Photobleaching test of FSS in the pores and selection of the concentration range

In order to study the multicomponent diffusion experimentally, we chose fluorescein sodium salt (FSS) as a marker electrolyte. Fluorescent intensity (gray value) is a direct measure of fluorescein concentration in the pores, under appropriate illumination conditions. We performed experiments on an inverted microscope (Leica DMI4000B, FITC) with fixed imaging parameters. By setting exposure, gain, and intensity values, respectively, 7 ms, 3, and 1, we set the laser intensity to be $\approx 350 \text{ W/m}^2$. The laser power is measured with an optical power meter (Thorlabs PM100D) and a standard photodiode power sensor (Thorlabs S120VC). Then with 1-minute-interval imaging, we observed that the fluorescein in these channel is not photobleached (Figure S1 (a,b)) for 7 minutes. Also, the averaged gray values over 7 minutes (Figure S1 (c)) show that the intensity increases linearly with the concentration of FSS up to 2 mM. Therefore, to use the normalized gray values in the pores as a direct measure of normalized FSS concentration, we conduct experiments with 2 mM FSS as the initial concentration in the pores. We note that the FSS concentration monotonically decreases once the experiment starts, and thus we never exceed the experimentally set maximum (2 mM FSS).



Figure 1 Photobleaching test for the fluorescein sodium salt (FSS) in the pores. (a) Image of 2 mM FSS in the pores under the selected imaging condition. Gray values of the ROIs (dashed boxes) were measured at different times. (b) 7-minute-measurements of the pore intensities plotted versus time for different FSS concentrations. The gray values remain nearly constant over 7 minutes with 1-minute-period imaging. (c) Gray values averaged over 7 minutes in the pores plotted versus FSS concentration. From 0.25 mM to 2 mM, the intensity in the pore shows linear increase with the FSS concentration. For larger concentrations of FSS, we observe loss of linearity between intensity and the concentrations, and thus we select 2 mM as the initial FSS concentration for all experiments.

Comparison between experiments and the coupled model with modified boundary condition

We performed the simulation of Eq. (3) by assuming that $c_2(0, \tau) = 0.06$ to quantitatively capture the effect of the concentration boundary condition at X = 0.

The results in Fig. 2 show that the quantitative agreement improves. To further improve the prediction, a full 3-dimensional model needs to be considered.



Figure 2 Comparison between experiments and the coupled with modified boundary conditions. Fluoroscein concentration $c_2(X, \tau)$ for $\beta = 1$, where $\beta = 1$ corresponds to the concentration of 4 mM NaCl in the main channel. The solid lines are results for the coupled electrolyte model (Eq. (3)), and the data points are obtained from experiments.