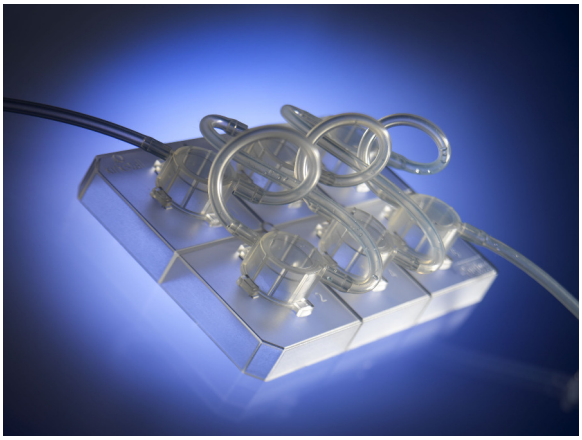

Figure 1: The QV900 System

2.1 Fluid dynamics

We firstly constructed, in COMSOL Multiphysics, a 3D representation of a single QV900 chamber with cells placed at the base of the chamber. The fluid flow velocity and pressure were solved for using the Navier-Stokes equations, assuming that the fluid is incompressible and is adequately represented as a Newtonian fluid:

$$\nabla \cdot \mathbf{u} = 0, \tag{1}$$

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u} \cdot \nabla)\mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u}, \tag{2}$$

where $\mathbf{u}$ is the velocity field, ρ is the fluid density, p is the pressure and μ is the dynamic viscosity.

2.2 Oxygen transport

The transport of oxygen through the fluid was modelled using a convection-diffusion equation:

$$\frac{\partial c}{\partial t} + (\mathbf{u} \cdot \nabla)c = D\nabla^2 c, \tag{3}$$

where c is the concentration of oxygen and D is the diffusion coefficient of oxygen in the fluid. For the purposes of this study, we choose the values of ρ, μ and D assuming the fluid is water. We model the cells as a thin layer of thickness h_c at the bottom of the chamber. Within the cells, oxygen diffuses with diffusion coefficient D_c and is consumed according to Michaelis-Menten kinetics:

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c - \frac{V_{\max} c}{K_m + c}, \tag{4}$$

where V_{max} is the maximum consumption rate and K_m is the Michaelis-Menten constant. It is assumed that there is no appreciable flow within the cells.

2.3 Initial and boundary conditions

Initially, the fluid velocity is zero in the chamber and the oxygen concentration is zero within the chamber and cell layer. At the inlet to the chamber, a parabolic velocity profile is assumed with the magnitude being derived from the volumetric flow rate Q, which can be controlled in experiments. No-slip conditions are imposed on all the walls of the chamber and at the outlet, we impose pressure with no viscous stress.

Regarding oxygen transport, we assume that the walls of the chamber and connecting tubes are impermeable. At the inlet we assume a constant supply of oxygen at concentration $c = c_i$ and at the outlet we impose a convective flux only. Continuity of concentration is assumed at the fluid/cell interface.

2.4 Connecting chambers

Our model was subsequently extended to include connected chambers. Chambers were connected in a simple way by joining the outlet of one chamber with the inlet of another via a short length of tubing. This allowed us to assess the effect of multiple connected chambers on the flow patters and oxygen concentration/shear stress experienced by the cells.

3 RESULTS AND CONCLUSIONS

Using our COMSOL model we simulated the fluid dynamics and oxygen transport for a range of flow rates, using experimentally measured parameter values relevant to HepG2 cells. In Figure 2 we display some steady state results for the single chamber case with $Q = 180\mu L/min$. As expected, the oxygen concentration in the chamber decreases with depth. Along the cell surface a gradient in oxygen concentration is established, with higher values closer to the inlet tube. Two flow recirculation zones are observed at the bottom of the chamber. The peak shear stress experienced at the cell surface is found to coincide with the centre of the chamber. Results from the other flow rates considered will be presented at conference.

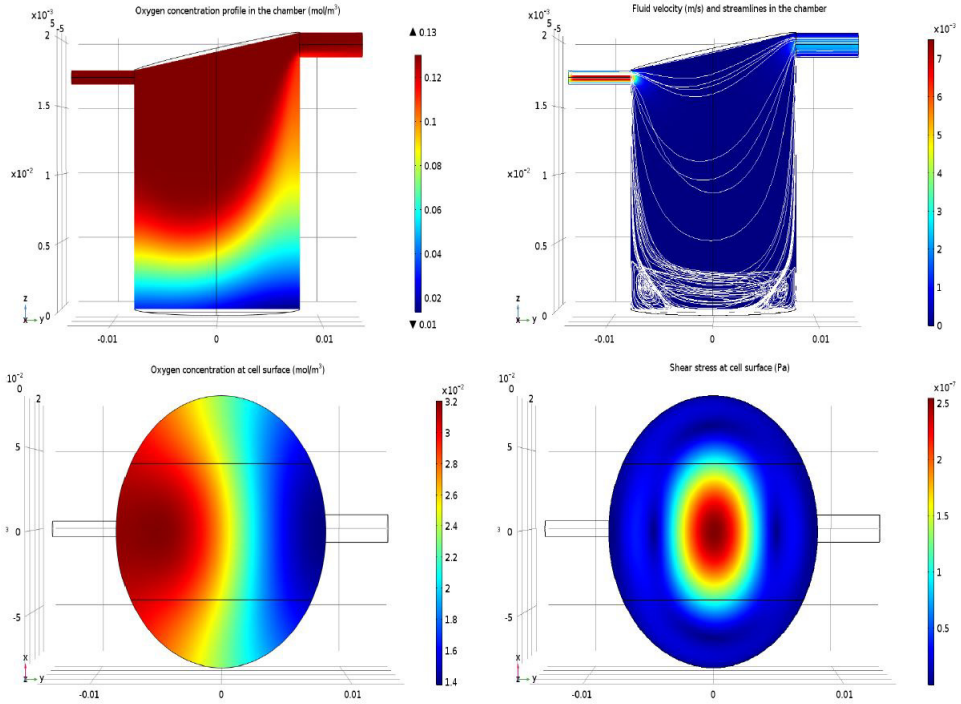

Figure 2: COMSOL results for $Q = 180\mu L/min$. Upper left: Oxygen concentration profile. Upper right: Flow profile. Lower left: Bird's-eye view of oxygen concentration at the cell surface. Lower right: Bird's-eye view of shear stress at the cell surface.

We sought to determine whether or not cell surface oxygen concentration gradients close to those observed along a hepatic sinusoid could be achieved using this system. Our results (not shown) indicate that such gradients cannot be obtained within a single chamber. However, by connecting chambers in a particular way, we can obtain gradients which are more physiologically relevant. Our findings, which are currently being validated experimentally, may have important implications in terms of devising appropriate cell culture systems for in vitro liver drug toxicity testing.

REFERENCES

[1] G. Mattei, S. Giusti, and A. Ahluwalia, Design Criteria for Generating Physiologically Relevant In Vitro Models in Bioreactors. *Processes*, 2:548-569, 2014.

[2] D. Mazzei, M. A. Guzzardi, S. Giusti and A. Ahluwalia, A Low Shear StressModular Bioreactor for Connected Cell Culture Under High Flow Rates. *Biotechnol. Bioeng.*, 106(1):127-137, 2010.