Computational Fluid Dynamics Investigation of a Cavity Micro-Bioreactor

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Abstract

In this paper, cavity aspect ratios and fluid inflow velocities of an idealized perfusion cavity micro-bioreactor for embryos were examined using computational fluid dynamics simulations. Changes to aspect ratios led to altered flow structures and concentration distributions of fluid solutes within the cavity. When cavity aspect ratios were decreased to less than unity, the resulting transport of solutes was impaired, but relatively low shear stresses were found to be imposed on the enclosed embryo. Conversely, when aspect ratios were greater to than unity, more efficient solute transport was observed, however detrimentally increased levels of shear stresses were imposed on the embryo. Variation in solute concentrations within the cavity became very small as cavity aspect ratios were increased to larger than four. Changes of inflow velocities of fluid (culture medium) into the bioreactor were also found to have substantial impact on the flow structures and solute concentration distributions within the cavity. With low inflow velocities, fluid movement in the bioreactor were dominated by diffusion, with impaired solute transport within the cavity and relatively low shear stresses imposed on the embryo. Conversely, high inflow velocities caused fluid flow in the bioreactor to be dominated by advection, promoting solute transport but leading to relatively high shear stresses. The flow Peclet number (Pe) can be used to distinguish whether the flow is advection dominant ($Pe > 1$) or diffusion dominant ($Pe < 1$). Overall, both parametric studies provided information that would be highly useful to the design of an optimized perfusion micro-bioreactor for early embryos. For example, one could select the optimum cavity shape to breed certain types of embryo based on the findings from the cavity aspect ratio study or make an informed choice in setting the suitable flow rates based on the findings from the fluid inflow velocity study.

Introduction

Embryo culture has been used as an important practical method in experimental embryology and assisted reproduction for several decades since the dawn of twentieth century [2]. The method is important to embryology in enabling an understanding of embryo growth, improving transgenic techniques and increasing the rate of success of embryo production in animal breeding as an adjunct to in-vitro fertilization (IVF) [5]. The method is also pivotal to human IVF programs. However, while embryo culture techniques have been optimized greatly in recent times, it has not reached its maximum potential. Cleavage rates and viability of preimplantation mammalian embryos are found to be reduced by in-vitro culture [6, 14]. Also, the viability of embryos produced by assisted reproduction techniques that include in-vitro embryo culture are relatively less efficient when compared to viability of in-vivo derived embryos [4, 12]. For these reasons, much work has been undertaken to increase the efficiency of the in-vitro embryo culture, mainly by improving culture techniques, formulating better culture media, and to a lesser extent by enhancing the designs of advanced production system such as

bioreactors so as to modify the physical culture environment that cultivate embryos [4, 12].

The vessels used in embryo culture have remained relatively under-developed compared to embryo culture techniques and culture media [1]. Therefore, there remains a substantial demand for further research into improving design and function for embryo culture systems. Some modification of existing static culture systems have included examples such as the perfusion flow system by Lim et al. [11], the Well-of-the-Well (WOW) culture system by Vajita et al. [17], the Glass Oviduct (GO) culture system by Thouas et al [16] and the development of new microchannel culture systems which utilizes microfluidic concepts [4, 12, 15]. All of these systems are showing promise results as alternative embryo culture systems.

With the same aim in mind, a theoretical model bioreactor design termed the cavity micro-bioreactor is proposed n this study. Its key feature employs the concept of the "niche environment", a micro-scaled environment designed to protect and promote embryo growth. The idea of this "niche environment" is inspired by the recurring theme in living tissues of local cavity environments that house small sub-populations of cells such as stem cells [3]. Apart from the recent work done on in the GO and WOW culture systems, the hypothesis that the "niche environment" is beneficial to embryo growth is also well supported by the findings of Lane and Gardner [9]. They found that by simply decreasing the incubation volume and thereby reducing a size of the controlling environment, improved embryo growth resulted. The increased density of embryos in this case was thought to have benefited from localized concentration of embryo-trophic growth factors.

An investigation into this approach involved constructing a micron-sized open cavity to accommodate an embryo. This permitted the culture medium to perfuse into the cavity from flow over the top of the cavity, enhancing solute transport. Investigating the feasibility of such a bioreactor design via computational fluid dynamics (CFD) techniques formed the main objective of this study. As a precursor to the actual study, the CFD modelling of the fluid flow and solute transport were initially tested and validated. Two design parameters: the cavity aspect ratio and perfusion rates of culture medium into the model bioreactor were varied to investigate how they may influenced the localised environment of the embryo. Outcomes of this study were hoped to provide an understanding of how controllable parameters affect embryo development to allowing optimisation of and improvements to actual laboratory and clinical bioreactors that may emanate from this work.

Methodology
Numerical Model
The *bioreactor* described in this paper was modelled as a two dimensional channel with a cavity situated in the middle of the

channel (Figure 1). The culture medium was directed to flow along the length of the channel from left to right. A spherical *embryo*, represented by the circle in the figure, was situated in the embryo-sized cavity consistent with the idea of a "niche environment". Oxygen uptake and lactate production from the embryo were modelled as a simplified representation of nutrient exchange as these are their predominant activities according to previous embryo metabolism studies [10]. Source and sink terms to simulate lactate production and oxygen uptake were introduced into the equations of transport for these chemical solutes.
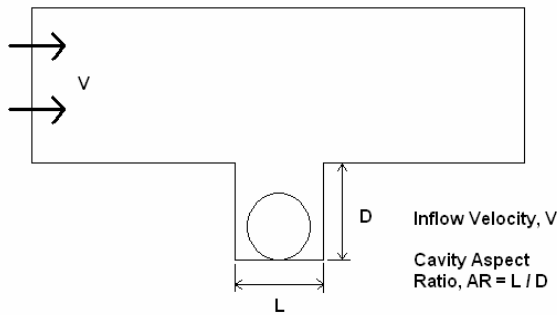

Figure 1: Schematic of the idealized cavity micro-bioreactor numerical model showing the two parameters to be studied.

The governing equations for the fluid continuity and momentum transports, as well as solute transport in the channel were given by Equation 1 to 3 below.

$$\nabla \cdot \overline{U} = 0, \tag{1}$$

$$\rho(\overline{U} \cdot \nabla)\overline{U} = -\nabla P + \mu \nabla^2 \overline{U}, \tag{2}$$

$$\overline{U} \cdot \nabla C = D \nabla^2 C, \tag{3}$$

where $\overline{U}$ is the flow velocity, P is the fluid pressure, ρ is the density of fluid, μ is the dynamic viscosity of fluid, C is the concentration of the solute and D is the diffusivity of the solute. As indicated above, oxygen and lactate concentrations in the cavity were chosen as the two dependent parameters employed to examine solute transport within the bioreactor. In addition, the shear stress on the embryo, which is known to affect the growth of a range of cell types, was selected as another important dependent parameter characterising the environment. To recap, these three dependent parameters were chosen because previous studies indicated that the mortality of the embryo was largely affected by the chemical composition of the culture medium, in particular oxygen and metabolic waste [2], as well as embryo shear stress [18].

In all the studies reported in this paper, a geometric model of the bioreactor was constructed using the commercial software package GAMBIT. This was used to produce an unstructured mesh. FLUENT was then used to solve the flow and solute equations based on a finite-volume formulation. Second-order discretization was used for the flow and equations. The solute transport of oxygen and lactate were solved using the species transport model built into the code. The flow was solved assuming a steady state with constant geometry because of the low Reynolds numbers involved and the time-scale for embryo growth is very much slower than the advection and diffusion flow time-scales.

Parametric Studies and Boundary Conditions
Previous studies on open cavity flow found that changing the cavity aspect ratio and the fluid flow velocity significantly altered the flow structure inside the cavity [13]. As this flow structure

theoretically regulates solute transports and shear stress distribution, the cavity aspect ratio (the ratio of length to depth) and the inflow velocity of culture medium of the bioreactor were chosen as the fluid mechanical parameters to be investigated in this paper. For each study, one parameter was varied while the other was kept constant to isolate the individual effects.

Material properties of the culture medium were kept constant for all parametric studies. The rates of oxygen uptake and lactate production applied in these studies corresponded to the experimental values reported for the growth of mouse embryos prior to the morula stage [8] (generally for in-vitro embryo culture, embryo was regarded to have matured when its growth reached the morula stage). The culture medium was set as having to pre-equilibrate with atmospheric 21% oxygen concentration as well as lactate concentration of 25mM before entering the bioreactor. The lactate concentration presented during the results post-processing stage referred to the net amount of lactate produced by the embryo alone.
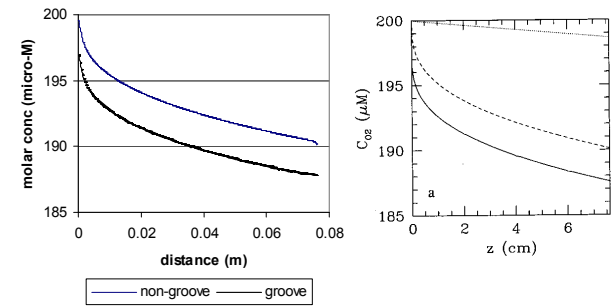
Code Validation

Figure 2: Oxygen concentration (20% inlet oxygen) comparison between grooved (solid line) and non-grooved (dashed line) perfusion bioreactor; (left) present simulations; (right) Horner's results [7].

A grid resolution study was undertaken. This indicated the selected grid size produced predictions within 3% of the grid independent solution (evaluated using Richardson extrapolation). The flow and mass transport models were validated by replicating the numerical study by Horner in analysing the oxygen and lactate transport of hematopoietic cells in a grooved perfusion flat-bed perfusion bioreactor [7]. Constant rates of oxygen and lactate transport were assumed in his analysis. As can be seen from the comparison in one of the results shown in Figure 2, good agreement was found between our results and Horner's results, providing confidence in the predictive ability of the model and code for the bioreactor studies.

Cavity Aspect Ratio Parametric Study
In this study, the aspect ratio (AR) of the cavity was varied from 4 to 0.25 to examine how this affects the fluid flow and the solute transport within the cavity. Other boundary conditions such as the inflow velocity and the embryo diameter were kept constant. The inflow velocity was set at 0.001 m/s corresponding to a flow Reynolds number of the order of 10^{-5} (depending on cavity dimensions).

Geometric Effects on Solute Concentration
It is shown in Figure 3 that lactate concentration in the cavity decreased rapidly when AR was varied from 0.25 to 0.5 and then gradually levelled off when AR was elevated from 1 to 4. Conversely the oxygen concentration in the cavity increased rapidly when the cavity AR was raised from 0.25 to 0.5 and then gradually levelled off as AR was increased to 4. It is speculated that solute concentration will change only slowly beyond a cavity AR of 4.
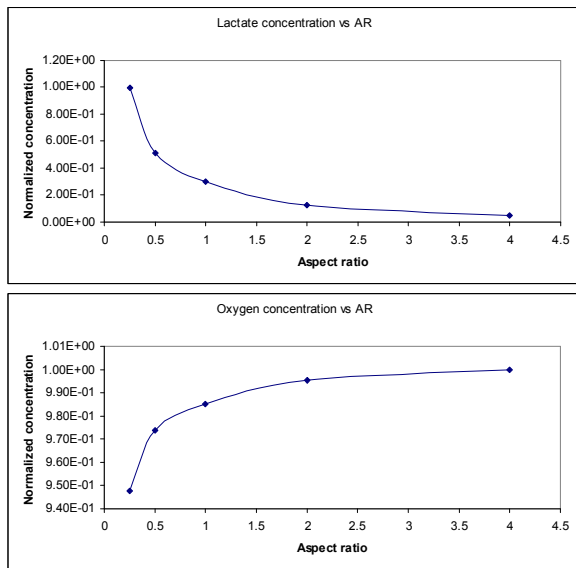
Figure 3: Lactate and oxygen concentration (normalized with respective highest concentration level) changes against cavity aspect ratio.

The initial rapid changes in the solute concentrations are consistent with the rapid change to the cavity topology as the cavity is transformed dramatically (Figure 4) as aspect ratio is varied. When the cavity AR > 1, the relatively wide opening allows the bulk flow to enter a sizable proportion of the cavity in turn producing strong flow circulations within. In particular, the bulk flow across the top of the cavity directly affects fluid flow below the level of the top of the embryo, so that it is bathed in fresh rather than recirculating fluid. As the speed of the main flow is fixed for this parametric study, the immediate flow and fluid environment of the embryo becomes primarily controlled by the main flow over the top of the cavity. This causes the solute concentration variation in the cavity to level off as cavity AR is increased to larger values.

For a cavity with AR < 1, the cavity opening is much smaller (relative to cavity depth). This has the immediate effect of

significantly reducing flow penetration directly into the cavity, and because of the low Reynolds number, the induced flow circulations reduce in strength rapidly with depth. At such low Reynolds numbers, solute transport is controlled by diffusion.
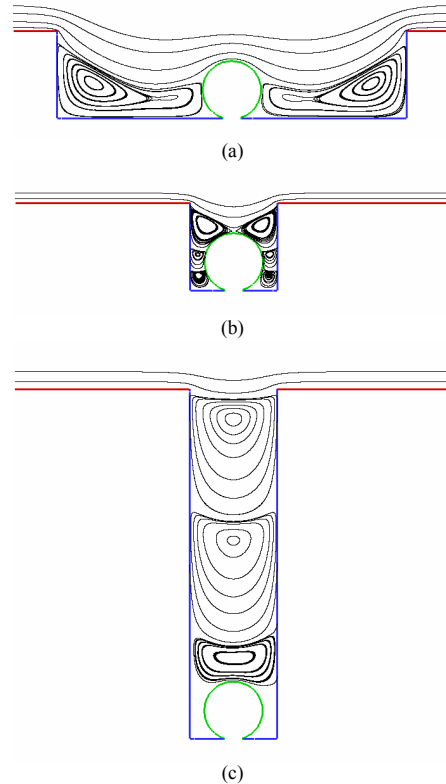

Figure 4: Streamline plots within the cavity for various cavity aspect ratios. (a) AR = 4.00; (b) AR = 1.00; (c) AR = 0.25. Note the streamfunction levels are not equi-spaced, but are chosen to highlight the recirculation regions.

Detailed examination of the solute concentration distribution near the embryo surface also provides some interesting insights.
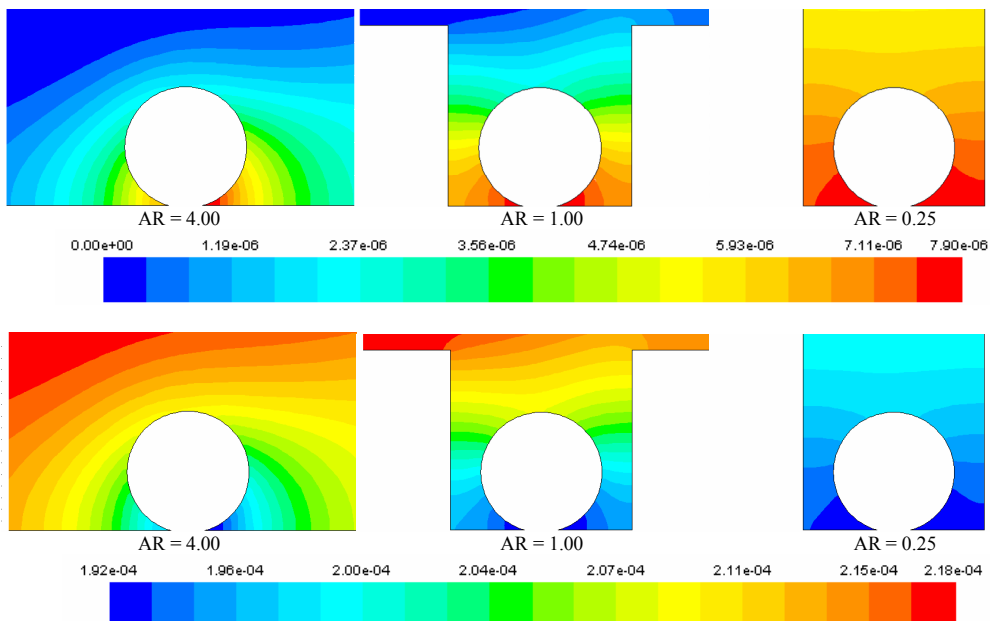

Figure 5: Lactate (top) and oxygen (bottom) concentration contour plots around the embryo for different cavity aspect ratios.

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Generally for all cases with different AR, the local solute concentration showed similar behaviour. For lactate, starting with high concentration level at the embryo bottom, the surface concentration level decreased gradually down to the minimum at a point on the top of the embryo; for oxygen, starting with low concentration level at the embryo bottom, the surface concentration level increased to maximum at a point on the top of the embryo (Figure 6). The only variation as cavity AR was changed was the overall concentration level. All these trend changes were closely related to solute concentration distribution surrounding the embryo, as can be seen from the close-up concentration contour plot around the embryo in Figure 5.

Of course, such trends of the embryo surface concentration distributions are not surprising, as the movement of fluid above the cavity constantly removed the lactate produced from the embryo surface. As lactate was being released at a constant rate from the embryo surface, this created a consistent lactate concentration gradient across the depth of the cavity (Figure 5). Likewise for oxygen, the movement of fluid in the main channel continuously supplied oxygen into the cavity. With the embryo surface modelled as an oxygen sink to absorb oxygen at a constant rate, a similar concentration gradient was produced across the depth of the cavity (Figure 5).

Several conclusions can be drawn from the lactate and oxygen modelling. Firstly, it was found that solute transport was impaired in deep cavities while it was significantly affected by perfusion in the main channel for shallow cavity. This observation is useful in deciding the nature of the bioreactor design; for example, if undesirable substrates produced by the embryo need to be removed, a shallow cavity (AR > 1) should be employed. Conversely, for the case where one needs to retain embryotrophic factors that promote embryo growth, a deep cavity (AR < 1) is preferably. This study is part of a larger program that will address the some of these issues experimentally.
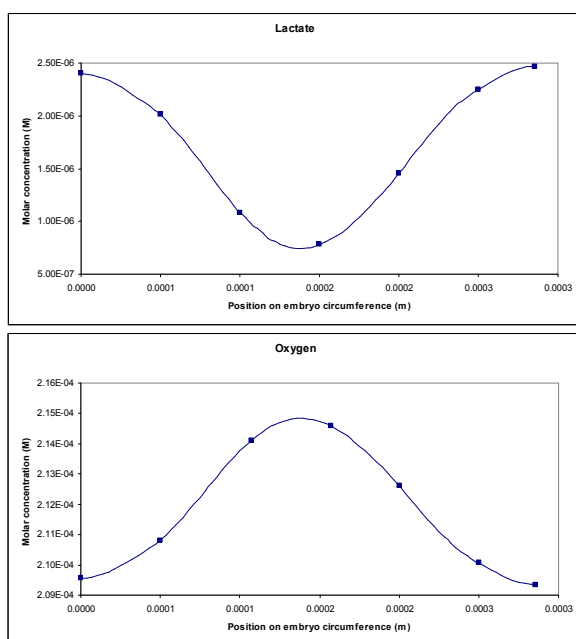

Figure 6: Lactate (top) and oxygen (bottom) concentration level on the surface of the embryo for cavity aspect ratio of 1.00.

Changes to the concentration level as cavity AR increased beyond 4 were minimal. This resembled the situation where fluid was flowing across an embryo located on a flat plate, again

implying that cavity aspect ratios larger than 4 have little effect on controlling solute concentrations within the cavity. Apart from that, solute concentration distributions around the embryo surface did not change by much as cavity AR changed except for overall concentration level. The effect of the large variation of solute concentration around the embryo surface is beyond the scope of the current study.

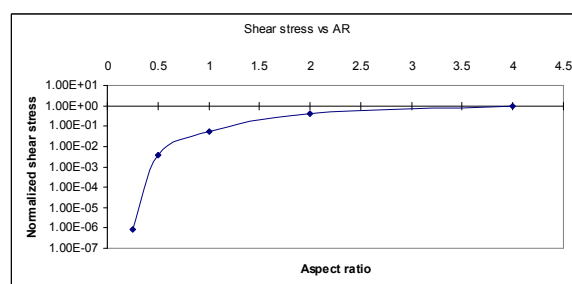**Geometry Effects on Embryo Shear Stress**

Figure 7: Embryo surface shear stress (normalized with largest stress level) change with respect to cavity AR.

The plot of embryo surface shear stress against AR (Figure 7) revealed that as AR increased, the shear stress increased exponentially. The shear stress indicated in the graphs referred to the averaged shear stress across the embryo surface. Shear stress increased sharply for cavity AR < 1, while this increase dropped off as cavity AR > 1 and appeared to stabilize for cavity AR larger than 4.

The reason for such changes again can be directly related to flow circulation topology changes when the cavity AR is increased from 0.25 to 4. For deep cavities, several strong flow circulations were found on top of the embryo while weaker, smaller flow circulations were found in contact with the embryo (Figure 4c). These flow structures are typically found in deep open cavities. For shallow and wide cavities, two large flow circulations develop, and the main flow in the channel is in contact with the embryo (Figure 4a). Weaker flow circulations induced low shear stress to the embryo in the deep cavity, while stronger flow circulations and the main flow in the channel in contact with the embryo induced large local shear stress on the embryo surface.

The maximum level of shear stress was found in a cavity with the maximum studied AR of 4. Thus one can reduce the shear stress exerted to the embryo by reducing the cavity AR. Reducing the cavity AR to less than unity can decrease the magnitude of shear stress greatly, while increasing the AR to more than 4 offers minimal changes to the shear stress experienced by the embryo. These observations are useful when considering the design of micro-bioreactors but clearly require more information of the effect of local and global shear on embryos at different times in their development.

Channel Inflow Velocity Parametric Study
The velocity of the culture medium was varied for this study. The inflow velocity was varied between 1 m/s and 1×10^{-11} m/s. For the initial investigation, the cavity aspect ratio was fixed at unity (square cavity).

Results and Discussions for Solute Concentrations
As shown in Figure 9, the plot of lactate concentration against Peclet number (Pe, here it served as an indication of the inflow velocity) revealed that the lactate concentration level increased as Pe was decreased to unity. When Pe was decreased to a value
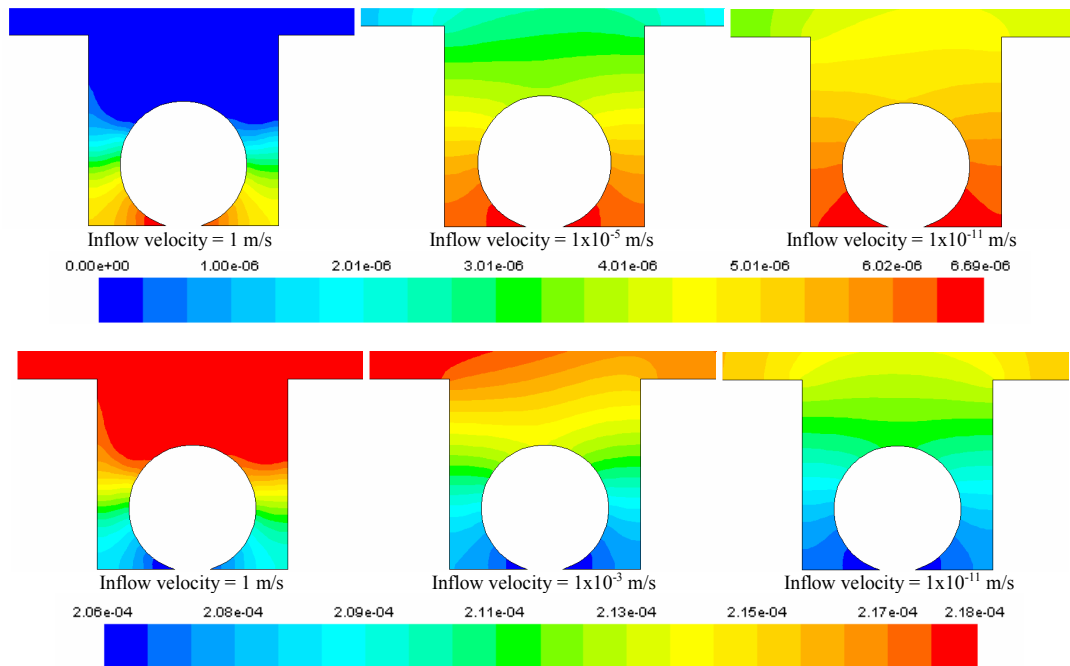
Figure 8: Lactate (top) and oxygen (bottom) concentration contour plots around the embryo for different inflow velocity.

much less than unity, solute concentration level stopped changing. A similar trend was observed for the changes in oxygen concentration level. Oxygen concentration level decreased as Pe decreased to unity. When Pe was reduced below unity, the concentration level began to level off and remained unchanged as Pe continued to decrease.
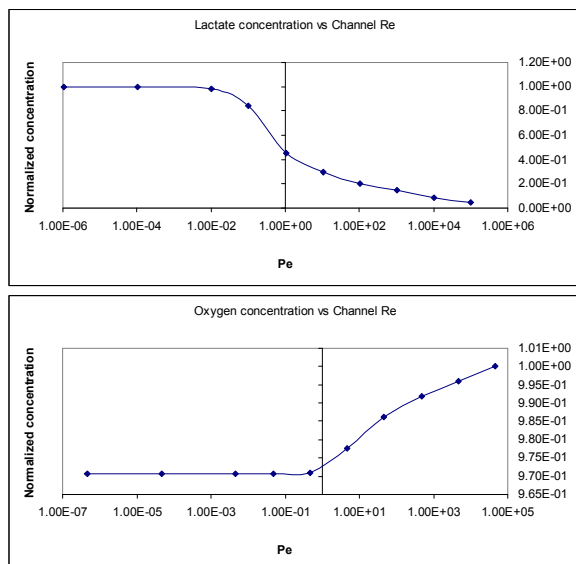

Figure 9: Lactate and oxygen concentration (normalized with respective highest concentration level) variation with Peclet number.

Focusing on the lactate distribution within the cavity (Figure 8), it can be observed that for the case of high Pe, almost half of the lactate within the cavity was advected out by the main flow. This was observed on the cavity lactate concentration contour plot for inflow velocity of 1 m/s, where the blue region that represent zero lactate concentration level dominated the upper half of the cavity. As the inflow velocity decreased, more lactate was

retained within the cavity, as evidenced by the concentration plot of the region of high lactate concentration level (denoted by the yellow, orange and red regions) increased as the inflow velocity decreased to 1×10^{-5} m/s. For cases of low inflow velocities (1×10^{-8} m/s to 1×10^{-11} m/s), it is found that lactate concentration distribution in the cavity remained unchanged as the inflow velocity decreased. Oxygen transport also exhibited a similar trend: with high inflow velocity, the cavity was filled with high oxygen concentration level. As the inflow velocity decreased, the oxygen level decreased and regions showing oxygen shortage (denoted by the green and blue regions in the oxygen concentration contour plots) increased.

These observations can be explained by the fact that as the inflow velocity decreased, the fluid flow changed from advection dominant to diffusion dominant. The plot of lactate concentration against Pe supported this claim. By definition, the fluid flow is advection dominant when $Pe > 1$ (this means rate of advection is higher than rate of diffusion), while it is diffusion dominant when Pe is less than unity (this means rate of diffusion is higher than rate of advection). As can be seen from the Pe against solute concentration plot (Figure 8), solute concentration changed consistently for $Pe > 1$; for $Pe < 1$, solute concentration increased or decreased to a certain level and then levelled off as Pe continued to drop. The reason for this is that the diffusivity of the culture medium was assumed to be constant, independent to the changes of temperature and solute concentration. This implied that as the flow became diffusion dominant, solute concentration was diffusing at a constant rate.

Even with different inflow velocities, solute concentration on the embryo surface in general had the same concentration distribution profile as shown in Figure 10: starting with a high concentration level at the embryo bottom, solute concentration decreased or increased gradually to a certain concentration level at the top of the embryo. Apart from concentration level changes, changing the inflow velocity did not alter the concentration profile on the embryo surface by much. This is true while the region that indicated the solute concentration changes (as shown in the cavity concentration contour plot, the region where the

concentration level changed from blue to red) covered the entire embryo. Outside this region, constant level of solute concentration is in contact with the top surface of the embryo, causing the solute concentration there to be unchanged. Summing up, it can be deduced that inflow velocity changes indirectly altered the solute concentration on the embryo surface by changing the solute distribution in the cavity.
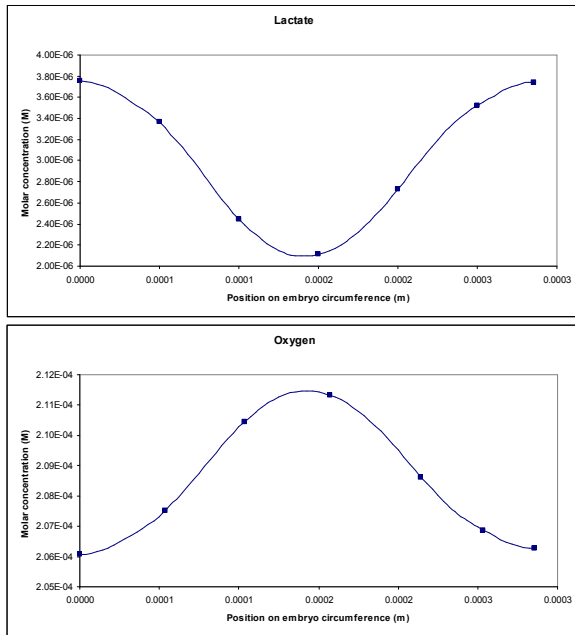

Figure 10: Lactate (top) and oxygen (bottom) concentration level on the surface of the embryo for inflow velocity of 1×10^{-5} m/s.

In general, in regard to inflow velocity changes, assuming that the diffusivity of the fluid was independent of solute concentration and temperature, solute transport variations with respect to inflow velocity in the bioreactor can be categorized into two types:

- Diffusion dominant for low inflow velocity
- Advection dominant for high inflow velocity

The governing parameter to distinguish these two types was the flow Peclet number (Pe). Using this parameter, one can predict the key inflow velocity that starts to keep the solute concentration level constant. The results documented here provided valuable insight in deciding on what inflow velocity range should be utilized in order to optimize the solute transport of the bioreactor. For example, to remove large amounts of unwanted waste products from the embryo, or to supply more oxygen to it, a high inflow velocity into the bioreactor is required.

By comparing the plots of Pe against solute concentration of lactate and oxygen, it is found that lactate concentration began to stabilize at a much lower Pe than oxygen. This is because oxygen has a higher diffusivity (3.2×10^{-9} m^2/s) than lactate (1.45×10^{-9} m^2/s). With higher diffusivity, oxygen diffusion is stronger than lactate diffusion at the same Pe. Coupled with the situation that diffusion becomes more dominant as Pe decreases, oxygen would exhibit a diffusion-related effect sooner than lactate.

Concentration distributions close to the embryo surface clearly show that cavity design is still insufficient to provide a uniform solute concentration distribution around the embryo. Biological experiments are needed to be performed to uncover whether variable concentration profiles simulated in this paper actually influence embryo development. If it is found to have detrimental effects, modifications to the bioreactor are required to ensure a more uniform solute concentration around the embryo or at least to not appreciably deplete valuable embryotrophic factors.

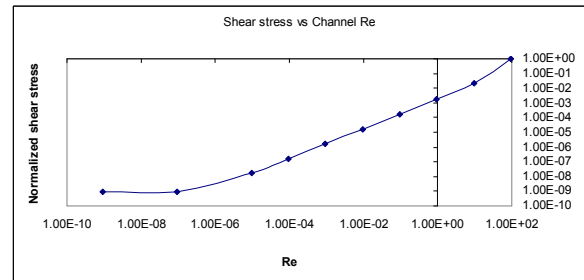### Results and Discussions for Embryo Shear Stress

Figure 11: Embryo surface shear stress (normalized with largest stress level) change with respect to flow Reynolds number.

The plot of embryo surface shear stress against flow Reynolds number (Re) as shown in Figure 11 revealed that as inflow velocity (denoted by the flow Re) decreased, shear stress on the embryo surface decreased at a constant rate. This trend continued until inflow velocity was reduced less than Re of 1×10^{-7}. The shear stress remained constant as the inflow velocity continued to decrease. This was expected, since as inflow velocity decreased, the flow past the cavity slowed down. This weakened the flow circulation in the cavity, inducing a lower shear stress across the embryo surface. When the inflow velocity was reduced further from 1×10^{-9} m/s, change of fluid movement in the cavity was too slow to produce any significant variation to the shear stress as the fluid flow in the cavity was approaching hydrostatic condition.

In general, decreasing the inflow velocity significantly lowered the shear stress on embryo surface. As evidenced from the shear stress versus Re graph, a logarithmic decrease of flow Reynolds number brought about a logarithmic decrease on the shear stress level. Not surprisingly, altering the flow velocity is therefore a very effective method to adjust the shear stress level on embryo surface.

Conclusion
Parametric studies on cavity aspect ratio and culture medium inflow velocity were undertaken to determine the feasibility of a new cavity micro-bioreactor for pre-implantation embryos. It was found that variation to controllable parameters appreciably altered the solute transport within the cavity and the shear stress on embryo surface considerably. High aspect ratio or high inflow velocity promoted solute transport but induced a high shear stress on the embryo, while low aspect ratio or low inflow velocity had the opposite effects. Solute concentration changes were only negligible when cavity aspect ratio was larger than four or when Peclet number (governed by the inflow velocity) was smaller than unity. These findings provide guidelines useful for improving the design of micro-bioreactors.

Future studies will be conducted to assess other design parameters of the cavity micro-bioreactor. They include changing the medium height, varying the starting concentrations of solutes and gases, studying different embryo growth characteristics and examining the scenario of having multiple cavities in close vicinity so that they interact. In addition, a three-dimensional model is under construction to examine the modifications that three-dimensional flow has in affecting the solute transport and the shear stress distribution on the embryo surface. Ultimately,

multi-parameter studies such as this are hoped to enable the development of more effective embryo culture systems for assisted reproduction laboratories.

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