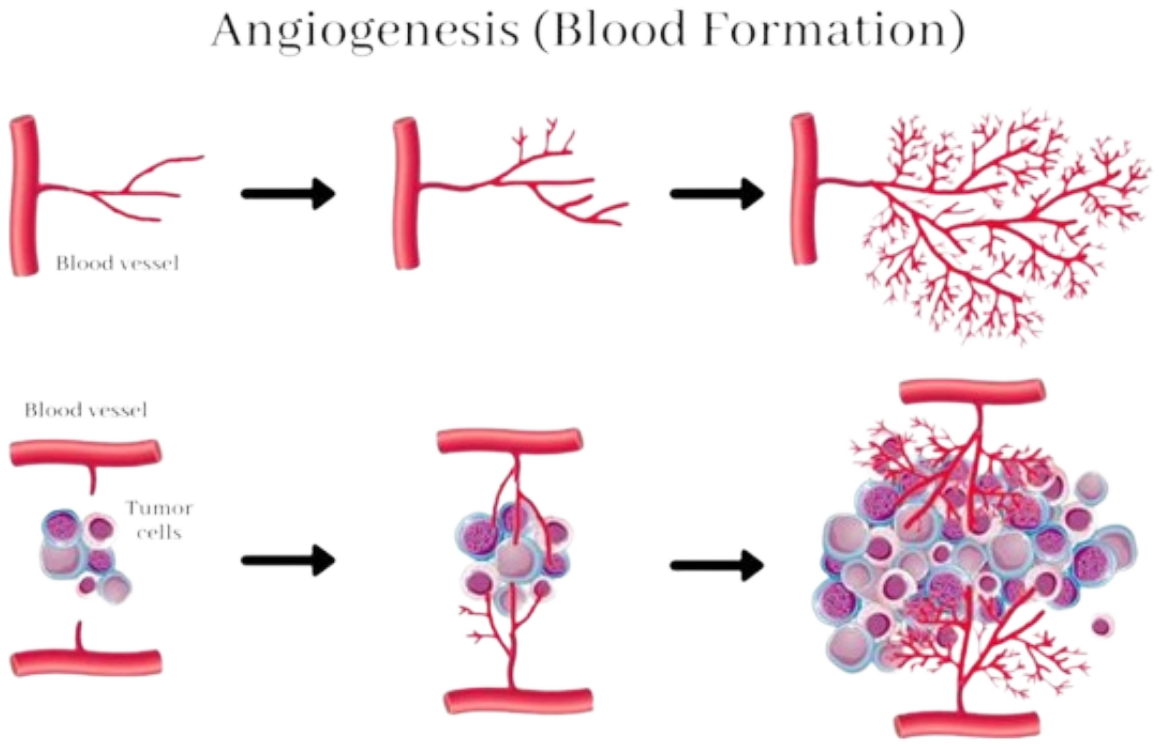
Modelling of Extracellular Vesicles behaviour

Overview

Computational modelling helped us to simulate extracellular vesicles behaviour inside the vascular system. Indeed, we have considered several administration methods such as local injection or intra-arterial injection. This model focuses on intra-arterial injection. Several problems arise when we talk about injecting extracellular vesicles in the blood system.

The first one is the reduction of blood supply around cancerous cells. It is mainly due to pressure increase in the interstitial environment that lead to reduction in diameter of capillaries. There is also an anarchic angiogenesis caused by the presence of the tumour. In fact when a tumor develops, the cell located in the center of it does not receive enough oxygen and nutrients. This state is called hypoxia. It makes the cancerous cells produce a factor (HIF-1α) that provokes the activation of angiogenesis. It finally leads to a disorganised production of capillaries.



From Madu, C.O., Wang, S., Madu, C.O., Lu, Y. (2020). Angiogenesis in Breast Cancer Progression, Diagnosis, and Treatment. Journal of Cancer, 11(15), 4474-4494. https://doi.org/10.7150/jca.44313.

Figure 1 : Blood vessel formation in presence of tumor cells.

The second one is the presence of a fibrosis around cancerous cells which is called the tumour stroma. It is made of fibroblasts and an extracellular matrix. This can hinder extracellular vesicles' progression to the cancerous cells.

For these reasons, it is interesting to simulate extracellular vesicles propagation inside the capillaries to have a better appreciation of the intra-arterial injection.

Objectives

Hence to understand the consequences of tumor development on the surrounding capillaries two models will be done :

1. A model to see pressure and speed differences between different cases of capillaries.
2. A model to see extracellular vesicles propagation through capillaries to the interstitial space.

Both of these models will be done with COMSOL Multiphysics (6.3 version) a finite element analyzer, solver and simulation software. More specifically, two modules will be mainly used :

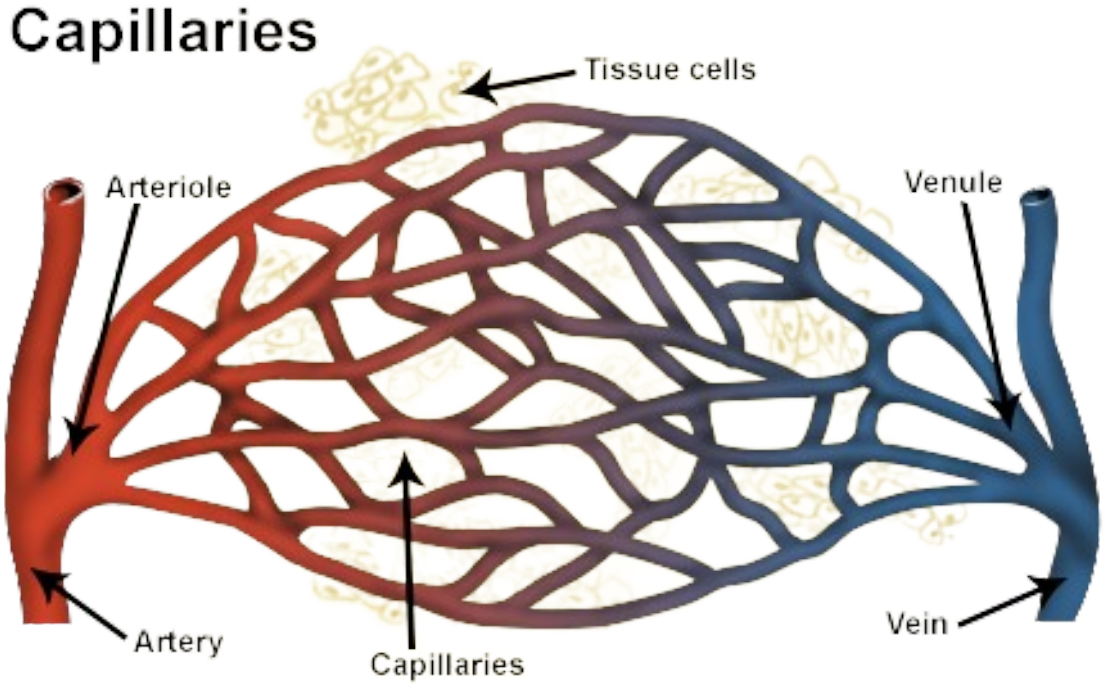
- Transport of diluted species from the chemical species transport branch, used to calculate the concentration field of a dilute solute in a solvent.
- Creeping flow interface to simulate fluid flow at a low Reynold number.

Every data used has been found in literature.

Development

Capillary network model

Most of the pancreatic ductal adenocarcinomas are located in the head of the pancreas (60~70%) vascularized by the superior and inferior pancreaticoduodenal arteries. It can also be present in the body and the tail (15% each) both vascularized by the splenic artery [1]. These arteries reduce in size to become arterioles and finally capillaries.



National Cancer Institute, National Institutes of Health - http://training.seer.cancer.gov/anatomy/cardiovascular/blood/classification.html

Figure 2 : Structure of blood system

This shrinking is accompanied by a modification of the vessel's walls. Arteries are more to endure the pressure of blood coming out of the heart, while arterioles are less elastic and more rigid. Most of the exchange between interstitial environments are done at capillaries levels. Three kinds of capillaries exist : continuous, fenestrated and sinusoidal.

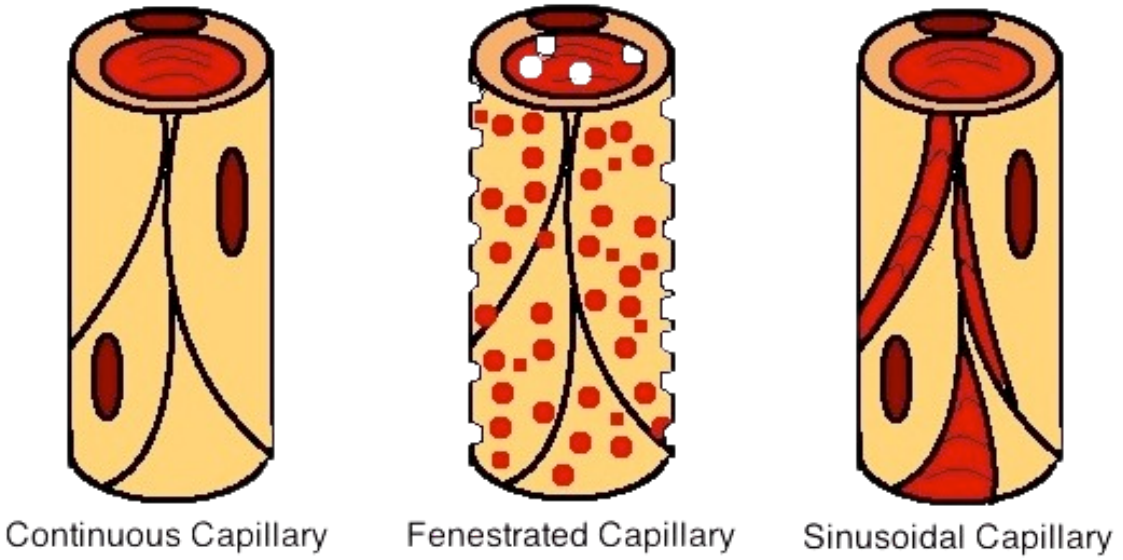


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Figure 3 : Each kinds of capillaries

We will focus on continuous capillaries that constitute most of the capillaries around the cancerous cells. The fenestrated ones are present in the surrounding but less than the continuous.

To simplify the model we will only keep arterioles. The goal is firstly to model a simple arteriole to take charge of the software.

Simple Arteriole Model

To simplify the computation the arteriole will be represented by a cylinder. Arterioles under normal conditions have a diameter around 30 micrometers [2] and a wall-to-lumen ratio around 0.4 [3].

$$WLR = \frac{2 \times wall\ thickness}{Lumen\ Diameter}$$

Figure 4 : Wall-to-lumen ratio formula

In our case we will take a diameter of 36 micrometers that hence lead to a 29.88 micrometers large arteriole lumen. To avoid edge effects we also choose a length of 1 millimeter.

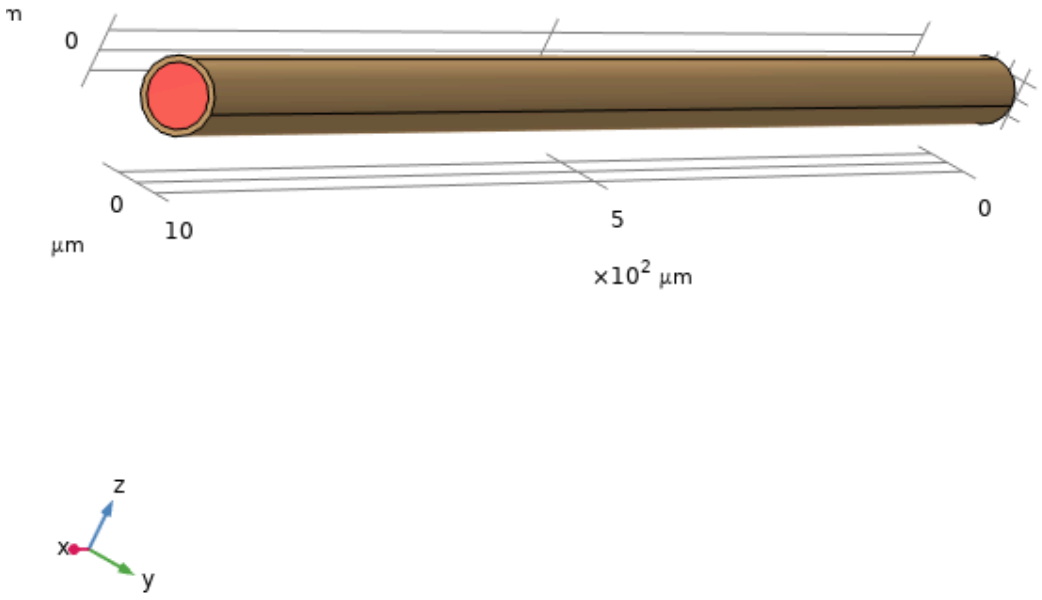

Figure 5 : The arteriole. In red the blood. In brown the wall.

As the Reynolds number for a blood flow inside an arteriole is relatively low (around 0,0006 [4]) it can be simulated with a Stokes flow (typically for Re much smaller than 1). Blood is a non-Newtonian fluid which means that its viscosity can change depending on the constraint. Hence, under pathological abnormalities such as tumor the viscosity can raise up to 8 cP [5]. The required parameters for Stokes flow are pressure and fluid velocity. For arteriole, pressure is around 35 to 40 mmHg and blood velocity between 1.2 and 2mm/s [6]. Nevertheless, the velocity can fluctuate depending on the distance from the center of the arteriole. Finally to define the blood in materials required basic properties : volumic mass and dynamic viscosity. Blood has a volumic mass of 1060 kg/m^3 [7] and a dynamic viscosity of 4 cP [5].

As it is a small part of the arteriole that is represented we will assume that pressure does not fluctuate all along the cylinder. By doing a stationary study we obtain the following results.

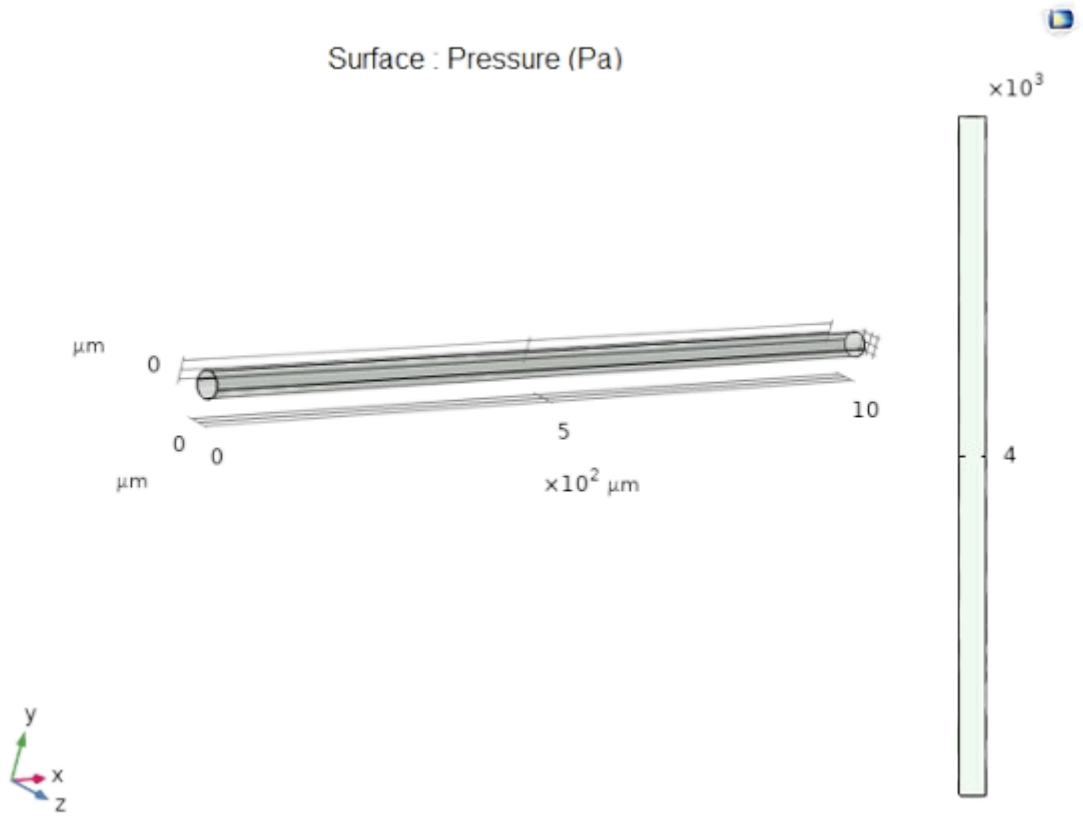

Figure 6 : Pressure distribution in the arteriole.

As expected the pressure is homogenous all along the cylinder.

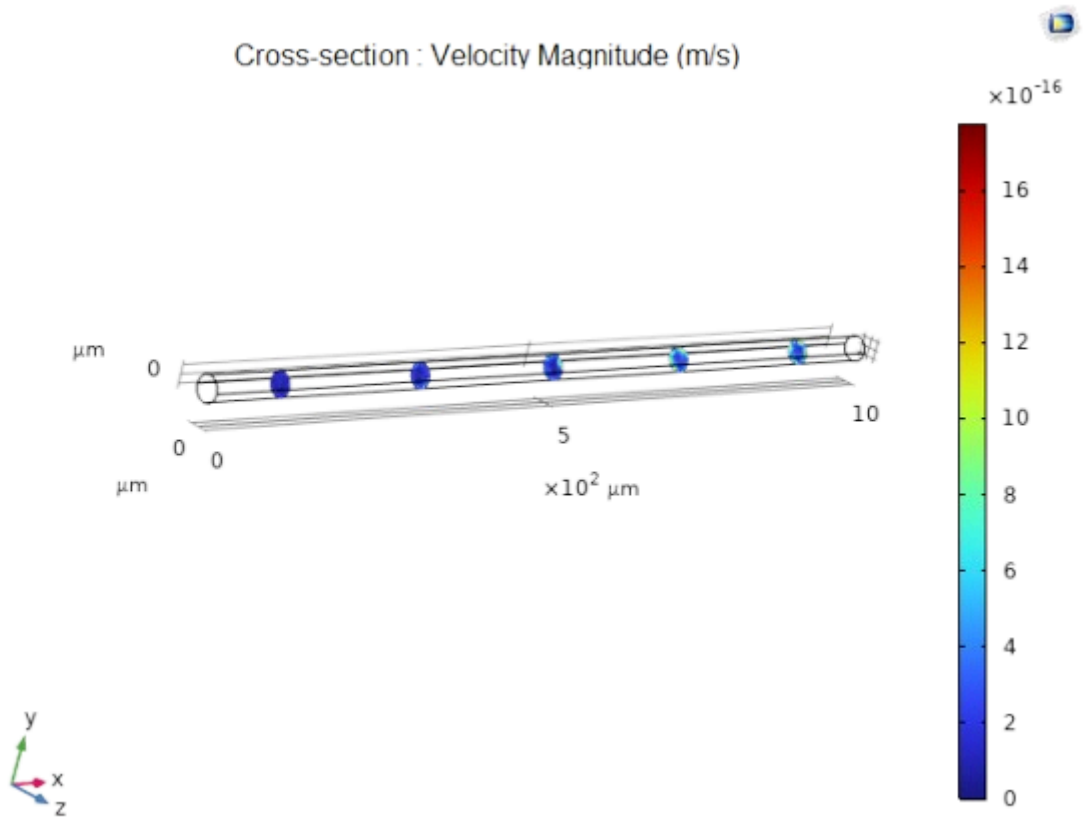

Figure 7 : Cross-section : Velocity magnitude in m/s.

As there is no difference in pressure between the entrance and the exit of the cylinder the velocity is very low everywhere in the cylinder. Seeing these results, we can move on to a more complete modeling.

Capillary and arteriole model

After modelling a simple arteriole the goal is now to model a complete arteriole capillary transition. To do so several parts are necessary : an arteriole, a capillary and a transition between them.

For the arteriole, the previous model will be kept as a basis. However the length of the arteriole will be reduced to 100 micrometers to simplify computations.

For the capillary, the goal is to represent a realistic form of capillary. Hence a straight line would not be accurate. Then it will be represented by a bent cylinder with a straight portion in the middle. Capillary diameter varies in presence of a tumour. In ordinary conditions capillaries are about 8 micrometers large [8]. When a tumor appears interstitial pressure increases which lead to a reduction in capillary diameters from 8 to 4 micrometers. As capillaries' wall thickness is about 0.2 to 0.3

micrometers it will not be represented in the geometry. To keep a realistic aspect the capillary section of the geometry will have a length around 50 micrometers.

Without a design module or external CAD software it was difficult to create a smooth and realistic transition between arteriole and capillary. Hence the most realistic way was to do a half-spheric transition on which capillary will start.

The initial goal was to simulate blood flow for several kinds of capillaries, to finally form a capillary network. Three kinds of capillaries have been tested.

Capillary	Normal	Cancerous	Anastomosed
Specificity	8µm diameter capillary A bent capillary	4µm diameter capillary A bent capillary	6µm diameter capillaries Anastomose of two capillaries in one

Figure 8 : Table of capillaries specificities.

Normal Capillary

With the previous parameters we obtain the following geometry for a normal capillary.

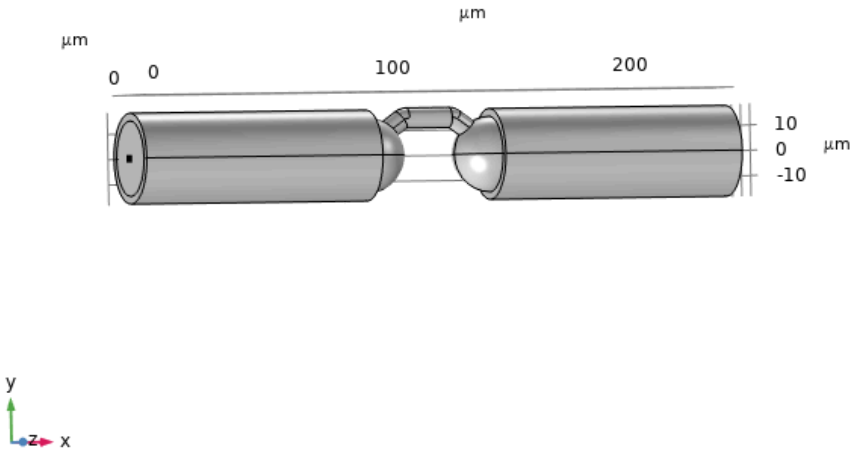

Figure 9 : Geometry of normal capillary.

For the Stokes flow an initial velocity field was necessary. In the geometry it appears consistent that the velocity field is only expressed on the x-axis. As blood velocity ranges from 1m/s near the aorta to 1mm/s in the capillary, the value chosen for the initial velocity field is 2.5 mm/s [6]. To model the blood flow, two boundary conditions are required. For the arteriole side as the previous model an initial pressure of 40 mmHg is chosen. For the veinule side the pressure imposed as a boundary is 10mmHg.

Another physic is implemented to model extracellular vesicles distribution. It is the transport of diluted species module. As extracellular vesicles sizes are ten to hundred times smaller than blood vessels involved in the geometry and because they are numerous, they can be considered as a diluted species in order to simplify the computation. In this model the initial concentration of species is 10µmol/m^3 which is around the concentration of extracellular vesicles produced in 2 days by a HEK 293 cell culture. Another parameter is the diffusion coefficient of the extracellular vesicles inside blood it can be calculated with the following formula :

$$D_{eff} = \frac{\varepsilon}{\tau} \times D_{free}$$

Figure 10 : Diffusion coefficient formula.

Considering the following values for blood porosity $\varepsilon = 0.2$, tortuosity $\tau = 1.4 - 1.6$ and $D_{free} = 1.12 \times 10^{-12} m^2/s$ [9][10], we get a diffusion coefficient of $1.6 \times 10^{-13} m^2/s$.

After computation we get the following results for velocity, pressure and concentration.

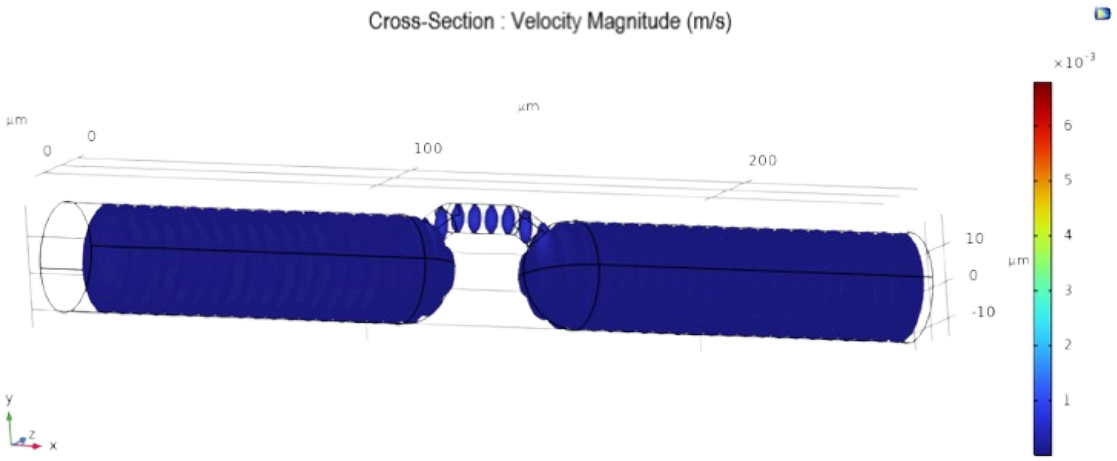

Figure 11 : Velocity for normal capillary.

We observe a small acceleration in the center of the capillary but blood flow velocity does not fluctuate a lot in this case.

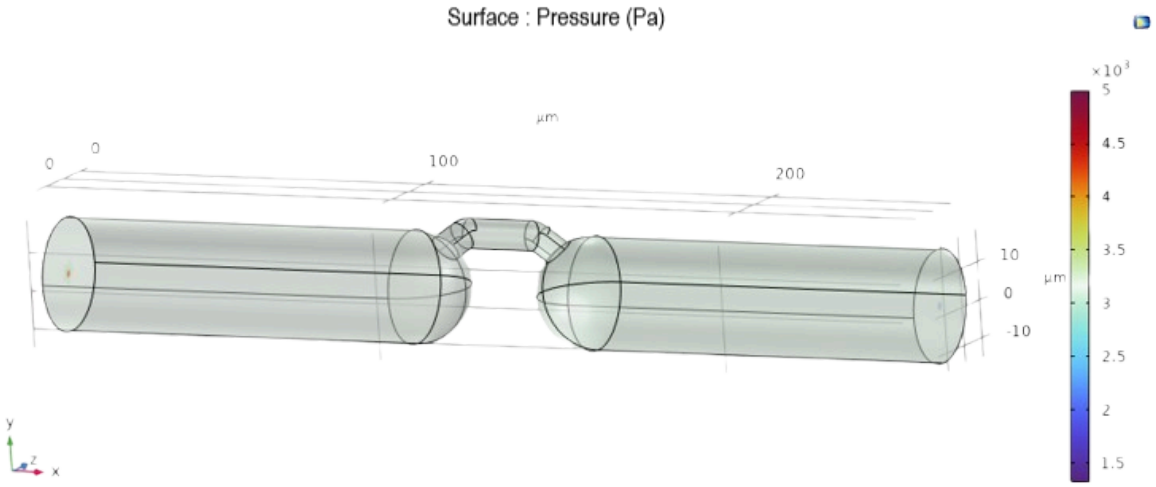

Figure 12 : Pressure for normal capillary.

The pressure difference observed is relatively low which is consistent with the small velocity increase in the capillary.

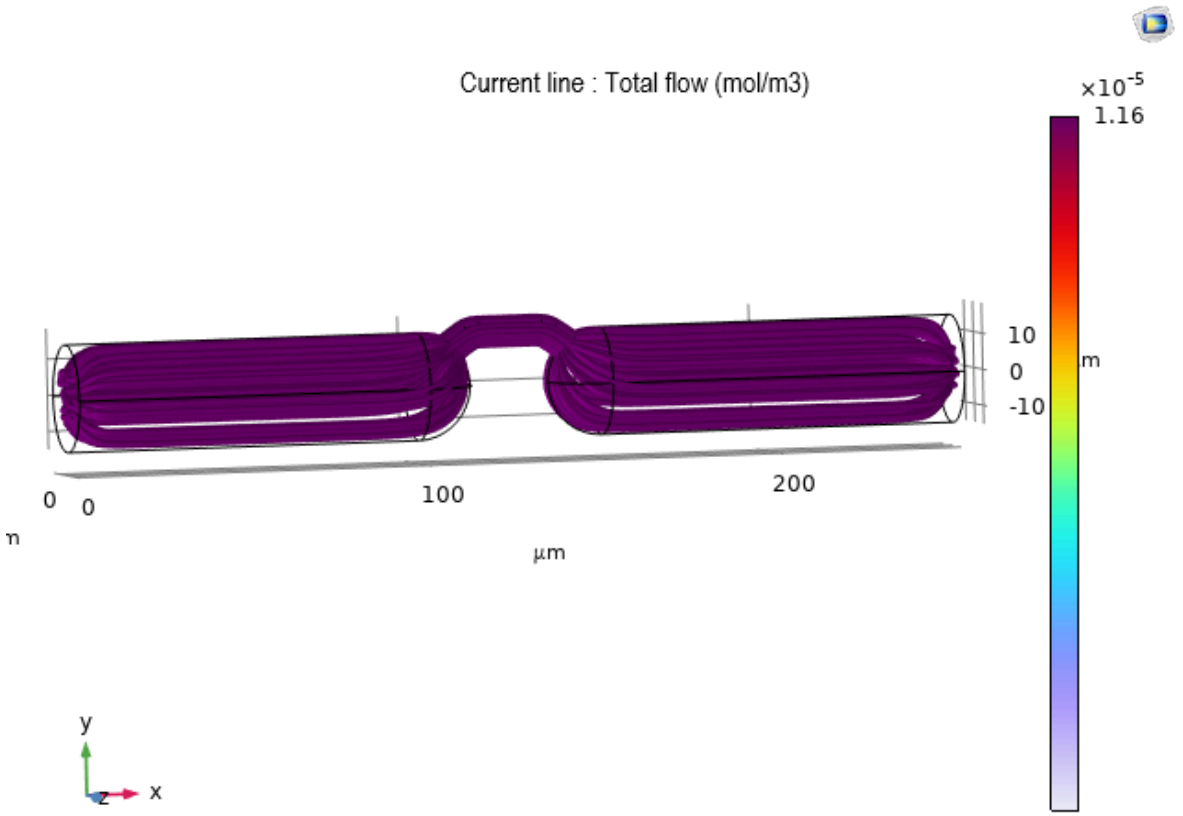

Figure 13 : Current line for normal capillary.

Current lines are parallel to each other which is consistent with the laminarity of the blood flow. We observe an uniform concentration of species all along the current line.

Now it has to be compared with the other cases.

Cancerous Capillary

As specified above, cancerous capillary is slightly smaller than the normal one with a diameter of 4 micrometers.

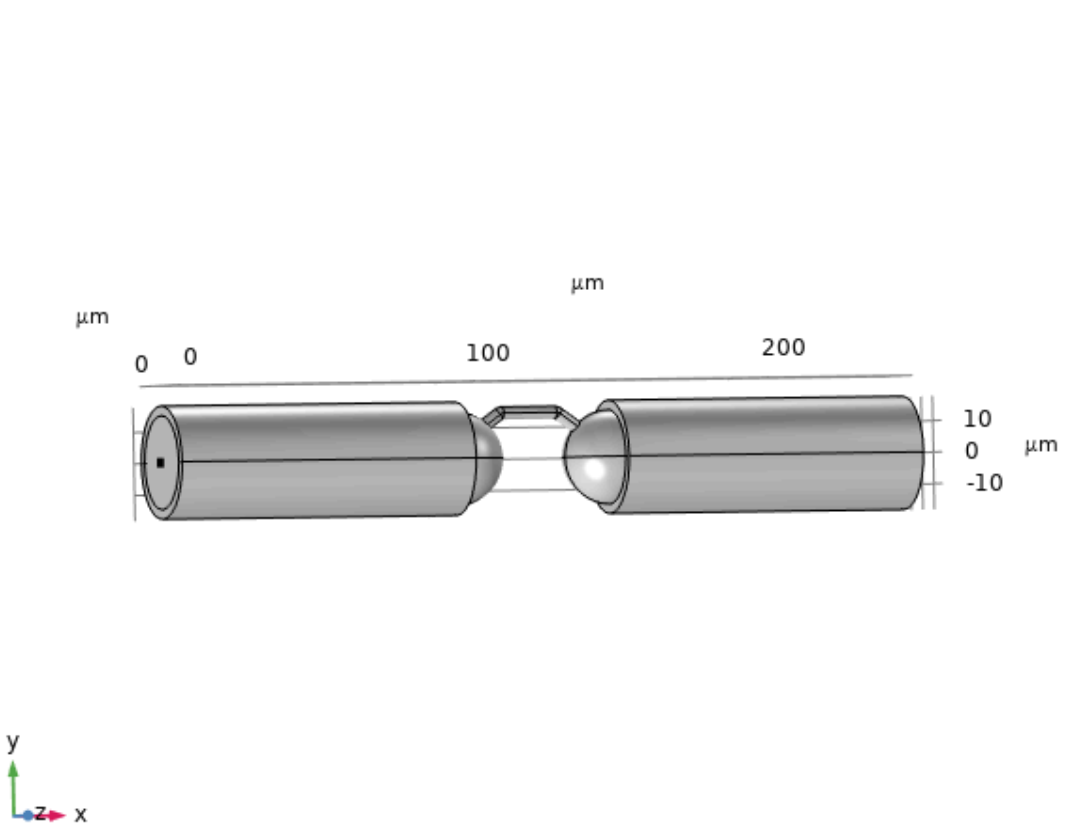

Figure 14 : Geometry for a cancerous capillary.

With the same flow parameters as before, we obtain the following results.

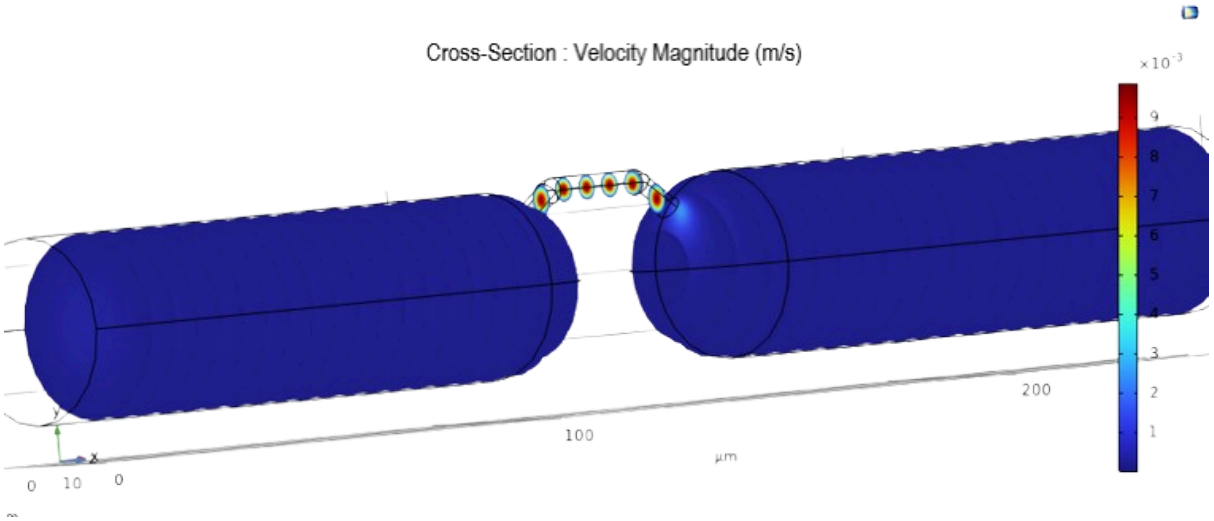

Figure 15 : Velocity for cancerous capillary.

A substantial acceleration is visible in the center of the capillary. It could possibly make it more difficult for the extracellular vesicles to propagate through the capillary membrane.

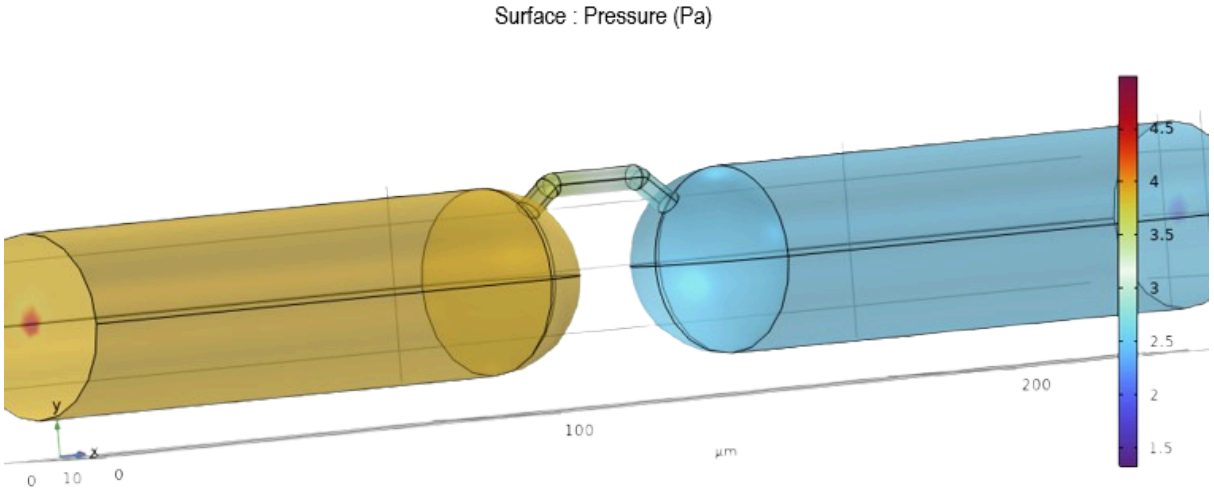

Figure 16 : Pressure for cancerous capillary.

The pressure difference observed before and after the capillary is consistent with the acceleration observed above. It is also one of the consequences of the presence of a tumor around vessels.

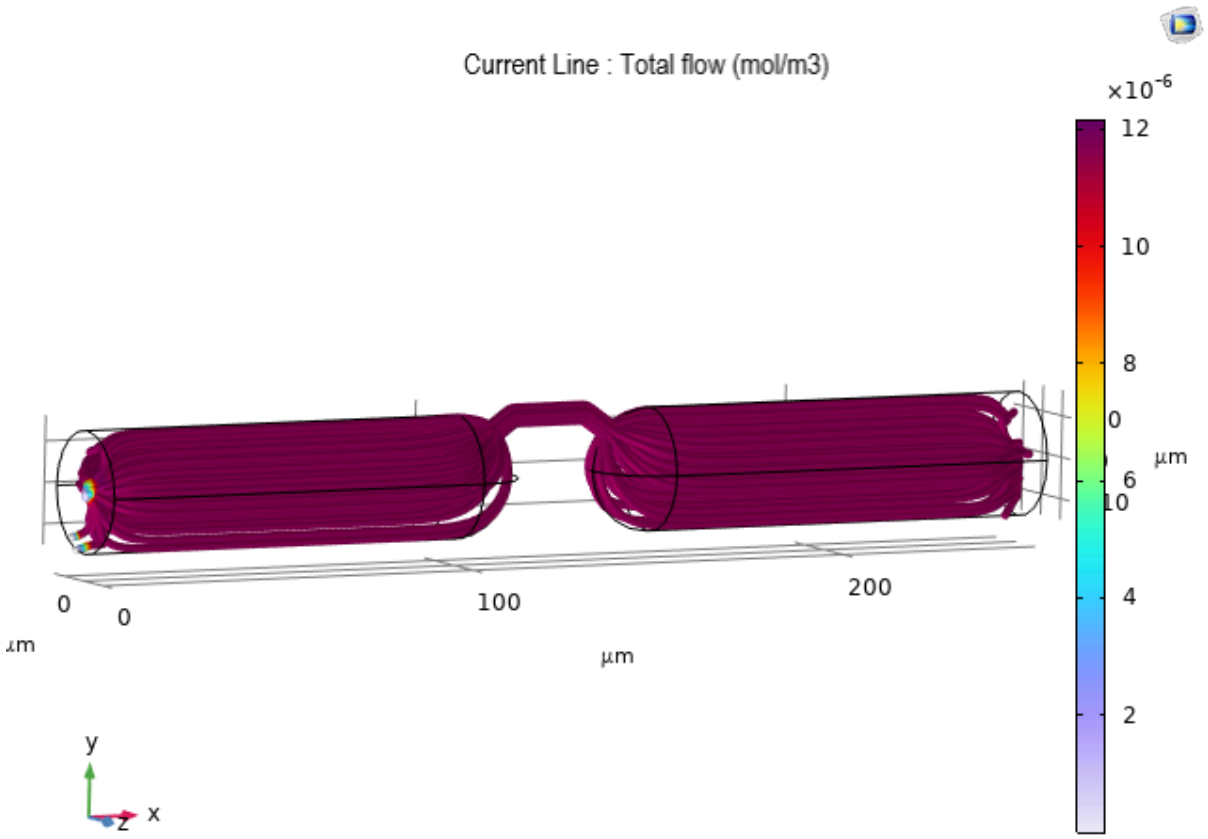

Figure 17 : Current line for cancerous capillary.

As for the normal capillary, the current lines observed are laminar and there is an homogeneous concentration of species in the system.

These results can be discussed as the deformation of the capillary should normally perturbate the blood flow.

Anastomosed Capillaries

In this case capillaries present an anastomosis, two capillaries became one. The diameter of the capillary is also reduced from 8 to 6 micrometers.

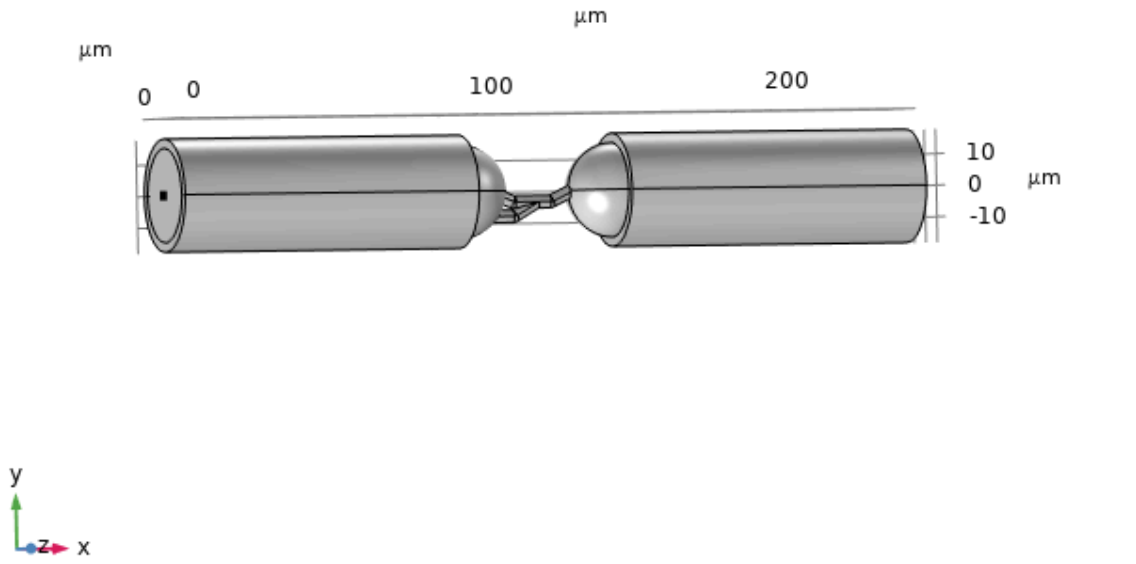

Figure 18 : Geometry for anastomosed capillaries.

We also keep the same flow parameters as before.

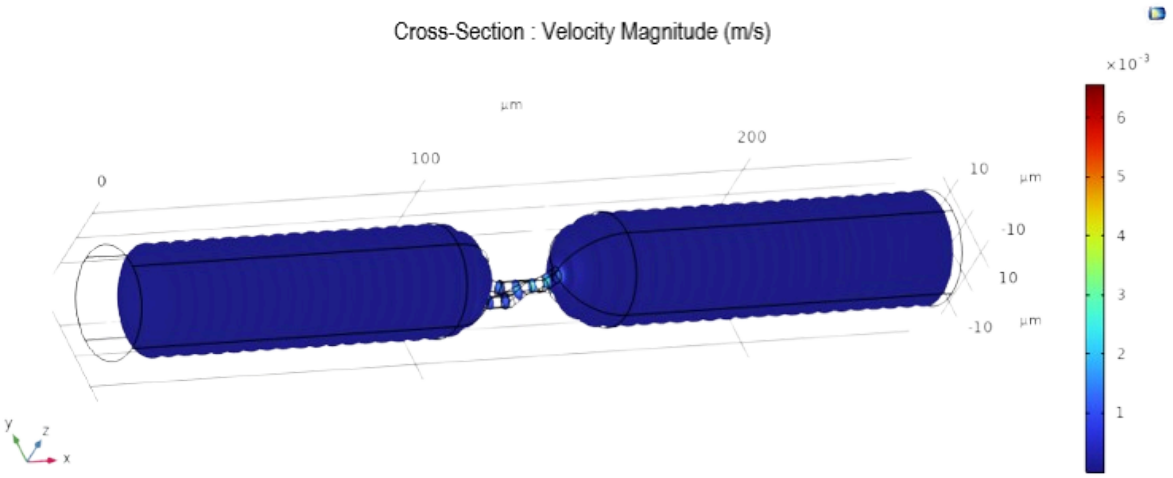

Figure 19 : Velocity for anastomosed capillaries.

As the cancerous capillary we can see a small increase in velocity in the center of the capillaries. Nevertheless, it is less important than for cancerous capillary.

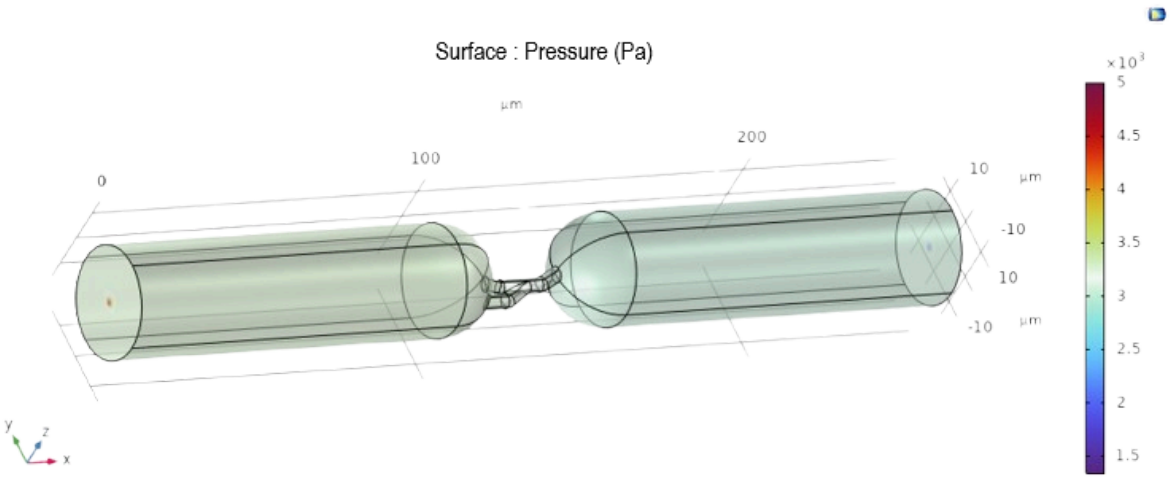

Figure 20 : Pressure for anastomosed capillaries.

Pressure difference is less prominent than for the cancerous capillary.

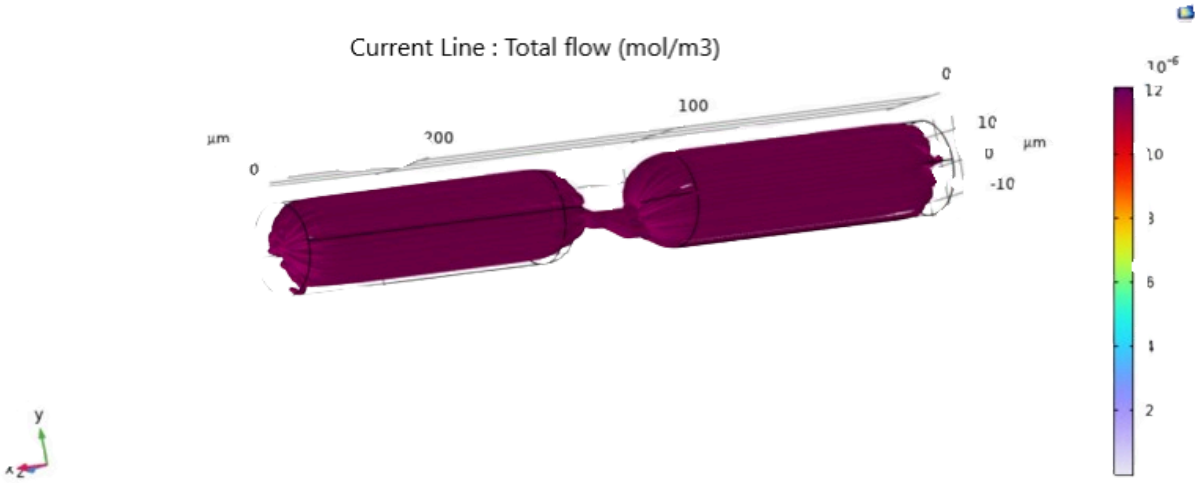

Figure 21 : Current line for anastomosed capillaries.

We also observe a laminar flow with the current line, which is also questionable due to the obstacle presented by the anastomosis.

These several models show blood flow aspects in different situations. The goal is now to model an interstitial environment - capillary interface. It will allow us to observe extracellular vesicles propagation through capillary to the interstitial environment.

Capillary-Interstitial environment interface model

Based on the previous results, the goal is now to elaborate a simple system to simulate capillary - interstitial environment interface.

The Stokes flow module will be kept with the same parameters for velocity. The boundary conditions for pressure will be 15 mmHg at the entrance and 10 mmHg at the exit.

To simulate the transport of diluted species through capillary wall and interstitial environment the transport of diluted species through porous media will be used. Three kinds of porous media will be defined.

To define porous media, two coefficients are required : tortuosity and porosity. The most simplified description of tortuosity is the arc-chord ratio: the ratio of the length of the curve (*C*) to the distance between its ends (*L*). Porosity is a measure of the fraction of void inside a material. It fluctuates between 0 and 1.

The blood with a diffusion coefficient identical to the previous model. It will be defined as a fluid.

The capillary wall with a diffusion coefficient calculated from the diffusion coefficient of the blood, the porosity and the tortuosity coefficients. For a capillary wall the porosity is about 0.2. The tortuosity is obtained through the Millington & Quirk model.

$$\tau_{F,i} = \varepsilon_p^{-\frac{1}{3}}$$

Figure 21 : Tortuosity formula.

Then, the interstitial environment. In this case the porosity equals to 0.3 which is slightly higher than for capillary wall. The other parameters are obtained with the same formula as for capillary wall.

The geometry is composed of three elements. We only consider half of the total system due to the symmetry of it. Capillary is represented by a 50 micrometers long and 2 micrometers large rectangle (in red). Capillary wall is represented by a 50 micrometers long and 0.4 micrometers large rectangle which is the thickness of cells composing capillary wall(in brown). The interstitial environment is represented by a 50 micrometers long and 20 micrometers large rectangle (in yellow).

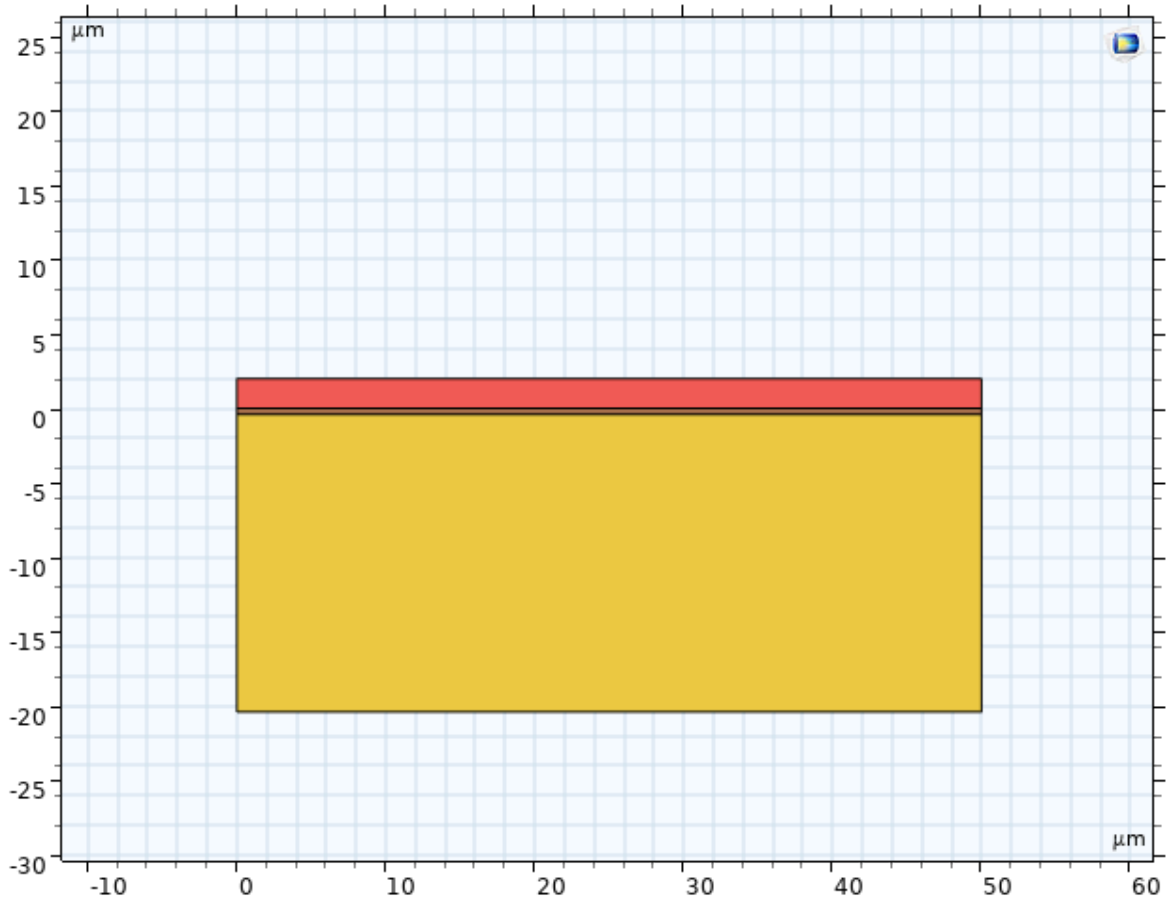

Figure 22 : Geometry of the interface model. In red the capillary. In brown the capillary wall. In yellow the interstitial environment.

The capillary entrance is situated on the left frontier of the capillary rectangle and the exit on the right one. The goal is to test several concentrations of species and several durations. To begin, we keep the same concentration of extracellular vesicles as above.

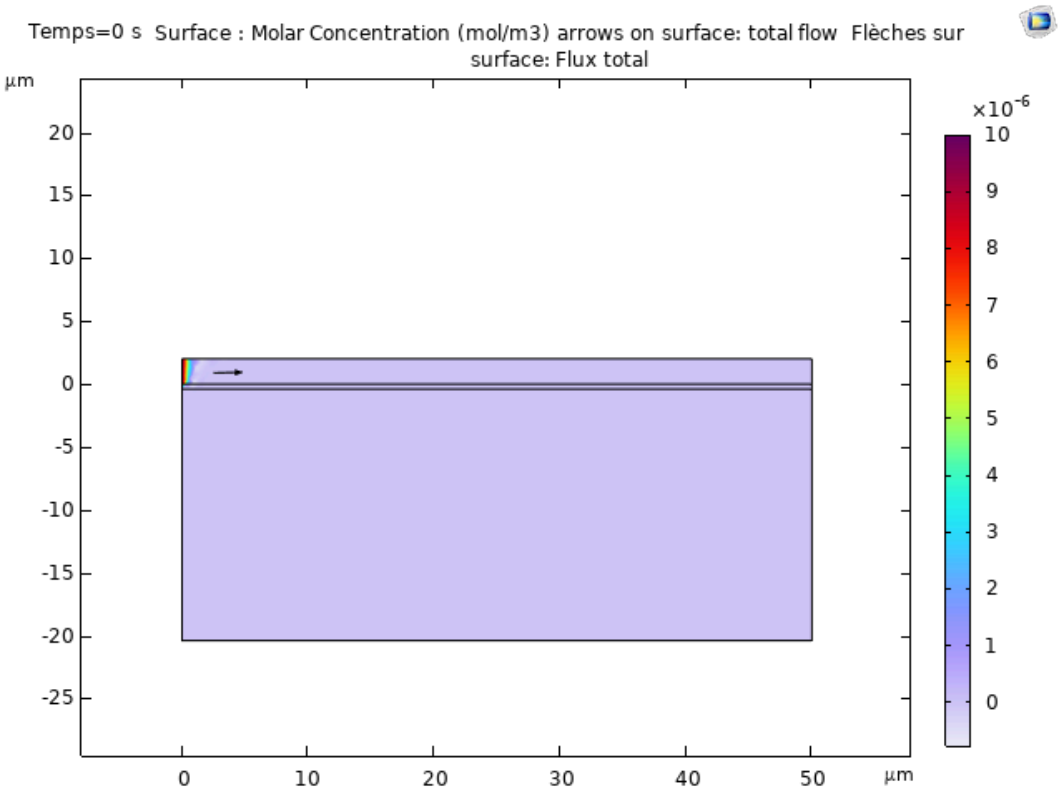

Figure 23 : Diluted species concentration in the system at t=0s.

At t=0s, the injection is done at the entrance of the capillary. Concentration of diluted species is at 0 mol/m^3 everywhere which seems normal.

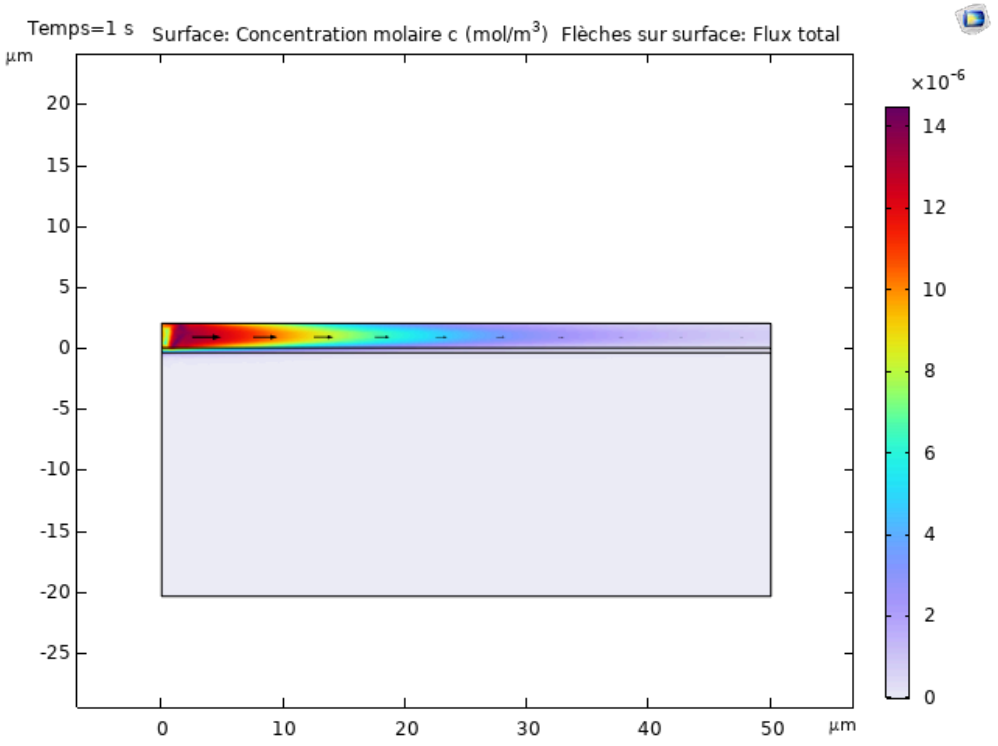

Figure 24 : Diluted species concentration in the system at t=1s.

At t=1s, we can already see the propagation of diluted species through capillary. Arrows represent the direction of the flow. At this time the concentration of diluted species outside of capillary is still equal to 0. It means that extracellular vesicles have not yet crossed the capillary wall.

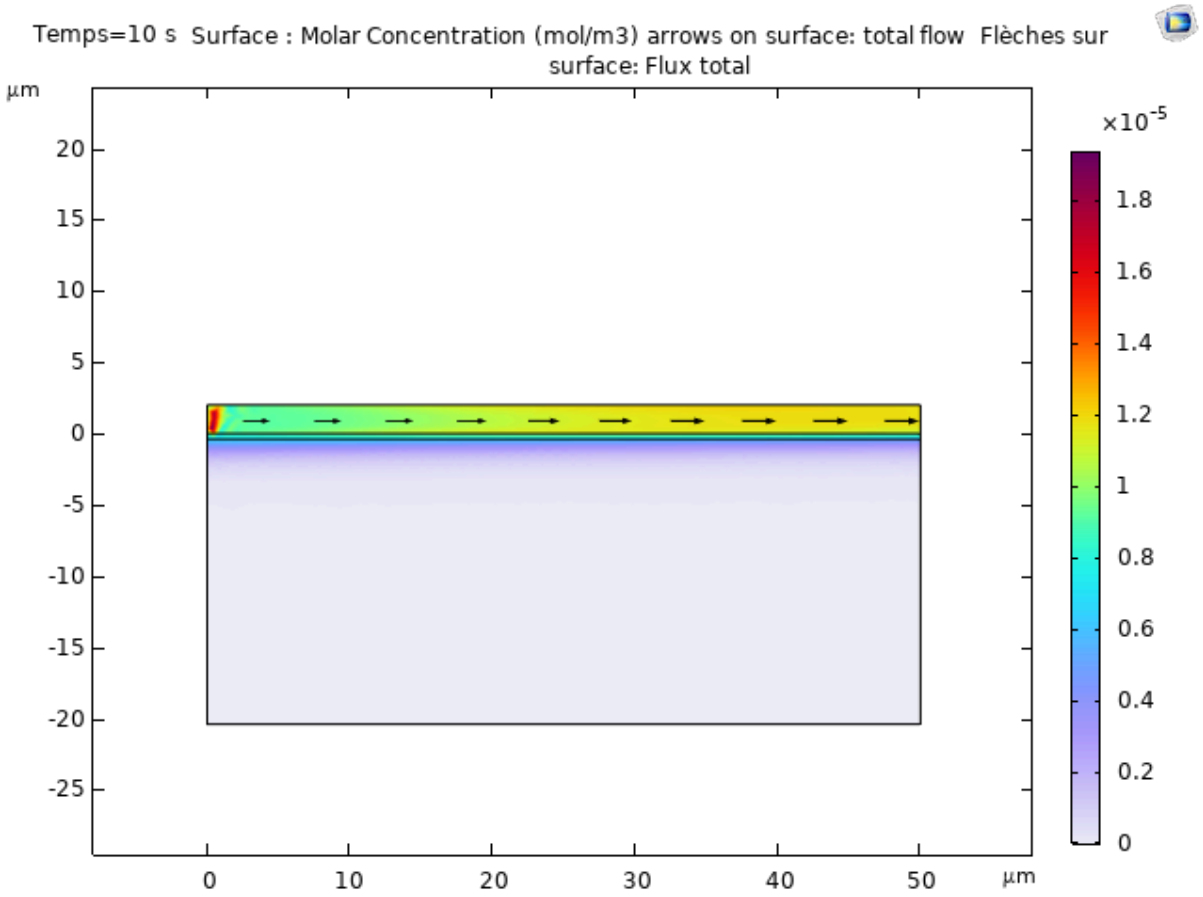

Figure 25 : Diluted species concentration in the system at t=10s.

After 10 seconds we can see an almost homogenous concentration inside the capillary. The species have started to cross the capillary wall. A very small concentration can be observed in the interstitial environment. If we extend the duration of the model until 6 minutes, we observe a greater concentration of species inside the interstitial environment and the depth of penetration reaches 10 micrometers.

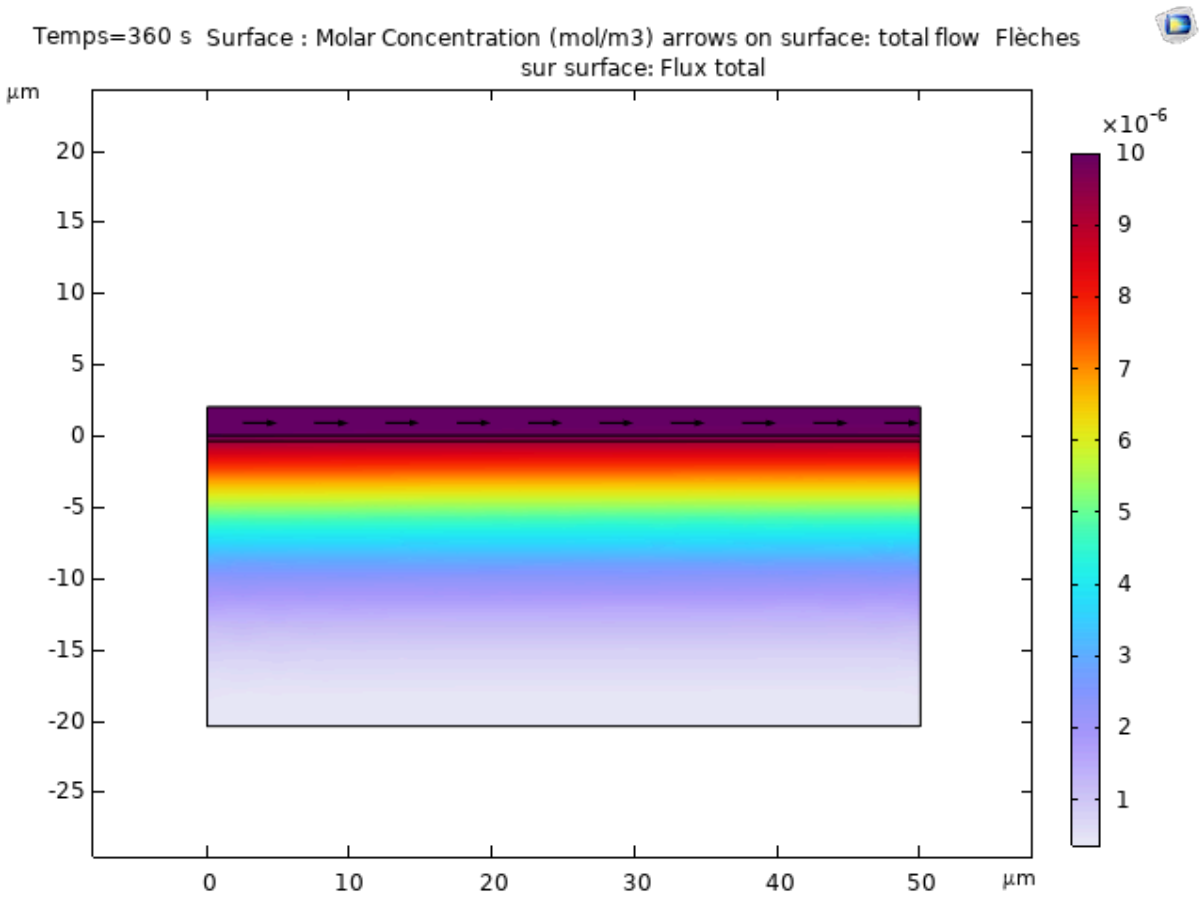

Figure 26 : Diluted species concentration in the system at t=360s.

The initial concentration of species does not influence the depth of penetration but only the concentration of species that have crossed the capillary wall. In this case we increase the concentration a thousand times.

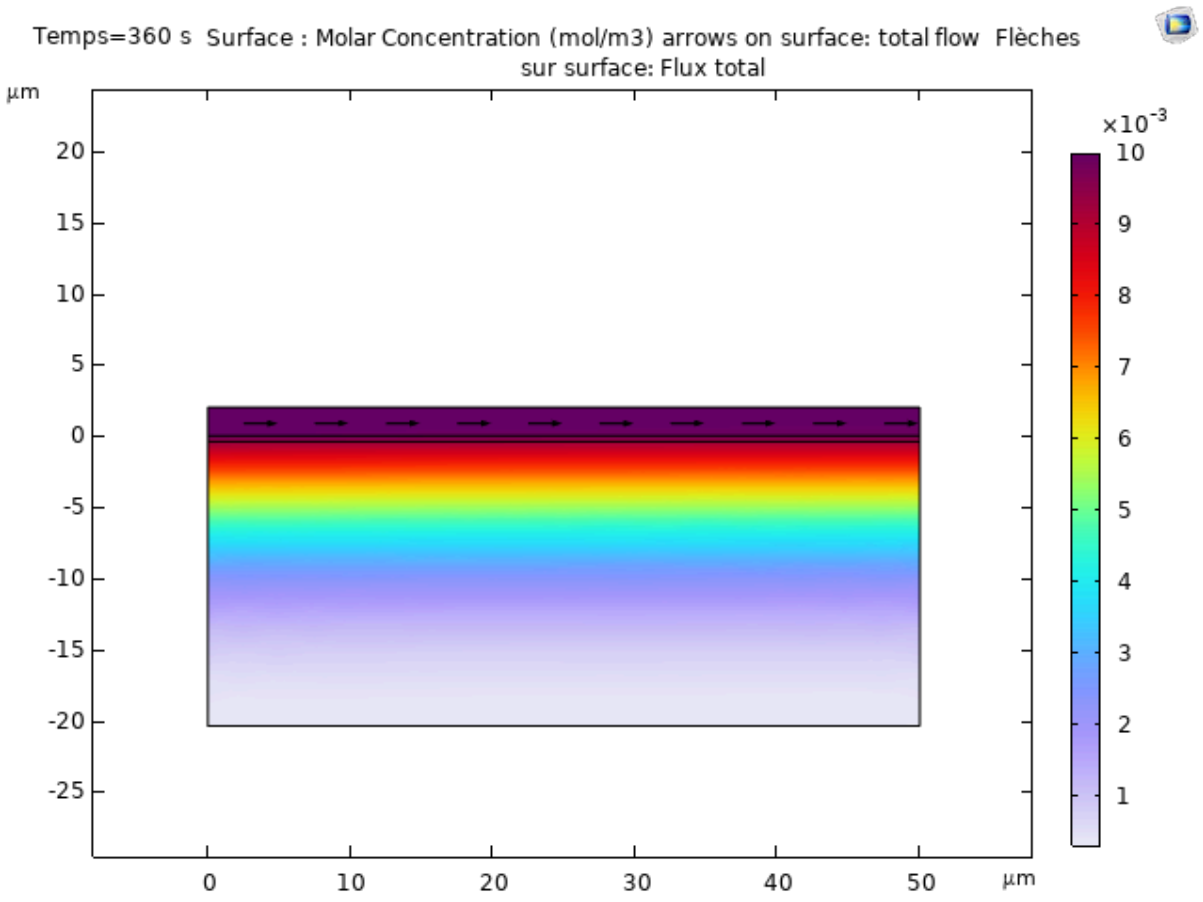

Figure 27 : Diluted species concentration in the system at t=360s.

Conclusion and Perspectives

These primary results confirm a possibility for extracellular vesicles to cross through the capillary wall in certain conditions. Nevertheless the results have to be moderated by the simplicity of the system and the assumptions. In fact, several aspects have not been taken into account. As said at the beginning, the cancerous cells are located under a dense fibrosis that could slow down or stop the extracellular vesicles from reaching the center of the tumor. Another point is the simplification done on geometry elements employed. In reality capillaries undergo several deformations and capillaries networks are more complex than just one capillary. All of these issues have to be taken into account to improve the model accuracy.

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