Fig. S-1 Cells are represented as deformable ellipsoids where each axis is represented by a Hookean spring with spring constant κ_1 in parallel with a Maxwell element consisting of viscous element μ and a Hookean spring κ_2. Fig from Palsson[17].

S1 Appendix

Modeling framework

In our model, there are two different cell types, macrophages and tumor cells, and two different signaling molecules, EGF and CSF-1. The model is written in the C programming language and it produces 3-D images using the graphic program openGL.

In this model, each cell is approximated as a deformable ellipsoids with constant volume, V. The ellipsoid has 3 axes and each axis of the ellipsoid has a Hookean spring, κ_2, in parallel with a Maxwell element, which consists of a viscous element, μ, in series with a Hookean spring, κ_1 (Fig S-1). The Maxwell element controls the relaxation of the axis once a force acting on it is removed.

κ_1 represents the initial elastic response of a cell to deformation, μ represents the steady deformation of a cell under constant force and κ_2 prevents the cells from being squished or stretched too much on any given axis. The three axes of the ellipsoids are represented with the vectors $\vec{a}$, $\vec{b}$ and $\vec{c}$. When one axis is stretched, another one must be compressed in order to preserve the volume of the ellipsoid. This generates a modifying force, F_{mod}, which can be calculated by solving equations 7 and 8 simultaneously.

$$\frac{dr_i}{dt} = \frac{\kappa_1(F_i - F_{mod})}{\mu(\kappa_1 + \kappa_2)} + \frac{dF_i/dt}{(\kappa_1 + \kappa_2)} - r_i \frac{\kappa_1 \kappa_2}{\mu(\kappa_1 + \kappa_2)} \qquad (7)$$

$$r_a r_b r_c = (r_a + \Delta r_a)(r_b + \Delta r_b)(r_c + \Delta r_c) = V/(\frac{4}{3}\pi) \qquad (8)$$

where r_i are the lengths of the different axes of the ellipsoid, i can be a, b or c, F_i is the total force acting on axis i and Δr_i is the change in length of axis i. When the force is removed the ellipsoid slowly relaxes back to its original shape.

Before the cell begins to move it polarizes and establishes a front and back. In the model the a axis of the ellipsoidal cells is always set to be oriented in the direction the cell is moving in. When the concentration of a signaling molecule around a cell is above a set threshold and the gradient is steep enough, the cell will orient it's a axis in the direction of the gradient, with some randomness, and start moving towards it. The gradient calculated is the relative gradient across the cell diameter. If the gradient is below a set threshold, the cells will choose a random direction with a bias towards the previous direction. The signaling molecule is EGF for tumor cells and CSF-1 for macrophages. When the unit-vector $\vec{a}$ of the ellipsoid is rotated in the new direction that the cell is moving in, the new $\vec{b}$ and $\vec{c}$ unit-vectors also need to be re-calculated. $\vec{b}$ and $\vec{c}$ were found using the Gram Schmidt process for an orthogonal basis where the old $\vec{b}$ and $\vec{c}$ vectors were used as the previous orthonormal basis. The lengths of the new axes a, b and c, (r_a, r_b and r_c) were calculated by finding where the new vectors would cut the surface of the old ellipsoid.

The deformability of the ellipsoids helps visualize the cells polarity and in practice represents the protrusion of cells along a chemical gradient. In these simulations, deformation of cells are not that important and using semi-hard spherical cells would have produced quantitatively similar results. We chose to use ellipsoidal cells for the following reasons: First, this model will be extended to simulate intravasation and extravasation of tumor cells where having deformable ellipsoidal cells is important because the cells will need to squeeze through the walls of the blood vessels. Second, using ellipsoidal cells does not add much to the computational time.

Forces

In order to determine the movement of each cell, all the active and exclusive forces acting on the cell are calculated using the following equation:

$$\mathbf{F}_{cell} = \mathbf{F}_{active} + \sum_N \mathbf{F}_{exclusive}, \qquad (9)$$

where N is the number of neighbouring cells that exert a exclusive force on the cell. Cells grab onto the extracellular matrix (i.e. collagen fibres) to generate an active force, $\mathbf{F}_{active}$, that moves them either in the direction of a gradient or in a random direction.

$$\mathbf{F}_{active} = \begin{cases} \mathbf{F}_{chemotax} & \text{if the gradient is above a set threshold,} \\ \mathbf{F}_{random} & \text{otherwise.} \end{cases}$$
$$(10)$$

$\mathbf{F}_{random}$ represents the random and exploratory behaviour of a cell, $\mathbf{F}_{chemotax}$ represents the force generated for a cell to chemotact in the direction of a gradient.

The a axis of the cell is always oriented in the direction that the cell is attempting to move, be it in the direction of the chemical gradient or in a random direction. Therefore, the direction of the active force is given as $\vec{\mathbf{F}} = F\vec{\mathbf{a}}$, where $\vec{\mathbf{a}}$ is the unit vector for the a axis. The magnitudes of the two active forces are given in Table 1.

There is an exclusive force between cells in close proximity to ensure they do not overlap. It is calculated from the following equation:

$$\mathbf{F}_{exclusive} = \begin{cases} 0 & \text{if } x > 0, \\ F_{compress}(-x)^{\frac{9}{5}} \cdot \frac{\vec{\mathbf{r}}_{ij}}{\|\vec{\mathbf{r}}_{ij}\|} & \text{if } x \leq 0. \end{cases} \quad (11)$$

$F_{compress}$ is the strength of the exclusive force, $x = \frac{d}{r_{cell}}$, where d is a measure of the distance between the surface of the two cells along the vector $\vec{r}_{ij}$ from the centers of cell$_i$ to cell$_j$ and r_{cell} is the radius of the cells (5 μm).

At each time step, all forces acting on every cell are calculated and then all the cells are moved at the same time according to the equation of motion. The cells move in a very low Reynolds number environment, so inertia can be neglected. Therefore, the active and exclusive forces are balanced by the drag force, resulting in the following equation of motion:

$$\frac{d\mathbf{x}_i}{dt} = \mathbf{v}_i, \quad (12)$$

$$\mathbf{v}_i = \frac{\mathbf{F}_{cell}}{\mu_{ecm}}. \quad (13)$$

$d\mathbf{x}_i$ is the change in position of cell i for a time step Δt = 0.01 min, $\mathbf{v}_i$ is the velocity of cell i and μ_{ecm} is the viscosity between the cells and the extracellular matrix. μ_{ecm} is estimated from experimental data of cell velocity and the force the cells apply to their surroundings. The shape of the cells does not change significantly and thus μ_{ecm} is assumed to be constant.

Concentration of signaling molecules

The tumor cells involved in the paracrine signaling loop with macrophages, secrete CSF-1 in response to a local EGF concentration above a set threshold. Similarly, macrophages secrete EGF in response to an above threshold local CSF-1 concentration. The concentration of the two signaling molecules is calculated on a 3-D grid where each side in a lattice cube is one cell diameter in length. Every time step, the concentration in all lattice cubes is used to find the local chemical concentration around each cell and to calculate the chemical gradients. Cells are free to move around independently of this grid and therefore each cell can partially occupy up to 8 different lattice cubes at a given point in time. This partial overlap is accounted for when calculating the local concentration around a cell and is also used to determine how the cell's secretion is distributed to the overlapping grid cubes. The local concentrations around the cells is calculated using the following equations:

$$[C]_{cell-q} = \sum_{i=l-1}^{i=l+1}\sum_{j=m-1}^{j=m+1}\sum_{k=n-1}^{k=n+1} \frac{S_{cell-q}^{ijk}}{S_{cell}}[C]^{ijk} \quad (14)$$

$$[E]_{cell-p} = \sum_{i=l-1}^{i=l+1}\sum_{j=m-1}^{j=m+1}\sum_{k=n-1}^{k=n+1} \frac{S_{cell-p}^{ijk}}{S_{cell}}[E]^{ijk} \quad (15)$$

where $[C]_{cell-q}$ is the CSF-1 concentration around macrophage q, the centre of macrophage q is located in a lattice cube with (x,y,z)-coordinates (l,m,n), $[C]^{ijk}$ is the CSF-1 concentration in lattice cube ijk, S_{cell} is the total surface area of a cell, S_{cell-q}^{ijk} is the segment of surface area of macrophage q that is located in

lattice cube ijk, $[E]_{cell-p}$ is the EGF concentration around tumor cell p, S_{cell-p}^{ijk} is the segment of surface area of tumor cell p that is located in lattice cube ijk and $[E]^{ijk}$ is the EGF concentration in lattice cube ijk. The local concentration around a cell needs to be above a certain threshold ($[E]_{th}$ for tumor cells or $[C]_{th}$ for macrophages) for the cell to begin secreting a signaling molecule.

The secretion of CSF-1 by tumor cells and EGF by macrophages can be approximated using Michaelis Menten kinetics:

$$\Omega'([E]_{cell-p}) = \frac{[E]_{cell-p}^n}{[E]_{th}^n + [E]_{cell-p}^n} \quad (16)$$

$$\Omega'([C]_{cell-q}) = \frac{[C]_{cell-q}^n}{[C]_{th}^n + [C]_{cell-q}^n} \quad (17)$$

where $\Omega'([E]_{cell-p})$ is the secretion of CSF-1 by tumor cell p in response to its local EGF concentration, $\Omega'([C]_{cell-q})$ is the secretion of EGF by macrophage q in response to its local CSF-1 concentration and $n \geq 1$. For large n this equation has a sharp transition threshold. Presuming that there is a sharp transition when these cells start secreting signaling molecules, equations 16 and 17 can be approximated with the following equations for simplicity:

$$\Omega([E]_{cell-p}) = \begin{cases} 0 & \text{if } [E]_{th} > [E]_{cell-p} \\ \frac{[E]_{cell-p}}{1+[E]_{cell-p}} & \text{if } [E]_{th} \leq [E]_{cell-p} \end{cases} \quad (18)$$

$$\Omega([C]_{cell-q}) = \begin{cases} 0 & \text{if } [C]_{th} > [C]_{cell-q} \\ \frac{[C]_{cell-q}}{1+[C]_{cell-q}} & \text{if } [C]_{th} \leq [C]_{cell-q} \end{cases}$$

Below a set threshold in the model ($[E]_{th}$ and $[C]_{th}$) there is no chemical secretion by the cells. Using a sigmoidal term instead of the transition threshold assumed here, does not affect the results qualitatively. The secretion of either EGF or CSF-1 from each cell must be distributed into all the lattice cubes that the cell is located in, therefore the secretion in each lattice cube becomes:

$$C_{sec}^{ijk} = \sum_{p\in(ijk)} \frac{S_{cell-p}^{ijk}}{S_{cell}} k_{sec-p}^{tumor} \mathscr{H}([E]_{cell-p} - [E]_{th}) \frac{[E]_{cell-p}}{1+[E]_{cell-p}} \quad (19)$$

$$E_{sec}^{ijk} = \sum_{q\in(ijk)} \frac{S_{cell-q}^{ijk}}{S_{cell}} k_{sec-q}^{macro} \mathscr{H}([C]_{cell-q} - [C]_{th}) \frac{[C]_{cell-q}}{1+[C]_{cell-q}} \quad (20)$$

where C_{sec}^{ijk} is the total amount of CSF-1 secreted by all tumor cells located in lattice cube ijk in each time step, the sum is over all tumor cells p that have some surface area (S_{cell-p}^{ijk}) in lattice cube ijk, k_{sec-p}^{tumor} is the CSF-1 secretion coefficient for tumor cell p and $\mathscr{H}$ is the Heaviside function. Similarly, E_{sec}^{ijk} is the total amount of EGF secreted by all macrophages located in lattice cube ijk in each time step, the sum is over all macrophages q that have some surface area (S_{cell-q}^{ijk}) in lattice cube ijk and k_{sec-q}^{macro} is the EGF secretion coefficient for macrophage q.

In each time step in the simulations, the change in concentration of the signaling molecules needs to be calculated with respect to the diffusion of the molecules, the secretion of signaling

molecules by cells and the depletion of signaling molecules at cell membrane and in the extracellular matrix. This is done using the following reaction-diffusion partial differential equations:

$$\frac{d[C]^{ijk}}{dt} = \mathscr{D}\nabla^2[C]^{ijk} + C_{sec}^{ijk} - k_{deg}^{csf^{ijk}}[C]^{ijk}$$

$$\frac{d[E]^{ijk}}{dt} = \mathscr{D}\nabla^2[E]^{ijk} + E_{sec}^{ijk} - k_{deg}^{egf^{ijk}}[E]^{ijk} \qquad (21)$$

where $\mathscr{D}$ is the diffusion coefficient for EGF and CSF-1 and $k_{deg}^{csf^{ijk}}$ and $k_{deg}^{egf^{ijk}}$ are the depletion coefficients for CSF-1 and EGF, respectively (further explained in section 3.3). The partial differential equations 21 are solved in each lattice cube in every time step using forward Euler's method with no flux boundary conditions.

In order for a tumor cell (macrophage) to start secreting CSF-1 (EGF) the local chemical concentration of EGF (CSF-1) has to be above a set threshold. However, in order for a cell to start migrating in the direction of the gradient both local concentration and steepness of gradient need to be above a set threshold in the model.

Ligand depletion

There are two different means of depleting the ligands in the model, local ligand depletion, k_{loc}, and external (global) ligand depletion, k_{ext}. Thus, the depletion coefficients in each lattice cube, ijk, are determined with the following equations:

$$k_{deg}^{csf^{ijk}} = k_{ext}^{ijk} + \sum_{q\in(ijk)}\frac{S_{cell-q}^{ijk}}{S_{cell}}k_{loc}^{csf},$$

$$k_{deg}^{egf^{ijk}} = k_{ext}^{ijk} + \sum_{p\in(ijk)}\frac{S_{cell-p}^{ijk}}{S_{cell}}k_{loc}^{egf}. \qquad (22)$$

where k_{loc}^{csf} is the CSF-1 local ligand depletion coefficient for macrophages and k_{loc}^{egf} is the EGF local ligand depletion for tumor cells. Equation 22 shows the cells can break down either EGF (tumor cells) or CSF-1(macrophages). The external ligand depletion coefficient, k_{ext}^{ijk}, is assumed to be uniform and the same for both EGF and CSF-1.

The local ligand depletion is a combination of three processes: 1) endocytosis, a portion of the bound ligands are internalized and degraded in the cell, 2) (MMP) degradation, the cells produce MMPs some of which bind to the cell membrane and break down ligands and 3) pinocytosis, macrophages engulf extracellular material. External ligand depletion occurs in the extracellular matrix and comes from two processes: i) degradation by enzymes secreted by the cell that diffuse freely through the extracellular matrix and ii) perfusion, the continuous addition and removal of material in the extracellular matrix caused by fluid flow. Assuming perfusion is constant, we can model it as an additional constant external ligand depletion. The external depletion constant is then a sum of those two processes.
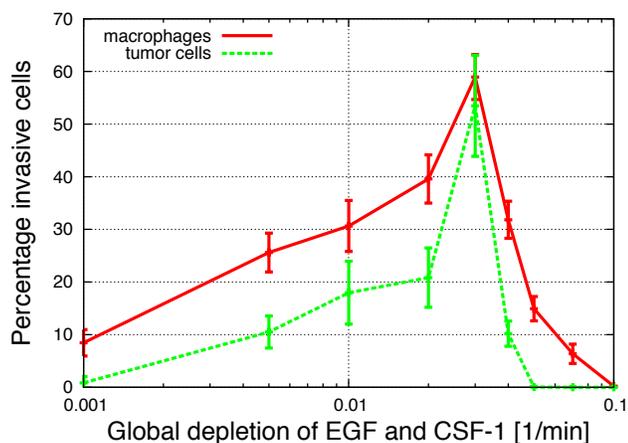
Fig. S-2 Increasing the global depletion of EGF and CSF-1 *in vitro*, results in a biphasic response. Initially, the number of invasive tumor cells and macrophages increased to a maximum around 0.03 min^{-1}. At higher global depletion rates, the number of invasive tumor cells and macrophages decreased to zero. When the global ligand depletion was too large, the macrophages could not detect the CSF-1 from the top because the CSF-1 concentration was below the detection threshold. See S4-Movie and S5-Movie for video with global depletion of 0.03 and 0.07 min^{-1} respectively.

S2 Appendix: Supplemental Results

Changes in global depletion of EGF and CSF-1 *in vitro*

Increasing the global depletion of EGF and CSF-1 resulted in a biphasic response in the number of invasive cells (Fig S-2). As the global depletion increased from zero, the percentage of invasive tumor cells and macrophages increased to a maximum. Increasing the depletion rate further resulted in a rapid decline in a number of invasive cells. When the global decay was low, the CSF-1 concentration built up close to the plate. As explained before, most macrophages could not detect an upwards CSF-1 gradient and thus very few cells invaded. As the global decay was increased, the CSF-1 gradient from the top was enhanced and more macrophages invaded followed by the tumor cells. However, when the decay was too high, the CSF-1 signal from the top was attenuated and the macrophages had difficulty detecting the gradient. At 0.1 min^{-1} global depletion rate neither macrophages nor tumor cells invaded.

Changing the EGF and CSF-1 concentration detection thresholds *in vitro*

As mentioned in the model description, in order for the cells in the simulations to be able to sense gradients, polarize and secrete signaling molecules, the concentration of the signaling molecule needs to be above a set concentration threshold. Increasing the CSF-1 concentration detection threshold for the macrophages resulted in a nearly constant percentage of collected tumor cells and macrophages until a threshold of 1nM was reached. At detection thresholds above 1nM the number of collected cells decreased rapidly until no cells were collected (Fig S-3 A). At higher CSF-1 detection threshold, it took the macrophages longer to detect
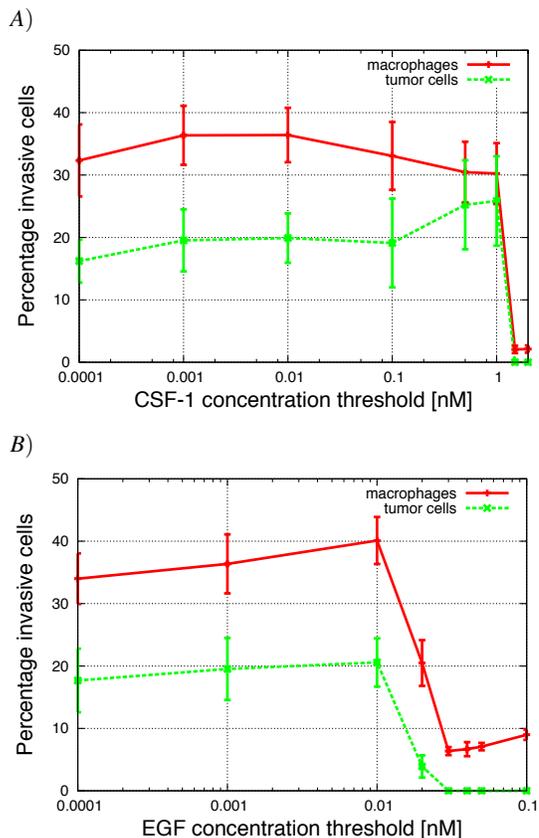

Fig. S-3 The concentration threshold is the concentration of signaling molecules around the cells needed for them to both start secreting their own signaling molecule and detect gradients. Increasing the A) CSF-1 concentration threshold and B) EGF concentration threshold results in a decreasing number of invasive cells until no cells became invasive.

the CSF-1 signal from above, and that could have given the tumor cells more time to get closer and follow the macrophages upwards. Increasing the EGF detection threshold had a slightly different effect on invasiveness than incresing the CSF-1 threshold. As the EGF detection threshold was increased to about 1 nM, the number of both invasive tumor cells increased slightly (Fig S-3 B). Further increase in the EGF threshold lead to a fast drop in the number of invasive cells. As before, the tumor cell–macrophage interaction helped the macrophages get out of the flat CSF-1 signal in the boundary region. However, for higher EGF thresholds, the tumor cells did not chemotact towards the macrophages and the macrophages got stuck in the boundary region and only around 8% of the macrophages invaded. This is similar to the situation in Fig 3 B where the EGF secretion was too low and there was no mechanical tumor cell macrophage interaction. At this point no tumor cells invaded because they could not detect the EGF signal.

Changing the external CSF-1 source from the media *in vitro*

The external source of CSF-1 in the experiments comes from the media located about 750–1,000 μm above the cells. To explore the effect that the CSF-1 source has on the invasiveness of cells, we systematically increased the CSF-1 source in the simulations
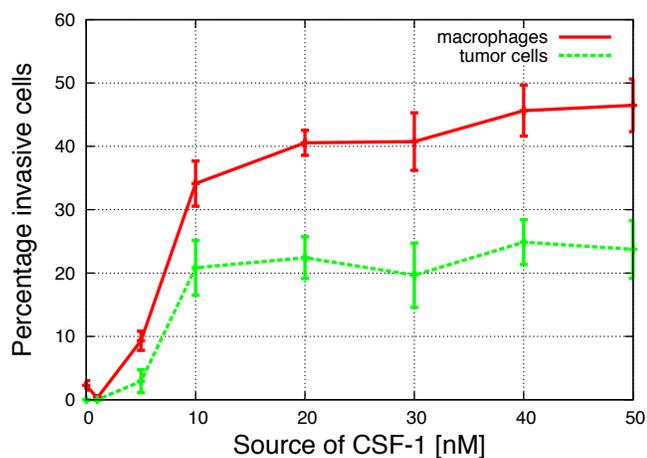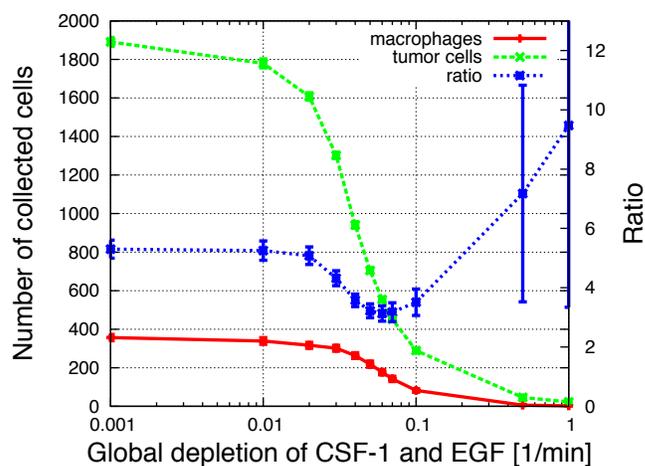
Fig. S-4 Increasing the concentration of CSF-1 in the media resulted in increased percentage of invasive cells. The media was located 750 μm above the cells. There was no further increase in the number of invasive cells as the CSF-1 concentration was increased past 40 nM mostly due to over-saturation of signal at the bottom.

Fig. S-5 Increasing the global depletion of EGF and CSF-1 for the *in vivo* simulations resulted in a decrease in the number of tumor cells and macrophages collected in the needle. The larger global ligand depletion caused the CSF-1 concentration to be below threshold for cells that were located further away from the needle opening. See S9-Movie for GLD = 0.01 min^{-1}.

(Fig S-4). As CSF-1 was increased from zero, the number of both invading tumor cells and macrophages increases. However, the increase in invasiveness was more rapid for macrophages. The higher the CSF-1 source, the less time it took for the macrophages to detect the CSF-1 gradient and thus some invaded sooner, often before a tumor cell could follow. Maximum cell invasion, 25% invasive tumor cells and 45% invasive macrophages, occurred when the CSF-1 source was 40 nM. Above this CSF-1 concentration, the percentage of invasive cells remained roughly constant because of over-saturation of CSF-1 at the bottom due to the no flux boundary condition.

Changing global depletion of EGF and CSF-1 *in vivo*

In these *in vivo* simulations, the global ligand depletion, GLD, represented the natural removal of the ligand, the degradation of the ligand by soluble MMPs and perfusion (removal of ligand from fluid flow). Therefore, the benchmark global depletion in these simulations was higher than in the *in vitro* simulations. Increasing the GLD of both EGF and CSF-1 had little effect at first, but once GLD increased past 0.01 min^{-1} the number of collected tumor cells and macrophages decreased rapidly and went to zero for high GLD (Fig S-5). Increased GLD lead to increased attenuation of the EGF from the needle, so only cells that were close to the needle detected the signal, whereas for cells further away the concentration was below threshold. The tumor cell/macrophage ratio remained ~ 5 until GLD > 0.01 when it decreased to a minimum of 3 for GLD = 0.05 min^{-1}. Above GLD of 0.1 min^{-1} less than 10 macrophages were collected in the needle, thus the ratio could not be accurately determined because the variation was too large.
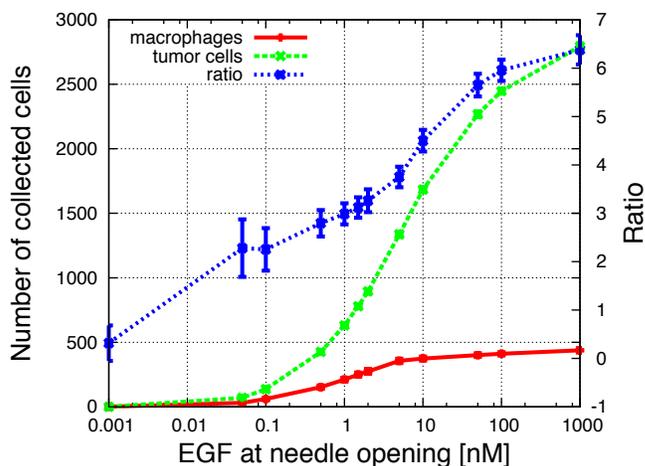
Fig. S-6 Increasing the concentration of EGF at the needle opening resulted in an increase in the number of tumor cells and macrophages collected in the needle. The number of collected tumor cells increased faster than the collected macrophages when the EGF concentration was increased, and thus the tumor cell/macrophage ratio increased.

Changing the EGF concentration in the needle *in vivo*

Increasing the EGF concentration at the needle opening in the simulations resulted in an increase in collected tumor cells (Fig S-6). The number of collected macrophages also increased because the CSF-1 signal from the tumor cells diffused outwards so the surrounding macrophages could follow the tumor cells to the needle. However, the number of collected tumor cells increased at a faster rate, and consequently the tumor cell/macrophage ratio increased. It should be noted that this is a semi-log plot so the ratio remains around 3 for EGF needle concentration between 0.5-1.3 nM. The higher concentration of EGF in the needle enabled cells

located further away from the needle opening to detect a gradient of EGF from the needle and chemotact towards it.