

Multi-scale modelling of cancer cell intravasation: the role of cadherins in metastasis

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Abstract

Transendothelial migration is a crucial process of the metastatic cascade in which a malignant cell attaches itself to the endothelial layer forming the inner wall of a blood or lymph vessel and creates a gap through which it enters into the bloodstream (or lymphatic system) and then is transported to distant parts of the body. In this process both biological pathways involving cell adhesion molecules such as VE-cadherin and N-cadherin, and the biophysical properties of the cells play an important role. In this paper, we present one of the first mathematical models considering the problem of cancer cell intravasation. We use an individual force-based multi-scale approach which accounts for intra- and inter-cellular protein pathways and for the physical properties of the cells, and a modelling framework which accounts for the biological shape of the vessel. Using our model, we study the influence of different protein pathways in the achievement of transendothelial migration and give quantitative simulation results comparable with real experiments.

1. Introduction

Metastasis is a crucial process in the growth of a cancer, enabling the primary tumour mass to spread to distant, secondary sites in the host. To colonize distant organs, the malignant cells need to get into a blood or lymph vessel, be transported in the vascular system and, eventually, attach to the inner wall of the vessel and escape from the vasculature. In this new location, the malignant cell proliferates to form a secondary tumour. Intravasation and extravasation are defined as the processes of a cell entering and leaving the vascular network, respectively. These are essential natural mechanisms used by specialized cells to travel to distant organs. However, the same mechanisms are used by cancer cells to create colonies and secondary tumours [13, 24]. Both intravasation and extravasation occur in a similar way. The cancer cell

attaches to the endothelial wall forming the vasculature and parts two of the endothelial cells to create enough space to go into (or out from, in the case of extravasation) the vascular network. This migration through the endothelial tissue is also known as transendothelial migration (TEM). The physical properties of the cell combined with the intra- and inter-cellular protein interactions that govern cell–cell adhesion are the driving forces of TEM. Formation and detachment of bonds involve the interactions of, among other molecules, N-cadherins and VE-cadherins and the activation of related protein pathways.

Modelling of cell–cell adhesion and biophysical properties have been widely studied by continuum models [4, 8, 9, 20, 35], individual-based models [7, 15, 29, 30, 32] and lattice-based [14] approaches, and cell rotation under flows has been previously modelled by Chotard-Ghodsniya *et al* [13] as a step in the extravasation process. Relatively

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little has been done up until now to study cell intravasation as a step in the metastatic cascade, and this still remains an open area. In this paper we introduce a force-based multi-scale mathematical model, which accounts for intra-cellular interactions and biophysical properties of the cell and the blood vessel, to show how intra-cellular protein pathways combined with physical forces could operate as the driving causes of cell intravasation in blood vessels. We simulate four cancer cell genotypes which differ in the adhesion protein pathways and study the influence on the time needed to achieve TEM when changing from one genotype to another.

The blood vessel wall consists of three distinct layers of tunics [2] from the inner wall to the outer wall. The *tunica intima* is composed of endothelium and rests on a connective tissue membrane rich in elastic and collagenous fibres. The *tunica media* makes up the bulk of the vessel wall and includes smooth muscle fibres and a thick layer of elastic connective tissue. Finally, the *tunica adventitia* attaches the vessel to the surrounding tissue. It is a thin layer which consists of connective tissue, elastic collagenous fibres and minute vessels which are the beginning of small capillaries which will help to irrigate the surrounding tissue. The *tunica intima* is the last cell layer that a cancer cell needs to cross to reach the vasculature. To achieve TEM, the metastatic cell needs to break, among other cellular adhesion molecules, the VE-cadherin bonds which hold the endothelial cells of the *tunica intima*. Whether these bonds are broken by the mechanical forces exerted by the malignant cell on the endothelial wall or whether there are other biological mechanisms involved is not yet completely elucidated. To study this problem, Qi *et al* [28] performed a transendothelial migration assay *in vitro*. They used matrigel chambers to create an artificial endothelial wall and showed how cancer cells undergoing TEM attached to the endothelial layer via N-cadherin proteins. After some time, the malignant cell was able to open a gap in the endothelial wall and, squeezing its body, pass through it. Later, they performed the same assay using cancer cells which were not able to express N-cadherin bonds. In this second experiment, they observed how TEM was significantly delayed even when the cancer cell was able to express other cell–cell binding proteins. These observations on how the specific behaviour of N-cadherin speeds up TEM raise the question of the existence of intra-cellular pathways related to N-cadherin which may help to alter the adhesion properties of endothelial cells.

VE-cadherin and N-cadherin are calcium-dependent adhesion molecules characterized by binding at the cytoplasmic tail with the same group of proteins to link the cell–cell bonds and the actin cytoskeleton. Both N- and VE-cadherins, when positioned in the intermembrane region, form homophilic cell–cell junctions. The cytoplasmic tail binds to the proteins of the catenin family $p120$ -catenin, α -catenin and β -catenin. At this position, α -catenin and β -catenin form a complex to link the actin filaments of the cytoskeleton and the cadherins. When bonds are released, caused by intra-cellular signalling or the effect of mechanical stress, the multi-protein complex is broken and the cadherins are internalized by endocytosis [26, 33].

Among the different intracellular pathways that can down-regulate cadherin-mediated adhesion, the Src pathway may

play a principal role in TEM. Src enzymes attach to the intracellular binding domain of cadherins and disrupt the bonds after phosphorylating the catenins [5, 19, 31, 38]. When studying TEM, Qi *et al* [28] showed that activation of the N-cadherin pathway was followed by an up-regulation of the concentration of Src enzymes at the zone of heterotypic contacts between the cancer and the endothelial cells. This interaction raises the question of a possible down-regulation of the VE-cadherin bonds of the endothelial cells forming the *tunica intima* after Src has been up-regulated via the N-cadherin pathway. Figure 1 shows a schematic diagram of this hypothesis. The cancer cell attaches to the endothelial wall via N-cadherin molecules. The expression of N-cadherin activates Src in both the cancer cell and the endothelial cells. In the cytoplasm of the endothelial cells, close to the apical part where the cancer cell is in contact, Src targets not only N-cadherin but also VE-cadherin. As a consequence, the bonds between endothelial cells are disrupted and TEM is initiated.

2. Material and methods

We study the disruption of the VE-cadherin-mediated bonds between the endothelial cells forming the *tunica intima* of the vessel. For this, we use a mathematical force-based multi-scale model which accounts for the biophysical properties of the cells and simplified protein pathways involved in TEM. The model is structured as follows. At the intra-cellular scale, the protein concentrations are governed by a system of ordinary differential equations (equations (4)–(19)). At the inter-cellular scale, the cell–cell forces are modelled by a modified Hertz model (equations (20)–(22)) where the intensity of the adhesive forces depends on the intracellular cadherins available to travel to the cell surface to form bonds. Finally, at the extra-cellular scale, the cells move according to a Langevin equation which accounts for the main biophysical characteristics of the multi-cellular system (equations (1) and (2)). In this section, we give a brief description of the model; a more detailed analysis is provided in the appendix.

Although under normal conditions cells adhere to each other via different types of cell adhesion molecules [21, 25, 34, 40], we include N-cadherin as the main cell–cell adhesion molecule expressed by the cancer cell. This simplification is partly required by the complexity of the system and motivated by the observations that, among other cell adhesion molecules, N-cadherin may play a principal role in TEM [27, 28]. Many of the parameters of the model have been extracted from the literature [18, 22, 29]. For the estimated parameters we have chosen realistic values in agreement with observed intracellular protein concentrations [30], and all of them can be in principle measured in experiments.

2.1. Intracellular scale

The cells in our model can attach to each other via VE-cadherin or N-cadherin molecules. Precisely which of these two binding proteins is used by an endothelial cell to bind to another cell will depend on the adhesion molecule that the adhering cell

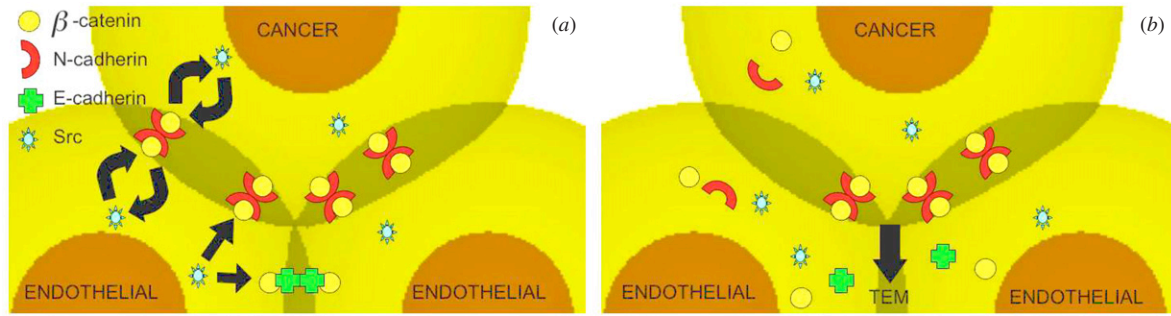


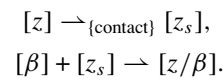
Figure 1. Diagram showing the intra- and inter-cellular protein pathways considered in the model. (a) The cancer cell comes into contact with the endothelial cells forming the wall and creates N-cadherin-mediated bonds. As a consequence of the activation of the N-cadherin pathway, the Src-kinase activity is up-regulated. (b) The β -catenin linked to the VE-cadherin and N-cadherin molecules is phosphorylated by the Src-kinases. After the β -catenin-VE-cadherin complex is phosphorylated, the VE-cadherin-mediated bonds holding the endothelial cells are down-regulated and TEM migration can be achieved.

(This figure is in colour only in the electronic version)

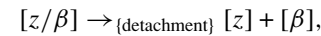
expresses at that precise moment. If it is the malignant cell that attaches to the endothelial cell, it will create an N-cadherin-mediated bond. If, on the other hand, the endothelial cell comes into contact with another endothelial cell, it will try to preserve the architecture of the *tunica intima* creating VE-cadherin-mediated bonds [27, 28].

When two cells of the system come into contact, the cadherins change into a stimulated state prior to forming cell-cell bonds. Following the observations of Chen *et al* [11], we consider that cadherins are transported to the intermembrane region to form bonds with the neighbours after forming a complex with β -catenin. This is translated into our model by considering the following possible states for VE- and N-cadherins: (a) free in the cytoplasm ($[E]$, $[N]$); (b) in the cytoplasm in a stimulated state before forming a complex with β -catenin ($[E_s]$, $[N_s]$) and (c) forming bonds at the intermembrane position ($[E/\beta]$, $[N/\beta]$).

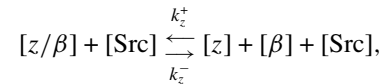
The expression of N-cadherin molecules forming bonds between the malignant cell and the endothelial cells activates the Src-kinase activity [27, 28]. In our model, these enzymes can target both the N-cadherin-mediated bonds between the cancer and the endothelial cells and the VE-cadherin-mediated bonds formed between two endothelial cells. Figure 1 shows the action of the Src kinases in the bonds of the cells forming the endothelial wall. The cancer cell comes into contact with the *tunica intima* and activates the kinases after forming N-cadherin-mediated bonds (figure 1(a)). The kinases not only target the N-cadherin- β -catenin complexes but also the VE-cadherin- β -catenin and disrupt the bonds between endothelial cells thereby facilitating TEM (figure 1(b)). When the cadherin bonds are disrupted, the β -catenin-cadherin complex is ruptured, the β -catenin becomes soluble β -catenin ($[\beta]$) and the cadherin is sequestered into the cytoplasm by endocytosis. It is known that in its soluble state, β -catenin can be degraded by different proteasome pathways [1]. To model this fact, we include a generic proteasome variable $[P]$ which degrades β -catenin after forming a complex with it ($[C]$). We also assume that both types of cadherins can be recruited to form bonds again. The main biochemical reactions affecting the cadherin adhesion pathways are the following (z denotes either E or N):



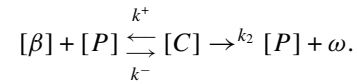
Cell-cell detachment can happen by a combination of physical stress, i.e.



and the action of tyrosine kinases:



where $[S_z]$ denotes the complex *Src-z-cadherin- β -catenin*. Finally, the generic *proteasome* variable accounts for the degradation of β -catenin giving a debris product ω :



These reactions will produce different adhesion dynamics depending on the type of the cell that is being observed. Following the observations of Qi *et al* [28], we assume that the malignant cell does not express VE-cadherin, and hence $z = N$ for the cancer cell.

In the case of the endothelial cells, there will be a set of equations where $z = N$ to explain the heterotypic bonds with the malignant cell and a set of equations where $z = E$ to explain the homotypic bonds between endothelial neighbours. The exact equations of the intracellular protein concentrations are given in appendix A.

2.2. Equations of motion

We model the cell movement by equations of motion, being slightly different depending on the type of cell. In the equation for the malignant cell, we take into account friction terms with the environment and with cells, adhesive-repulsive forces between cells, random movement and directed movement:

$$\underbrace{\frac{\Gamma_{cs}^f v_c}{\text{substrate friction}}}_{\text{substrate friction}} + \underbrace{\sum_{j \text{ nn } c} \frac{\Gamma_{cj}^f (v_c - v_j)}{\text{cell-cell friction}}}_{\text{cell-cell friction}} = \underbrace{\sum_{j \text{ nn } c} \frac{F_{cj}}{\text{interaction forces}}}_{\text{interaction forces}} + \underbrace{\frac{f_c(t)}{\text{random movement}}}_{\text{random movement}} - \underbrace{\frac{\hat{F}_c}{\text{directed movement}}}_{\text{directed movement}}. \quad (1)$$

On the left-hand side of the equation, where \underline{v}_c is the velocity of the cancer cell at time t , the first term accounts for the friction with the substrate (i.e. the different tunics and protein networks surrounding the cancer cell). The second term accounts for the friction with the membranes of the closest neighbours. We assume that this term also accounts for the friction caused by other cell–cell adhesion molecules not explicitly included in the model (details of the tensors $\underline{\Gamma}_{cs}^f$ and $\underline{\Gamma}_{cj}^f$ are given in appendix A). The sums are over the nearest neighbours in contact with the malignant cell (denoted as nn). On the right-hand side, the first term models the forces between the cancer cell and the endothelial cells (explained in detail in appendix A). This term accounts both for the repulsion forces, when cells come too close up to compression, and for the adhesive forces created by the VE-cadherin molecules. The second term models the random contribution to the movement caused by the exploration of the cancer cell of the nearby space. The third term is the directed movement which leads the cancer cell towards the vessel. Before the cell has crossed the endothelial wall, it is not directly exposed to the stream. Therefore we assume that flow forces may be negligible. At the moment when the cell has crossed the endothelial wall, the stream will exert a force on the cell. However, since we are studying only the process comprised from the approach of the cell to the endothelial wall up to the precise instant when the cell has crossed the wall (i.e. before the stream exerts a force on the cell), we do not account for any flow term. Endothelial cells forming the *tunica intima* move according to the force balance equation:

$$\underbrace{\underline{\Gamma}_{cs}^f \underline{v}_i}_{\text{substrate-friction}} + \sum_{j \text{ } nn \text{ } i} \underbrace{\underline{\Gamma}_{ij}^f (\underline{v}_i - \underline{v}_j)}_{\text{cell-cell friction}} = \underbrace{\sum_{j \text{ } nn \text{ } i} \underline{F}_{ij}}_{\text{interaction-forces}} + \underbrace{f_i(t)}_{\text{random-movement}} + \underbrace{\hat{F}_{c,i}}_{\text{response-forces}}. \quad (2)$$

The first two terms are defined in an analogous way as for equation (1) with the difference that the subindex i refers to the endothelial cell and the subindex j can refer to another endothelial cell or to the malignant cell. On the right-hand side, the first term models the adhesive and repulsive forces between the endothelial cell and the neighbours in contact. The second term models the random spatial fluctuations of the tissue forming the blood vessel. The third term is only active when the malignant cell comes into contact with the endothelial wall and models the response to the force exerted by the malignant cell in the endothelial cells (*directed movement* in (1)).

2.3. Coupling of cell parameters to intra-cellular molecule concentrations

The malignant cell moves towards the *tunica intima* driven by interactions with its local surroundings. When not in contact with other cells, surface adhesion molecules, such as integrins [10, 17, 39], allow the cell to move through the medium via a combination of random movement and directed movement. In our model, this type of propulsion is recorded

in the random force term and the directed force term of equation (1). When the migrating cell uses other cells as a substratum for movement, as in the case of crossing the *tunica intima*, other adhesion molecules such as N-cadherins play the role of the integrins. These bonds via N-cadherin proteins exert a resultant force on the neighbouring cells in the opposite direction to the movement of the malignant cell, explained by the response forces in equation (2). We model this using weights depending on the density of N-cadherin adhesion molecules shared between the cancer cell and each endothelial cell in contact, i.e.

$$\underline{\hat{F}}_{c,i} = - \frac{[N/\beta]_{c,i}}{\sum_{cnni} [N/\beta]_{c,i}} \underline{\hat{F}}_c, \quad (3)$$

where the subscripts denote the pair of cells sharing the N-cadhering bonds. Recall that equations (1) and (2) combined with (3) are a balanced system ($\underline{F}_c + \sum_i \underline{\hat{F}}_{c,i} = 0$) as required for consistency.

The adhesion forces between the endothelial cells forming the *tunica intima* are controlled by the density of VE-cadherin in the cell membrane within the cell–cell contact zone. Following our previous work [29] we take as the adhesion energy surface density $W_s \varrho_m = 200 \mu\text{N m}^{-1}$, so that the surface receptor density is $\varrho_m = 200 \mu\text{N m}^{-1} W_s^{-1}$. We use this value as a maximum density of the cadherin- β -catenin complex in the membrane and define the actual density by

$$\varrho = \frac{[E/\beta]}{E_T} \varrho_m.$$

Figure 2 shows the resulting force function depending on the different ϱ_m^{ij} values. By modifying the intra-cellular concentration of β -catenin, the cells can control the concentration of $[E/\beta]$ -complexes and thereby the strength of the inter-cellular adhesion force.

3. Results and discussion

In this section, we present the simulation results of the intra-cellular model and the force-based model. For initial conditions, we set a configuration of endothelial cells in the form of the *tunica intima* of a small blood vessel (about 25 cells of the perimeter). Between the endothelial cells, the bonds are formed by VE-cadherins. In these cells, initially, the N-cadherin concentration is sequestered in the cytoplasm. We place a cancer cell a few microns away from the vessel. Due to mutations that affect its adhesive system, the cancer cell cannot express VE-cadherin but is only able to express N-cadherin. The malignant cell will move towards the blood vessel governed by the directed movement force term in equation (1) until it comes into contact with the endothelial cells forming the *tunica intima*.

3.1. Multi-scale simulations

Figure 3 shows the spatio-temporal dynamics of the multi-scale simulations. The colouring of the cells denotes the different types of protein they are using when forming bonds,

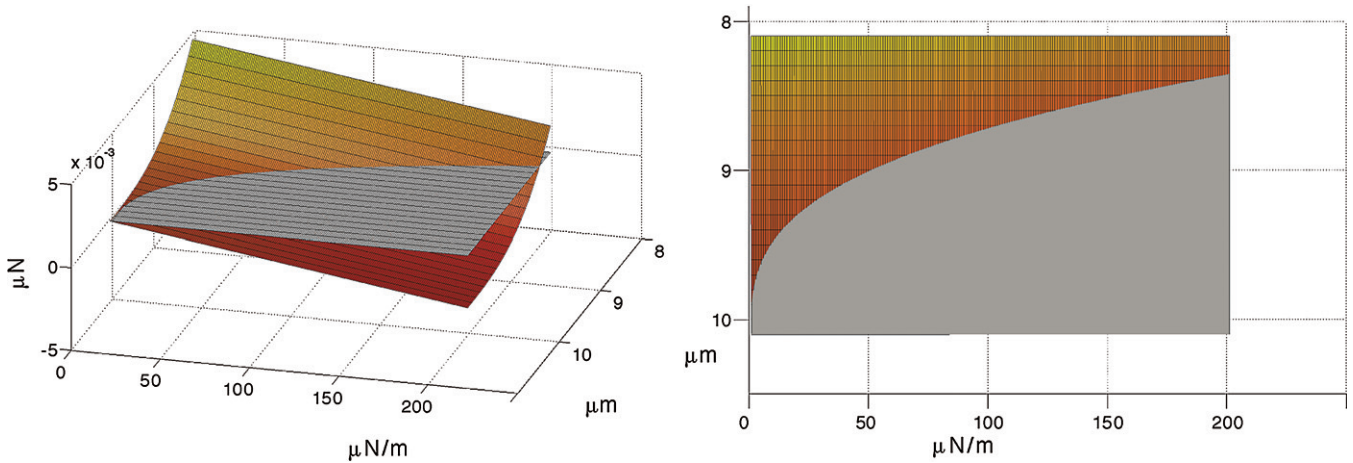


Figure 2. The left plot shows the force function between two cells. The variables are cell–cell distance (in μm) and adhesion energy per unit of area in contact (in $\mu\text{N m}^{-1}$). On the right, the plain zone determines the zone where adhesive forces act.

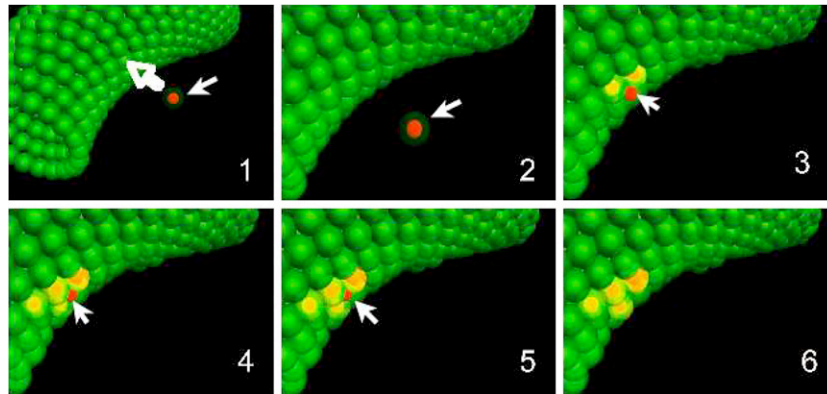


Figure 3. Plot showing the spatio-temporal evolution dynamics of a malignant cell (red nucleus coloured cell, marked with a full arrow) approaching a blood vessel to undergo TEM. The initial green colour of the endothelial cells forming the blood vessel denotes the $[E/\beta]$ concentration. When the malignant cell attaches to the vessel, the VE-cadherin bonds are disrupted and new N-cadherin bonds are formed (yellow). After some time, the malignant cell manages to disrupt the endothelial bonds enough to open a gap in the vessel and undergo TEM. Time scales in the frames correspond to the intracellular simulations of figure 4 at time 1 = 0 min, 2 = 50 min, 3 = 100 min, 4 = 200 min, 5 = 300 min, 6 = 400 min.

i.e. green is used for VE-cadherin bonds and yellow for N-cadherin bonds. The malignant cell approaches the *tunica intima* and attaches to the endothelial cells. At the moment when contact occurs (frame 3, $t \approx 100$ min), the malignant cell and the endothelial cells start forming heterotypic N-cadherin bonds (yellow). As time evolves, it can be observed how the VE-cadherin bonds (green) between the endothelial cells are disrupted and the malignant cell is able to open a gap in the endothelial wall and undergo TEM (frame 6, $t \approx 400$ min).

From the assumptions of the model, a malignant cell that is only able to express N-cadherin therefore only induces the formation of N-cadherin bonds with the endothelial cells. At this stage within the endothelial cells, the two types of cadherins start competing for the soluble β -catenin. Figure 4 shows the intra-cellular dynamics of the cadherin and β -catenin proteins corresponding to the intracellular simulations of figure 3. The plot on the left shows the intracellular protein dynamics of an endothelial cell to which the cancer

cell attaches. At the moment when the malignant cell comes into contact with the endothelial cell ($t \approx 100$ min), the VE-cadherin concentration forming bonds between the endothelial neighbours decreases. This is initially caused by the degradation of the VE-cadherin- β -catenin complexes by the Src enzymes. At time $t \approx 450$ min, due to a combination of the degradation of the VE-cadherin bonds by the Src activity and the physical forces exerted by the malignant cell on the wall, the bonds between endothelial cells are totally disrupted and the VE-cadherins are internalized in the cytosol. As a consequence, soluble β -catenin is up-regulated. The plot on the right shows the intracellular protein dynamics of the cancer cell. When it attaches to the endothelial wall at time $t \approx 100$ min, the N-cadherin in the cytosol is stimulated and binds to β -catenin. This complex is transported to the membrane to form heterotypic bonds with the endothelial cells. At time $t \approx 450$ min, the cancer cell succeeds in opening a gap in the *tunica intima* and undergoes TEM. As a consequence of this intravasation and losing contact with the *tunica intima*, the

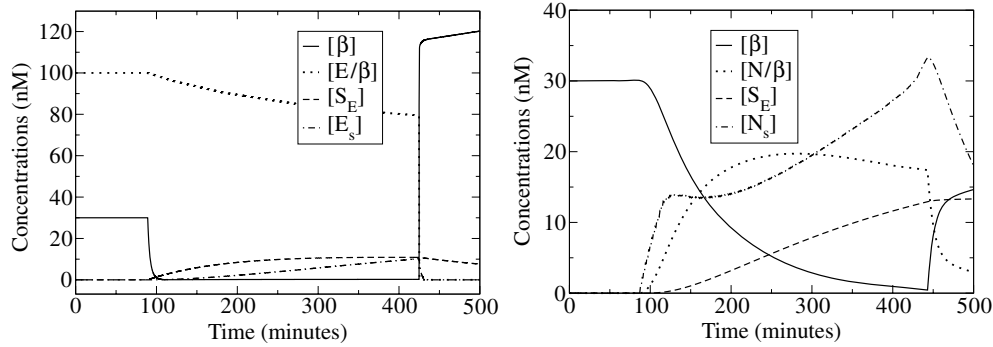


Figure 4. The plots in the left figure show the temporal evolution of the intra-cellular protein concentrations of an endothelial cell to which the cancer cell attaches at time $t = 100$ min. As time evolves, the VE-cadherin concentration at the surface of the cell is partially decreased by the action of the enzymes. At $t \approx 450$ min the cancer cell disrupts the bonds formed by the endothelial cells and, as a consequence, the VE-cadherin at the surface of the endothelial cell is dramatically decreased. The plot on the right shows the protein concentrations of the cancer cell when attaching to the endothelial wall. At time $t = 100$ min, the cell comes into contact with the tunica intima and the N-cadherin travels to the membrane to form heterotypic bonds. As a combination of the biochemical pathways and the physical forces, the cancer cell undergoes TEM at time $t \approx 450$ min.

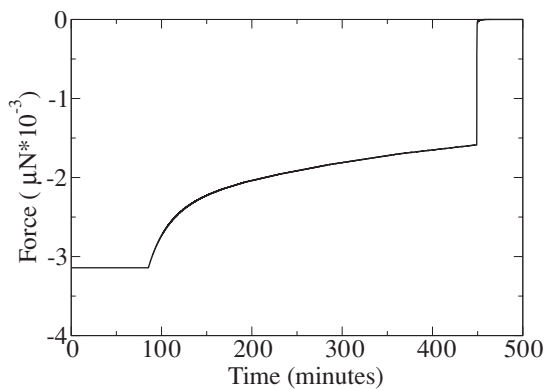


Figure 5. Plot showing the time evolutionary dynamics of the adhesion force of the same endothelial cell as that in figure 4 with the neighbours forming the vessel. It can be observed that the intensity of the adhesive forces decreases in time.

N-cadherin molecules forming bonds in the intermembrane position are internalized into the cytosol.

Figure 5 shows the time evolution dynamics of the adhesive forces between the endothelial cell to which the cancer cell attaches at time $t \approx 100$ min and its endothelial neighbours forming the *tunica intima*. It can be observed that the strength of the adhesive forces decreases up to detachment simultaneously as the VE-cadherin concentration is down-regulated in the left plot of figure 4. The slow reduction of the adhesive forces is caused by the intracellular action of the Src enzymes. At time $t \approx 450$ min the cancer cell undergoes TEM, thereby breaking the bonds between the endothelial cells and the adhesive forces disappear. In this simulation, detachment occurs by a combination of physical and biological causes. Cell detachment via physical forces has been previously studied in experiments using atomic force microscopy in [6, 36].

3.2. Influence of the protein pathways in TEM

For a better understanding of how the intracellular pathways and physical forces influence TEM, we now make

experimentally testable predictions concerning the behaviour of four different genotypes of the malignant cell. In each genotype, we ‘knocked out’ a biological pathway related to the cadherin-mediated adhesion and performed multi-scale simulations. The genotypes are characterized by its capacity of creating N-cadherin-mediated bonds with the *tunica intima* and by the capacity of inducing a detachment of the endothelial–endothelial bonds by Src activity. The ability for creating N-cadherin-mediated bonds is modelled by changing the values of the β -catenin–N-cadherin binding rate, v_N (in equations (7), (6), (12) and (16)). The ability of inducing Src activity within the cells forming the endothelial wall is modelled by changing the *Src*–*E-cadherins* binding rate, k_z^+ , in equations (9), (18) and (19). The initial parameter values of all the genotypes are taken from table 1 and the different variations of ‘knock-outs’ in the pathways are specified in the definition of each genotype.

3.2.1. Genotype N-Off, Src-Off. In the first genotype, the cancer cell cannot induce the formation of N-cadherin- β -catenin complexes and it does not induce the activation of the Src pathway in the endothelial cells after coming into physical contact. Therefore, the malignant cell undergoes TEM as a consequence of the physical forces exerted after pushing the underlying substrate. This genotype is equivalent to setting the intracellular parameters $k_z^+ = 0$ in the endothelial cells (equations (18) and (19)) and $v_N = 0$ in the endothelial and cancer cells (equations (6), (7), (12) and (16)).

3.2.2. Genotype N-On, Src-Off. In the second genotype, the contact of the malignant cell with the endothelial wall induces N-cadherin-mediated bonds ($v_N = 100 \text{ min}^{-1}$ in both the cancer and the endothelial cells) but does not activate the Src pathway in the endothelial cells. In this case, the cell will undergo TEM by the pulling forces exerted on the N-cadherin focal contacts with the endothelial cells. To obtain this genotype, we set the intracellular parameters $k_z^+ = 0$ in the endothelial cells (equations (18) and (19)).

Table 1. The table shows the parameter values used in the model. Wherever possible, the parameters were taken from the literature. \diamond Those intra-cellular parameters not taken from experimental data were assumed to be of such a magnitude as to maintain the β -catenin steady value at 35 nM as observed by Lee *et al* [22].

Parameter	Definition	value
Physical parameters		
R_0 [18]	Cell radius	$5 \mu\text{m}$
E [18]	Young modulus	1 kPa
ν [18]	Poisson ratio	1/3
γ_{\parallel} [18]	Parallel friction constant	$24 \mu\text{N min } \mu\text{m}^{-1}$
γ_{\perp} [18]	Perpendicular friction constant	$24 \mu\text{N min } \mu\text{m}^{-1}$
ρ_m [18]	Adhesion energy	$200 \mu\text{N m}^{-1}$
γ [18]	Cell-medium friction constant	$24 \mu\text{N min } \mu\text{m}^{-1}$
D_c	Cancer cell diffusion constant	$10^{-12} \text{ cm}^2 \text{ s}^{-1}$
D_e	Endothelial cell diffusion constant	$10^{-15} \text{ cm}^2 \text{ s}^{-1}$
$\ F_c\ $	Directed movement force modulus in figure 6(a)	150 μN
$\ F_c\ $	Directed movement force modulus in figure 6(b)	120 μN
Biological parameters		
$k_2 \diamond$ [29]	β -catenin degradation rate in proteasome	0.03 min^{-1}
$k_{2,E}$	Src-E- β disruption rate	100 min^{-1}
$k_{2,N}$	Src-N- β disruption rate	10 min^{-1}
k_m [22]	β -catenin production rate	0.01 nM min^{-1}
P_T [29]	Proteasome total concentration	0.335 nM
E_T [29]	VE-cadherin total concentration	100 nM
$N_T \diamond$	N-cadherin total concentration	100 nM
$Src_T \diamond$	Src total concentration	33 nM
$k_E^+ \diamond$	Src-[E/ β] binding rate	10 min^{-1}
$k_N^+ \diamond$	Src-[N/ β] binding rate	1 min^{-1}
v_E [29]	[E/ β] transport to the membrane rate	100 min^{-1}
$v_N \diamond$	[N/ β] transport to the membrane rate	100 min^{-1}
$\rho_{c,z} \diamond$	Cell-cell contact stimuli	200
$\rho_{d,z} \diamond$	Cadherin-mediated bonds' disruption	200
$\alpha_z \diamond$	Stimulated cadherin to the non-stimulated transition rate	1 min^{-1}

3.2.3. *Genotype N-Off, Src-On.* The third cancer cell genotype when coming into contact with the endothelial wall induces the activation of the Src pathway in the endothelial cells but does not form N-cadherin-mediated bonds. In this case, the cell achieves TEM by a combination of the exerted force by pushing the underlying substrate and the disruption of VE-cadherin bonds due to the activity of Src kinases. This is equivalent to set $v_N = 0$ in the intracellular parameters of the cancer and endothelial cells (equation (6)).

3.2.4. *Genotype N-On, Src-On.* The last cancer cell genotype creates N-cadherin-mediated contacts ($v_N = 100 \text{ min}^{-1}$ in both the cancer and the endothelial cells) and activates the Src pathway in the endothelial cells. This genotype achieves TEM as a combination of pushing forces exerted on the underlying substrate, pulling forces exerted on the focal contacts with the endothelial cells and degradation of the VE-cadherin bonds between endothelial cells caused by Src activity. The parameter values of this genotype are given in table 1.

To generate realistic results comparable with experimental data, the directed movement force strength was chosen to obtain speeds in the range of the observations of Wolf and Friedl [39]. Table 2 shows the time spent to achieve TEM for each of the different genotypes when using different speeds of migration. We consider the genotype that cannot express N-cadherin nor induce Src activity in the endothelial cells as a

control variable (*N-Off, Src-Off*). This is motivated by the fact that it achieves TEM by using only physical forces without the direct interaction of any protein pathway. The last column of table 2 gives the percentage of changes in the time for achieving TEM with respect to this genotype. It can be seen that the genotypes expressing either N-cadherin (*N-On, Src-Off*) or Src (*N-Off, Src-On*) can achieve TEM faster than the control genotype (*N-Off, Src-Off*). The fastest migration values are achieved for the genotype combining both molecules (*N-On, Src-On*).

Figure 6 shows two different profiles of the resulting migration velocity of the cancer cell obtained from the multi-scale simulation results. The plot at the top represents the migration speeds of the four different genotypes when travelling towards the endothelial wall at an approximate speed of $5 \mu\text{m min}^{-1}$ (obtained under a migration force $\|F_c\| \approx 150 \mu\text{N}$ and a friction constant of $\gamma = \gamma_{\parallel} = \gamma_{\perp} = 24 \mu\text{N min m}^{-1}$; see equations (23) and (24) in appendix A for a detailed explanation of these parameters). At time $t \approx 100 \text{ min}$, the malignant cell comes into contact with the endothelial wall and the migration speed is considerably reduced. At time $t \approx 450 \text{ min}$, the cell succeeds in crossing the wall and recovers its initial speed. In these simulations, all the genotypes achieve TEM. It can be observed that the genotypes expressing either N-cadherin (red) or activating Src (green), or both of them (blue), achieve TEM faster than the control genotype (black).

Table 2. The table shows the results of the sensitivity analysis performed to study the variation of the time spent to intravasate for different migration speeds. The first column gives the mean migration speed of the cell when approaching the *tunica intima*. The second column gives the time spent from the moment of getting into contact with the *tunica intima* up to achieving TEM. The last column gives the time spent to achieve TEM with respect to the control genotype (*N-Off, Src-Off*) in percentages.

Migration speed ($\mu\text{m min}^{-1}$)	Time (min)	Percentage
<i>N- Off, Src- Off</i>		
5	400	100
9	213	100
13	13	100
<i>N- Off, Src- On</i>		
5	390	97
9	202	94
13	12	92
<i>N- On, Src- Off</i>		
5	363	90
9	192	90
13	12	92
<i>N- On, Src- On</i>		
5	340	85
9	190	89
13	10	76

External interactions such as a higher ECM concentration and the friction produced by other cell–cell adhesion molecules are biological factors that can also influence TEM. These factors can be modelled by increasing the cell–substrate friction force and the cell–cell friction force respectively. The plot at the bottom of figure 6 shows the speed profiles of a cancer cell when increasing the friction with the ECM, the friction with the neighbour cells ($\gamma = \gamma_{\parallel} = \gamma_{\perp} = 240 \mu\text{N min } \mu\text{m}^{-1}$) and the magnitude of the directed movement force ($\|F_c\| \approx 1000 \mu\text{N}$). With these parameter values, the migration profile of the genotypes changes. In this case, TEM is only achieved by the genotype expressing both N-cadherin and activating Src (blue). Nevertheless, for cell migration speed values close to experimental observations [17, 39], most of the simulations show a profile closer to (a) than to (b) of figure 6. In most of the cases, all the genotypes achieve TEM with a small time delay. Figure 7 shows a comparison of the control cell (*N-Off, Src-Off*), labelled as C, with the genotype (*N-On, Src-On*), labelled as G, for the different migration speed values 7, 6 and 5 $\mu\text{m min}^{-1}$ (obtained after setting: $\|F_c\| \approx 170, 150$ and $120 \mu\text{N}$, and $\gamma = \gamma_{\parallel} = \gamma_{\perp} = 24 \mu\text{N min } \mu\text{m}^{-1}$). In each case, the control cell takes longer to achieve TEM than the genotype, preserving a profile similar to that observed at the top plot of figure 6. It can be seen that the difference in time spent achieving TEM prevails near to constant when comparing the control and the genotype (magenta–yellow \approx purple–brown \approx cyan–green \approx 40 min).

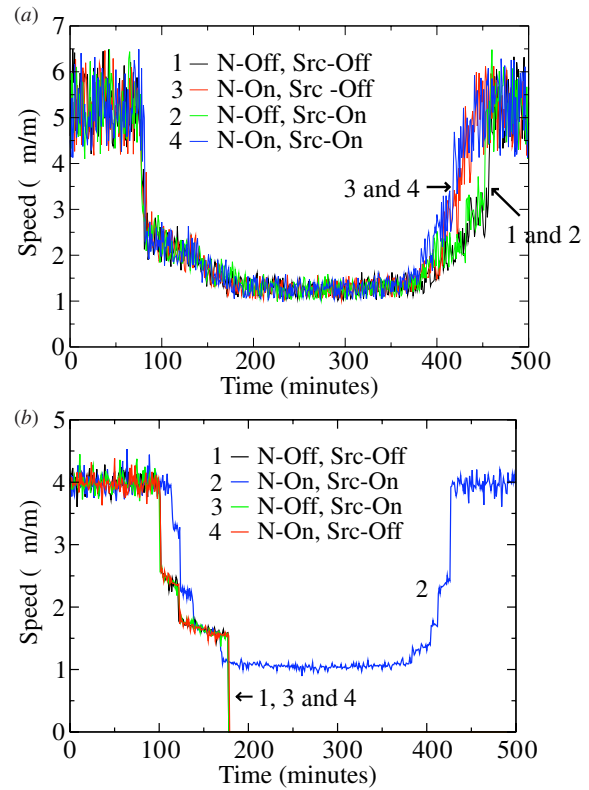


Figure 6. Plots showing two different profiles of migration assays. When the cancer cell reaches the wall at time $t \approx 100$ min, the reaction force slows down the cell velocity. The initial migration speed is recovered when the cell manages to open a gap after disrupting the VE-cadherin bonds between endothelial cells at $t \approx 450$ min. In (a), all the different genotypes succeed in crossing the endothelial wall. As observed by Qi *et al*, the genotypes expressing N-cadherin achieve TEM faster than the genotypes not expressing N-cadherin [28] (in this simulation, $\gamma = \gamma_{\parallel} = \gamma_{\perp} = 24 \mu\text{N min } \mu\text{m}^{-1}$ and $\|F_c\| \approx 120 \mu\text{N}$). In (b), the plot shows how, when increasing the friction with the environment by a factor of ten ($\gamma = \gamma_{\parallel} = \gamma_{\perp} = 240 \mu\text{N min } \mu\text{m}^{-1}$) and the magnitude of the directed movement force ($\|F_c\| \approx 1000 \mu\text{N}$), only the genotype expressing N-cadherin and inducing Src activity undergoes TEM.

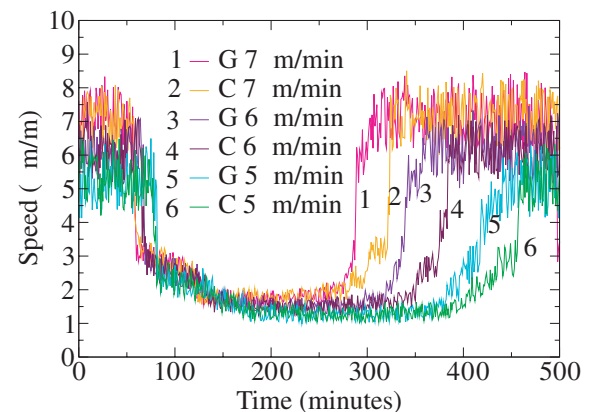


Figure 7. Plot showing the influence of the cell genotype on the time spent to achieve TEM for three different migration speeds. For all migration speeds (7, 6 and 5 $\mu\text{m min}^{-1}$, obtained after setting: $\|F_c\| \approx 170, 150$ and $120 \mu\text{N}$ and $\gamma = \gamma_{\parallel} = \gamma_{\perp} = 24 \mu\text{N min } \mu\text{m}^{-1}$), the control cells (C) take on average around 40 min longer to achieve TEM than the genotypes expressing N-cadherin and activating the Src pathway (G).

4. Conclusion and outlook

The achievement of metastasis in cancer depends crucially on the ability of malignant cells to successfully undergo intravasation and extravasation. These processes are conditioned and controlled by cell–cell adhesion proteins and the biophysical properties of the cell. Qi and collaborators [28] observed how N-cadherin-mediated cell adhesion of the cancer cell to the endothelial wall was able to speed up the process of TEM. Furthermore, they also showed that when using the same line of cancer cells but with defective N-cadherin, TEM was significantly delayed. This protein interaction may disrupt the adhesion of the endothelial cells in merely a non-physical way.

The fact that the cells forming the endothelial wall bind to each other using principally VE-cadherin adhesion molecules, and the fact that VE-cadherin and N-cadherin share the same proteins to bind the cytoskeleton to the cell–cell bonds, might be an important factor in the achievement of TEM. One of the possible explanations of how easier achievement of TEM is related to this is an intra-cellular competition of VE-cadherin and N-cadherin for some of the common intra-cellular binding proteins at the invasive tip of the cancer cell in contact with the endothelial wall. After a disruption of the VE-cadherin-mediated bonds between the endothelial cells, the contact of the malignant cell with the endothelial wall at the junction zone of two endothelial cells may induce the formation of a new N-cadherin bond of the endothelial cell with the cancer cell instead of a recovery of the old VE-cadherin bonds between endothelial cells. To study this hypothesis, we chose β -catenin as one of the principal proteins involved in the formation of cadherin-mediated adhesion. This choice is motivated by two facts. First, β -catenin binds to α -catenin in a complex which works as a link of the cadherin to the cytoskeleton and therefore is a determinant protein providing physical support to the cell and probably related to the necessary physical interactions that might be needed to disrupt the VE-cadherin bonds. Second, it is known that the N-cadherin expression is correlated with the activation of the β -catenin pathway [40] after interacting with enzymes of the Src family. To our knowledge, the existence of a unique enzyme of the Src family with the capability of targeting both N-cadherin and VE-cadherin has not been demonstrated as yet. In our model, we included this possibility of predicting the effect of bond detachment in the endothelial wall after the Src enzymes are activated as a consequence of expressing N-cadherin-mediated bonds. In this case, the model predicts a faster intravasation motivated by the interaction of the Src intracellular pathways and not only by merely physical forces.

Nevertheless, from the results of our simulations, it can be concluded that physical interactions play a major role in TEM. The observations of the stability analysis of table 2 and the profiles observed in figures 6 and 7 indicate that the type and intensity of the mechanical forces exerted on the *tunica intima* may be considered as the driving mechanism of TEM. It can also be concluded that not only the physical and biological characteristics of the endothelial cells and *tunica intima* influence TEM, but also other external factors such as focal

adhesions between the malignant cell and the surrounding environment (density of ECM, activity of integrins, etc) that can modify the speed of cell migration and the intensity of the mechanical forces exerted on the endothelial wall. These results highlight the importance of developing realistic multi-scale models that account for the main characteristics of the systems.

Appendix A

In this section, we give a detailed explanation of the equations governing the intracellular protein concentrations, the cell–cell force interactions between endothelial cells and other cellular parameters.

Intracellular equations

The intracellular protein reactions give the following system of equations for the malignant cell:

$$d_t[N] = -c_N(t)[N] + d_N(t)[N/\beta] + \alpha_N[N_s] + k_{2N}[S_N], \quad (4)$$

$$d_t[N_s] = c_N(t)[N] - v_N[N_s][\beta] - \alpha_N[N_s], \quad (5)$$

$$d_t[N/\beta] = v_N[N_s][\beta] - d_N(t)[N/\beta] - k_N^+[N/\beta](Src_T - [S_N]) + k_N^-[S_N], \quad (6)$$

$$d_t[\beta] = -v_N[N_s][\beta] + d_N(t)[N/\beta] - k^+[\beta](P_T - [C]) + k^-[C] + k_m + k_{2N}[S_N], \quad (7)$$

$$d_t[C] = k^+[\beta](P_T - [C]) - k^-[C] - k_2[C], \quad (8)$$

$$d_t[S_N] = k_N^+[N/\beta](Src_T - [S_N]) - k_N^-[S_N] - k_{2N}[S_N]. \quad (9)$$

The equations for the endothelial cells include in addition the VE-cadherin activity:

$$d_t[E] = -c_E(t)[E] + d_E(t)[E/\beta] + \alpha_E[E_s] + k_{2E}[S_E], \quad (10)$$

$$d_t[E_s] = c_E(t)[E] - v_E[E_s][\beta] - \alpha_E[E_s], \quad (11)$$

$$d_t[E/\beta] = v_E[E_s][\beta] - d_E(t)[E/\beta] - k_E^+[E/\beta](Src_T - [S_N] - [S_E]) + k_E^-[S_E], \quad (12)$$

$$d_t[N] = -c_N(t)[N] + d_N(t)[N/\beta] + \alpha_N[N_s] + k_{2N}[S_N], \quad (13)$$

$$d_t[N_s] = c_N(t)[N] - v_N[N_s][\beta] - \alpha_N[N_s], \quad (14)$$

$$d_t[N/\beta] = v_N[N_s][\beta] - d_N(t)[N/\beta] - k_N^+[N/\beta](Src_T - [S_N] - [S_E]) + k_N^-[S_N], \quad (15)$$

$$d_t[\beta] = -v_N[N_s][\beta] - v_E[E_s][\beta] - k^+[\beta](P_T - [C]) + k^-[C] + k_N^-[S_N] + k_E^-[S_E] + k_m + d_N(t)[N/\beta] + d_E(t)[E/\beta] + k_{2N}[S_N] + k_{2E}[S_E], \quad (16)$$

$$d_t[C] = k^+[\beta](P_T - [C]) - k^-[C] - k_2[C], \quad (17)$$

$$d_t[S_N] = k_N^+[N/\beta](Src_T - [S_N] - [S_E]) - k_N^-[S_N] - k_{2N}[S_N], \quad (18)$$

$$d_t[S_E] = k_E^+[E/\beta](Src_T - [S_N] - [S_E]) - k_E^-[S_E] - k_{2E}[S_E]. \quad (19)$$

We observe that we have ‘abused’ our notation by not changing the names of the variables for the different types of cells, but it should be clear that the first set of equations belong to the intracellular pathways of a cancer cell and the second set belongs to an endothelial cell, and they are completely independent as there is no interchange of proteins between cells. Definitions and values of the parameters are given in table 1. Many of the parameter values have been taken from biological and physical literatures and the rest have been estimated in order to maintain the soluble β -catenin steady-state concentration nearby the value of 35 nM observed in experiments by Lee *et al* [22].

The functions $c_z(t)$ and $d_z(t)$ measure the amount of cadherin stimulated to form bonds by physical contact to neighbour cells, i.e.

$$c_z(t) = \sum_{\text{new contacts}} a_{cj}(t) \rho_{c,z},$$

where $a_{cj}(t)$ measures the new area of the membrane in contact with the neighbours and $\rho_{c,z}$ is a dimensionless parameter that reflects the rate at which the $[z]$ concentration is stimulated when induced by cell–cell contact stimuli. Observe that the backward reaction to this process is explained in equations (5) and (11) where α_z is the rate at which the cadherins pass from the stimulated ($[z_s]$) to the initial soluble state ($[z]$). Therefore if contact stimulus occurs but there is not enough β -catenin to form bonds, the cadherins can go back to the initial non-stimulated state. Conversely, when detachment occurs, the function $d_z(t)$ is

$$d_z(t) = \sum_{\text{new detachments}} a_{dj}(t) \rho_{d,z}.$$

Here, $a_{dj}(t)$ measures the contact area lost in cell–cell detachments at time t with cell j and $\rho_{d,z}$ is the rate at which the adhesion complex is dissociated.

The functions $a(t)_{c,j}$ and $a(t)_{d,j}$ determine the area stimulated to interchange the cadherin from the cytosol to the membrane and from the membrane to the cytosol respectively. We define these functions as

$$a_{c,j}(t) = \begin{cases} \frac{\partial}{\partial t} \hat{a}(t)_j, & \text{if } \frac{\partial}{\partial t} \hat{a}(t)_j > 0, \\ 0, & \text{otherwise,} \end{cases}$$

and

$$a_{d,j}(t) = \begin{cases} \left\| \frac{\partial}{\partial t} \hat{a}(t)_j \right\|, & \text{if } \frac{\partial}{\partial t} \hat{a}(t)_j < 0, \\ 0, & \text{otherwise,} \end{cases}$$

where $\hat{a}(t)_j$ is the approximated proportion of an area in contact with the cell j at time t , calculated by the spherical caps in contact (this approximation has also been used by Galle *et al* [18]). Hence both, attachment and detachment of cells lead to an exchange of cadherins between the membranes in the contact zone of the interacting cells.

The biophysical model of a single cell

We assume that both the cancer and the endothelial cells can be parameterized by the same biophysical properties. We model them as isotropic elastic objects and parameterize them by cell-kinetic, biophysical and cell-biological parameters that

can be experimentally measured. We now describe below the key features of this modelling approach.

Cell–cell shape.

The shape of the endothelial cells forming the *tunica intima* can vary depending on the radius of the vessel. For small vessels they show elongated shapes similar to a ring while for larger vessels they look closer to a sphere [37]. We assume that an individual cell in isolation is spherical and characterize the cell shape by its radius R , and does not differ largely from a compact shape within the vessel wall. As the model accounts for the main biophysical characteristics of the system, we believe that this simplification does not affect the scenarios and conclusions of this paper.

Cell–cell interaction.

With a decreasing distance between the centres of two cells (e.g. upon compression), both their contact area and the number of adhesive contacts increase, resulting in an attractive interaction [6, 36]. On the other hand, if cells are spheroidal in isolation, a large contact area between them significantly stresses their cytoskeleton and membranes. Furthermore, experiments suggest that cells only have a small compressibility (the Poisson numbers are close to 0.5 [3, 23]). In this instance, both the limited deformability and the limited compressibility give rise to a repulsive interaction. We model the combination of the repulsive and attractive energy contributions by a modified Hertz model [18, 32], where the potential V_{ij} between two cells of radii R_i and R_j is given by

$$V_{ij} = \underbrace{(R_i + R_j - d_{ij})^{5/2} \frac{1}{5\tilde{E}_{ij}} \sqrt{\frac{R_i R_j}{R_i + R_j}}}_{\text{repulsive contribution}} + \underbrace{\epsilon_s}_{\text{adhesive contribution}}. \quad (20)$$

The first term of the equation models the repulsive interaction, the second term the adhesive interaction and \tilde{E}_{ij} is defined by

$$\tilde{E}_{ij}^{-1} = \frac{3}{4} \left(\frac{1 - \sigma_i^2}{E_i} + \frac{1 - \sigma_j^2}{E_j} \right). \quad (21)$$

Here, E_i, E_j are the elastic moduli of the cells i, j and σ_i, σ_j are the Poisson ratios of the spheres. $\epsilon_s \approx \rho_m A_{ij} W_s$, where $W_s \approx 25k_B T$ (T : temperature, k_B : Boltzmann constant) is the energy of a single bond, A_{ij} is the contact area between cells i and j and ρ_m is the density of surface adhesion molecules in the contact zone, in our case the density of VE-cadherin [12] (for details see the end of section 2). The interaction force results from derivation of the potential function

$$\underline{F}_{ij} = -(\partial V_{ij} / \partial d_{ij})(d(d_{ij})/dx, d(d_{ij})/dy, d(d_{ij})/dz). \quad (22)$$

Tensors and random movement

The tensors $\underline{\Gamma}_{kj}^f$ and $\underline{\Gamma}_{ks}^f$ used in equations (1) and (2) denote cell–cell friction and cell–substrate friction, respectively. The cell–substrate friction tensor takes the form

$$\underline{\Gamma}_{cs}^f = \gamma \underline{I}, \quad (23)$$

where \underline{I} denotes the identity matrix and γ is the friction coefficient of the medium. The cell–cell friction tensor is [16]

$$\underline{\Gamma}_{kj}^f = \gamma_{\parallel}^{(kj)} \underline{n}_{kj} \underline{n}_{kj} + \gamma_{\perp}^{(kj)} (\underline{I} - \underline{n}_{kj} \underline{n}_{kj}), \quad (24)$$

where \underline{x}_k and \underline{x}_j are the positions of the centres of mass of the cells, and $\underline{n}_{kj} = \frac{\underline{x}_j - \underline{x}_k}{|\underline{x}_j - \underline{x}_k|} \underline{n}_{kj}$. \underline{n}_{kj} here denotes the dyadic product, i.e. it is a 3×3 -matrix. $\gamma_{\parallel}^{(kj)}$ and $\gamma_{\perp}^{(kj)}$ are the parallel and perpendicular friction constants, respectively.

The *random movement* terms in (1) and (2) model the random component in the cell movement

$$\langle \underline{f}(t) \underline{f}(t') \rangle \approx 2 \hat{\Gamma} \delta(t - t')$$

and zero mean

$$\langle \underline{f}(t) \rangle = 0.$$

$\hat{\Gamma}$ denotes the amplitude of the autocorrelated noise and may depend on the friction term as well as on the diffusion constant D [16]. D characterizes the free random movement of isolated cells in the medium. For the cancer cell, we took $D \approx 10^{-12} \text{ cm}^2 \text{ s}^{-1}$ [15, 29]; in contrast, the endothelial cells will tend to move less and preserve the vessel architecture, and we assume $D = 10^{-15} \text{ cm}^2 \text{ s}^{-1}$. The rest of the parameter values are given in table 1.

Appendix B

In this section, we give a brief study of the fixed points of the intracellular system to show that the steady state of the proteins is in agreement with two biological observations when a cell is isolated from the neighbours: (a) the cadherins remain sequestered in the cytosol [11] and (b) the steady state of soluble β -catenin remains stable close to the value of 35 nM [22].

When a cell is isolated from the neighbours, $d_z(t) = c_z(t) = 0$. For the parameter values of table 1, the steady state of the system is a stable fixed point that preserves the cadherins in the cytosol:

$$[S_z] = 0, \quad [z_s] = 0, \quad [z] = z_T, \quad [z/\beta] = 0,$$

where $z_T = [z_s] + [z] + [z/\beta]$. When two cells come into contact, in absence of the Src activity, the cadherin will travel to the cell membrane to form bonds and increase the adhesion forces:

$$[S_z] = 0, \quad [z_s] = 0, \quad [z] = 0, \quad [z/\beta] = z_T.$$

If there exists Src enzymatic activity, considering not all the enzymes have been saturated (i.e. $Src_T > [S_N] + [S_E]$), the cell adhesion forces will be dependent on the steady-state concentration of cadherins at the intermembrane position:

$$[z/\beta] = \frac{k_z^- [z]}{k_z^+ ([S_N] + [S_E] - Src_T)}. \quad (25)$$

Therefore, a high Src activity (i.e. $k_z^+ \gg k_z^-$) leads to a decrease in the adhesion properties of the *tunica intima*.

The steady states of the proteasome system and the soluble β -catenin are

$$[C_o] = \frac{k_m}{k_2} \quad (26)$$

and

$$[\beta_o] = \frac{k_m/k_2(k^- + k_2)}{k^+(P_t - k_m/k_2)}, \quad (27)$$

respectively. From (27), it is required that $P_t > [C_o]$ which is satisfied for the chosen parameter values. The soluble β -catenin concentration at the steady state is controlled by the degradation rate of the β -catenin-proteasome complex. For the chosen parameter values, it is a stable fixed point and the concentration remains close to 35 nM as observed in experiments [22].

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