

Figure S1. Schematic of computational model baseline showing unidirectional flow entering from the left (orange), exiting from the right (maroon), with open boundary conditions on the other faces and a flux condition around the cell.

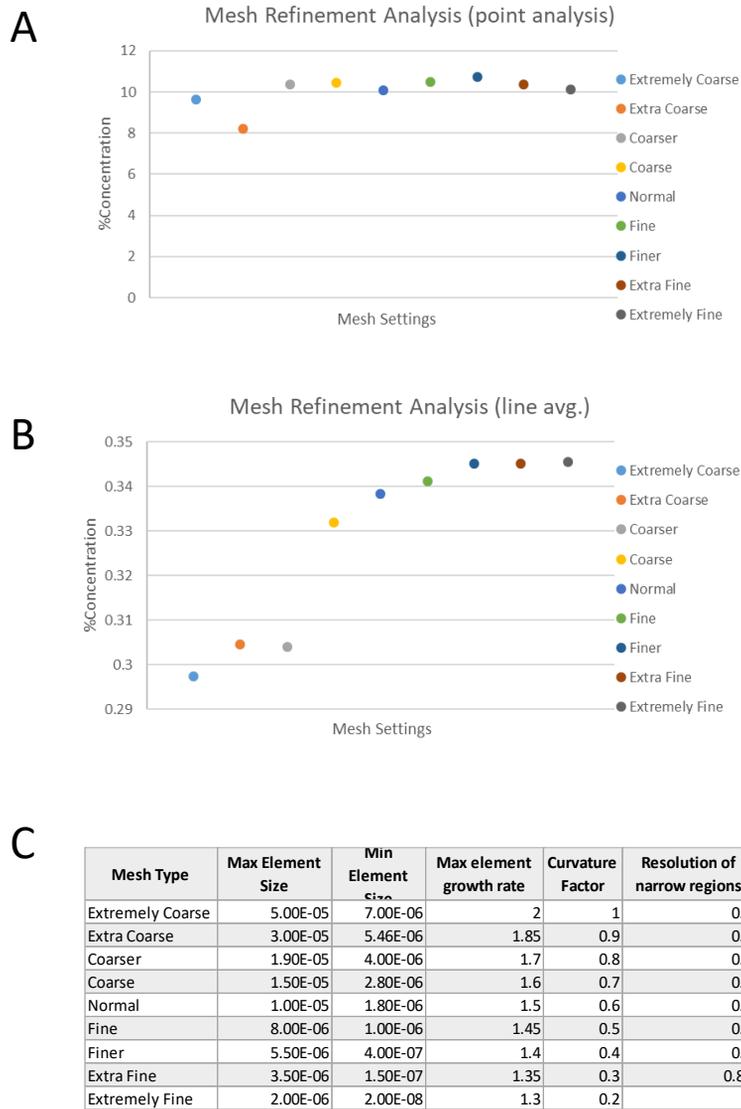


Figure S2. Mesh refinement analysis. A) point analysis of %concentration showing convergence occurring after the normal mesh is employed B) line average analysis of %concentration showing convergence at the finer mesh. C) table of mesh parameters for each mesh refinement. Overall, finer mesh was optimized based on convergence of solution and solution time.

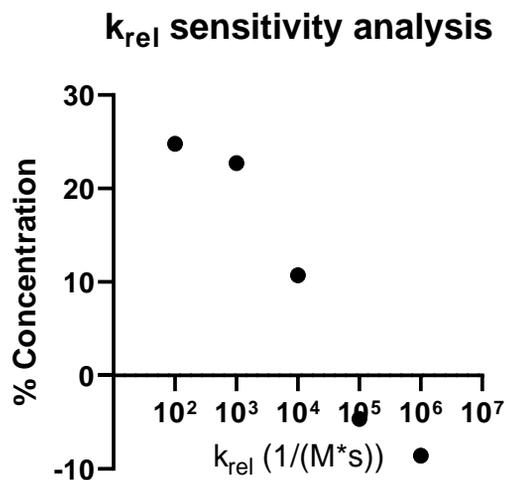


Figure S3. Sensitivity analysis of k_{rel} . Variable tested by changing order of magnitude and running COMSOL simulation. A large effect can be seen on the resulting %concentration.

Parameter Effects on Concentration Profile

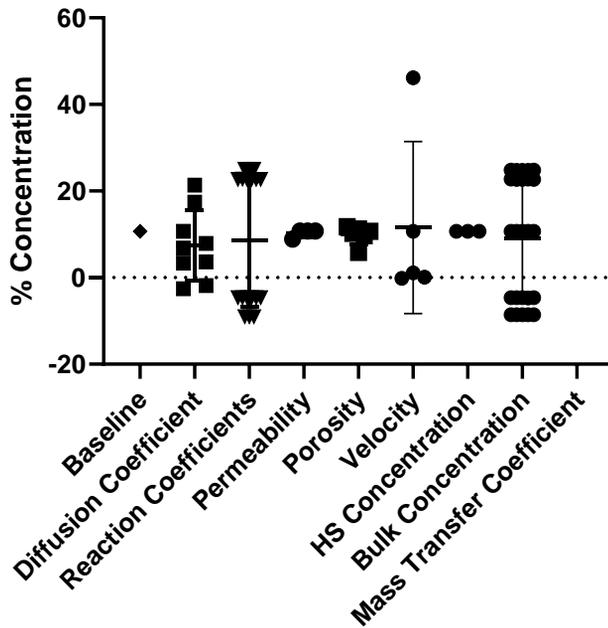
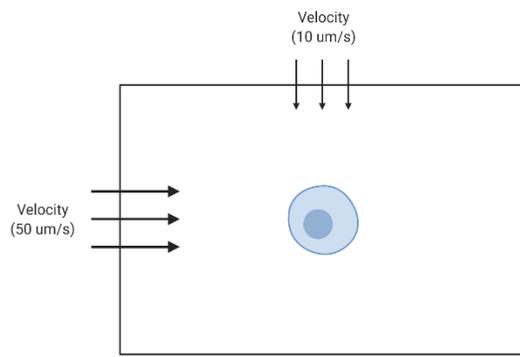
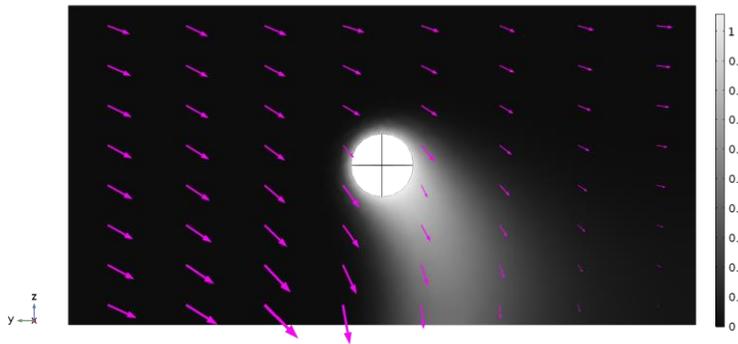


Figure S4. Parametric sweeps of input variables and their corresponding effects on %concentration. Velocity has the largest impact on %concentration outcomes. Diffusion coefficient and reaction coefficients also impact the %concentration magnitude but have less impact on %concentration variability.



Slice: Concentration (mol/m^3) Arrow Volume: Velocity field



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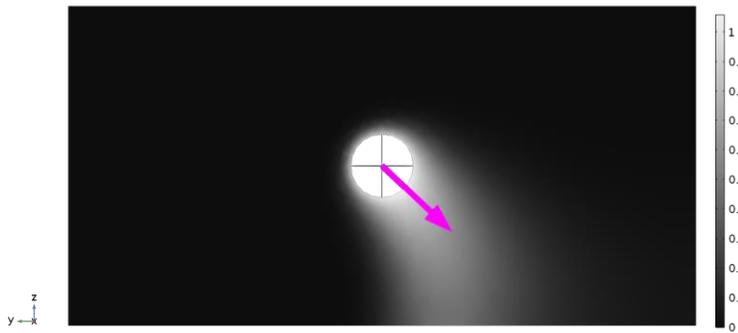


Figure S5. **Additional multidirectional flow modeling** and results with higher magnitude in the x-direction than y-direction.

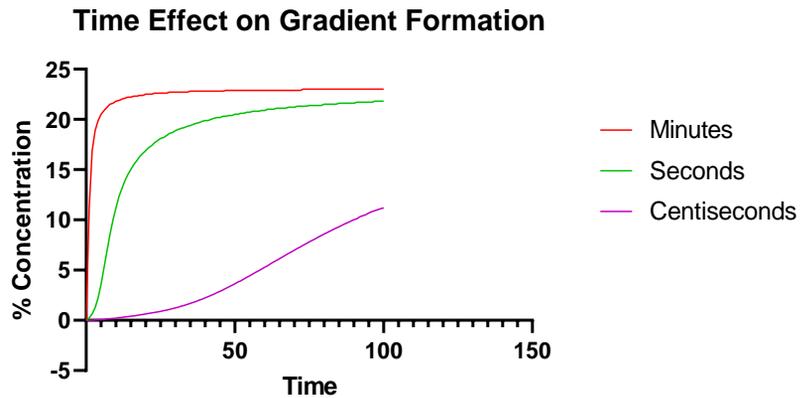


Figure S6. **Time effect on gradient formation for baseline condition.** The gradient can be seen to develop on the centisecond scale and reach an equilibrium around two minutes.

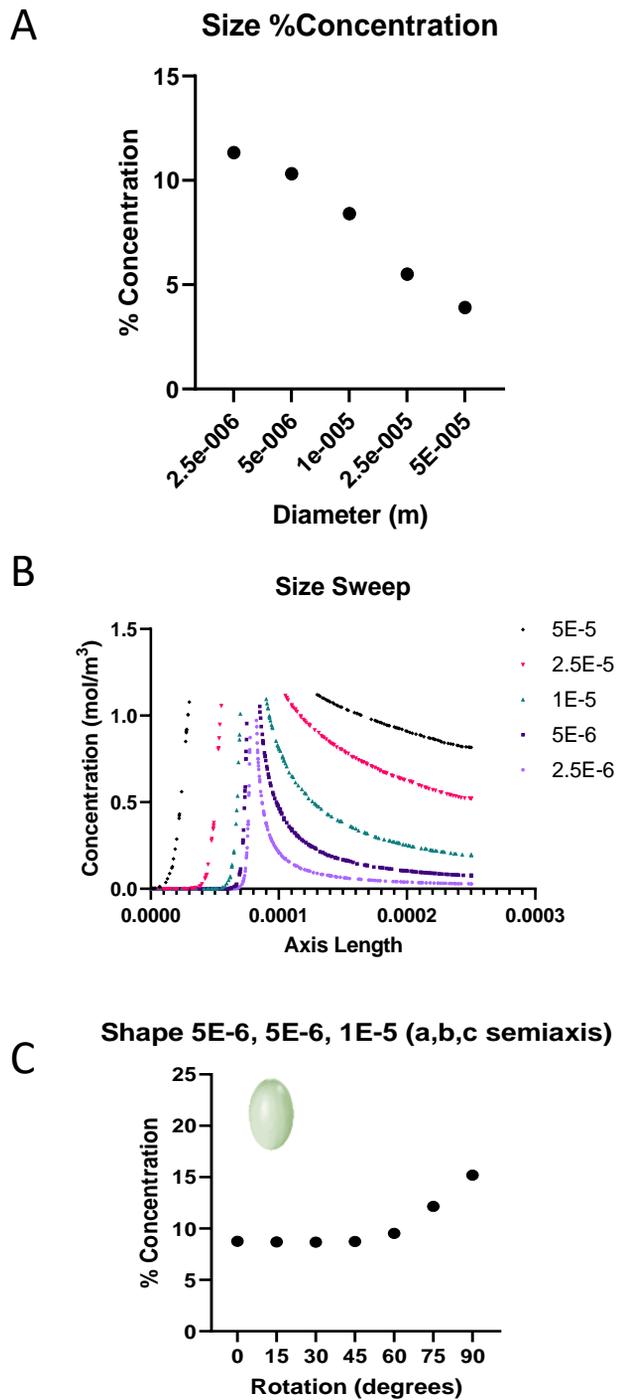


Figure S7. **Cell size and orientation impact gradient formation.** As the cell increases in size, the %concentration decreases (A) and as the cell aligns with the direction of fluid flow, the %concentration increases (B,C).

Autologous gradient formation under differential interstitial fluid flow environments

Supplemental methodology. Descriptions of further methodology below correspond to the figures within the primary manuscript file.

Modulation of transport parameters yields expected responses in gradient formation

Parametric sweeps of the model are performed for each variable of interest based on physiologically-relevant values that could be observed. These variables are reaction coefficient, permeability, diffusion coefficient, velocity, and porosity. Steady-state solutions only are computed as we are interested in comparing the parameter contributions on concentration gradient formation. Time dependent solutions are considered later. Point analysis was performed to obtain values for % concentration calculation (Equation 9) for each parametric sweep and a 'yz' slice was taken to show chemokine gradient formation. Line graphs were generated by taking model results of bound chemokine [hs_cxcl12] along a line drawn through the model geometry in the axis of flow.

Multidirectional flow and its impact on gradient formation around a single cell.

Geometry for the control volume was changed from baseline to add partitions on the top and bottom boundaries where flow inlets or outlets could be applied. For this figure, multiple inlets and outlets were used simultaneously, either on the side boundaries or the top and bottom boundaries. The first run was done with outlets on either side and inlets on both the top and the bottom boundaries. The second run had an inlet on the side and top and an outlet on the opposite side and bottom. Inlet velocities were changed between 10 $\mu\text{m/s}$ and 50 $\mu\text{m/s}$.

Transient solutions of gradient formation around single cells based on physiological time-dependent changes to superficial flow rate

Simulations were performed with a time dependent solver configuration for unsteady state solutions. The time scale was set to seconds and functions were created to show a range of velocities that could happen due to tumor formation or other circumstances such as CED treatment which included a ramp function ($f(x) = 0.25 * t$), a custom step function, and a sinusoidal function ($f(x) = \sin\left(\frac{t}{5}\right) + 0.5$). These were input into the model by changing the inlet condition to the baseline velocity (10 $\mu\text{m/s}$) multiplied by the desired function at each time

step. The simulations change in velocity over time depending on the phenomena being observed, such as pulsatile flow, resulting in different concentration gradient formations. Boundary conditions, geometry, and meshing all stayed consistent with baseline.

Background concentration reduces the concentration gradient around single cell

Geometry for the control volume was altered to include a partition down the middle to assign concentration boundary conditions. The model is based on the control volume being centered at coordinates (0,0,0) such that the sphere is located on the positive end of the y axis. Boundary conditions for mass transport were set on the inlet boundary at $5E-4 \text{ mol/m}^3$ (500nM), on the wall boundaries before the partition at $y + 5E-5 \text{ mol/m}^3$ (which essentially sets up a gradient of concentration down this boundary that goes from $5E-4 \text{ mol/m}^3$ to 0 mol/m^3 based on the y axis values), and on the outlet boundary at 0 mol/m^3 . This creates a concentration gradient that starts at the inlet and forms around the cell over time until it hits the partition in the middle of the geometry (Supplemental Figure). 500nM was chosen so that the background concentration would completely cover the cell taking into account the binding reactions in the model. The model is further adapted to provide a temporal component that ‘switches on’ the background concentration once the cell has had time to establish its own concentration gradient and then ‘switches off’ after a set time. In this way we can look at the effects of background concentration gradient on the developed pericellular gradient and then what happens when that background gradient gets removed. A parametric sweep was then defined to change the position of the sphere along the y axis.

Background Concentration Calculation

In order to figure out the point at which the background concentration would negate the gradient formation, an equation was made based on the baseline condition:

$$y = \frac{(1.0518 - (0.94992 + x))}{(1.0518)} * 100 \quad (10)$$

Where y is % concentration and x is the background concentration of bound CXCL12 in mol/m^3 . At $x = 0$, % concentration is about 10% and it gets negated once the background concentration reaches a total of 1 mol/m^3 where the % concentration drops to $<5\%$.

Concentration gradients felt by an invading cell beyond the tumor border at varying distance

Geometry was changed to incorporate a 'tumor border' by adding a set of circles upstream of the single cell with locations based on a histological sample. Boundary conditions were set the same as the 3D model with the addition of flux conditions around some of the added circles corresponding to the percentage of cells expressing CXCL12 from the same histology samples. A parametric sweep was done with the single cell at different positions along the y axis ranging from $0.6\text{E-}4 \text{ m}$ to 0 m (0 to $1.2\text{E-}4 \text{ m}$ away from the tumor border). Velocity was also changed between $1\text{E-}6$, $1\text{E-}5$, and $1\text{E-}4 \text{ m/s}$.

Concentration gradients depend on location along a morphologically accurate tumor cell

Two parametric sweeps are performed, one that modulates diameter of the sphere from $2.5 \mu\text{m}$ to $50 \mu\text{m}$ and one that changes the long axis of an ellipsoid and also rotates it in order to understand how shape and orientation affect gradient formation. In addition, meshes of cells that have been imaged from in vivo and in vitro conditions are imported into the model and concentration gradients across them observed. Boundary conditions for Figure 2 are the same as above. Geometry of the control volume has been changed on the size parameter sweep to include the larger sphere diameters. Dimensions for this are $300 \mu\text{m} \times 150 \mu\text{m} \times 150 \mu\text{m}$ (LxWxH). The sphere geometry is also changed to an ellipsoid so that each semiaxis can be defined individually to create the various shapes used in this simulation. Point evaluation is used to calculate % concentration for the size parameter sweep while a line evaluation is used for the ellipsoid rotation changes. This line evaluation is performed by creating one line through the upstream portion of the ellipsoid and one line through the downstream portion and taking the max values along each line which correspond to the concentrations on the surface of the ellipsoid, similar to previous.

Cell imaging and 3D rendering

The cells used in the are eGFP-GL261 (murine glioblastoma) cell. The GL261 cell was embedded in a collagen-hyaluronan gel, placed on a coverslip, and imaged with a Zeiss LSM800 confocal microscope and 63x plan-apochromat objective. Z stacks were acquired at a step size of 0.34 μm from the top of the cell to the bottom (36.38 μm total). Acquired images were then opened in ImageJ where they were processed to remove background signal if needed, converted to binary, and had noise outliers removed. Using the 3D viewer plugin in ImageJ, images were displayed as a surface with a resampling factor of 1 and threshold of 50. These surfaces were then exported to a .stl file and opened with Meshmixer, an open-source meshing software. The imported surfaces were made into a solid using the “make solid” tool and a mesh applied based on modeling accuracy and minimal amount of triangles in the mesh. If needed, the mesh was simplified and then the “inspector” tool used to check for any discontinuity or errors before exporting again as a finalized .stl file. In COMSOL, the .stl files were imported as mesh parts and used as the cell geometry for their respective runs.