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## **PERFORMANCE EVALUATION OF FOUR CELL FLOW CHAMBERS: HOW WELL IS STRESS CONTROLLED AT A CELLULAR LEVEL?**

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### **ABSTRACT**

Fluid structure interactions at the cellular level are poorly understood yet they appear to be universal across tissue types and may hold the key to unraveling mechanisms of mechanotransduction at a cellular and subcellular level. Due to practical difficulties in studying cells *in situ* during normal physiologic activity, cell perfusion chambers have been developed to simulate physiologic fluid flow *in vitro*. While this approach has obvious advantages for unraveling cell signaling pathways in mechanotransduction, little is known with regard to how well these *in vitro* flow profiles emulate actual physiologic flow. The purpose of this computational study was to compare the local stress imparted through fluid flow in four cell perfusion chambers. From the computational models, in each chamber, varying velocity components cause the local shear stress imparted to the cells to vary as a function of location, and in fact only a limited number of cells are exposed to target stress. Due to differences in flow regimes between the four chambers, comparison between experimental data obtained using different perfusion chambers may be inappropriate.

### **INTRODUCTION**

Due to practical difficulties in studying fluid flow *in situ* during normal physiologic activity [Sorkin 2004], cell perfusion chambers have been developed to simulate such physiologic fluid flow and to observe cellular responses *in vitro*. In particular, the pressure driven parallel-plate perfusion chamber design has been implemented and optimized for application of known fluid shear stresses and correlation to cell activity and adaptation. Variations of the parallel-plate chamber design have become commonplace in cell biological research and provide a basis for current *in vitro* modeling of many physiologic flow regimes including those relevant to bone articular cartilage, connective tissue, vascular endothelium,

leukocyte recruitment, as well as pathologies specific to renal dysfunction, and respiratory distress. In addition, flow perfusion chambers have been implemented to characterize cell-biomaterial interactions, improve tissue engineered implants, and develop novel biomedical applications. While this approach has obvious advantages for investigating effects of fluid shear in many biomedical arenas, it is not known how well these *in vitro* flow chambers perform, *e.g.* in achieving a desired stress at the cell level and in emulating physiologic flow regimes. Furthermore, no published study to date has reported how flow regimes induced through different, commercially available, flow chamber designs compare.

Hence, the purpose of this study was to compare flow regimes in three commercially available cell flow/perfusion chambers and a fourth custom-designed chamber to evaluate their efficacy in providing a defined flow regime and shear stress to cultured cells. For each chamber, the principal velocity component and local shear stress imparted through fluid flow were calculated for a target shear stress including 0-10 dyn/cm<sup>2</sup> used typically for osteoblast stimulation and 20 dyn/cm<sup>2</sup> used for endothelial cell studies. Special attention was paid to local flow regimes in the vicinity of cells within the chambers. The basic approach was to apply computational models for fluid flow along with computational fluid dynamics for flow simulations, while taking particular care to create fluidic models that accurately represent the chamber design to be modeled.

### **NOMENCLATURE**

$\Delta\tau$  = change in shear stress

### **METHODS**

Computational fluid models were created for three commercial cell flow/perfusion chambers (FCS, Oligene GmbH, Berlin, Germany; FCS2, Bioptechs, Butler, PA; RC-

30HV, Warner Instruments, Hamden, CT) and a fourth custom-geometry chamber in order to understand the specific aspects of each design as well as to prepare models for a computational fluid analysis. The chambers were reproduced using a three-dimensional modeling program (Pro/Engineer, PTC, Needham, MA) that allows for exact reconstruction (including exact dimensions) of chamber components and assembly of those components to mimic the workings of the actual device. For each chamber, the principal velocity, mass flow rate, and pressure were determined at the inlet and outlet for a corresponding maximum desired shear stress of  $10 \text{ dyn/cm}^2$  and  $20 \text{ dyn/cm}^2$  at the location where cells are placed within the chamber. The perfusion medium was idealized as similar to water at  $310 \text{ K}$  and, accordingly, was assigned comparable fluid properties: viscosity =  $0.01 \text{ dyn-s/cm}^2$  and density =  $993 \text{ kg/m}^3$ . Simulations were then produced under steady flow conditions with a convergence criterion of  $0.0001$  for the solution.

## RESULTS

In each chamber, the *velocity component* of the flow field varies with location across the surface where cells are seeded. As a result, the local shear stress imparted to the cells varies as a function of location as well. Furthermore, the *range* of shear stresses imparted to the cells varies from chamber to chamber for each perfusion chamber studied; in fact, inter-chamber differences in flow profile and shear stress are dramatic. In *chamber 1* (Fig. 1), for a target stress of  $10 \text{ dyn/cm}^2$ , the total range of shear found at cellular monolayer is  $2.04 \text{ dyn/cm}^2$ . In *chamber 2* (Fig. 2), the total range of shear is found to be  $5.89 \text{ dyn/cm}^2$ . *Chamber 3* (Fig. 3) shows the widest range of imparted stresses, revealing a range of shear stress of  $5.51 \text{ dyn/cm}^2$ . In the fourth chamber (Fig. 4), the results for shear stress and velocity are strikingly different from that of the previous three commercial chambers. The range of shear stress found within the section is drastically lower than previous chambers, where a total range of shear stress,  $\Delta\tau$ , that would be imparted to the monolayer is  $1.75 \text{ dyn/cm}^2$ . The fourth chamber was subjected to PIV experimentation in order to visualize and validate the velocity profile predicted in the computational models. The measured particle velocities are found both above and below the calculated profile, which holds true across the entire width of the chamber, although on average the measured particle velocities tend to be less than the predicted values. The general trend of the velocity profile is parabolic, which is expected for this fluid geometry. Measured values are within 5% of the computer model predictions, which validates the values calculated using the CFD models.

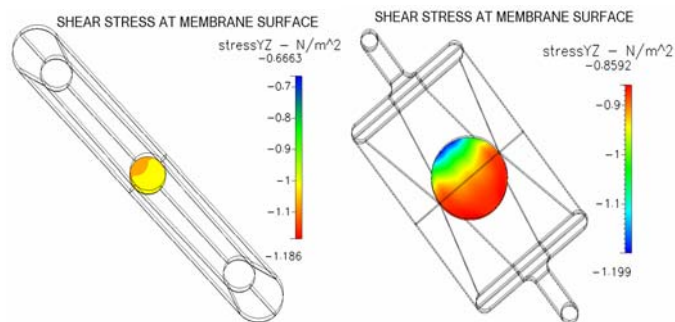


Fig. 1: Chamber 1 – shear stress

Fig. 2: Chamber 2 – shear stress

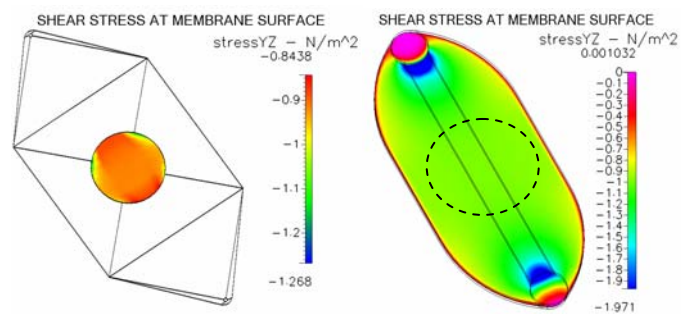


Fig. 4: Chamber 3 – shear stress

Fig. 4: Chamber 4 – shear stress

## DISCUSSION

In conclusion, the results of this study challenge the basic premise of *in vitro* mechanotransduction studies, *i.e.* a controlled flow regime is applied to impart a defined mechanical stimulus to cells, even if it is not always possible to insure that the flow regimes are purely physiologic. In fact, flow regimes found in commercially available perfusion chambers are not constant and shear stresses that are imparted to cells are location dependent at the cellular level. Hence, cells on one side of a chamber insert may experience a different stress than those on the opposite side. This complicates the elucidation of cellular mechanisms of mechanotransduction. Furthermore, these flow fields differ between chambers as well; according to their geometry and set flow rate. This further exacerbates meaningful elucidation of mechanotransduction mechanisms through comparison of studies conducted with different chamber designs. At the very least, this study underscores the importance of calibrating devices to achieve stress magnitudes near targeted stress levels. From a broader perspective, by coupling computational fluid dynamics with cell biology, new approaches can be developed to overcome limitations of the current technology. Thereby, the impact of *in vitro* studies will be increased and data from different laboratories will be able to be compared, which could amplify the impact of cell biology research.

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## REFERENCES

- Allen, F.D. et al., *J. Biomech* 33 (2000) 1585-1591.
- Donahue, T.L. et al., *J. Biomech* 36 (2003) 1363-71.
- Frangos, J. et al., *Biochem Biophys Res. Comm.* 280 (2001) 481-5.
- Hung, C.T. et al., *Clin Orth Rel Res.* 313 (1995) 256-269.
- Jacobs, C.R. et al., *J. Biomech.* 31 (1998) 969-976.
- Knothe Tate, M.L. and Knothe U., *J. Biomech* 33 (2000) 247-254
- Sorkin, A.M. et al., *Am. J. of Phys: Cell Phys.* In Press, Online Publication 2004.