Simulation of Chemotractant Gradients in Microfluidic Channels to Study Cell Migration Mechanism *in silico*

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Abstract: Cell migration of endothelial cells along gradients is an important process in vivo and an interesting target for cancer therapeutics. Microfluidics offer very powerful tools to study such migration processes in detail in the lab. In order to optimize microfluidic systems multiphysics simulations are very well suited. In this study, we describe a model to simulate molecular gradients in a diffusion based microfluidic gradient generator and how a cell senses these gradients via cell receptors. The results show the importance of incorporating the binding reaction into the model and that there is a large difference between the molecular gradient in solution and the gradient sensed by the cell. The approach presented here allows capturing of the whole process from gradient formation with a microfluidic channel to binding of molecules to cell receptors and allow a better prediction of parameters for cell experiments.

Keywords: Cell migration, Cell-receptor interaction, microfluidic, molecular gradient, VEGF

1. Introduction

Directed cell migration along molecular, chemotractant gradients in solution plays an important role in many in vivo processes, from early embryogenesis to wound healing to cancer [1]. One particular process is angiogenesis, during which new blood capillaries are formed to reestablish or improve blood circulation in a certain part of the body in response to e.g. low oxygen levels. To activate and control this process in vitro is a crucial step for tissue engineering larger organ constructs that can be successfully transplanted. Angiogenesis is furthermore a key component in cancer biology, where neovascularization facilitates cancer growth, and will eventually lead to metastases formation. The ability to limit neovascularization of tumors is a promising target in cancer therapy and a better understanding of the underlying processes might help to improve them.

During angiogenesis, endothelial cells migrate from existing blood capillaries along a chemotractant gradient and in successive steps form new blood capillaries. One very potent migration stimulant for endothelial cells is vascular endothelial growth factor (VEGF) that is recognized by cells via special cell surface receptors. VEGF is secreted by cells exposed to low oxygen levels and diffuses out from that area through the tissue and extracellular matrix, resulting in a concentration gradient forming with a high VEGF concentration at its origin. Endothelial cells will migrate along this gradient towards the oxygen depleted area, form new blood capillaries and reestablish normal physiological oxygen levels. [2]

The ability for a cell to sense a chemical gradient depends on a difference in cell receptor occupation along the cell. The difference between the number of VEGF molecules at front and the back needs to be large enough to evoke a cell response. To study the underlying cell biological processes in detail and see how they can be influenced, demands a robust and flexible in vitro cell culture system capable of forming controlled gradients. One emerging technology that is suitable for this task is microfluidics, which is capable of forming molecular gradients with spatial and temporal resolution unmet by other techniques. For the work presented here, we used a diffusion based microfluidic gradient generator, because it enables us to form gradients on the cellular length scale without exposing the cells to shear stress. This is especially important when working with endothelial cells since they are very shear stress sensitive.

In this study, Comsol Multiphysics are used to simulate interactions of the cell receptor VEGFR-2 and a VEGF-A gradient formed within a microfluidic network. It is building upon the earlier results focused on the design of the microfluidic network. The aim was to find suitable parameters, such as inlet concentration and flow velocity, for cell experiments and understand processes that cannot be studied otherwise.

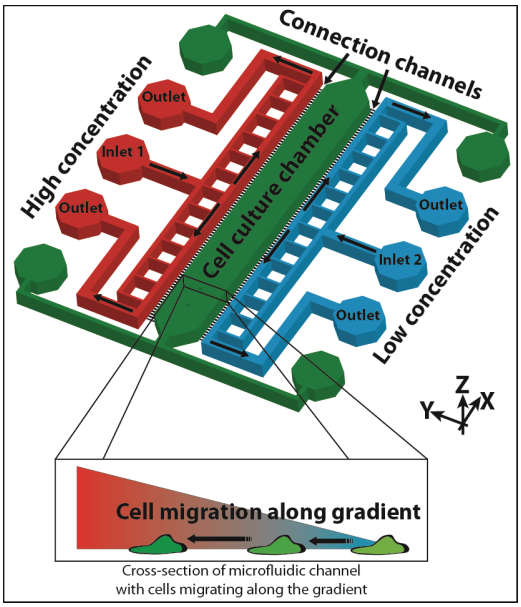

Figure 1. Principle of the microfluidic setup used to study cell migration along chemotractant gradients.

2. Governing equations

In order to mimic the complexity of the system and have a model that closely resembles the properties of the microfluidic channel, a three-dimensional model approach was used. The microfluidic network was designed with AutoCAD2010 and imported into Comsol 4.2, before a model cell geometry was added directly in Comsol to have full control over its position and size. Therefore, it was possible to use a parametric sweep function to change the position of the cell, thus mimicking a cell moving along a concentration gradient.

The whole channel geometry is meshed with free tetrahedral structures at element sizes between 30 and 100 µm. This is sufficient to model the concentration and flow of VEGF within the microfluidic channels and simultaneously keeping the simulation time at a reasonable level. To increase the precision close to the cell surface a boundary layer mesh with 4 layers is added at the cell surface.

Navier Stokes equations for incompressible fluids are used to model the flow in the system. Due to the small dimensions of microfluidic channels, the viscous forces are typically much larger than the inertial forces which is expressed in a low Reynolds number, hence the flow was modeled as laminar. The boundary conditions for inlet 1 and 2 are defined as normal inflow velocity (u_{inlet}), whereas the outlet boundary condition is set to "Pressure, with no viscous stress".

The concentration of soluble VEGF in the channels is solved with mass balance equations from the transport of diluted species module. Transport of VEGF occurs via diffusion, diffusion coefficient D_{VEGF}, and convection; the convection part is coupled to the velocity field from the Navier Stokes equations solved in the laminar flow module. The initial concentration of VEGF ($c_{VEGF\_t=0}$) in the channel is set to 0 and VEGF enters the system through inlet 1 (c_{VEGF_inlet1}) (Figure 1). The inlet concentration (c_{VEGF_inlet1}) is changed with a parametric sweep function to study the effects of different inlet concentrations on gradient formation and cell sensing. The concentration at inlet 2 is always 0 and VEGF is transported out of the model domain by fluid flow through the outlets. Furthermore, a flux boundary conditions is used to model transport to and from the cell surface (eq. 1).

$$Inward\ flux = -(r_{on} + r_{off}) \qquad (eq.1)$$

The binding of VEGF to its cell surface receptor is modeled as a surface reaction that is limited to the area of the cell. On the cell surface two probing areas (2x5x30 µm) are defined, "cell front" and "cell back", to study differences of VEGF binding to its receptor at the front and back of the cell under gradient conditions (Figure 2). The difference between both probes ($\Delta\int cs_{VEGF}$) is the main readout from the model to estimate cell responses for a given gradient condition, as cells need to sense a difference over their membrane in order to be able to respond. The reaction kinetics of the VEGF-to-receptor binding are coupled to the amount of soluble VEGF through the flux boundary condition in the transport of diluted species module (eq. 1).

Binding rate:

$$r_{on} = k_{on} \cdot c_{VEGF} \cdot VEGFR_{free} \cdot VEGFR_{density} (eq.2)$$

Release rate:

$$r_{off} = k_{off} \cdot VEGFR_{bound} \cdot VEGFR_{density} \qquad (eq.3)$$

The density of available VEGF receptor binding sites is defined by $VEGFR_{density}$, the ratio of free receptor sites by $VEGFR_{free}$ (ranging

from 0=no free sites to 1=all sites free) and the ratio of bound receptor sites by $VEGFR_{bound}$. VEGF binds to the receptor in a one-to-one manner (site occupancy 1) and the affinity of the binding is defined by the association constant k_{on} and the dissociation constant k_{off}. It is assumed that there is no surface bound VEGF (cs_{VEGF}) at the beginning of the simulation. Furthermore, surface bound VEGF does not react any further on the surface and a no flux boundary conditions on the edge of the cell is used to restrain surface bound VEGF to the cell surface. Figure 2 shows an overview about the surface reaction model and table 1 gives the initial values for the different parameters.

The calculations of the model are carried out in a two-step process. The first step calculates the stationary solution for the fluid flow field and the second step calculates the time-dependent solution for the chemical transport and reaction. For the first step, a fully coupled approach is used with an iterative GMRES linear solver. The second step is calculated with a segregated method with one part solving for VEGF concentration in solution with an iterative linear GMRES solver and one part solving for VEGF concentration on the surface with a direct linear PARDISO solver.

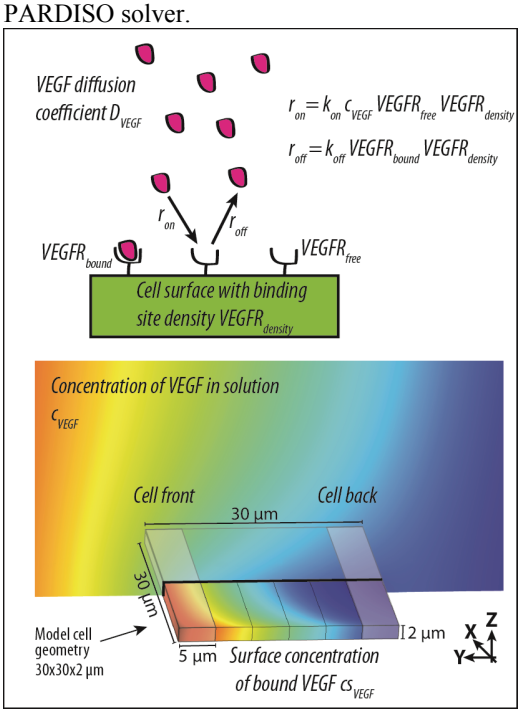

Figure 2. Surface reaction model used to simulate VEGF binding to cell receptors.

Parameter	Value	Unit	Description
c_{VEGF}	calculated	mol/m^3	VEGF concentration in solution
$c_{VEGF\ t=0}$	0	mol/m^3	VEGF concentration in the network at t=0
$c_{VEGF\ inlet\ 1}$	$1.25 \cdot 10^{-5}$ – $1.25 \cdot 10^{-8}$	mol/m^3	VEGF concentration at inlet 1 (parametric sweep)
cs_{VEGF}	calculated	mol/m^2	VEGF concentation bound to the surface
$cs_{VEGF\ t=0}$	0	mol/m^2	VEGF concentation bound to the surface at t=0
$VEGFR_{density}$	$3.4 \cdot 10^{-9}$	mol/m^2	VEGF receptor density [2]
$VEGFR_{free}$	calculated	Ratio 0-1	Ratio of free VEGF receptors
$VEGFR_{bound}$	calculated	Ratio 0-1	Ratio of bound VEGF receptors
u_{inlets}	$6.25 \cdot 10^{-5}$	m/s	Flow velocity at inlet 1 & 2
D_{VEGF}	$2 \cdot 10^{-6}$	cm^2/s	VEGF diffusion constant [2]
k_{on}	$3.6 \cdot 10^{6}$	L/mol·s	Association rate constant VEGF-VEGFR [2]
k_{off}	$2 \cdot 10^{-6}$	1/s	Dissociation rate constant VEGF-VEGFR [2]
$Cell_{position\text{-}y}$	-800 -400 0 400 800	µm (from the center of the channel)	Cell position in y-direction (parametric sweep in study)

Table 1. Variables and parameters used in the simulation model.

3. Results

3.1 Gradient formation

The microfluidic network, excluding the cell geometry, was simulated under stationary conditions to study the overall capability of the system to form molecular gradients and their dependence on flow speed. Figure 3A shows the relative concentration of c_{VEGF} (color coded) in the microfluidic network in x-y direction. The gradient changes along the x-axis and diminishes completely at the sides. Therefore, cell studies should be limited to the area indicated in the figure where the gradient properties are nearly constant, as evaluated with additional simulations (data not shown). Figure 3B shows gradient profiles along a line in y-direction in the center of the main chamber for different flow rates at inlet 1 and 2. The gradient is steeper for higher flow velocities, but the linear portion of the gradient is larger for lower flow velocities.

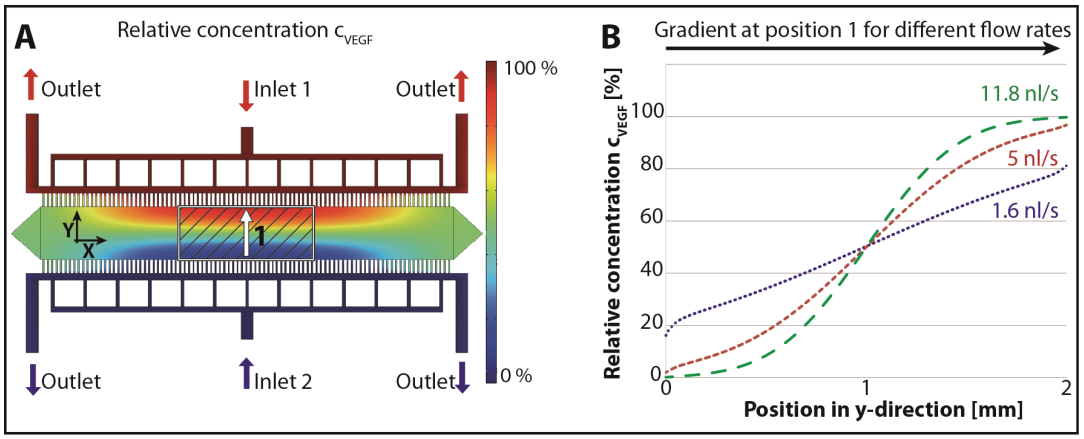

Figure 3. Gradient in solution generated by microfluidic diffusion gradient network. A) Color coded VEGF concentration throughout the whole network (inlet flow rate 5 nl/s). B) Concentration line profiles of the gradient at the center of the main chamber (position 1) for different inflow rates.

3.2 Surface reaction at different positions

In the second set of simulations, the cell geometry was included and the binding of VEGF to its receptor was modeled as a surface reaction. The position of the model cell on the y-axis was changed with a parametric sweep function and its position on the x-axis was kept constant in the center of the channel. The different positions are indicated in figure 4A. For all simulations, the inflow rate was kept at 5 nl/s and the surface reaction was simulated over 24 hours with a step size of 120s.

Figure 4B shows the color coded c_{VEGF} concentration in the x-y plane at a height of 3 micrometers throughout the main channel. The cell has a height of 2 micrometer, thus the plane is 1 micrometer above the cells. There is a clear depletion of c_{VEGF} directly over the cell and the gradient profile is disturbed. To illustrate this effect further, line profiles are taken in the x-y plane throughout the whole channel at the position indicated with the dotted line.

The line profiles (figure 4C/D) show the expected linear shape seen in previous simulations, but have a dip directly above the cells. This dip gets more pronounced at lower inlet concentrations 4D, where it also increases from position 5 to 1.

The extension of this local depletion in z-direction for position 3 is illustrated in figure 5. In figure 5B, VEGF concentration in the y-z plane is shown. The plane is placed in the center of the channel and going right through the middle of the cell. The line in the image indicates the position of the line profiles shown in 5C/D at the center of the cell. The line profiles show the concentration (c_{VEGF}) over the height of the channel for different time points and two different inlet concentrations.

At t=10min nearly no VEGF has reached the center of the channel, due to the long distance from the source channel. With progressing time, VEGF concentration increases and reaches steady state conditions before t=24h, as expected from other simulations on gradient formation. The approaching VEGF front is, however, not homogenous throughout the height of the channel; the VEGF concentration near the cell is lower than further up in the channel. Again, the differences are more pronounced for an inlet concentration of 1.25×10^{-8} than for 1.25×10^{-6} mol/m^3. At the higher concentration, the main depletion effect is limited to a height of 40 micrometer and nearly completely gone in the center of the channel. For the lower concentration the effect is extending much further into the channel and first starts to diminish at 100 micrometer.

To investigate further how the depletion will affect the gradient sensed by a cell, the integrated surface concentration $\int cs_{VEGF}$ from the front and the back of the cell were calculated. Figure 6 shows the data over time for all five cell positions. The slopes of the integrated surface concentration $\int cs_{VEGF}$, as well as the steady state concentration differ for the five positions, but equilibrium is reached within the simulation time frame for all of them. Figure 6B shows a

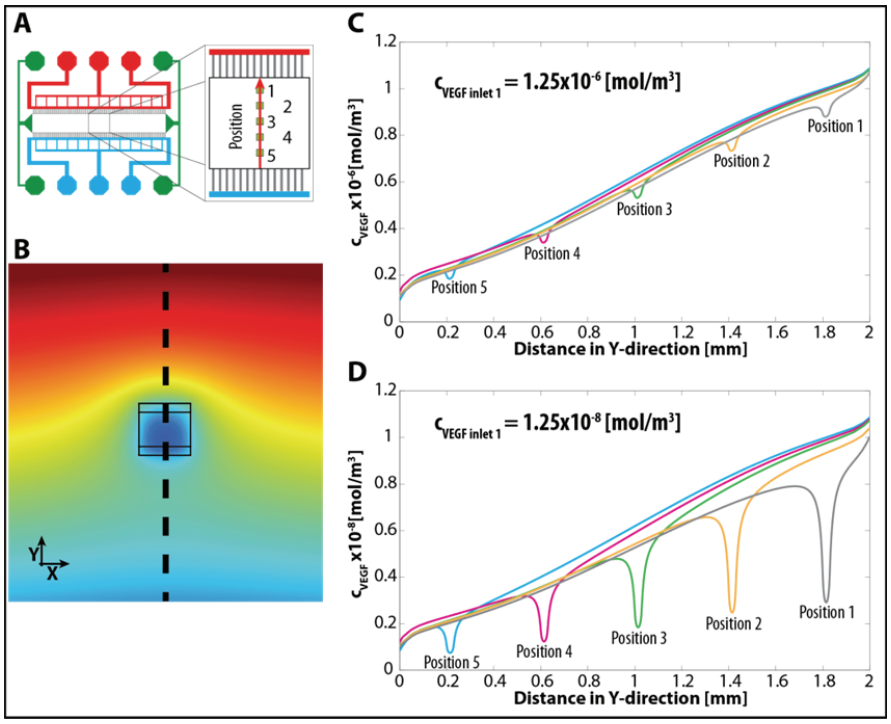

Figure 4. Local depletion of VEGF in solution in a plane in x-y direction at a height of 3 µm.

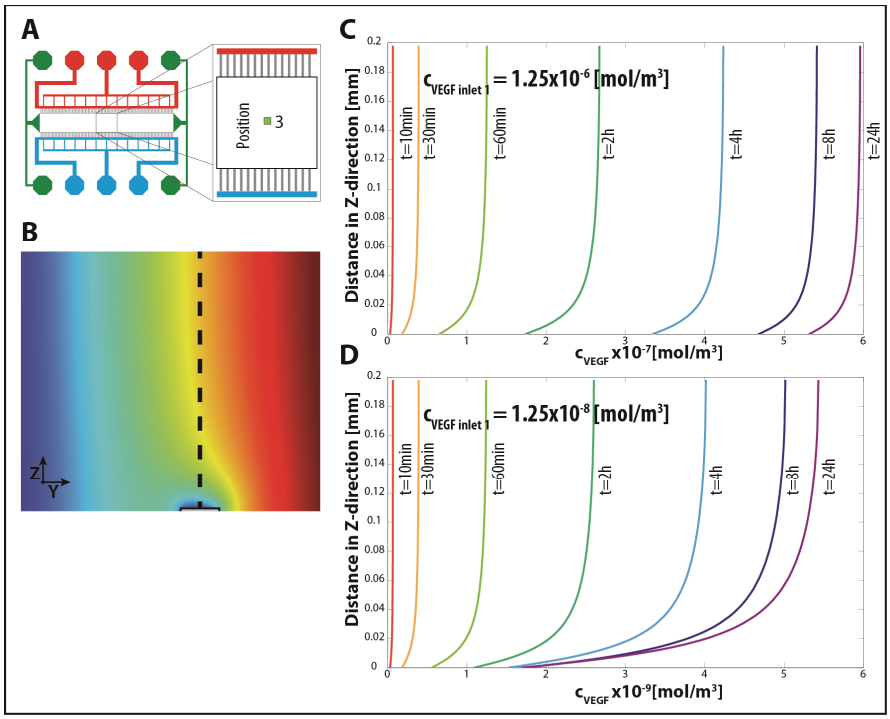

Figure 5. Extension of local depletion of VEGF in solution in z-direction

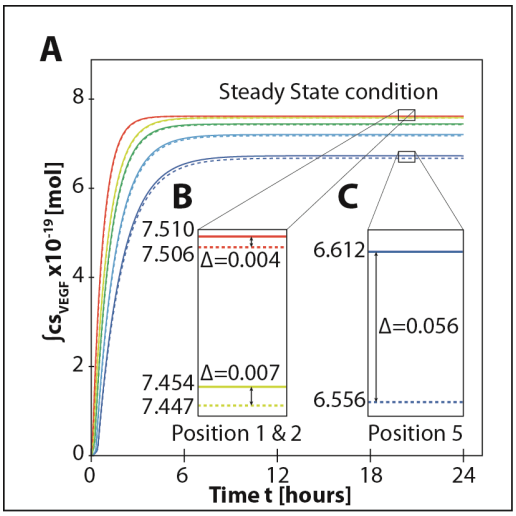

Figure 6. Integrated surface concentration over time at the front (solid line) and back (dashed line) of a cell at different position (as defined in Figure 4) in the channel for an inlet concentration of $1.25x10^{-7}$

magnification of the steady state condition at position 1 & 2 and figure 6C at position 5. The difference between the front and the back of the cell changes for the different positions, it is highest for position 5 ($5.6x10^{-21}$ mol) and lowest for position 1 ($0.4x10^{-21}$ mol). For each position a difference between the front and the back of the cell can be observed. Furthermore, the receptors are not saturated for any position at an inlet concentration of $1.25x10^{-7}$ mol/m^3, as saturation for the probing area is $7.82x10^{-19}$ mol.

3.4 Effect of different inlet concentrations

From the previous simulations, it was clear that the inlet concentrations had a large effect on the size of the depletion zone around a cell. Therefore, additional simulations were carried out to see how the inlet concentration of inlet 1 would influence $\Delta\int cs_{VEGF}$ between the front and the back of the cell at different positions.

Figure 7A shows $\Delta\int cs_{VEGF}$ plotted against the inlet concentration for all 5 positions. The shape of the curves for the different positions is similar, but the amplitude of $\Delta\int cs_{VEGF}$ differs; it is highest for position 5 (nearest to the sink) and lowest for position 1 (nearest to the source). For high inlet concentrations $\Delta\int cs_{VEGF}$ is smallest and increases with decreasing inlet concentrations, until reaching a maximum between 1.25 and $4x10^{-7}$ mol/m^3 ($c_{VEGF\ inlet\ 1}$). For lower inlet concentrations there is a sharp decrease of $\Delta\int cs_{VEGF}$.

In order to see how strong the migration stimulus for a cell would be along the channel, $\Delta\int cs_{VEGF}$ was plotted against the position along the y-direction. Figure 7B shows the data for an inlet concentration of $1.25x10^{-7}$ mol/m^3. It is clear that the difference between the front and the back of the cell is not constant along the channel. There is a drop in $\Delta\int cs_{VEGF}$ from 9.16 to $3.85x10^{-21}$ mol over the whole channel, which is most pronounced towards the source channel with a high concentration of VEGF.

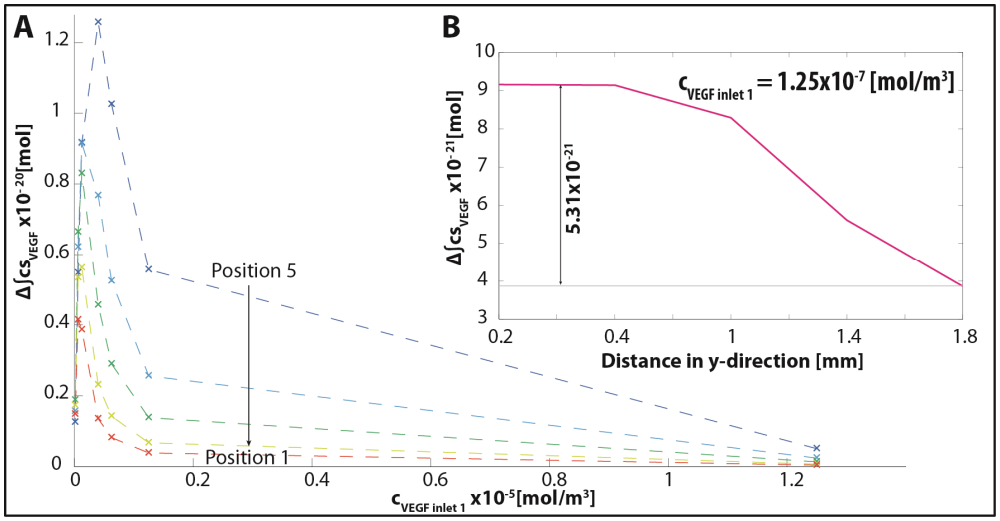

Figure 7. Difference in integrated surface concentration between the front and the back of the cell in dependence of inlet concentration and cell position

4. Discussion

Comsol simulations of the microfluidic network were initially used during the design process of the chip prior to production to ensure optimal functionality [3]. The model was then extended and developed further to the version presented here to study binding of VEGF to its cell receptor in gradients. This model is used to estimate cell migration behavior in response to chemical gradients and find optimal experimental conditions for cell experiments. The optimal inflow rate found in the initial simulations was 5 nl/s, where the gradient is steep enough and linear over a large part of the cell culture chamber to ensure constant cell stimulation, and we used this flow rate in all new simulations.

The ability to predict VEGF concentrations in liquid throughout the microfluidic network is interesting, but it is even more important to study how such a gradient will be sensed by the cell. Our simulations showed that the cell is influencing gradient properties and locally deplete VEGF in solution. The cell is acting as a sink for VEGF molecules and for low inlet concentration the effect is more pronounced, because the total number of molecules is small. The depletion is extending in all directions and this local alteration is both affecting the cell itself, but could potentially also effect other cells in close proximity. We did not perform any multi-cell simulations, but it can be assumed that this would extend the effect even further, because each cell will act as an individual sink.

This depletion effect has some severe consequences for the way a cell perceives the VEGF gradient, as shown with the $\Delta\int cs_{VEGF}$ data. This data shows that the difference between the front and the back of the cell is changing over the length of the channel. If the VEGF gradient would be directly imprinted onto the cell surface, $\Delta\int cs_{VEGF}$ should be constant for a linear gradient, because the slope would be the same for all positions. Due to the depletion, however, the concentration of VEGF in solution directly above a cell is altered and does not longer have a linear shape. If the concentration of VEGF in solution is very low, depletion will have a large effect and $\Delta\int cs_{VEGF}$ gets lower, because the cell is in the center of the depletion dip shown in figure 4. If the concentration of VEGF in solution is very high, the receptors on the cell surface will saturate and also result in a minimal difference between the front and the back. In-between those two extreme conditions, there is an optimum inlet concentration for which $\Delta\int cs_{VEGF}$ is largest at all positions. The extent of the depletion is depending both on the position along the channel and the inlet concentration, because both alter the number of VEGF molecules available directly above the cell. Moving the cell towards the source channel will increase the concentration, as will a higher concentration of VEGF in the inlet, at least to some degree as the gradient steepness will increase.

The extent of local depletion and its effects on cell surface gradients could not be predicted without incorporating the cell into the model and this data enables us to fine tune experimental parameters in a much more precise manner. It should however be noted that the model presented here does not cover the whole signaling mechanism of VEGF on cells, but is focused on the most potent stimulation of endothelial cells by VEGF-A binding to VEGF receptor 2. There are, however, other VEGF forms and receptors that will influence the interaction, for example VEGF-A also binds to VEGF receptor 1 without stimulating cell migration. [2] Nevertheless, we feel that our approach is capable of representing VEGF cell signaling in an appropriate way and is much more predictive than simulation solely based on concentration gradients in solution.

Overall, this study demonstrates the ability to model cell microenvironments and their interactions with great detail, by coupling fluid dynamics with the transport of chemotractant molecules that interact with modeled cell surfaces. Previous approaches have modeled either the gradient formation [4] or the cell-molecule interaction [2], [5]. By combining the two into a single model it is possible to study for example effects of flow velocity and local depletion on a single cell level, while still modeling the whole microfluidic network.

5. Conclusions

The model developed in this study allowed us to simulate the gradient sensed by a cell in a microfluidic network much better than with previous approaches. There is a clear difference between the gradient in solution and the gradient

on the cell surface, which has severe consequences for cell experiments. The difference in receptor-bound signaling molecules between the front and back of a cell varies non-linearly as a function of cell position across the gradient, as well as inlet concentration of signaling molecules. With the model from this study suitable experimental parameters can be predicted with higher certainty and experiments carried out in a more efficient ways.

6. Acknowledgements

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