Modelling the Formation of Capillaries

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1 Vasculogenesis and Angiogenesis

Vasculogenesis and angiogenesis are two different mechanisms involved in the development of blood vessels. The former process mainly occurs when the primitive vascular network is formed. It consists in the aggregation and organisation of the endothelial cells, the main bricks of the capillary walls. The latter consists in the formation of new vessels which only sprout from an existing capillary or post-capillary venule. Angiogenesis already intervenes in the embryo to remodel the initial capillary network into a mature and functional vascular bed comprised of arteries, capillaries, and veins. Angiogenic remodelling co-ordinates with the establishment of blood flow and can occur through sprouting, i.e., by the formation of new branches from the sides of existing capillaries (see Figure 1a) or intussusception, i.e., by internal division of the vessel lumen (see Figure 1b).

The main role of angiogenesis is however during the adult life when it is involved in many physiological processes, for instance, the vascularization of the ovary and the uterus during the female cycle, of the mammary gland during lactation and wound healing. However, angiogenesis also plays a fundamental role in many pathological settings, such as tumors, chronic inflammatory diseases like rheumatoid arthritis and psoriasis, vasculopathies like diabetic microangiopathy, degenerative disorders like atherosclerosis and cirrhosis, tissue damage due to ischemia. We explicitly mention that though during the adult life angiogenesis is the main process of capillary formation, vasculogenesis can still occur.

It is possible to divide the angiogenic process into well differentiated stages which sometimes partially overlap. We will briefly describe them in the following, though the interested reader can find more information on the process in the recent reviews by Bussolino et al. [BA03] and by Mantzaris et al. [MW04]. These stages are regulated by precise genetic programmes and are strongly influenced by a chemical factor called vascular endothelial growth factor (VEGF)

1. The first stage is characterised by changes in the shape of the endothelial cells covering the walls of the blood vessel, by the loss of interconnection between endothelial cells, and by the reduction of vascular tonus. This in particular induces an increase in the vessel permeability.
2. The stage of progression is characterised by the production of proteolytic enzymes (serine-proteins, iron-proteins) which degrade the extracellular matrix surrounding the capillary facilitating the cellular movement and by the capacity of the endothelial cells, to proliferate and to migrate chemotactically, i.e. up the gradient of suitable chemical factors, toward the place where it is necessary to create a new vascular network.
3. The stage of differentiation is characterised by the exit of the endothelial cells from the cellular cycle and by their capacity of surviving in sub-optimal conditions and of building themselves primitive capillary structures, not yet physiologically active.
4. In the stage of maturation, the newborn vessel is completed by the formation of new extracellular matrix and by the arrival of other cells named pericytes and sometimes of fat muscle cells and . During this phase a major role is played by some molecules called angiopoietins leading to the development of the simple endothelial tubes into more elaborate vascular tree
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Fig. 1. Different modalities of angiogenesis: (a) sprouting and (b) intussusception.

composed of several cell types. In fact, they contribute to the maintenance of vessel integrity through the establishment of appropriate cell-cell and cell-matrix connections.

5. After the formation of the vascular network, a remodelling process starts. This involves the formation of anastomosis between capillaries, the loss of some physiologically useless capillaries, and remodelling of the extracellular matrix.

The inductors of angiogenesis, e.g. VEGF, cause the endothelial cell to migrate, to proliferate and to build structures which are similar to capillaries even when they are cultivated in vitro on an extracellular matrix gel. This phenomenon is called \textit{angiogenesis in vitro}.

A further process leading to vessel formation is \textit{arteriogenesis}, a process triggered by the occlusion of an artery. In order to overcome the problems of possible formation of ischemic tissues, the pre-existing arteriolar connections enlarge to become true collateral arteries. In this way, bypassing the site of occlusion, they have the ability to markedly grow and increase their lumen providing an enhanced perfusion to region interested by the occlusion. It has to be remarked that the formation of collateral arteries is not simply a process of passive dilatation, but of active proliferation and remodelling.

2 In Vitro Vasculogenesis

Vasculogenesis can be obtained in vitro using different experimental set-ups, substrata, and cell-lines, as reviewed in [VV01]. This is an important experiment performed not only to understand the mechanisms governing the angiogenic process, but also to test the efficacy of anti-angiogenic drugs and, in principle, to build the initial vascular network necessary to vascularise the tissues grown in vitro.

In order to understand the subsequent modelling and results we here describe the experimental set-up used in [SA03]. A Petri dish is coated with an amount of Matrigel, a surface which favours cell motility and has biochemical characteristics similar to living tissues, which is 44 ± 8 µm thick. Human endothelial cells from large veins or adrenal cortex capillaries (HUVEC) are dispersed in a physiological solution which is then poured on the top of the Matrigel. The cells sediment by gravity onto the Matrigel surface and then move on the horizontal Matrigel surface giving rise to the process of aggregation and pattern formation shown in Figure 2.

The process lasts 12-15 hours and goes through the following steps:

1. In the first couple of hours endothelial cells have a round shape. It seems that they choose a direction of motion and keep migrating with a small random component until they collide
with the closest neighbors (see Figures 2a, b). This effect is called in biology cell persistence and is related to the inertia of the cell in rearranging its cytoskeleton, the ensemble of fibers (e.g., actin and microtubules) which drive cell motion. The direction of motion, however, is not chosen at random, but it can be shown to be correlated with the location of areas characterised by higher concentrations of cells. It is interesting to remark that in this phase cells move much faster than later on when activation of focal contacts and interactions with the substratum increase. This type of motion, called amoeboid, can be compared with the exhibition of a gymnast with a quick sequence of jumps of the cells from handle to handle using few “arms” at a time (see Figure 3).

2. After collision, the cells attach to their neighbours eventually forming a continuous multicellular network (Figure 2c). The number of adhesion sites increases and the cells achieve a more elongated shape multiplying the number of adhesion sites with the substratum. The motion is much slower and resembles that of a mountain-climber which uses as many footholds as possible (see Figure 3b) grabbing new adhesion sites and detaching from old ones one at a time. This type of motion is named mesenchymal.

3. The network slowly moves as a whole, undergoing a slow thinning process (Figure 2d), which however leaves the network structure mainly unaltered. In this phase the mechanical interactions among cells and between cells and the substratum become important.
Finally, individual cells fold up to form the lumen of the capillary, so that one has the formation of a capillary-like network along the lines of the previously formed bidimensional structure.

The interested reader can look at a movie of an experiment (partial for easier downloads) at the web site of the EMBO journal (embjournalm.npgjournals.com/content/vol22/issue8/index.shtml) as a supplementary material to [SA03].

As the motion of cells in the first phase seems to be well established toward the region characterised by higher cell densities and kept till the cells encounter other cells, the main question is how the cells feel the presence of other cells. In fact, the evidences above suggest the presence of both a mechanism of persistence in cell motion and a mechanism of cross-talk among cells. As a matter of fact, endothelial cells in the process of vascular network formation exchange signals by the release and absorption of Vascular Endothelial Growth Factors-A (VEGF-A) which are also essential for their survival and growth. Moreover, autocrine/paracrine secretion of VEGF-A by endothelial cells has been shown to be essential for the formation of capillary beds. This growth factor can bind to speciﬁc receptors on the cell surface and induce chemotactic motion along its concentration gradient.

In order to quantify both cell persistence and the chemotactic behavior in cell motion Serini et al. [SA03] performed a statistical analysis of the cell trajectories. Referring to Figure 4, they measured the angle $\varphi$ between two subsequent displacements relative to the same trajectory, which gives a measure of the persistence, and the angle $\theta$ between the velocities and the concentration gradients at the same point simulated starting from the distribution of cells and taking into account that VEGF-A, like similar soluble molecules, is degraded by the environment in a finite time, mainly through oxidation processes. The angle $\theta$ then gives a measure of the chemotactic behavior.

In order to test the importance of chemotactic signalling mechanisms Serini et al. [SA03] also performed some experiments aimed at extinguishing VEGF-A gradients spreading from individual endothelial cells plated of Matrigel by adding a saturating amount of exogenous VEGF-A. Indeed, saturation of VEGF-A gradients resulted in a strong inhibition of network formation.

The same statistical analysis mentioned above was repeated in saturating conditions. In this case, the diagram for $\varphi$ shows that cell movement maintains a certain degree of directional persistence, while the diagram for $\theta$ shows that in saturating conditions the movement is completely decorrelated from the direction of simulated VEGF gradients showing the importance of VEGF in the process.

The final configuration achieved in the experiments is a capillary-like network which can be represented as a collection of nodes connected by chords. The amazing thing is that over a range of values of seeded cell density extending from 100 to 200 cells/mm², the mean chord length measured on the experimental records is approximately constant and equal to $\ell \approx 200 \pm 20 \mu$m.

It is interesting to notice that capillary networks characterised by typical intercapillary distances ranging from 50 to 300 $\mu$m is instrumental for optimal metabolic exchange, so that the characteristic size of the network in vitro is biologically functional: a coarser net would cause necrosis of the tissues in the central region, a finer net would be useless.
Fig. 4. Trajectories of some cells in the field of chemoattractant (a) and sample trajectory (b). Arrows indicate concentration gradients. The angles $\varphi$ and $\theta$ refer respectively to persistence and chemotaxis.

A pathological situation in which the dimension of the capillary network changes has been described by Ruhrberg et al. [RG02]. In fact, they observed that mice lacking heparin-binding isoforms of VEGF-A form vascular networks with a larger mesh (see Figure 5). This is related to the fact that the binding of some of the isoforms with lower or higher molecular weight affects the effective diffusivity of the chemical factor. Therefore VEGF plays a role in defining the mesh size and, in particular, different isoforms (with different diffusivities) can lead to different mesh size. As will be discussed in Section 4.1, the model proposed in [GA03, SA03] predicts that the size of the network is related to the product of the diffusion constant and the half-life of the chemical factor, so that if the effective diffusion increases the typical size of the network chords increases.

If on one hand the chord length is nearly independent from the density of seeded cells in a certain range, on the other hand it is observed that outside this range one does not have a proper development of vascular networks. In fact, below a critical value about $100\text{ cells/mm}^2$ the single connected network shown in Figure 6b breaks down in groups of disconnected structures as shown in Figure 6a. On the other hand at higher cell densities, say above $200\text{ cells/mm}^2$ (Figure 6c) the mean chord thickness grows to accommodate an increasing number of cells. For even higher values of initial density, the network takes the configuration of a continuous carpet of cells with holes, called lacunae (Figure 6d). This configuration is not functional. In fact, cells do not even differentiate to form the lumen in the chords.
We end this section by mentioning that the generalization of this phenomenon to a three dimensional set-up is not standard as it might seem, because in this situation cells are surrounded by extracellular matrix (the network of fibers, e.g. fibronectin, collagen, vitronectin, filling part of the extracellular space) and their possibility of ameboid motion is limited. A movie showing the motion of a cell in a three-dimensional extracellular matrix can be viewed at [www.bloodjournal.org/cgi/content/full/2002-12-3791/DC1](http://www.bloodjournal.org/cgi/content/full/2002-12-3791/DC1). In fact, to move in the gel they have to cleave the extracellular matrix via the production of matrix degrading enzymes, which alter the environment the cells move into. However, this does not exclude the possibility of existence of a layer between two strata characterised by a reduced amount of extracellular matrix so that ameboid motion can still occur. This might be the case of the so-called sandwich experiments in which a second Matrigel layer is placed on the top of the cells after seeding them over the layer at the bottom of the Petri dish. This gives a preferential direction of motion for the cells, the horizontal plane.

3 Modelling Vasculogenesis

The objective of the mathematical model presented in this section is to simulate in silico the entire course of events occurring during vasculogenesis, i.e. a chemically dominated phase characterised by an initial ameboid motion mainly affected by gradients of endogenous chemoattractants and a subsequent mesenchymal motion in which chemotactic effects are still important but mechanics dominates because of cell anchoring to the substratum and of the subsequent development of stresses.

To deduce the model we will consider the following three compound system:

1. the ensemble of cells, dealt with as a continuum;
2. the substratum, e.g. Matrigel;
3. the physiological liquid, which will be considered as a passive constituent with negligible interactions with the others;

in addition to the chemical factor(s) influencing the process.

We will develop the model in the framework of mixture theory (see, for instance, [AM03]), suitably adapted to the biological setting. One can then generally write for the three constituents above the following equations related to mass and momentum balance.
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\( n = 62.5 \)

\( n = 125 \)

\( n = 250 \)

\( n = 500 \)

Fig. 6. Dependence of the types of structures formed varying the density of seeded cells.

\[
\begin{align*}
\frac{\partial \rho_c}{\partial t} + \nabla \cdot (\rho_c \mathbf{v}_c) &= \Gamma_c, \\
\frac{\partial \rho_s}{\partial t} + \nabla \cdot (\rho_s \mathbf{v}_s) &= \Gamma_s, \\
\frac{\partial \rho_\ell}{\partial t} + \nabla \cdot (\rho_\ell \mathbf{v}_\ell) &= \Gamma_\ell, \\
\frac{\partial}{\partial t} (\rho_c \mathbf{v}_c) + \nabla \cdot (\rho_c \mathbf{v}_c \otimes \mathbf{v}_c) &= \nabla \cdot \mathbf{T}_c + \mathbf{f}_c + \mathbf{f}_c + \Gamma_c \mathbf{v}_c, \\
\frac{\partial}{\partial t} (\rho_s \mathbf{v}_s) + \nabla \cdot (\rho_s \mathbf{v}_s \otimes \mathbf{v}_s) &= \nabla \cdot \mathbf{T}_s + \mathbf{f}_s + \mathbf{f}_s + \Gamma_s \mathbf{v}_s, \\
\frac{\partial}{\partial t} (\rho_\ell \mathbf{v}_\ell) + \nabla \cdot (\rho_\ell \mathbf{v}_\ell \otimes \mathbf{v}_\ell) &= \nabla \cdot \mathbf{T}_\ell + \mathbf{f}_\ell + \mathbf{f}_\ell + \Gamma_\ell \mathbf{v}_\ell, 
\end{align*}
\]

where \( c \) stands for cell, \( s \) for substratum and \( \ell \) for liquid. Referring to the \( i \)-th constituent \((i = c, s, \ell)\), \( \Gamma_i \) is the production rate, \( \mathbf{T}_i \) is the partial stress tensor, \( \mathbf{F}_i \) is the body force acting on the \( i \)-th constituent, \( \mathbf{f}_i \) is the momentum supply, related to the local interactions with the other constituents, and \( \rho_i \) and \( \mathbf{v}_i \) are the density and velocity of the \( i \)-th constituent. In particular, we notice that the density of cell, i.e. the mass of cells per unit area, can be written as \( \rho_c = m_c n \) where \( m_c \) is the mass of a cell, and \( n \) is the number of cell per unit area (the in vitro process is two-dimensional).
If the mixture is closed, then overall mass and momentum balance implies that
\begin{align}
\Gamma_c + \Gamma_s + \Gamma_I = 0, \\
\mathbf{f}_c + \Gamma_R \mathbf{v}_c + \mathbf{f}_s + \Gamma_I \mathbf{v}_s + \mathbf{f}_I \mathbf{v}_I = 0.
\end{align}

(7)

(8)

In normal conditions endothelial cells replicate every one or two days, but this process is inhibited even further in the experimental environment. Therefore, rather than to apoptosis or mitosis the right hand side of (1) takes into account the possible change of number of cells on the substratum. This can be due to the detachment of cells from the substratum, which seems to occur during some experiments by Vaillhé et al. [VL98/99], or to the sedimentation and then accumulation of cells on the substratum which may occur over a time of the order of one hour. As a consequence the last term on the right hand side of (4) takes into account of the gain/loss of momentum due to the gain/loss of mass.

We will however neglect this phenomenon here. We will also assume that the extracellular matrix is neither produced, nor degraded and therefore we can write
\begin{align}
\Gamma_c = \Gamma_s = \Gamma_I = 0, \\
\mathbf{f}_I = 0, \\
\mathbf{f}_s = -\mathbf{f}_c := \mathbf{f}_n,
\end{align}

(9)

(10)

which shows the character of internal (interaction) force of the force \( \mathbf{f}_n \) exerted by the cells on the substratum.

### 3.1 Diffusion Equations for Chemical Factors

Before studying in detail the persistence equation (4) and the substratum equation (5) let us focus on the diffusion of chemotactic factors which is governed by the usual reaction-diffusion equations. We will distinguish between \textit{endogenous} chemical factors, i.e. those produced by the cell themselves, and \textit{exogenous} chemical factors, i.e. those introduced by other components, in our case mostly from the outside. From the experimental viewpoint this can be achieved by adding to the substratum gellly sponges or gelly “spaghettis” impregnated with chemical substances with the properties to attract endothelial cells, like VEGF, or repel endothelial cells, like semaphorines. This is done because we have in mind possible applications to tissue engineering where it is important to understand how to govern the characteristics of the network acting from outside the system.

The diffusion of the different chemotactic factors is then governed by
\begin{align}
\frac{\partial c}{\partial t} &= D \nabla^2 c - \frac{c}{\tau} + \alpha(p_c), \\
\frac{\partial c_a}{\partial t} &= D_a \nabla^2 c_a - \frac{c_a}{\tau_a} + s_a(t) H_a(x), \\
\frac{\partial c_r}{\partial t} &= D_r \nabla^2 c_r - \frac{c_r}{\tau_r} + s_r(t) H_r(x),
\end{align}

(11)

(12)

(13)

where \( c \) is the concentration of endogenous vascular endothelial growth factors-A (VEGF-A) produced by endothelial cells, \( c_a \) is the concentration of exogenous chemoattractant, which might still be VEGF-A, and \( c_r \) is the density of exogenous chemorepellent.

In (11) the chemoattractant is produced by the endothelial cells at a rate \( \alpha \) and degrades with a half life \( \tau \). In (12) and (13) the chemical factors are released at a rate \( s_a(t) \) and \( s_r(t) \) in some domains identified by the indicator functions \( H_a \) and \( H_r \), which is constantly equal to 1 in the region where the chemical factor is released and vanishes outside it. Convection is neglected for the smallness of the fluid velocity.

The model in [GA03, SA03] contains a production term \( \alpha(p_c) = a p_c \) in the reaction-diffusion equation for the chemical factor. This implicitly means that each cell always produces a constant amount of chemoattractant independantly of the what happens around. The model in [TA05] assumes a more general functional form of the production term based on the following consideration:
the chemoattractant is a mean of communication and survival for the cell itself and its neighbours. It is known that upon contact cell activate mechanotransduction pathways involving the cell-to-cell junctions and transmembrane proteins like cadherins. This may lead to a downregulation of the production of the chemical factor, because when cells reach an aggregate state, there is no need to communicate and recruit new cells with the release of more chemical factors. In particular, VEGF communication means can be substituted with contact cadherin-cadherin signalling. At present this is a phenomenological hypothesis and we are not aware of any experimental evidence supporting or counter-proving it. It would however be interesting to do experiments in this direction.

Specifically, the simulation to follow use

$$\alpha(\rho_c) = \frac{a\rho_c}{1 + b\rho_c}$$

with $a > 0$ and $b \geq 0$, so that for $b \neq 0$ $\alpha(\rho_c)$ has a maximum production $\frac{a}{2b}$ at $\rho_c = \frac{1}{2b}$ and goes to zero for $\rho_c \to +\infty$.

3.2 Persistence Equation for the Endothelial Cells

Focusing on the cell population, we will use the mass balance equation (1) and the momentum balance equation for the cellular matter, which using (1), (9) and (10) can be simplified to

$$\frac{\partial v_c}{\partial t} + v_c \cdot \nabla v_c = -\frac{1}{\rho_c} (\nabla \cdot T_c + F_c - f_n).$$

(15)

It must be noticed that in most biological phenomena inertia is negligible. In fact, velocities are of the order of $\mu$m per second. Actually, the left hand side of Eq.(15) should be understood as the “inertia” of the cells in changing their direction of motion, i.e. cell persistence.

The right hand side of (15) also contains the fundamental chemotactic body force

$$F_{chem} = \rho_c \beta(c) \nabla c,$$

(16)

where $c$ is the concentration of a particular chemical factor and $\beta(c)$ measures the intensity of cell response which can include saturation effects, e.g.,

$$\beta(c) = \frac{\beta}{1 + \frac{c}{c_M}}; \quad \sigma \beta(c) = \beta(1 - c/c_M)^+,$$

(17)

where $c_M$ is constant and

$$f_+ = \begin{cases} f & \text{if } f > 0; \\ 0 & \text{otherwise}; \end{cases}$$

is the positive part of $f$. The linear dependence of the force on $\rho_c$ corresponds to the assumption that each cell experiences a similar chemotactic action so that the momentum balance in integral form depends on the number of cells in the control volume and the related local equation on the local density. If one has all three chemical factors mentioned in the previous section, then

$$F_{chem} = \rho_c \left( \beta \nabla c + \beta_x \nabla c_x - \beta_y \nabla c_y \right).$$

Finally, the partial stress tensor give an indication of the response of the ensemble of cells to stresses. Several constitutive equations can be formulated, but unfortunately no experimental data are available on the mechanical characteristics of ensemble of cells. It can be argued that because the cytosol is a watery solution containing a lot of long proteins contained in a viscoelastic membrane, the ensemble of cells might behave as a viscoelastic material. However, we can expect that the characteristic times of the viscoelastic behavior are much smaller than those related to cell motion (minutes with respect to hours), so that viscoelastic effects can be considered negligible. On the
other hand, plasticity should probably be taken into account to describe the breaking of cell-to-cell adhesion bonds.

In absence of experimental evidence, in the following the simplest constitutive equation possible

\[ T_c = -p(\rho_c)I, \]  

will be considered, corresponding to an elastic fluid. This assumption implies, for instance, that the ensemble of cells can not sustain shear, which, of course, is not true.

As we shall see however the presence of \( T_c \) is particularly important in describing the formation of lacunae.

Equation (15) then specializes in

\[ \frac{\partial \mathbf{v}_c}{\partial t} + \mathbf{v}_c \cdot \nabla \mathbf{v}_c = \frac{1}{\rho_c} \nabla p(\rho_c) + \beta(c) \nabla c - \frac{1}{\rho_c} \mathbf{f}_n. \]  

The exact form of \( \mathbf{f}_n \), the interaction force with the substratum, will be specified in the following section.

3.3 Substratum Equation

Focusing on the substratum, which is an inert intricate web of long fibers on which cell move, we can certainly state that inertial effects can be neglected. From Eq. (5), the force balance equation for the substratum then writes

\[ \nabla \cdot \mathbf{T}_s + \mathbf{f}_n + \mathbf{F}_s = 0 \]  

where in particular \( \mathbf{F}_s \) is the anchoring force to the Petri dish, and \( \mathbf{f}_n \) is the interaction force exerted by the cells on the substratum.

We observe that Eq. (20) works in the two-dimensional layer. The procedure to obtain the two-dimensional reduction of the stress balance equation is often not so clear in the literature. For this reason we will report it in detail in the Appendix to this chapter. The forces appearing in (20) derive from the interaction with the cells and with the Petri dish at the top and the bottom of the layer.

We assume that the interaction force between the substratum and the cells includes an elastic and a viscous contribution. During the ameboid motion the interaction force acting on the cells is of viscous type, which implies a weak interaction between the cells and the substratum, characterised by a quick removal of the bonds and formation of new bonds, with weak deformation of the substratum. We can model this force as

\[ \mathbf{f}_{visc} = -\gamma \rho_c (\mathbf{v}_s - \mathbf{v}_c), \]  

where \( \mathbf{v}_s = \frac{d\mathbf{u}_s}{dt} \) and \( \mathbf{u}_s \) is the displacement of the substratum.

The elastic contribution takes into account of the fact that after some time cells attach to the substratum with a strong bond. If the cell anchors in \( \mathbf{u}_c \) and then moves to \( \mathbf{u} \) we can assume that the elastic force is proportional to \( \mathbf{u} - \mathbf{u}_c \). This change of behavior characterises the transition between the chemotactic and the mechanical phase. In other words, this force is absent in the initial ameboid motion and starts when the motion becomes of mesenchymal type, i.e. when cells start attaching to the adhesion molecules of the Matrigel. If we assume that there exists a characteristic time \( t_{th} \) needed to anchor to the adhesion sites on the substratum and characterising the transition between a purely ameboid phase and a mesenchymal phase we can write

\[ \mathbf{f}_{elast} = -\kappa \rho_c (\mathbf{u}_s - \mathbf{u}_c) H(t - t_{th}), \]  

where \( \kappa \) is the anchoring rigidity and \( H \) is the Heaviside function.
Another interesting hypothesis can be that ameboid motion stops when cells come in contact, so that the strongly reduced velocity allows for a better link with the adhesion molecules of the substratum. This phenomenon could be included in the previous set-up assuming that

\[ f_{st} = -\kappa(\rho_c)\rho_c (u_s - u_c), \]

where in particular \( \kappa(\rho_c) \) vanishes below a given value \( \rho_{c,b} \) of cell density, e.g., \( \kappa(\rho_c) = \kappa \delta(\rho_c - \rho_{c,b}) \).

However, if the pulling is strong enough, then some adhesion bonds could break and so the inclusion of plastic phenomena should be considered, but this is not done here and is a possible interesting development.

Referring to the two dimensional reduction of the substratum equation described in the Appendix, we need to consider the adhesion of the substratum to the Petri dish, which can be taken to be proportional to the displacement, i.e.

\[ f_{ext} = -\frac{s}{h}u_s, \]

where \( h \) is the substratum thickness. We explicitly mention that this force does not act on the cellular constituent, so it must not be considered in the momentum equation for the cells.

\[ H(\tau) = \begin{cases} 1 & \text{if } \tau > 0; \\ 0 & \text{otherwise}; \end{cases} \]

Fig. 7. Network formation in absence of substratum interaction. In particular, \( \beta = 1, \gamma = 0, a = 1, \) and \( b = 0. \)

4 In Silico Vasculogenesis

According to the deduction above the mathematical model writes as follows
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\[
\frac{\partial n}{\partial t} + \nabla \cdot (n \mathbf{v}_c) = 0, \quad (23)
\]

\[
\frac{\partial \rho_c}{\partial t} + \nabla \cdot (\rho_c \mathbf{v}_s) = 0, \quad (24)
\]

\[
\frac{\partial \mathbf{v}_c}{\partial t} + \mathbf{v}_c \cdot \nabla \mathbf{v}_c = \frac{1}{n} \nabla p(n) + \beta \nabla c + \beta_s \nabla c_s - \beta_r \nabla c_r + \gamma m_c (\mathbf{v}_c - \mathbf{v}_s) - \kappa m_c (\mathbf{u}_c - \mathbf{u}_s) H(t - t_{kh}), \quad (25)
\]

\[
\nabla \cdot \mathbf{T}_c + \gamma m_c n (\mathbf{v}_c - \mathbf{v}_s) + \kappa m_c n (\mathbf{u}_c - \mathbf{u}_s) H(t - t_{kh}) - \frac{\kappa}{h} \mathbf{n}_s = 0, \quad (26)
\]

\[
\frac{\partial c}{\partial t} = D \nabla^2 c - \frac{c}{\tau} + \alpha (\rho_c), \quad (28)
\]

\[
\frac{\partial c_s}{\partial t} = D_h \nabla^2 c_s - \frac{c_s}{\tau_s} + s_0 (t) H_s (\mathbf{x}), \quad (29)
\]

\[
\frac{\partial c_r}{\partial t} = D_r \nabla^2 c_r - \frac{c_r}{\tau_r} + s_r (t) H_r (\mathbf{x}), \quad (30)
\]

where we prefer to use the number of cells per unit area \( n = \rho_c/m_c \), because this is the quantity which is given and changed in the experiments.

The experiments described in Section 2 start with a number of cells randomly seeded on the Matrigel. To reproduce the experimental initial conditions we will always start with the following cell distribution

\[
n (\mathbf{x}, t = 0) = \frac{1}{2\pi r^2} \sum_{j=1}^{M} \exp \left[ - \frac{(\mathbf{x} - \mathbf{x}_j(\omega))^2}{2 r^2} \right], \quad (31)
\]

\[
\mathbf{v} (\mathbf{x}, t = 0) = 0, \quad (32)
\]

Each Gaussian has width of the order of the average cell radius \( r \simeq 20 \mu m \), so that from the mathematical point of view it represents a cell. Then \( M \) Gaussian bumps are centered at random locations \( \mathbf{x}_j \) distributed with uniform probability on a square of size \( L \) (in the experimental set-up \( L = 2 \) mm). The initial velocity is null, because cells sediment from above on the horizontal surface. Unless when specified, periodicity is imposed at the boundary of the domain.

4.1 Neglecting Substratum Interactions

As a first example we consider the formation of the vascular network in isotropic conditions, under the action of endogenous chemical factors only and neglecting the mechanical interactions with the substratum.

The model then reduces to that proposed in [AG04, GA03, SA03]

\[
\frac{\partial n}{\partial t} + \nabla \cdot (n \mathbf{v}_c) = 0, \quad (33)
\]

\[
\frac{\partial \mathbf{v}_c}{\partial t} + \mathbf{v}_c \cdot \nabla \mathbf{v}_c = \frac{1}{n} \nabla p(n) + \beta \nabla c - \gamma \mathbf{v}_c, \quad (34)
\]

\[
\frac{\partial c}{\partial t} = D \nabla^2 c - \frac{c}{\tau} + \alpha (\rho_c). \quad (35)
\]

The result of a simulation is shown in Figure 7. Let us now consider the information encoded in the coupling of the first two equations above with the diffusion equation (13). This can be understood in the simplest way if we neglect pressure and assume for a moment that diffusion is a faster process than pattern formation, so that the dynamics of \( c \) is “slaved” to the dynamics of \( n \) and the derivative term \( \partial c/\partial t \) can be neglected in a first approximation. Then it is possible to formally solve the diffusion equation for \( c \) and to substitute it in the persistence equation, so that one can write (for \( b = 0 \))
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Fig. 8. Dependence of the specific network structure on the initial conditions.

\[ \frac{\partial v_c}{\partial t} + v_c \cdot \nabla v_c = \frac{a\beta}{D} \nabla \left( \ell^{-2} - \nabla^2 \right)^{-1} n, \]  

(36)

where

\[ \ell := \sqrt{D\tau}. \]  

(37)

The appearance in the dynamical equations of the characteristic length \( \ell \) suggests that the dynamics could favor patterns characterised by this length scale. As a matter of fact, if we rewrite the right hand side of (36) in Fourier space as

\[ \frac{a\beta}{D} \frac{ik}{k^2 + \ell^{-2} \eta k}, \]

we observe that the operator \( \frac{ik}{(k^2 + \ell^{-2})} \) acts as a filter, which selects the Fourier components of \( n \) having wave numbers of order \( \ell^{-2} \) damping the components with higher and smaller wavenumbers.

Experimental measurements of the parameters gives \( D \sim 10^{-7} \text{cm}^2 \text{s}^{-1} \) and \( \tau = 64 \pm 7 \text{min} \) and therefore \( \ell \sim 100 \mu \text{m} \), which is in good agreement with experimental data.

The process of network formation is then understood in the following way. Initially, non zero velocities are built up by the chemotactic term due to the randomness in the density distribution. Density inhomogeneities are translated in a landscape of concentration of the chemoattractant factor where details of scales \( \ell \) are averaged out. The cellular matter move toward the ridges of the concentration landscape. A non-linear dynamical mechanism similar to that encountered in fluid dynamics sharpens the ridges and emptys the valleys in the concentration landscape, eventually producing a network structure characterised by a length scale of order \( \ell \). In this way, the model provides a direct link between the range of intercellular interaction and the dimensions of the structure which is a physiologically relevant feature of real vascular networks.

The results of the simulations are shown in Figure 8 which shows how the precise network structure depends on the initial conditions which is randomly set. However, at glance the general features seem to be independent on the precise form of the initial condition and compare well with the experimental results shown in Figure 2.

Changing the effective diffusion of the chemical factors lead to the results shown in Figure 9, which agree with the observation that larger effective diffusivities lead to vascular networks with a larger mesh (see [RG02] and Figure 5).

The model is also able to reproduce the dependence of the characteristics of the structure on the density of seeded cells. In fact, as experimentally known (see Section 2) on one hand the chord length is nearly independent from the density of seeded cells in a certain range, on the other hand it is observed that outside this range one does not have a proper development of vascular networks. Varying the density of seeded cells one can put in evidence the presence of a percolative-like transition at small densities and a smooth transition to a “Swiss-cheese” configuration at large density.
Fig. 9. Dependence of the network characteristic size on $l = 100, 200, 300 \mu m$.

In fact, also in the simulation below a critical value $n_c \sim 100$ cells/mm$^2$ the single connected network (Figure 10b) breaks down in groups of disconnected structures (Figure 10a). On the other hand at higher cell densities, say above 200 cells/mm$^2$ (Figure 10c), the mean chord thickness grows to accommodate an increasing number of cells. For even higher value of seeded cell density, the network takes the configuration of a continuous carpet with holes (Figure 10d). This configuration is not functional.

Methods of statistical mechanics have been used in [CD04, GA03] to characterise quantitatively the percolative transition. They concluded that the transition occurring in the neighbourhood of $n_c \sim 100$ cells/mm$^2$ falls in the universality class of random percolation, even in the presence of migration and dynamical aggregation. This is confirmed by the fractal dimension of the percolating cluster ($D = 1.85 \pm 0.10$). In fact, both the value obtained on the basis of the experiments and that obtained on the basis of the numerical simulations ($D = 1.87 \pm 0.03$) are close to the theoretical value expected for random percolation ($D = 1.896$). Actually, a bi-fractal behavior seems to appear at small scales, but we will not enter in details referring to [CD04] for further details.

The presence of a percolative transition in the process of formation of vascular networks is not obvious, and is linked to the average constancy of the chord length. As a matter of fact, there are at least two ways of accommodating an increasing number of cells on a vascular-type network. The first one is to privilege connectivity with respect to chord lengths as in Figure 11a. The second one corresponds to the opposite behaviour. In this case, when the number of cells is too low, enforcing the constraint on the chord length makes it impossible to achieve side-to-side connectivity leading to sets of disconnected clusters as in Figure 11b.

It appears that Nature in this case chose to privilege network size, probably because widely spaced capillary networks, like the one in Figure 11a, would not be able to perform their main function, i.e., to supply oxygen and nutrients to the central part of the tissues.

The same mechanism might in principle explain the formation of lacunae. If the number of cells doubles, then there are two ways of accommodating the new cells. Either placing them in a more homogeneous way, forming smaller polygons, as in Figure 11d, or addressing the new cells next to the others, as in Figure 11b. In the first case the size of the polygons halves, in the second case it remains nearly the same, but the chords thicken. It seems that, the same reasoning used in the percolative transition, can be repeated here. Nature prefers to keep the size of the network as far as possible. Eventually, this leads to the formation of lacunae.

In this situation, the presence of the pressure term in the model is crucial as it avoids overcompression in the chords and allows to reproduce the transition to the “Swiss-cheese” configuration experimentally observed for high cell densities (Figure 6d). In addition, it avoids the blow up of solutions characteristics of many chemotaxis models [K05]. In fact, neglecting the mechanical interactions among overcrowding cells allows them to overlap in the points of maximum of the chemotactic field causing the blow up of the solution. From the physical point of view it is easy to realize that the pressure term avoids overcrowding. In fact, among other things, Kowalczyk [K05] proved that it is enough that

$$\exists n \quad \text{such that} \quad \forall n > n, \quad p'(n) > \text{const}.$$
to assure the boundedness of solutions in any finite time.

In order to study the formation of lacunae starting from a continuous monolayer of cells Kowalczyk et al. [KG04] also studied the linear stability properties of the model (35) finding that chemotaxis with the related parameters (motion, production, degradation) is the key destabilising force while pressure is the main stabilizing force.

4.2 Substratum interactions

In this section we will include the mechanical interactions with the substratum still assuming only endogenous chemotaxis. The model, introduced in [TA05], is a particular case of Eq. (35) without \( c_r \) and \( c_a \).

The effect of mechanical stretching obtained in the simulation is compatible with what observed in vitro, namely pulling on the extracellular matrix, the cells deform the substratum (see Figure 13). However, if the substratum is too rigid or if cell adhesion is too strong, then it is very hard for the cell to form a chord. In the limit case of very stiff substrata as that in Figure 12, then the morphogenic process leads to the formation of lacunae rather than chords.

The mechanical interactions seem also to play a fundamental role in guaranteeing the stability of the network.

Figure 13a shows the contour plot of the norm of the stress tensor relative to the Matrigel...
at the final stage of the network formation, corresponding to the cellular density shown in Figure 13b. It can be observed that the stress is concentrated in thin stripes edging the cords and surrounding the cellular density holes. At present to our knowledge there are no measurement of Matrigel displacement done during the process of in vitro vasculogenesis, though we think that they could be done disseminating the substratum with microsphere and monitoring their displacements.

In order to estimate the relative importance of chemotaxis versus mechanics, one can compute the $L^1$ norm over the domain. Figure 14 shows that in the first instant of the simulations the chemotactic force grows more rapidly than the elastic one, so that in the first period chemotactic effects result being prevalent. After that, the elastic force grows, till a substantial equilibrium is reached.

In order to understand the role of cell adhesivity and substratum stiffness, Figure 12 should be compared with Figure 15 which presents a moderate interaction ($\kappa = 0.2$ compared with $\kappa = 1$ in Figure 12) and with Figure 7 which is obtained in absence of any interaction with the substratum. We can also observe that, as expected, say that if the anchoring force is too weak, and therefore the chemotactic action is always prevailing, one has an acceleration in the formation of the chords.

We end this section by pointing out the effect of different VEGF production rates. Figure 15a shows the formation of the vascular network using the usual production term $\alpha(n) = an$ with $a = 1$. In addition, in the interaction force $\kappa = 0.2$ and $\gamma = 0$. On the other hand, Figure 15b is obtained for the same values as Figure 12a but consider the effect of contact dependent production of VEGF. In fact, as already mentioned cells might produce less VEGF because upon aggregation, there is no need to recruit new cells communicating with the release of more chemical factors. Rather than the standard linear production the function $\alpha(n)$ is expressed by (14), with $a = 30$ and $b = 0.2$. It can be noticed that the results of the two simulations present some differences. In particular, though the topology of the network is very similar, the saturation in the production term leads to neater structures.

Though we will not show it here, the model is still able to reproduce the transitions occurring at low and high densities, similarly to what obtained in Figure 10.
Fig. 12. Influence of the mechanical interactions on the network formation for \( \kappa = 1 \). The other parameters are as in Figure 12b. The results can be compared with those in Figure 7 which refer to no interaction with the substratum (\( \kappa = 0 \)). The initial condition is the same as that shown in Figure 7.

Fig. 13. Plot of the norm of the Matrigel stress tensor (a) and the corresponding density of endothelial cells (b) at the final stage of network formation. Level curves denote increasing values from blue to red.
Fig. 14. Evolution of the magnitude of the chemotactic (blue line) and elastic (red line) forces averaged over the domain for the simulation reported in Figure 15.

4.3 Exogenous control of vascular network formation

We now consider the case in which the formation of capillary networks is externally controlled by the use of exogenous chemoattractant ($c_a$) and chemorepellent ($c_r$), neglecting however substratum interaction, a problem studied by Lanza et al. [LA05].

Because diffusion is a much faster process than cell aggregation, the model can be simplified and be written as

$$\frac{\partial n}{\partial t} + \nabla \cdot (n \mathbf{v}_c) = 0,$$  \hspace{1cm} (38)

$$\frac{\partial \mathbf{v}_c}{\partial t} + \mathbf{v}_c \cdot \nabla \mathbf{v}_c = \beta \mathbf{v}_c + \beta_a \nabla c_a - \beta_r \nabla c_r - \gamma \mathbf{v}_c + \frac{1}{n} \nabla p(n),$$ \hspace{1cm} (39)

$$D \nabla^2 c_a - \frac{c_a}{\tau_a} + \alpha n = 0,$$ \hspace{1cm} (40)

$$D_a \nabla^2 c_a = \frac{c_a}{\tau_a} + s_a(t) H_a(x) = 0,$$ \hspace{1cm} (41)

$$D_r \nabla^2 c_r = \frac{c_r}{\tau_r} + s_r(t) H_r(x) = 0.$$ \hspace{1cm} (42)

Of course in some particular cases it may be possible to integrate (41) and/or (42) so that the relative solution can be directly substituted in (39).

We have already remarked several times that the diffusion equation (40) introduces a characteristic length $\ell = \sqrt{D_r}$ related to the size of the chords in the network structure. In the same way the other two diffusion equations (41) and (42) are characterised by two natural lengths $\ell_a = \sqrt{D_a}$ and $\ell_r = \sqrt{D_r}$, related with the ranges of action of the exogenous chemoattractant and chemorepellent, respectively. We will then show that within these ranges the effect of the exogenous chemical factors strongly influence the structure of the network. On the other hand, outside these ranges endogenous chemotaxis governs the formation of a more isotropic network.

From the practical point of view this means that having decided where to put the “spaghettis” or the “sponges” saturated with chemical factors one can identify some strips around them where the effect of the exogenous chemical factors will be felt, as shown in Figure 16.

As a first example consider the case in which the exogenous chemoattractant is located on two opposite sides of the domain, situation which can be realised putting some sponges impregnated with chemoattractant on the border of the Petri-dish. In this case Eq. (42) slightly modifies as there is no source term and the concentration of chemoattractant in the sponges (assumed constant in time) represents the proper boundary condition for (42)

$$c_a(x = 0, y, t) = c_a(x = L, y, t) = c_0, \quad \forall y \in [0, L], \forall t \geq 0.$$ \hspace{1cm} (43)
Fig. 15. Snapshots of the process of capillary network formation taken at different times as predicted by the chemomechanical model with $\kappa = 0.2$, $\gamma = 0$, and $a = 1$ and $b = 0$ in (a) and $a = 30$ and $b = 0.2$ in (b). The initial condition is the same as that shown in Figure 7.
Fig. 16. Cartoon explaining the effect of the ranges of influence of the chemotactic factors. In pink the chemorepellent, in green the chemoattractant, in red a possible capillary network.

together with periodic boundary conditions on the other two sides $y = 0, L$. Actually, in this case Eq. (41) can be readily solved so that the concentration

$$c_a = \tilde{c}_b \frac{e^{x/\ell_a} + e^{(L-x)/\ell_a}}{1 + e^{L/\ell_b}},$$

can be directly substituted in (39).

Fig. 17. Network formation influenced by an exogenous chemoattractant. In (a) it is placed on the right and on the left of the domain, and in (b) it is placed in the center of the domain. Bars indicate the value of $\zeta_a = 0.1$, i.e., the order of magnitude of the range of action of the exogenous chemoattractant.

In the simulation presented in Figure 17 the exogenous and endogenous chemoattractant were the same, so that $\ell_a = \ell = 0.196$ mm. Figure 17a then shows that in a range $\ell$ from the sides $x = 0$
and $x = L$ capillaries tend to organise perpendicularly to the sides. At a distance of order $\ell$ they branch giving rise to a capillary network very similar to the one obtained in the endogenous case. The final structure resembles the capillary network between arteries and veins. In spite of that the comparison is simply qualitative as the mechanisms governing the remodelling of capillaries is probably different from that modelled here.

In Figure 17b the chemoattractant is placed in the center $x_0$, i.e. $H_d(x) = \delta(x - x_0)$. This forms a circular zone influenced by the chemical factors characterised by the formation of capillaries more or less arranged in the radial direction.

On the other hand, the simulation in Figure 18 shows very clearly the action of chemorepellents. In particular, in Figure 18a it is placed in the center of the domain. Cells then move away from the central region (more or less in a radial direction) accumulating in a moving circumference with faster cells catching up slower ones. This process generates a circular capillary loop connected with the more isotropic structure outside it. The final size of the circumference and therefore of the circular capillary loop corresponds to the range of the chemorepellent. In fact, in the simulation the values of the parameters give $\ell_r = 0.31$ mm, which is close to the theoretical value $0.316$ mm.

In Figure 18b the chemorepellent is placed in a central axis parallel to the $y$-axis. Also in this case cells move away from the central axis, along $x$ accumulating on two lines parallel to the $y$-axis at a distance close to the range of the chemorepellent. In this way a capillary parallel to the stripe of chemorepellent is formed and connects with the outer network structure.

Again the size of the capillary-free region is nearly twice the range of action of the chemorepellent, actually a bit smaller ($0.54$ mm with respect to the theoretical value $0.532$ mm).

In Figure 18c three $1$ mm long stripes of chemorepellent are placed half a millimeter from each other. Again, cells are repelled from the stripes moving in a perpendicular way and aligning in the “corridors” forming capillaries parallel to the stripes. Outside the region influenced by the chemorepellent, the capillaries coalesce and connect to the external network.

In general, we can then say that chemoattractants induce in their range of actions the formation of capillaries which tend to run perpendicularly to the source of chemoattractants, while chemorepellent induce the formation of capillaries which tend to run parallel to the source of chemorepellent, at a distance from the source of the order of magnitude of the range of action, as sketched in Figure 16.

5 An Angiogenesis Model

As already mentioned in Section 1 another important process leading to the formation of vascular networks is angiogenesis, the recruitment of blood vessels from a pre-existing vasculature. Though this is a physiological process occurring, for instance, in wound healing, we will here focus on tumour induces angiogenesis, one of the most dangerous pathological aspects.

In fact, one of the crucial milestones in tumour development is the so called angiogenic switch, i.e. the achieved ability of the tumour to trigger the formation of its own vascular network. In order to achieve this, the tumour cells first secrete angiogenic factors which in turn induce the endothelial cells of a neighbouring blood vessel to degrade their basal lamina and begin to migrate towards the tumour. As they migrate, the endothelial cells develop sprouts which can then form loops and branches through which blood circulates. From these branches more sprouts form and the whole process repeats forming a capillary network. The biological process is described in more details in [2].

In the literature there are several angiogenic models. Some of them are discussed in [BD04, MW04, LS03] where the interested reader can find more references. We here focus on a procedure introduced by Chaplain and Anderson [CA00] and by Sleeman and Wallis [SW02] to reproduce realistic capillary networks induced by a tumour.

Specifically, Chaplain and Anderson [CA00] study the problem focusing on the evolution of

- the endothelial cell density per unit area ($n$) at the tip of the capillary sprouts;
- the concentration $c$ of Tumour Angiogenic Factors (TAF), e.g. VEGF;
Fig. 18. Network formation influenced by an exogenous chemorepellent. In (a) the chemical factor is placed in the center, while in (b) it is placed on the central axis of the domain. In (c) three \( \frac{L}{2} \)-long stripes of chemorepellent are placed at a reciprocal distance \( \frac{L}{4} \). Bars indicate the value of \( r = 0.1 \), i.e. the order of magnitude of the range of action of the exogenous chemorepellent.

- the concentration \( f \) of fibronectin, which, as already mentioned, is an important constituent of the extra-cellular matrix;

and focus on a fixed region outside the tumour.

As already seen in the previous section, the motion of the endothelial cells (at or near a capillary sprout-tip) is influenced by chemotaxis in response to TAF gradients. The chemotactic drift velocity can be taken to be of the form

\[
v_{\text{chemo}} = \beta(c) \nabla c,
\]

where the receptor-kinetic law of the form
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\[ \beta(c) = \beta_0 \frac{c_M}{c_M + c}, \]  

(45)

is assumed, to reflect the fact that a cell’s chemotactic sensitivity decreases with increased TAF concentration.

Also interactions between the endothelial cells and the extracellular matrix are found to be very important and to directly affect cell migration toward regions with larger amounts of extracellular matrix. The influence of fibronectin on the endothelial cells can then be modeled by the haptotactic drift velocity

\[ \mathbf{v}_{\text{haptot}} = w(f) \nabla f, \]  

(46)

where \( w(f) \) is the haptotactic function. In the following, it will be taken to be constant, \( w(f) = w_f \).

We observe that the cell velocity obtained by the sum of (44) and (46)

\[ \mathbf{v}_c = w(f) \nabla f + \beta(c) \nabla c, \]  

(47)

might be obtained from Eq. (15) neglecting its left hand side and the effect due to the partial stress tensor for the cellular constituent of the mixture. In fact, considering the interaction force as given by (21) with \( \mathbf{v}_s = 0 \) corresponding to an undeformable substratum and

\[ \mathbf{F}_c = \rho_c \hat{w}(f) \nabla f + \rho_c \tilde{\beta}(c) \nabla c, \]  

(48)

(see also (16)) one has

\[ \rho_c \hat{w}(f) \nabla f + \rho_c \tilde{\beta}(c) \nabla c - \rho_c \gamma \mathbf{v}_c = 0, \]  

(49)

which by a suitable definition of the coefficients leads to (47). In this way the usual chemotactic closure can be understood as a limit velocity obtained balancing the chemotactic and haptotactic “pulling” with the “drag force” related to the difficulty of moving in the extracellular-matrix and of removing old adhesion sites looking for new ones.

In their model Chaplain and Anderson [CA00] omit any birth and death terms because of the fact that they are focusing on the endothelial cells at the sprout-tips (where there is no proliferation) and that in general endothelial cells have a long half-life, on the order of months. On the other hand they add a random motility of the endothelial cells so that the equation for the endothelial cell density \( n \) can be written as

\[ \frac{\partial n}{\partial t} + \nabla \cdot [(\beta(c) \nabla c + w_f \nabla f) n] = k_n \nabla^2 n. \]  

(50)

The evolution of the concentration of TAF and fibronectin is assumed to satisfy locally

\[ \frac{\partial c}{\partial t} = -\delta_e n c, \]  

(51)

\[ \frac{\partial f}{\partial t} = \gamma n - \delta_f n f, \]  

(52)

where \( \delta_e \) and \( \delta_f \) are the uptake coefficients from the endothelial cells and \( \gamma \) is the production rate of fibronectin by the cells.

As initial conditions the concentration of fibronectin is taken to be constant, while the concentration of TAF, which is produced by the tumor located at the boundary of the domain, is taken to be satisfying

\[ k_c \nabla^2 c - \frac{c}{\tau} = 0, \]  

(53)

where \( k_c \) is the diffusivity of TAF and \( \tau \) its half life. The concentration of TAF at the boundary is a constant value where the tumor is located, e.g., in a central interval of one side or on the entire side, simulating a large tumor. Elsewhere no-flux boundary conditions are applied.
Therefore, the TAF secreted by the tumor diffuses into the surrounding tissue and sets up the initial concentration gradient between the tumour and any pre-existing vasculature, which is responsible for the directionality in the formation of the new capillaries. Later on, endothelial cells uptake TAF. However, diffusion is neglected.

The model (50, 52) is considered to hold on a square spatial domain of side $L$ with the parent blood vessel (e.g. limbal vessel) located along one side of the domain and the tumour located on the opposite side, either over the entire length or over part of it. Cells, and consequently the capillary sprouts, are assumed to remain within the domain of tissue under consideration and therefore no-flux boundary conditions of the form

$$
\mathbf{n} \cdot [-k_n \nabla n + n \left( \beta \nabla c + u_f \nabla f \right)] = 0,
$$

are imposed on the boundaries of the square, where $\mathbf{n}$ is the outward unit normal vector.

The aim of the technique used by Chaplain and Anderson [CA00] and by Sleeman and Wallis [SW02] which develops in the framework of reinforced random walk, is to follow the path of the endothelial cells at the capillary sprout tip in a discrete fashion. In order to do that they used a discretized version of the continuous model above. They then used the resulting coefficients of the five-point stencil of the standard central finite-difference scheme to generate the probabilities of movement of an individual cell in response to the chemoattractant gradients and to diffusion. We briefly sketch the procedure in two dimensions, because the generalisation to three dimensions is technical.

If $P_0$ is related to the probability of the cell of being stationary and to the probability of cells of moving away from the node $(i, j)$ to one of its neighbours, $P_1$ is related to the probability of new cells coming from the node to the right, and similarly for the others, one can write

$$
n_{j,k}^{i+1} = P_0 n_{j,k}^i + P_1 n_{j+1,k}^i + P_2 n_{j-1,k}^i + P_3 n_{j,k+1}^i + P_4 n_{j,k-1}^i, \tag{54}
$$

$$
\gamma_{j,k}^i = (1 - \Delta t \phi c_{n,j,k}^i) \gamma_{j,k}^i, \tag{55}
$$

$$
f_{j,k}^i = (1 - \Delta t \phi c_{n,j,k}^i) f_{j,k}^i - \Delta t \gamma_{j,k}^i, \tag{56}
$$

where, for instance,

$$
P_0=1 - \frac{4 \Delta t k_{n,j,k}}{\Delta x^2} + \frac{\Delta t}{\Delta x^2} \frac{\beta_0 c_{M} M_{c,j,k}}{c_{M} + c_{j,k}^i} \left[ (c_{j+1,k}^i - c_{j-1,k}^i)^2 + (c_{j,k+1}^i + c_{j,k-1}^i)^2 \right], \tag{58}
$$

$$
P_1 = \frac{4 \Delta t k_{n,j,k}}{4 \Delta x^2} \left[ \frac{\beta_0 c_{M} M_{c,j,k}}{c_{M} + c_{j,k}^i} \left( c_{j+1,k}^i + c_{j-1,k}^i + c_{j,k+1}^i + c_{j,k-1}^i - 4 c_{j,k}^i \right) \right] \tag{59}
$$

$$
+ w_f (f_{j+1,k}^i + f_{j-1,k}^i + f_{j,k+1}^i + f_{j,k-1}^i - 4 f_{j,k}^i), \tag{60}
$$

$$
P_1 = \frac{\Delta t}{\Delta x^2} \left[ \frac{\beta_0 c_{M} M_{c,j,k}}{c_{M} + c_{j,k}^i} \left( c_{j+1,k}^i - c_{j-1,k}^i \right) + w_f (f_{j+1,k}^i - f_{j-1,k}^i) \right]. \tag{61}
$$

In particular, if there is no chemical gradient, the situation is isotropic and the probabilities $P_1 - P_4$ of moving in any direction are equal. Even in this case, the extraction of a random number will decide whether the tip cell will stay still or will move to a particular neighbouring node rather than another. On the other hand, in presence of a chemical gradient the random walk becomes biased, because the cell has higher probabilities to move up the gradients of chemical factors.

In addition to that, the discretized set-up allows to include some phenomena difficult to describe using a model based on partial differential equations, e.g., capillary branching and anastomosis, i.e., the formation of capillary loops. In particular, Chaplain and Anderson [CA00] assumed that the density of endothelial cells necessary to allow capillary branching is inversely proportional to the distance from the tumour and proportional to the concentration of TAF. However, in order to branch a minimal distance from the previous diramation is needed, and of course there must be enough space in the discretized space to allow the formation of a new capillary. This assumption is
consistent with the observation that the distance between successive branches along the capillaries decreases when the tumour is approached. This phenomenon is called brush border effect, an effect well described by the model and the simulation, as shown in Figure 19.

In this approach it is even easier to describe anastomosis. When during their motion a capillary tip meets another capillary, then they merge to form a loop. If two sprout tips meet, then only one of the original sprouts continues to grow.

As shown in Figure 19, the capillary network build in this way look very realistic and compare well with what is observed experimentally. A three-dimensional animation of the angiogenic process is available at the web site www.maths.dundee.ac.uk/sanderso/3d/index.html.

![Fig. 19. Typical tumour induced capillary network as reproduced by the procedure in (??).](image)

6 Future Perspective

This chapter has been devoted to the presentation of some modelling approaches aimed at the description of the formation of capillary networks. We have shown some of the models available in the literature to reproduce in silico such structures. However, in our opinion this is only a first step. In fact, important developments would be achieved interfacing these models with others considering on one hand phenomena occurring at the cellular scale and on the other hand macroscopic effects and interactions with the surrounding environment. For instance, in the first framework, one could

- consider that along the developed capillary network the permeability of the vessel wall changes, resulting in an increased perfusion and interstitial pressure;
- consider that new-born vessels are immature and therefore subject to mechanical collapse due, for instance, to the pressure exerted by the tumour growing around them;
- consider in more detail the remodelling process giving rules to close unnecessary branches (see for instance ?? and references therein);
- consider adhesive properties of endothelial cells (see [MN05]);
- consider more closely the receptor dynamics involved for instance in the amplification of the chemotactic signal or in its saturation;
- consider the protein cascade linked to the VEGF-receptor to closely link the action (VEGF) to the reaction (motion and proliferation).

In the latter framework one could, for instance,
• simulate the diffusion of drugs in the capillary network as done by Stephanou et al. [SM05] and the diffusion of drugs and nutrients in the tissue;
• link the approach above with the one dealing with the development of tumour cords ?? and reviewed in this volume in the chapter by Fasano, Bertuzzi and Gandolfi to develop multiscale models of vascularized tumours;
• describe the oxygenation of tissues and the link between capillary distribution, hypoxic regions and remodelling.

Of course, the two directions are not mutually exclusive. For instance, in order to describe properly the perfusion of nutrients and drugs, one should take into account of the changes in wall permeability, the remodelling process can give rise to the formation of temporary hypoxic regions which trigger back the formation of new vessels.

Actually, developing models which spans from the sub-cellular to the tissue level is in our opinion one of the most fascinating problems in theoretical medicine to be developed in the future.

Appendix: 2D Reduction of the Substratum Equation for the Vasculogenesis Model

In describing the response of the substratum to the pulling of endothelial cells in the vasculogenesis process, we can exploit the fact that the size of the Petri dish is at least one order of magnitude larger than the thickness of the layer ($\approx 50 \mu m$). For this reason, it is convenient to reduce the force balance equation considering the substratum as two-dimensional.

We start from the equilibrium equation for the Cauchy three-dimensional continuum, which reads as

$$\nabla \cdot \mathbf{T} + \mathbf{f} = 0,$$  \hspace{1cm} (62)

where, for instance, $\mathbf{T}$ is a $3 \times 3$ tensor and $\nabla$ operates in the three-dimensional space.

Integrating over the thickness of the substratum, i.e., over the interval $[0, h]$, one obtains

$$\int_{0}^{h} \nabla \cdot \mathbf{T} \, dz + \int_{0}^{h} \mathbf{f} \, dz = 0.$$  \hspace{1cm} (63)

Confining the attention to the first term and using the tensorial notation, one can write

$$\int_{0}^{h} \nabla \cdot \mathbf{T} \, dz = \left( \int_{0}^{h} \frac{\partial T_{ij}}{\partial x_j} \, d x_3 \right) \mathbf{e}_i, \quad i, j = 1, 2, 3,$$  \hspace{1cm} (64)

where we have set $(x, y, z) = (x_1, x_2, x_3)$ and $(i, j, k) = (e_1, e_2, e_3)$ for sake of clarity and Einstein convention is used. In particular, it results

$$\int_{0}^{h} \frac{\partial T_{ij}}{\partial x_j} \, d x_3 = \int_{0}^{h} \left( \frac{\partial T_{i1}}{\partial x_1} + \frac{\partial T_{i2}}{\partial x_2} \right) \, d x_3 + \left[ T_{i3} \right]_0^h = \frac{\partial}{\partial x_i} \int_{0}^{h} T_{i1} \, d x_3 + \frac{\partial}{\partial x_3} \int_{0}^{h} T_{i2} \, d x_3 + \left[ T_{i3} \right]_0^h, \quad i = 1, 2, 3.$$

Defining the mean stresses per unit of length

$$T_{ij} := \int_{0}^{h} T_{ij} \, d x_3, \quad i, j = 1, 2, 3,$$  \hspace{1cm} (67)

Eq. (64) rewrites in the form
\[ \int_0^h \nabla \cdot \mathbf{T} \, dx_3 = \left( \frac{\partial T_{11}}{\partial x_1} + \frac{\partial T_{12}}{\partial x_2} + [T_{i3}]_0^h \right) \mathbf{e}_n. \]  

(68)

The second term in the left-hand side of (63) can be treated analogously

\[ \int_0^h f \, dz = \left( \int_0^h \tilde{f}_i \, dx_3 \right) e_i = \tilde{f}_i e_i. \]  

(69)

It is now convenient to split equation (63) into the following system:

\[ \nabla \cdot \mathbf{T} + \mathbf{f} + \left[ \left( \frac{T_{13}}{T_{13}} \right) \right]_0^h = 0, \]  

(70)

\[ \left( \frac{\partial T_{31}}{\partial x_1} + \frac{\partial T_{32}}{\partial x_2} \right) + \tilde{f}_3 + \left[ T_{33} \right]_0^h = 0, \]  

(71)

where

\[ \mathbf{T} := \begin{pmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{pmatrix}, \quad \text{and} \quad \mathbf{f} := \begin{pmatrix} \tilde{f}_1 \\ \tilde{f}_2 \end{pmatrix}, \]  

(72)

which define the reduced stress tensor and the reduced forcing term and \( \nabla \) is the operator on the plane. Note that the main formal difference between (62) and (70) consists in the boundary terms which appear in the second equation.

Assuming for simplicity that the substratum behaves like an elastic material

\[ \mathbf{T} = 2\mu \mathbf{E} + \lambda (\text{tr} \mathbf{E}) \mathbf{I}, \]  

(73)

where \( \mu, \lambda \) denote the Lamé coefficients and \( \mathbf{E} = \frac{1}{h}(\nabla \mathbf{u} + \nabla \mathbf{u}^T) \), by integrating (73) over the thickness of the substratum one has

\[ \int_0^h T_{ij} \, dx_3 = 2\mu \int_0^h E_{ij} \, dx_3 + \lambda \left( \int_0^h E_{kk} \, dx_3 \right) \delta_{ij}, \quad i, j = 1, 2, 3, \]  

which, after the obvious definition of the mean strains

\[ E_{ij} := \frac{1}{h} \int_0^h \tilde{E}_{ij} \, dx_3, \]  

yield

\[ T_{ij} = 2\mu h E_{ij} + \lambda h E_{kk} \delta_{ij}, \quad i, j = 1, 2, 3. \]  

This relation can be formally rewritten by the same splitting adopted in (70)

\[ \mathbf{T} = 2\mu h \mathbf{E} + \lambda (\text{tr} \mathbf{E} + E_{33}) \mathbf{I}, \]  

\[ T_{3j} = 2\mu h E_{3j} + \lambda h E_{kk} \delta_{3j}, \quad j = 1, 2, 3, \]  

(74)

where

\[ \mathbf{E} := \begin{pmatrix} E_{11} & E_{12} \\ E_{21} & E_{22} \end{pmatrix}, \]  

represents the reduced strain tensor and \( \mathbf{I} \) denotes here the \( 2 \times 2 \) identity matrix. Thanks to the symmetry of \( \mathbf{T} \) and to Eq. (67), one has \( T_{3j} = T_{j3} \), then Eqs. (74) effectively allow to express the constitutive relation of \( T_{ij} \) for all \( i, j = 1, 2, 3 \).

Finally, integrating the usual kinematic relation over the thickness of the substratum gives
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\[
\int_{0}^{h} E_{ij} \, dx_3 = \frac{1}{2} \left\{ \int_{0}^{h} \frac{\partial u_i}{\partial x_j} \, dx_3 + \int_{0}^{h} \frac{\partial u_j}{\partial x_i} \, dx_3 \right\} = \frac{1}{2} \left\{ \frac{\partial}{\partial x_j} \left( \int_{0}^{h} u_i \, dx_3 \right) (1 - \delta_{j3}) + [u_i]_0^h \, \delta_{j3} \right\} + \frac{\partial}{\partial x_i} \left( \int_{0}^{h} u_j \, dx_3 \right) (1 - \delta_{i3}) + [u_j]_0^h \, \delta_{i3} \right\} = \frac{1}{2} \left\{ \frac{\partial}{\partial x_j} \left( \int_{0}^{h} u_i \, dx_3 \right) + \frac{\partial}{\partial x_i} \left( \int_{0}^{h} u_j \, dx_3 \right) + \left( [u_i]_0^h - \frac{\partial}{\partial x_j} \int_{0}^{h} u_i \, dx_3 \right) \delta_{j3} + \left( [u_j]_0^h - \frac{\partial}{\partial x_i} \int_{0}^{h} u_j \, dx_3 \right) \delta_{i3} \right\} ,
\]

which, introducing the mean displacements,

\[
u_i := \frac{1}{h} \int_{0}^{h} u_i \, dx_3 ,
\]

allows to write

\[
E_{ij} = \frac{1}{2} \left\{ \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} + \left( \frac{1}{h} [u_i]_0^h - \frac{\partial u_i}{\partial x_j} \right) \delta_{j3} + \left( \frac{1}{h} [u_j]_0^h - \frac{\partial u_j}{\partial x_i} \right) \delta_{i3} \right\} ,
\]

and then split it into the system

\[
E = \frac{1}{2} (\nabla u + (\nabla u)^T)
\]

\[
E_{3j} = \frac{1}{2} \left[ \frac{\partial u_3}{\partial x_j} + \frac{1}{h} [u_3]_0^h + \left( \frac{1}{h} [u_3]_0^h - \frac{\partial u_3}{\partial x_j} \right) \delta_{j3} \right] , \quad j = 1, 2, 3 ,
\]

with \( u := (u_1, u_2)^T \), and in particular

\[
E_{33} = \frac{1}{h} [u_3]_0^h .
\]

Substituting (81) into (74) yields

\[
T = \mu h (\nabla u + (\nabla u)^T) + \lambda h \left( \nabla \cdot u + \frac{1}{h} [u_3]_0^h \right) I ,
\]

\[
T_{3j} = \mu h \left( \frac{\partial u_3}{\partial x_j} + \frac{1}{h} [u_3]_0^h \right) + h \left( \lambda \nabla \cdot u + \mu + \frac{\lambda}{h} [u_3]_0^h - \mu \frac{\partial u_3}{\partial x_j} \right) \delta_{j3} , \quad j = 1, 2, 3 ,
\]

using this result, Eq.(70) finally specialize to

\[
h \mu \nabla^2 u + h(\lambda + \mu) \nabla (\nabla \cdot u) + f + \left[ \begin{array}{c} T_{13} \\ T_{23} \\ T_{33} \end{array} \right] + \lambda \nabla u_3 \right)_0^h = 0 ,
\]

\[
h \mu \nabla^2 u_3 + f_3 + \left[ \begin{array}{c} T_{13} \\ T_{23} \\ T_{33} \end{array} \right] + \mu \left( \frac{\partial u_1}{\partial x_1} + \frac{\partial u_2}{\partial x_2} \right) \right)_0^h = 0 .
\]

Note that Eqs.(85) and (86) are mutually independent with respect to the variables \( u \) and \( u_3 \), provided the respective forcing terms \( f \) and \( f_3 \) do not depend on \( u_3 \) and on \( u_1, u_2 \). In this
hypothesis, we can restrict our analysis to equation (85), which suffices by itself to obtain a two-dimensional model of the substratum, but we need to characterise the boundary term

\[
\left[ \left( \frac{T_{13}}{T_{23}} \right) + \lambda \nabla u_3 \right]_0^h,
\]

via the new variable \( u \). For this, we refer to the original three-dimensional problem and we make the following assumptions:

1. a no-slip condition of the substratum at the lower basis, which can be interpreted as its adhesion to the underlying Petri dish

\[
\begin{align*}
\dot{u}_1 &= \dot{u}_2 = \dot{u}_3 = 0 & \text{for } x_3 = 0; \\
\end{align*}
\]

2. because of cells motion, an imposed shear stress and a zero normal stress at the upper basis, coming from the hypothesis that cells move on the surface of the substratum without penetrating it

\[
\left( \frac{T_{13}}{T_{23}} \right) = t_{\text{cell}}, \quad T_{33} = 0 & \text{ for } x_3 = h. 
\]

Equation (88) gives then \( \nabla u_3 = 0 \) for \( x_3 = 0 \) which, together with equation (89), allows to rewrite the boundary term (87) as

\[
\begin{align*}
t_{\text{cell}} + \lambda \nabla u_3 |_{x_3=h} - \left( \frac{T_{13}}{T_{23}} \right) |_{x_3=0}.
\end{align*}
\]

Furthermore, using the linear elastic constitutive relation (73) we obtain

\[
\nabla u_3 = -\left( \frac{\partial \dot{u}_1}{\partial x_3}, \frac{\partial \dot{u}_2}{\partial x_3} \right)^T + \frac{1}{\mu} \left( \frac{T_{13}}{T_{23}} \right),
\]

which yields

\[
\begin{align*}
\lambda \nabla u_3 |_{x_3=h} &= -\lambda \left( \frac{\partial \dot{u}_1}{\partial x_3}, \frac{\partial \dot{u}_2}{\partial x_3} \right)^T |_{x_3=h} + \frac{\lambda}{\mu} t_{\text{cell}},
\end{align*}
\]

so that Eq. (90) specializes to

\[
\begin{align*}
\frac{\mu + \lambda}{\mu} t_{\text{cell}} - \lambda \left( \frac{\partial \dot{u}_1}{\partial x_3}, \frac{\partial \dot{u}_2}{\partial x_3} \right)^T |_{x_3=h} - \left( \frac{T_{13}}{T_{23}} \right) |_{x_3=0} = 0,
\end{align*}
\]

and Eq.(85) becomes

\[
\begin{align*}
h\mu \nabla^2 u + h(\lambda + \mu) \nabla (\nabla \cdot u) + f + \frac{\mu + \lambda}{\mu} t_{\text{cell}} - \lambda \left( \frac{\partial \dot{u}_1}{\partial x_3}, \frac{\partial \dot{u}_2}{\partial x_3} \right)^T |_{x_3=h} - \left( \frac{T_{13}}{T_{23}} \right) |_{x_3=0} = 0.
\end{align*}
\]

The procedure above holds for any three-dimensional linear elastic body. The thin layer assumption has not been used yet, but its application allows to approximate the displacements \( u \) as a linear function of \( x_3 \)

\[
\begin{align*}
\dot{u}_i (x_1, x_2, x_3) &= \varphi_i (x_1, x_2) x_3, \quad i = 1, 2, 3
\end{align*}
\]

where we used the no-slip condition at the interface between the substratum and the Petri dish. According to Eq. (80), then
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\[ u_i = \frac{1}{h} \varphi_i \int_0^h x_3 \, dx_3 = \frac{h}{2} \varphi_i, \]

which gives \( \varphi_i = \frac{2}{h} u_i \) and then

\[ \tilde{u}_i (x_1, x_2, x_3) = \frac{2}{h} u_i (x_1, x_2) x_3. \] (91)

Thanks to (91) we can now express

\[ \left( \frac{\partial \tilde{u}_1}{\partial x_3} \right) \bigg|_{x_3 = h} = \frac{2}{h} \left( \frac{u_1}{u_2} \right) \] (92)

\[ \left( \frac{\partial \tilde{u}_2}{\partial x_3} \right) \bigg|_{x_3 = h} = \frac{2}{h} \left( \frac{u_1}{u_2} \right) \] (93)

\[ \left( \frac{\partial \tilde{E}_{13}}{\partial x_3} \right) \bigg|_{x_3 = 0} = 2\mu \left( \frac{E_{13}}{E_{23}} \right) \bigg|_{x_3 = 0} = \frac{2\mu}{h} \left( \frac{u_1}{u_2} \right), \] (94)

and finally obtain

\[ h\mu \nabla^2 \mathbf{u} + h(\lambda + \mu) \nabla (\nabla \cdot \mathbf{u}) + \mathbf{f} + \mathbf{f}_{\text{cell}} - \frac{s}{h} \mathbf{u} = 0. \] (95)

where

\[ \mathbf{f}_{\text{cell}} = \frac{\mu + \lambda}{\mu} \sigma_{\text{cell}}, \quad s = 2(\mu + \lambda). \]

We remark that (95) is characterised by the presence of body forces which actually derive from the boundary conditions. In fact, the term \( \sigma_{\text{cell}} \) comes from the cell pulling at the top surface and the terms \( -\frac{s}{h} \mathbf{u} \) is a consequence of the shear stress produced at the lower boundary of the substratum by the adhesion of the Petri dish. In Section 4.3 \( \sigma_{\text{cell}} = F_{\text{visc}} + F_{\text{elast}} \).

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References


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