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Evaluation of a Groove Bioreactor for Stem Cell Expansion: Effects of Inhibitory Signalling

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Abstract

This computational fluid dynamics study investigates the distribution of inhibitory signals secreted from a stem cell population in a groove bioreactor. Two bioreactor configurations are considered, the first with a single groove of 400 μm, and the second with two grooves of 200 μm. For the second configuration, the effect of the distance between the upstream and downstream groove is also quantified. It is found that the flow past the larger groove is able to fully penetrate the groove, greatly reducing the concentration of inhibitory signalling within the groove. In contrast, the flow past the smaller grooves is only able to partially enter the groove, and a recirculation zone is set up, leading to larger concentrations of inhibitory signalling within these grooves.

Introduction

The ability to grow homogeneous stem cell populations outside of the body is a critical step towards producing clinically relevant quantities for cell therapy protocols [5]. Perfusion bioreactors are commonly used to optimise stem cell expansion due to their ability to provide a continuous flow of nutrients to a stem cell population [7].

The culturing of haematopoetic stem cells in perfused systems offers an additional challenge because they are non-adherent, and care must be taken to ensure they are not flushed through the system. A promising approach to trap these cells within a culturing device is based upon the use of multiple wells, or grooves, perpendicular to the flow within a perfused channel [2, 4, 6]. A further complication is the negative feedback signalling inherent in haematopoetic stem cell cultures that limits expansion of the population [1, 3]. Minimising the concentration of inhibitory signal within the cell culture is crucial to ensure that expansion of the population is not compromised.

In this study we investigate the effect of inter-gap distance δ on the inhibitory signalling concentration that cells are exposed to in each groove, and evaluate whether a single larger groove, or smaller multiple grooves, is more effective at mitigating the effects of the inhibitory signalling.

Method

The bioreactor under consideration here consists of a perfused rectangular channel of height $h = 200$ μm and width $w = 5$ mm. Cell culture medium of density $\rho = 1000$ kg/m^3 and viscosity $\mu = 8.9 \times 10^{-4}$ Pa.s is perfused at a rate of $Q = 1$ mL/day through the channel. Grooves, or wells, are present on the bottom surface of the bioreactor, running perpendicular to the flow direction. Cells are seeded within these grooves, allowing perfusion to continually renew the cell culture medium without also washing out the cells from the bioreactor. Two bioreactor configurations are considered in this study. The first configuration consists of two grooves of width 200 μm and of height 200 μm (figure 1), identical to the geometry of Sandstrom et al. [6]. In this configuration, the two grooves are separated by the distance δ μm. The second configuration consists of a bioreactor with a single groove of width 400 μm. The total groove surface area, where the cells are situated, is the same for both configurations.

The Navier-Stokes and continuity equations are solved using the commercial finite-volume software ANSYS-CFX 15.0 (ANSYS, Canonsburg, PA, USA). The smaller grooves are considered to contain a uniformly distributed, fixed population of $X = 10^5$ cells secreting a model inhibitory signal at a rate of $r_\phi = 1.94 \times 10^{-6}$ pg/cell.day [3]. The larger groove is considered to contain a uniformly distributed, fixed population of 2×10^5 cells secreting at the aforementioned rate. The model inhibitory signal is considered to have a diffusivity of $D = 6.5 \times 10^{-11}$ m^2/s, similar to that of known inhibitors of haemopoeitic stem cell expansion [1]. The distribution of the inhibitory signal within the bioreactor is determined by the transient advection-diffusion equation

$$\frac{\partial \phi}{\partial t} + \boldsymbol{u} \cdot \nabla \phi = D \nabla^2 \phi. \tag{1}$$

The gradient of inhibitory concentration ϕ normal to the surface of each groove is given by

$$\frac{\partial \phi}{\partial n} = \frac{r_\phi X}{AD}, \tag{2}$$

where n is the coordinate normal to the surface, and the groove surface area $A = 10^{-6}$ m^2 for the smaller grooves, and $A = 2 \times 10^{-6}$ m^2 for the larger groove. At all other boundaries the normal gradient of ϕ was set to zero.

Because the width of the perfused channel is much greater than the channel height, the computational domain used for the simulations is a two-dimensional slice in the flow direction (figure 1). A typical mesh consisted of approximately 35,000 nodes. A mesh resolution study was performed to ensure mesh independent results were obtained. At the domain inlet, a uniform velocity of $\bar{V} = 1.125 \times 10^{-5}$ m/s was specified to give a flow rate

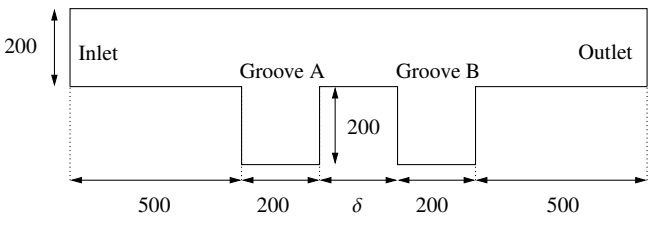
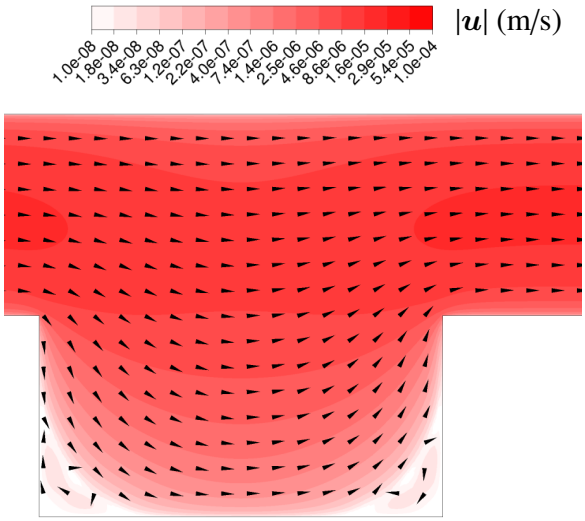


Figure 1. Schematic of the computational domain for the double-groove configuration. All dimensions are in μm.



a) Single groove configuration

b) Double groove configuration

Figure 2. Steady-state contours of inhibitory velocity magnitude $|\boldsymbol{u}|$ and velocity direction $\boldsymbol{u}$ in the bioreactor for a) the single 400 μm groove configuration, and b) the double 200 μm groove configuration with inter-gap distance $\delta = 100$ μm. The contour scale is logarithmic, and the arrows represent direction only.

of $Q = 1$ mL/day. The inlet and outlet lengths were fixed at 500 μm, and tests were performed to show that these lengths were sufficient to ensure that the concentration within each groove was unaffected by the inlet and outlet boundaries.

Simulations were run for a total time of 1 hour, with constant time-steps of Δt = 5 seconds. Steady-state was reached after about 10 minutes of cell culture, much shorter than the typical cell culture period of 8 - 16 days. The transient equations were solved here to facilitate the addition of a growth model at a later date, where due to the two-way coupling between inhibitory signalling and cell growth a steady-state solution is not reached within the cell culture period [1]. Tests were performed with different time-step sizes to ensure that the steady-state solution was independent of time-step size.

Results

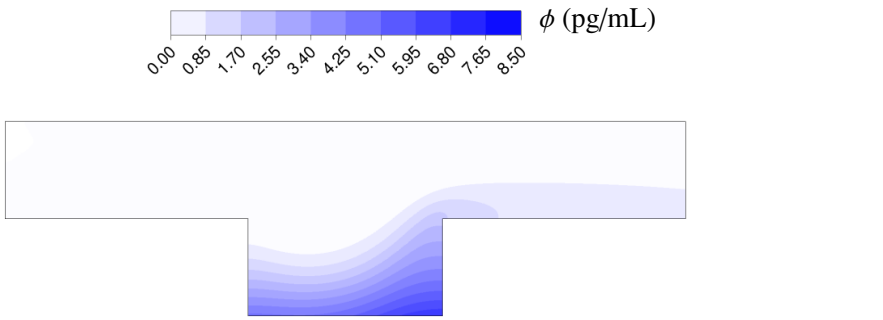
The steady-state flow inside the bioreactor is shown for the two bioreactor configurations in figure 2. The flow over the single 400 μm groove is able to penetrate completely into the groove, facilitating the removal of inhibitory proteins away from the cells and out of the groove. In contrast, the flow over the two 200 μm grooves is only able to partially penetrate the groove, and as a result a recirculation zone is present in each groove. The presence of a recirculation zone is not ideal for mitigating the effects of inhibitory signalling, because there is no convective transport available to remove inhibitory proteins from the groove. Instead, diffusion is the only mechanism available to transport inhibitory proteins out of the smaller grooves and away from the cells.

The steady-state distribution of the inhibitory signal ϕ is shown in figure 3 for the two bioreactor configurations, and various inter-gap distances δ. The highest concentration of inhibitory signal ϕ within each groove is found at the downstream corner for the single groove configuration, whereas the highest concentration of ϕ in the double groove cases is found in the upstream corners. This difference can be explained by the direction of the flow in the grooves for each configuration. Due to the recirculation zone present in the smaller grooves, the flow at the bottom surface is in the opposite direction to the channel flow, and there is an accumulation of ϕ at the upstream corner. There is no such recirculation zone for the larger groove case, and the flow causes an accumulation at the downstream corner.

When the inter-distance gap is large in the double groove configuration, for example $\delta = 400$ μm, the distribution of ϕ within each groove is similar, though there is slightly higher levels of ϕ in groove B in comparison to groove A. As the inter-gap distance decreases, this effect is amplified. There is also a slight increase in the concentration within groove A as the inter-gap distance decreases. However, in the single groove configuration, the concentration at the bottom of the groove is much less than for all the two-groove cases depicted.

To determine how the inhibitory concentration varies within each groove, figure 4 shows the variation of ϕ along the bottom of each groove for various inter-gap distances δ. The inhibitory concentration ϕ decreases monotonically from the front of the groove to the back for all of the 200 μm grooves shown, due to the recirculation zone present. For the 400 μm groove, the concentration reaches a minimum at approximately 100 μm from the front edge, then increases thereafter. For the double groove configuration, The presence of a groove immediately downstream of groove A only slightly increases the inhibitory concentration throughout the groove. This result is expected, given that the Peclet number for this bioreactor configuration is Pe$= \bar{V}h/D = 34.6$, convective effects are dominant, and there is minimal diffusive transport of inhibitory signal opposite to the direction of the imposed flow. Because of the relatively high convective effects, the presence of a groove immediately upstream of groove B results in a significant increase in inhibitory signal in groove B. The concentration in the 400 μm groove is well below the concentration in each of the 200 μm grooves.

Figure 5 shows the average concentration within each groove as a function of inter-gap distance δ, along with the average concentration for the 400 μm groove. When the inter-gap distance is small, $\delta = 10$ μm, the downstream groove experiences an average concentration $\sim 16\%$ higher than the upstream groove. At the largest inter-gap distance of 400 μm, this decreases to $\sim 5\%$. This suggests that expansion of a sub-population of cells within the downstream groove will be significantly compromised by the inhibitory signalling from the upstream population. For all of the cases considered the average concentration within the smaller grooves is at least 40% greater than the average concentration in the single, large groove.



a) Single groove configuration

b) Double groove configuration, δ = 10 μm

c) Double groove configuration, δ = 25 μm

d) Double groove configuration, δ = 50 μm

e) Double groove configuration, δ = 400 μm

Figure 3. Steady-state contours of inhibitory concentration ϕ in the bioreactor for a) the single 400 μm groove configuration, and the double 200 μm groove configuration with inter-gap distance b) $\delta = 10$ μm, c) $\delta = 25$ μm, d) $\delta = 50$ μm, and e) $\delta = 400$ μm.

Conclusions

The flow through two configurations of a grooved bioreactor is modelled to evaluate the effect of groove size and inter-groove distance on the levels of secreted inhibitory signals present in the bioreactor. For the bioreactor configuration with two grooves, the presence of the upstream groove significantly increases the concentration within the downstream groove, due to the dominance of convective transport within the bioreactor. Increasing the distance between each groove lessens this effect slightly, but elevated levels of concentration are still present in the downstream groove. This suggests that the expansion of a sub-population of cells downstream of other sub-populations

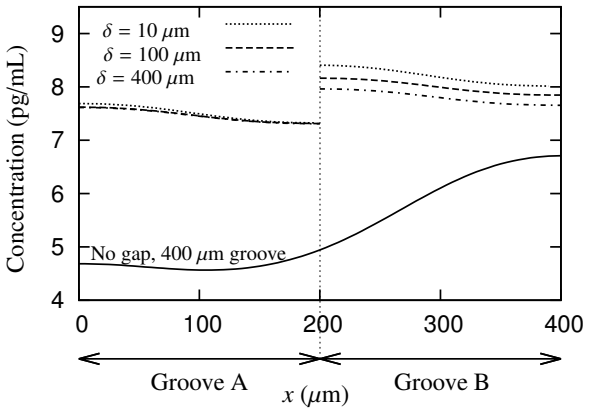


Figure 4. Variation of inhibitory concentration ϕ along the bottom of the grooves, for both bioreactor configurations and various inter-gap distances δ.

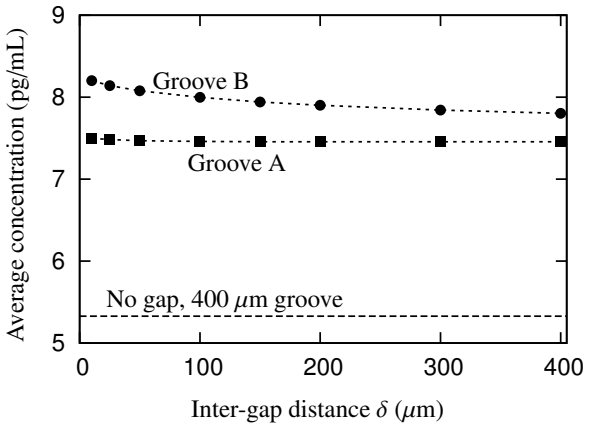


Figure 5. Effect of inter-gap distance δ on average concentration of ϕ at the bottom of each slit for the double groove configuration. Also shown is the average value for the single groove configuration.

will be significantly compromised.

The concentration of inhibitory signal within the large groove is approximately 45% less than the concentration within either of the two small grooves, suggesting that this configuration is best for mitigating the effects of inhibitory signalling and therefore enhancing the expansion of a population of haemopoeitic stem cells.

Future work will focus on predicting the cell expansion within grooves using an appropriate growth model [1, 3], and to also investigate the effect of groove depth on flow regimes and subsequent cell expansion within the bioreactor.

Acknowledgements

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References

[1] Berry, J.D., Godara, P., Liovic, P. & Haylock, D., Optimal Mitigation of Paracrine Inhibitory Signalling in Haemopoietic Stem Cell Cultures, *in preparation*.

[2] Horner, M.,Miller, W.,Ottino, J. and Papoutsakis, E., Transport in a Grooved Perfusion Flat-Bed Bioreactor for Cell Therapy Applications, *Biotechnol. Prog.*, **14**(5), 689-698.

[3] Kirouac, D.C., Madlambayan, G.J., Yu, M., Sykes, E.A., Ito, C. & Zandstra, P.W., Cell-Cell Interaction Networks Regulate Blood Stem and Progenitor Cell Fate, *Mol. Sys. Biol.*, **5**, 2009, 293.

[4] Korin, N., Bransky, A., Khoury, M., Dinnar, U. & Levenberg, S., Design of Well and Groove Microchannel Bioreactors for Cell Culture, *Biotech. Bioeng.*, **102**(4), 2009, 1222–1230.

[5] Lee, E.-J., Godara, P. & Haylock, D., Biomanufacture of Human Platelets for Transfusion: Rationale and Approaches, *Exp. Hematol.* **42**, 332-346.

[6] Sandstrom, C.E., Bender, J.G., Miller, W.M. & Papoutsakis, E.T., Development of Novel Perfusion Chamber to Retain Nonadherent Cells and its Use for Comparision of Human "Mobilized" Peripheral Blood Mononuclear Cell Cultures With and Without Irradiated Bone Marrow Stroma, *Biotech. Bioeng.*, **50**(5), 1996, 493–504.

[7] Walker, G.M., Zeringue, H.C. & Beebe, D.J., Microenvironment Design Considerations for Cellular Scale Studies, *Lab Chip*, **4**(2), 2004, 91–97.