Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Figures

Figure 1. (a) Flow velocity profiles obtained from 3D computational simulation using COMSOL. (b-e). Each image is taken from the each condition. Even the oocytes under 20 min slope were exposed to the flow over 20 minutes, the oocyte still look viable morphologically.

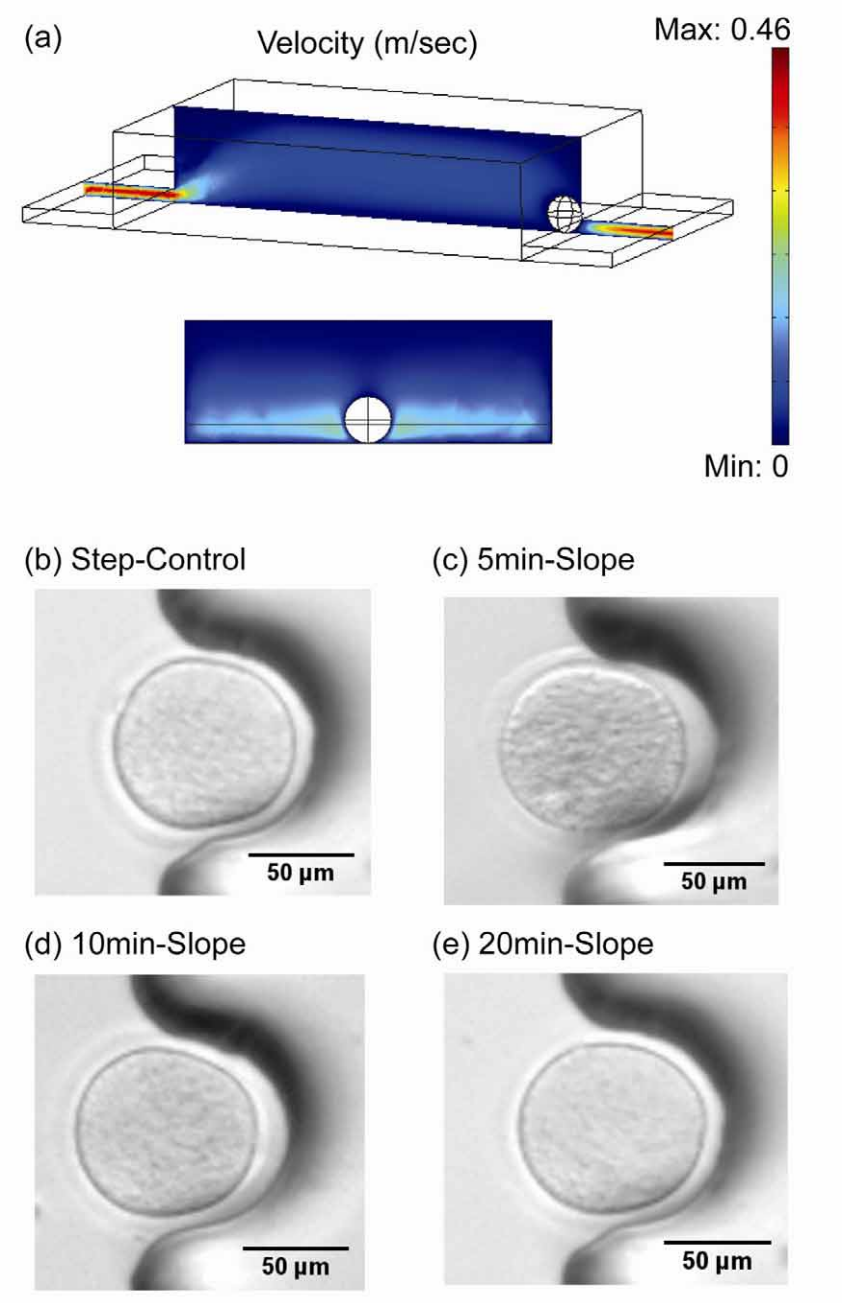

Figure 2. Curve fitting. A representative plot of volume versus times for mouse oocytes exposed to 1.5M PROH at 23 °C. The calculated data represents the best fit for L_p, P_s, and σ.

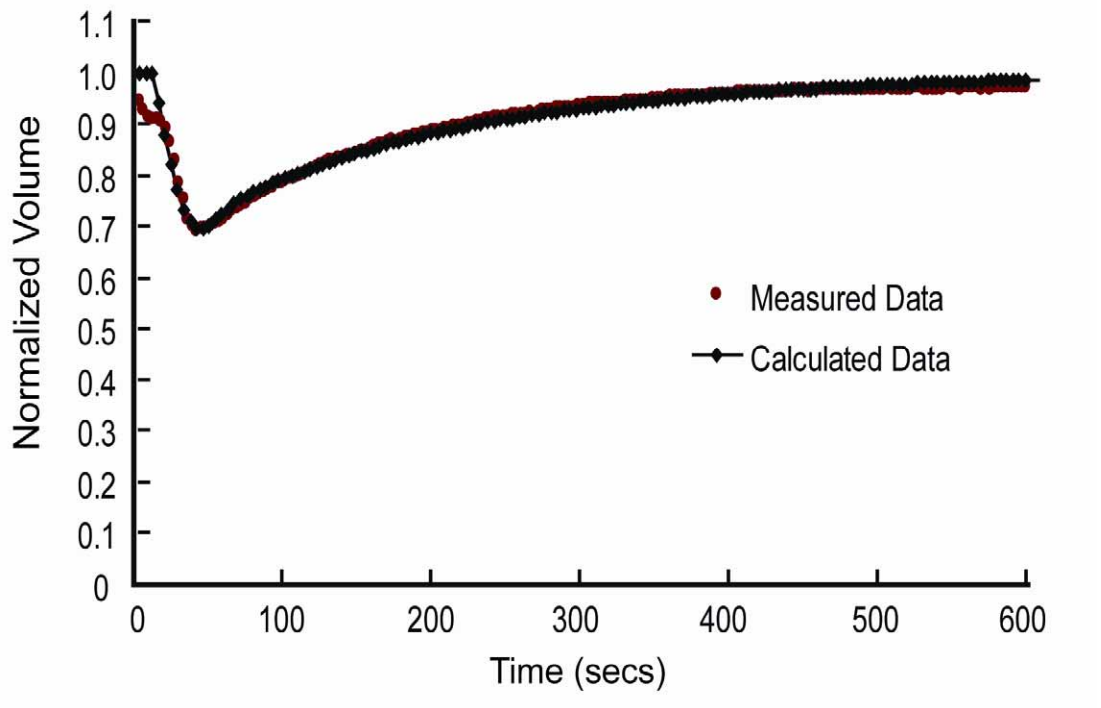

Figure 3. From simulations, we identified 3 major factors influencing the outcome: the slope time (time until the concentration reaches the max CPA concentration), the initial condition of the concentration, and the overshoot of the concentration with respect to the target value. These results are compared to a slope function with a slope time of approximately 3.25 minutes, 0% initial condition, and 0% overshoot, which were demonstrated to achieve cell equilibrium in 15 minutes, while the relative cell volume change temporarily reached at maximum of 18%. (a), (b) Effect of Concentration Overshoot (O.S.) on the maximum volume change and recovery time. (c), (d) Effect of Initial Concentration (I.C.) on the maximum volume change and recovery time. (e) We also found that the initial condition of concentration has a strong influence on the maximum relative cell volume change. Changing the initial condition however has only a small effect on the equilibrium time. This also implies that even with optimal concentration functions, the tradeoff between volume change and total CPA loading time to equilibrium still exists.

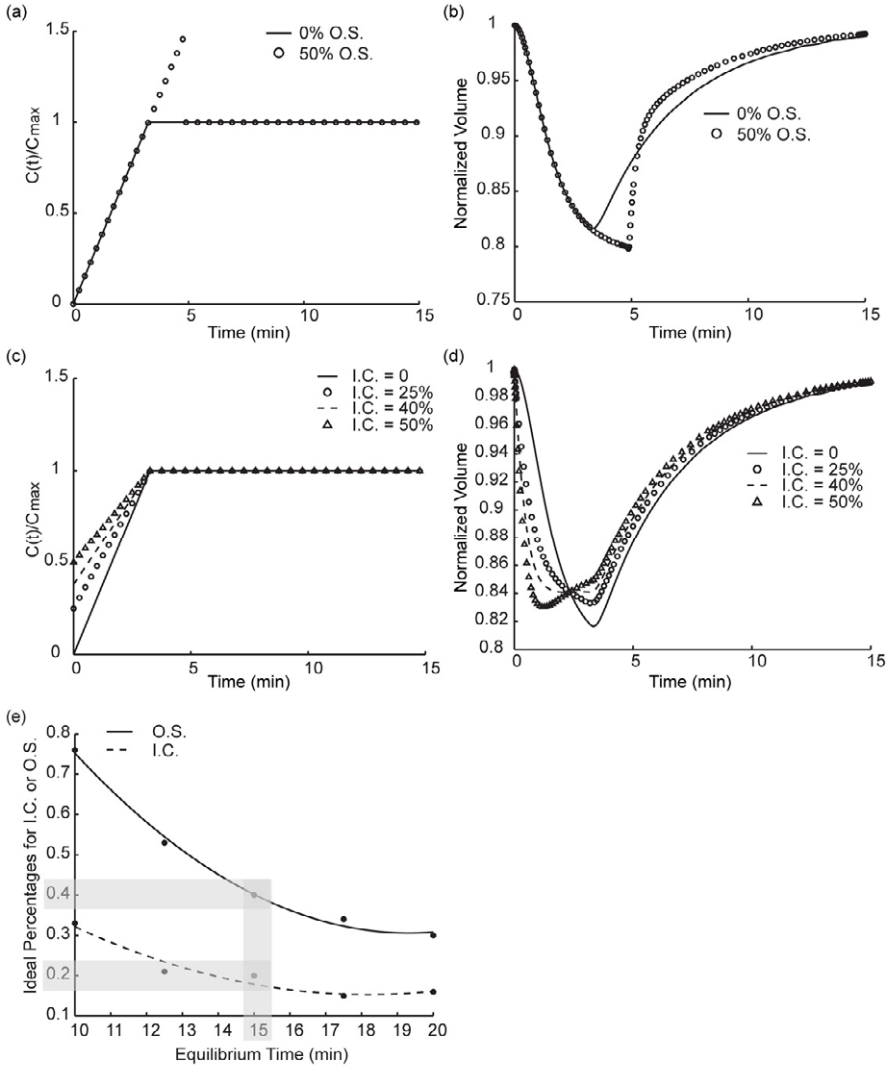

Figure 4. To understand how the L_p, P_s and σ affect the volume change during the cryo process a Matlab code was written to compare 50%, 75%, 125% and 150% of the experimentally derived value of these three parameters (L_p = 0.5022 (um/min/atm), P_s = 19.164 (um/min) and σ= 0.5). Therefore for each of the three plots below, two of these values were held constant and one was changed. (a) L_p is variable (b) P_s is variable and (c) σ is variable.

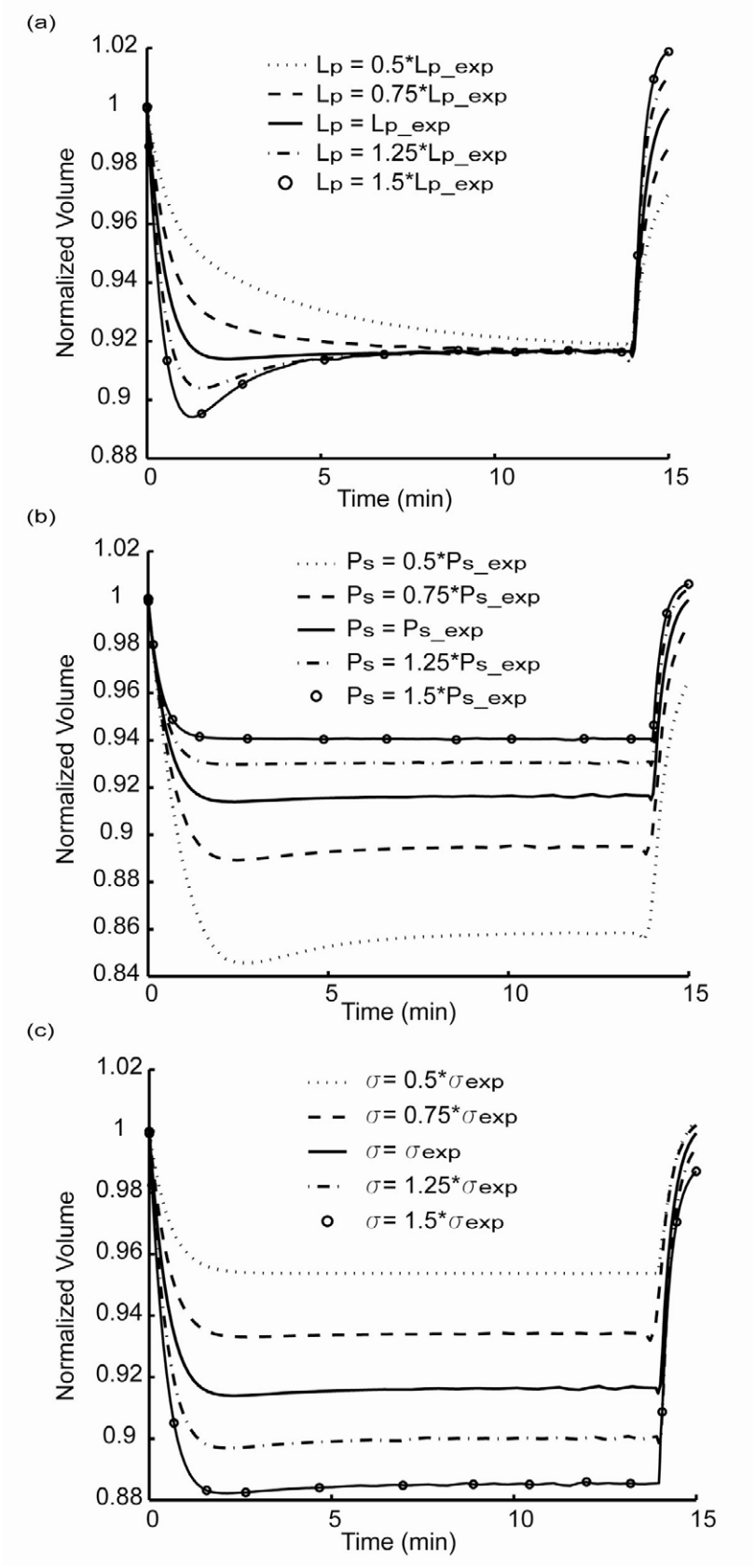

Figure 5. A tradeoff between the maximum allowable volume change (a) and the time to equilibration (b) are necessitated for the determination of the optimal "slope time" of the concentration function in a linear profile. As the "slope time" becomes larger, maximum allowable volumes, ε, decreases (a) while equilibration time increases (b).

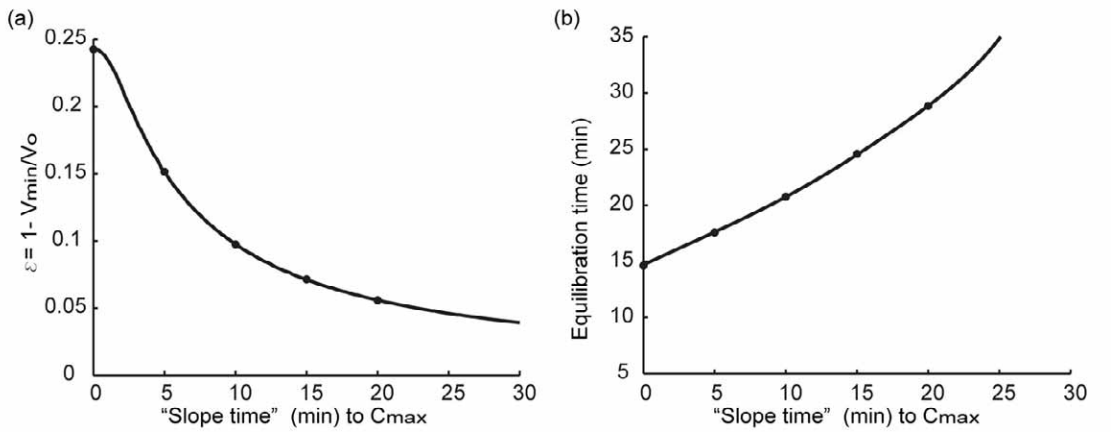

Figure 6. Size variance in the initial oocytes versus minimal volume. It is possible that differences in the initial diameter of the cell influence the volumetric changes. For the purpose of this study we selected oocytes with initial diameter in a narrow range around 75µm (average diameter is 74.4±1.8 µm in the case of the control).