Modelling and simulating the dynamics of in-stent restenosis in porcine coronary arteries

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5. Modelling smooth muscle cells migration in response to vascular injury using the Cellular Potts Model

In-stent restenosis (ISR), being an unwanted vascular response, initiates due to arterial injury caused by balloon angioplasty and stent deployment. Migration and proliferation of smooth muscle cells (SMC) are the primary factors that are responsible for the development of neointima in the early phases of injury [150]. The migration of SMCs is linked to the cell cycle state of the cell [151]. Under physiological conditions, SMCs remain in a quiescent state (G0 of the cell cycle) however, after arterial injury, some of these cells change their phenotypes and enter into G1 stage [100]. Studies have shown that the migration of SMCs is directly linked to the cell cycle state of the cell where SMCs in the later phase of G1 stage (G1b) tend to exhibit greater migratory activities [151].

Once a cell enters into a cell cycle, an increase in the cell volume along with an increase in the cell viscosity takes place [151]. The cell becomes less adhesive to its neighbours in the later phase of

This chapter is a further continuation of the master thesis work done by Ioana Niculescu at computational Science section, UvA [152]. A full detailed model description and new results will be submitted to a journal soon. Ioana Niculescu will be a co-author in that submission.
G1 (G_{1b}) phase (before entering into Mitosis) and this may result in cell migration \[151\]. Fukui et al. \[151\] observed that most of the migrating SMCs were in the later G1 phase of the cell cycle and this seems to be consistent with other studies related to drugs like Rapamycin or Paclitaxel which have been used to block the cell cycle progression. These studies also reported that cell migration was also blocked due to the presence of these drugs \[150,153,154\].

In previous chapters, the effects of different factors (injury score, strut shape, drug elution from the stent, endothelium recovery rate, origin of the endothelium recovery etc.) on ISR development have been shown. However in those studies, the vascular reaction of the vessel in response to injury was initiated by breaking the internal elastic lamina (IEL) layer. According to \[69\], this may not be the case in real situations and neointima can still develop in the presence of un-broken IEL. There seems to be a direct correlation between the IEL stretch, based on the angle between the IEL and the stent strut, with the final neointima in the porcine animal experiments \[69\]. However, our previous chapters do not include such a property. This was mainly limited due to modelling approach taken to model the vascular tissue using the agent based model (ABM), where cells were just point based particles and act as independent entities by responding separately to different levels of stimuli. The IEL layer in the ABM was created using a monolayer of cells which were densely packed into each other and the potential function was tuned in such a way that IEL cells slightly overlap each other in the state of equilibrium.

Several modelling approaches (continuum, cell-based, lattice based) have been adapted in the past decades to model and to study different aspects of ISR both at tissue and cellular levels. However, every approach has its own versatile features to capture a specific problem but it may at the same time fail to follow other distinct features that are also observed. Continuum models such as finite element (FE) have been proven to adequately represent the natural behaviour of vascular tissue in terms of structural stresses due to stent deployment. Moreover, the sites of high structural stresses have been correlated with the neointimal tissue growths using follow-up studies \[155\]. Despite the fact that continuum approaches offer the best solution in terms of estimating structural analysis of the vascular tissue, these models reach their limits when cell-cell and cell-environment interactions become important. Moreover, to our knowledge, there has been no study where cell growth and migration of cells can be incorporated using FE. Although all the \textit{in silico} models are limited to some extent, the cell based (off lattice) or lattice based models (Cellular Automata (CA) or Cellular Potts Model (CPM)) can
simulate the dynamics of the functions occurring at the cellular level in a relatively easy way. Agent based or cell based model (lattice free) used in the first few chapters of this thesis are very robust in terms of cellular dynamics where the interaction between cells is rule based. However, that model is less suitable to capture SMC migration through the IEL layer.

Right after the vascular injury caused by the stent and the balloon angioplasty, SMCs from the vascular medial wall have been observed to migrate through the small openings (fenestrae) of the IEL into the lumen where they start to proliferate [156,157,158]. The 2D SMC model (presented in chapter 2, 3 & 4 of this thesis) for tissue dynamics does not include fenestrations in the IEL layer. Fenestration in the IEL can be created in our agent based tissue model by tuning the equilibrium distance between IEL cells. A similar kind of tuning of the IEL layer has already been implemented in the ISR3D model (chapter 6) where stent deployment produces stretch on the IEL layer. Moreover, a deeper penetration of the stent produces larger gaps between the IEL cells. The 3D model also allows manually breaking of the IEL layer in order to compare the neointimal growth results with a scenario where the IEL remains intact. Despite of the fact that the fenestration in the IEL can be implemented and can be tuned in order to replicate the natural fenestration in the IEL layer, the underlying SMCs cannot migrate through these fenestrations. They can only start to grow and enter the G2/S/M phase of the cell cycle and once a cell divides into two daughter cells, one of them may appear into the lumen. There is no real crawling of the cells in the agent based ISR models (both in 2D and 3D). Instead cells try to remain densely packed (the state of equilibrium) based on the potential function associated with the relevant cell types.

On the other hand, CPM allows modelling cell migration by tuning the adhesion and cohesion energies between different cells types. This model permits both the migration through the IEL fenestrae and proliferation of cells at the same time, which makes it far more attractive than the ABM model where cells can only proliferate first and then pop-up into the lumen. However, CPM model lacks to replicate the mechanics (stress strain relationship) of the vascular tissue.

In this chapter, the involvement of SMCs migration towards the development of neointimal tissue has been highlighted. The trauma initiated by the stent deployment has been observed to activate SMCs migration from the medial layer into the lumen where they give rise to neointima. A hypothesis has been developed based on the relationship observed between the number of migrated SMCs and the injury scores. This chapter also
Modelling SMCs migration in response to injury using CPM highlights the connection between the number of initial migrated SMCs and the growth speed of neointimal growth.

### 5.1 2D Cellular Potts Model of ISR

The Cellular Potts method [159], being a lattice based computational modelling method, is widely used to study the collective behaviour of cellular structures. Each cell in CPM has its own identity $\sigma$ and is represented as a set of lattice sites sharing the same index. In addition to its unique identifier, each cell has a specific cell type ($\tau > 0$). Therefore, every lattice site or pixel $(i,j)$, where $i$ and $j$ represent the coordinates axis, is a part of one specific cell that has its own identity $\sigma (i,j)$. Moreover, that specific cell also belongs to a particular cell type $\tau (i,j)$. This modelling framework usually allows having a couple of cell types. Moreover, all biological cells are immersed in a medium that represent a very special cell type. Any lattice site that does not belong to any biological cell type is considered as medium.

The classical implementation of the CPM employs a modified Metropolis Monte-Carlo algorithm [160]. The dynamics of the cells is described by an effective energy function called "Hamiltonian" which usually is the sum of the terms representing cell adhesion and volume or surface constraints. Once the Hamiltonian is defined, the system evolves by applying the following procedure at each Monte Carlo Step (MCS):

A number of lattice sites equal to the size of the lattice are randomly visited. One MCS consists of as many index-change attempts as the number of pixels in the lattice [160]. Each randomly visited lattice site tries to copy its cell index into a randomly chosen neighbour in the next nearest neighbourhood. The difference in the total energy of the system $\Delta H$ caused by the index change attempt is calculated. The probability of the accepting an index change attempt is based on the following transition rule:

$$
Probablity \left( \sigma (i,j) \rightarrow \sigma (i',j') \right) = \begin{cases} 
1, & \Delta H < 0 \\
e^{-\frac{\Delta H}{T}}, & \Delta H \geq 0 
\end{cases}
$$

where $\sigma (i,j)$ is the cell index of the lattice site $(i,j)$ and $\sigma (i',j')$ is the index of the first or second order randomly selected neighbour lattice $(i',j')$. The parameter $T$ is known as temperature and represents the fluctuations in the system, resulting in a complete freeze of the system when $T$ approaches 0. The above equation narrates that the index change attempt is always accepted if $\Delta H$ is less than zero. Otherwise system follows the Boltzmann
probability to accept or reject an index change attempt.

There are at least two basic components in a typical Hamiltonian for every variation of the 2D CPM: bond energy and surface area energy

\[ H = H_{\text{bond}} + H_{\text{area}} \]

The cell-cell and cell-medium interactions take place through bounding energies. The bounding energy between two cells is proportional to the size of the interface between both cells and is given by the formula:

\[ H_{\text{bond}} = \sum_{(i,j) \neq (i',j')} J_{\sigma(i,j), \sigma(i',j')} \left( 1 - \delta_{\sigma(i,j), \sigma(i',j')} \right) \]

where \( J_{\sigma(i,j), \sigma(i',j')} \) is the bond energy between two neighbouring cells, where \( \delta_{ij} \) is the Kronecker delta, which eliminates contributions from the neighbouring lattice sites belonging to the same cell.

**Figure 5.1**: A benchmark 2D un-stented vessel composed of contractile SMCs (pink color) and two laminas. IEL is shown in yellow whereas EEL is drawn in blue.

The area energy term in the Hamiltonian is given by:

\[ H_{\text{area}} = \lambda \sum (a_\sigma - A_\sigma)^2 \]

where \( a_\sigma \) represents the current area of the cell \( \sigma \), while \( A_\sigma \) is the target area. The factor \( \lambda \) is the stiffness of the cell area. Any deviation of the current area of the cell from the target area (in case a cell is stretched or compressed) penalises the Hamiltonian by increasing the total cell energy.
For modelling ISR in two dimensions using CPM, the Tissue Simulation Toolkit (http://tst.sourceforge.net/) was used. A 2D vessel, placed on a rectangular dish (1124 x 1330 lattice sites) corresponding to a vessel length of 1.5 mm, is shown in figure 5.1. The size of one lattice site is kept equivalent to 1.334 µm. The vascular medial wall thickness and the lumen width are chosen to be at 120 µm (90 lattice sites) and 1 mm (750 lattice sites) respectively. The thickness of the medial wall is taken from the analysis of typical porcine histological coronary artery sections and has already been used in our previous papers [59,91]. In order to make a vessel, the layers of IEL and external elastic lamina (EEL) are created first and then the space between both laminas is filled by seeding SMCs. The target area of SMCs is set to 250 lattice sites.

**Table 5.1: Simulation parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>25</td>
<td>Effective temperature of the simulation</td>
</tr>
<tr>
<td>nr_smcs</td>
<td>700</td>
<td>the number of SMCs in the vessel</td>
</tr>
<tr>
<td>Nr_iel</td>
<td>282</td>
<td>The number of IEL cells</td>
</tr>
<tr>
<td>Nr_eel</td>
<td>322</td>
<td>The number of EEL cells</td>
</tr>
<tr>
<td>λ_SMC</td>
<td>50</td>
<td>The compressibility of cells</td>
</tr>
<tr>
<td>λ_Laminas</td>
<td>50</td>
<td>The elasticity parameter of laminas (IEL, EEL)</td>
</tr>
<tr>
<td>J_SMC_SMC</td>
<td>70</td>
<td>Energy between synthetic SMCs</td>
</tr>
<tr>
<td>J_SMC_Medium</td>
<td>50</td>
<td>Energy between synthetic SMCs and medium</td>
</tr>
<tr>
<td>J_SMC_IEL</td>
<td>50</td>
<td>Energy between synthetic SMCs and IEL</td>
</tr>
<tr>
<td>J_SMC_EEL</td>
<td>50</td>
<td>Energy between synthetic SMCs and EEL</td>
</tr>
<tr>
<td>J_qSMC_qSMC</td>
<td>30</td>
<td>Energy between quiescent SMCs</td>
</tr>
<tr>
<td>J_qSMC_Medium</td>
<td>60</td>
<td>Energy between quiescent SMCs and Medium</td>
</tr>
<tr>
<td>J_qSMC_IEL</td>
<td>100</td>
<td>Energy between quiescent SMCs and IEL</td>
</tr>
<tr>
<td>J_IEL_IEL</td>
<td>60</td>
<td>Energy between quiescent SMCs and EEL</td>
</tr>
<tr>
<td>J_IEL_IEL</td>
<td>20</td>
<td>Energy between IEL cells</td>
</tr>
<tr>
<td>J_IEL_Medium</td>
<td>50</td>
<td>Energy between IEL and Medium</td>
</tr>
<tr>
<td>J_EEL_EEL</td>
<td>5</td>
<td>Energy between EEL cells</td>
</tr>
<tr>
<td>J_EEL_Medium</td>
<td>30</td>
<td>Energy between EEL and Medium</td>
</tr>
<tr>
<td>Moment activation</td>
<td>20000</td>
<td>The Monte Carlo step in which quiescent SMCs become synthetic</td>
</tr>
<tr>
<td>Relaxation</td>
<td>20000</td>
<td>The number of Monte Carlo steps in which vessel is initialised and stent is deployed.</td>
</tr>
<tr>
<td>MCS</td>
<td>500000</td>
<td>Total Simulation time in Monte Carlo Steps</td>
</tr>
</tbody>
</table>

The medium properties do not vary inside and outside of the vessel. The energy between the elastic laminas (IEL and EEL) and
medium is kept constant. A full list of parameters including the energies between different cell types is shown in Table 5.1.

5.1.1 Elastic laminas

The monolayers of elastic laminas, both IEL and EEL, were assigned with different cell types in the simulation and both exhibit different energies with the other cell types (Table 5.1). The centre of mass of every two cells in the laminas were connected by an elastic spring which means that the centres of mass of two cells is constrained to stick to a target distance. To create and maintain a mono-layered membrane of laminas, every cell in the lamina has two links which are connected to the next neighbours on both sides (right and left) belonging to the same cell type. Moreover, the energy between the lamina cells belonging to the same cell type is chosen to be very low so that those cells stick to each other and form a cohesive membrane. In order to further model the process of stent deployment, the boundary lamina cells (the first and the last) on both sides of the domain were fixed to the lattice. This was extremely necessary to fix the vessel from both sides to the lattice otherwise the force exerted by the stent would have pushed the whole vessel outward, making it impossible to capture the influence the stent related stretch on the laminas and underlying SMCs.

Every link adds a new term $H_{\text{elastic}}$ to the Hamiltonian. The factor $H_{\text{elastic}}$ is given by:

$$H_{\text{elastic}} = \sum_{k=\text{link}} \lambda_{\text{elastic}}(k) \times \left( l(k) - L_T(k) \right)^2$$

where $k$ is a link, $l(k)$ is the current length of the link, $L_T(k)$ is the specified target length of the link. The term $\lambda_{\text{elastic}}(k)$ is the elasticity parameter and results in a stiffer link if the value of the $\lambda_{\text{elastic}}(k)$ is higher. The length of a link $l(k)$ is estimated by calculating the absolute distance between the centres of mass of two cells which were connected by the link.

In equilibrium, the distance between the IEL cells (fenestration) is kept very small. However due to the stretch caused by stent deployment, this distance becomes enlarged near the stent struts which allows the medial SMCs to migrate through them. The assumption of having a almost no holes in the IEL allowed us to inhibit the SMC proliferation in the un-stented vessel. The migration of SMCs only takes place if the SMC finds a hole in the IEL, which is certainly to be a result of the stretch due to stent deployment.
5.1.2 Stent deployment

The process of stent deployment was modelled by deploying two square stent struts into the vessel. Stent struts were modelled as a special type of cell whose boundaries form a barrier and other cell types were not allowed to extend protrusions into the struts. For the current study, cells were prevented to stick to the struts by assigning high energy to the struts, thus modelling the stent material as non-adhesive.

Stent struts were deployed in the middle of the vessel length as an initial condition. During the stent deployment, the vascular wall was composed of quiescent (contractile) SMCs, However, once struts were deployed, SMCs change their phenotype to synthetic. The strut size was chosen to be at 90 µm. The deployment process was modelled by perceiving $\Delta H$ as a force. The force field was gradually applied on the tissue perpendicular to the horizontal axis until it reaches to a specified deployment depth. The force filed width was set equivalent to the strut thickness and was only effective at the locations (the centre of the vessel) where struts will be deployed (figure 5.2). At those locations in the tissue, the force field was applied to all the cells which pushes the vessel outwards.

The process of force field was implemented by biasing the $\Delta H$ of all the index change attempts. If an index change attempt was in the direction opposing the deployment direction, this was discouraged by adding a positive value F to the $\Delta H$, and otherwise encouraged by subtracting F from $\Delta H$. No restrictions were applied to the index change attempts in the direction perpendicular to the direction of deployment. The direction of force was always perpendicular to x-axis (horizontal axis) however, the direction of the deployment for both struts (upper and lower) was opposite to each other.

For the lower strut, the deployment depth increases in the positive direction of the vertical axis (y-axis) whereas for the upper strut, it was the other way around and the deployment depth increases in the opposite direction of the vertical axis (Figure 5.2). This rule can be stated as:

For lower strut:

$$\Delta H = \begin{cases} \Delta H + sgn(j_p - j) \times F, & \text{lattice site } (i,j) \in \text{force field} \\ \Delta H, & \text{otherwise} \end{cases}$$

For upper strut:
\[ \Delta H = \begin{cases} \Delta H + sgn(j - j_p) \times F, & \text{lattice site } (i, j) \in \text{force field} \\ \Delta H, & \text{otherwise} \end{cases} \]

where \( sgn \) is the sign function, \( j_p \) is the \( y \)-coordinate of the lattice which tries to copy itself in the lattice with coordinate \( j \) and \( F \) is the force.

The force field remains switched on until the desired deployment depth is achieved. Next, the struts are placed in the vessel and the force field is switched off. The magnitude and the duration of the applied force remain constant for all simulations presented in this study.

![Figure 5.2: Stent deployment procedure. (A) Pre stented vessel. (B) A force field is initialized at the location where stent struts will be deployed and this causes the vessel to deform from those locations. (C) Force field is kept switched on until the desired deployment depth is reached. (D) Stent struts are placed inside the vessel and the force field is switched off.](image)

### 5.1.3 SMC migration and proliferation

In order to understand the growth of SMCs as a function of time, a relationship between real time and CPM steps should be built first. The time is determined by relating the cell growth speed in the CPM with the cell cycle duration of porcine SMCs. A typical cell cycle length of aortic porcine SMCs is 32 hours [48]. Therefore,
relating a typical porcine cell cycle (32 hours) to CPM steps results in $2850 \text{ MCS} = 32 \text{ hours}$ ($2138 \text{ MCS}$ for 24 hours (1 days)).

In the current model, all SMCs change their phenotype immediately after the stent deployment and are allowed to grow only if they obey certain rules. If a specific cell has less than two neighbours and has space available to grow, the target area of that cell is gradually increased. If a cell is allowed to grow, the target area of that specific cell is increased by two lattice sites every 12.23 minutes. The cell doubles its target area in $2850 \text{ MCS}$ (32 hours real time) and then it is divided into two daughter cells where half of its lattice sites are assigned with a new cell index (a new daughter cell). The other half of the cell lattice sites keeps the properties of the mother cells.

Once a cell starts to grow by increasing its target area and if there is any gap in the IEL adjacent to that cell, the cell is pushed towards the empty space in the IEL layer and we call it as a migration of the medial SMCs into the lumen. The migration and proliferation of SMC outside the EEL is not allowed in our simulation. Therefore, the only gap SMC can find is through the large fenestrations of the IEL due to stent deployment, so they grow and migrate through those fenestrae.

Different injury levels were identified by measuring the angle between the IEL and the base of the stent (figure 5.3). Injury levels were categorised into three injury scores, based on Gunn’s injury score, to observe their possible effects on the SMCs migration and proliferation (Table 5.2). The stent causes stretch on the IEL allowing the fenestrae to become bigger depending on the deployment depth of the stent (injury score 1 & 2). However, the IEL may break in response to excessive stent deployment (injury score 3), therefore, the rupture of the IEL in our model is permitted by removing IEL links based on the link length. If the length of any IEL link exceeds twice of its target link length, we remove that specific link. After rupture, the IEL undergoes a period of elastic recovery until it gets to 0% strain. This elastic recovery allows the IEL to retract until it reaches to equilibrium state (figure 5.3C).
**Figure 5.3:** Injury score and angle of IEL stretch. (A) Injury score 1 where the angle is below 45°, (B) Injury score 2 where IEL angle is above 45° and (C) Injury score 3 where IEL ruptures. The red cells represent synthetic SMCs, whereas yellow and blue linings represent IEL and EEL layers respectively.

**Table 5.2:** Injury score versus deployment depth information based on Gunn Injury Score [69]

<table>
<thead>
<tr>
<th>Injury Level</th>
<th>Description</th>
<th>Deployment Depth (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury Score 1</td>
<td>IEL stretch &lt; 45°</td>
<td>≤ 40 µm</td>
</tr>
<tr>
<td>Injury Score 2</td>
<td>IEL stretch &gt; 45° and IEL stretch &lt; 70°</td>
<td>&gt; 40 µm and ≤ 90 µm</td>
</tr>
<tr>
<td>Injury Score 3</td>
<td>IEL is broken</td>
<td>&gt; 90 µm</td>
</tr>
</tbody>
</table>

In order to identify SMCs migration and proliferation as a function of the injury, we run simulations with three injury scores as mentioned in Table 5.2. Each simulation was run five times to obtain the standard variation in the results. In this thesis, we present preliminary results based on three deployment depths: 40 µm (Injury score 1), 90 µm (Injury score 2) and 190 µm (Injury score 3). For a detailed publication (in preparation), we intend to explore the full range of injury scores: Injury 1 (10, 20, 30, 40
µm), Injury 2 (50, 60, 70, 80, 90 µm) and injury 3 (100, 110, --190 µm or higher). We do not break the EEL (injury score 4 as identified by Gunn et al. [69]) in this study because then cells will start to proliferate outside of the vessel. In physiological conditions, a thick layer of tunica adventitia surrounds EEL. Our current 2D CPM model does not include such adventitial tissue.

5.2 Preliminary Results

Results for five simulations per deployment depth were obtained from the model. Due to the stent deployment, the fenestrae in IEL become larger near the stent struts thus allowing the underlying synthetic SMCs to migrate through the IEL and enter into the lumen where they start to proliferate. Figure 5.4 shows a snapshot of the cells migrating through the IEL layer. The black arrows indicate those cells which have entered into G1 and started migration. Figure 5.5A shows the growth of neointima due to SMCs proliferation at a follow-up time point (3 weeks after stenting). The growth of SMCs stops when the lumen is completely occluded (Figure 5.5B). Note that this is due to fact that there is no other stopping mechanism (coupling with flow, re-endothelialisation and nitric oxide release) except contact inhibition present in the current model.

To understand the effect of injury on SMCs migration, the number of migrated SMCs which crossed the IEL barrier within the first week after stenting were counted in each simulation. The migrated SMCs can only be seen in the simulations with injury score 1 and 2. However, this migration went down in injury score 3 where the IEL is broken. This happened due to elastic recovery of the lamina (retraction) and the fenestrae in the intact IEL became smaller again, thus reducing the SMC migration. Therefore, we also counted those inner most SMCs which became exposed after IEL breakage and considered them as migrated cells. These exposed cells can immediately enter into the cell cycle and give rise to neointima.
Figure 5.4: Migration of SMCs through the IEL fenestrae. Black arrows show the SMCs that were migrating through the IEL gaps. Deployment depth is 90 µm.

Figure 5.5: Progression of neointima at follow-up. The stent was deployed at 90 µm. (A) Partial occlusion of the lumen, (B) Complete occlusion of the vessel due to SMCs proliferation.

The averaged results of the number of migrated SMCs through the IEL as a function of time are shown in Figure 5.6. It is clear
from Figure 5.6 that migration of the SMCs increases with the increase in the vascular injury caused by the stent. Additionally, the migration of SMCs among all injury groups appears to be stopped with in the first one week after the injury. This is due to the fact that once an SMC migrates through a pore of the IEL, it starts to proliferate at that location, thus blocking the passage for other SMCs through that specific pore. Figure 5.6 also highlights a significant increase in the number of migrated SMCs in the injury score 3 when compared to the other two injury scores.

Figure 5.6: Number of SMCs migrated as a function of time for all three injury scores.

Apart from the migration analysis as a function of injury, figure 5.7 demonstrates a direct relationship between the injury scores and the total number of neointimal cells as a function of time after stenting. Neointimal cells include both the number of migrated SMCs from the media into the lumen as well as the ones proliferated in the lumen. It is also clear from Figure 5.7 that the rate at which SMCs proliferated increases with the injury, resulting in a faster neointimal growth with a deeper injury (injury score 3). We only evaluated the neointimal cell growth until the first 10 days after stenting because there is no stopping mechanism (except contact inhibition) in the current tissue model that can inhibit or stop cell proliferation. Neointimal growth completely stops when the lumen is completely occluded (Figure 5.5B). We observed an exponential neointimal growth during the first week after stenting, however, the growth trends enter into a linear regime as the time further progresses (Figure 5.7). In order to further understand the effect of injury on the neointimal growth response, we applied exponential regression to the neointimal cells count data using the equation:
\[ N(t) = N_0 \cdot e^{rt} \]

where \( N_0 \) is the initial number of neointimal cells, \( t \) is the time after stenting and \( r \) is the growth rate. Since we only observe an exponential growth until the first 6 days after stenting and trends start to become linear afterwards (data not shown), therefore, fitting the data until a later time point (after 6 days) with an exponential curve will result in a poor quality fitting. We observed the best fits \( (R^2 \approx 0.99) \) for all injury scores using the neointimal growth data until the first 6 days. Therefore in this case, our fitted time interval is 6 days.

Evaluation of the fitting data, tabulated in Table 5.3, reveals that the growth rates for injury 1 and injury 2 appear to be the same. However for injury score 3, a slower growth rate, \( r = 0.53/\text{day} \) is obtained. A positive correlation is also observed between the injury scores and the \( N_0 \). Injury score 1 resulted in a slightly lower \( N_0 \) (\( N_0 = 0.75 \)) when compared to Injury score 2 (\( N_0 = 1.85 \)). Moreover, the initial number of the neointimal cells in injury score 3 was found to be much higher (\( N_0 = 11 \)) when compared to the other injury groups. This evaluation of \( r \) and \( N_0 \) further suggests that the difference in the neointimal cell growths between injury scores 1 & 2 is mainly due to the difference in the number of

Figure 5.7: The growth of the neointimal tissue as a function of injury score.
initial migrated SMCs which resulted in a slightly different $N_0$ however the growth rate was observed similar in both cases.

**Table 5.3:** Growth rate ($r$) and initial number of cells ($N_0$) obtained from exponential fitting performed at $6^{th}$ day fitted interval.

<table>
<thead>
<tr>
<th>Description</th>
<th>Growth Rate ($r$) per day</th>
<th>$N_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury 1</td>
<td>0.6</td>
<td>0.75</td>
</tr>
<tr>
<td>Injury 2</td>
<td>0.6</td>
<td>1.85</td>
</tr>
<tr>
<td>Injury 3</td>
<td>0.53</td>
<td>11</td>
</tr>
</tbody>
</table>

If we put one single SMC on a dish and allow it to grow and proliferate, the SMC will divide into two daughter cells in 32 hours. The doubling of cells every 32 hours results in a growth rate $r = 0.52$/day. When we compare this value of $r$ with the ones obtained from the neointimal growth fittings performed until $6^{th}$ day fitting interval, it is clear the growth rate in injury scores 1 & 2 is higher than this value, however, unrestricted growth rate ($r = 0.52$/day) is comparable to the fitted growth rate observed in injury score 3 ($r = 0.53$/day). But it should also be noted that the growth rate equals 0.52/day is true only for the case where there is no contact inhibition and cells are free to grow. But in case of vessels, some of the cells until day 6 may return to quiescent state depending on if they were contact inhibited or not. Then why the growth rate is still so high?

The reason behind this increased growth rate is that the exponential fitting was applied to the overall neointimal cell count, including those migrating into lumen as a function of time. Let's assume, there is one neointimal cell at day 1 and that cell is already in a G1 phase of the cell cycle. This cell will divide into two daughter cells on the next day. This will produce a growth rate of 0.52/day (assuming the cell cycle of 32 hours). But based on figure 5.6 (injury scores 1 & 2), another process of SMCs migration from the medial layer into the lumen is also active at the same time. If you assume that there will be two migrated cells on the $2^{nd}$ day, these cells will also be counted as neointimal cells. Therefore, based on this example, the neointimal cell count on day 2 will be four instead of two (one parent and the other daughter). This addition of migrated SMCs in the neointimal cells shows up as an increase in the fitted growth rate. However, in case of injury 3, there were not so many migrated SMCs which were being added at later time points, therefore the growth rate in that case resembles very well with the ideal growth speed of 0.52/day. Fitting the neointimal cell count data to longer fitted time intervals reveals that the fitted growth rate goes down with an increase in the fitted time interval for all injury scores (Figure 5.8A). This is due to fact that as the cell count increases, most of
the cells are turned back to G0 due to contact inhibition. We also observed a positive correlation between \( N_0 \) and fitted time interval for all injury scores (figure 5.8B). A drop in the quality of fitting \( (R^2) \) with the increase in the fitted time interval has been observed and shown in figure 5.8C. It is clear from figure 5.8C that the best fits for all injury scores were observed at 6\(^{th}\) day fitted time interval.

![Figure 5.8](image_url)

**Figure 5.8:** Exponential regression analysis on the averaged neointimal cell count from all injury scores as a function of fitted time interval. (A) Growth rate resulting from exponential fitting for all three injury scores, (B) Initial number of neointimal cells \( (N_0) \) obtained from the fitting. (C) Quality of the fitting \( R^2 \). Grey solid line: Injury score 1, Black solid line: Injury score 2 and Grey dashed line: Injury score 3.

**5.3 Discussion**

There were few main important goals associated with this study; the first one was the development of a CPM tissue model that can replicate a biological function happening in the injured arteries. The second aim was to incorporate different levels of injury scores as described by Gunn et al. [69] based on the condition of the IEL layer (stretched or broken). The third aim was to observe a relationship between injury scores and the initial number of synthetic SMCs migrated through the IEL. The results presented in this chapter clearly shows the involvement of the migrated SMCs towards the growth of neointima where a higher number of migrated SMCs always result in a higher neointimal cell growth.
In the current chapter, we hypothesize that the migration of SMCs through the fenestrae of IEL into the lumen is needed to start neointima formation and that the number of migrated SMCs correlates with the injury score. The SMCs that immediately after stenting migrate into the lumen form an ‘initial condition’ for the early exponential growth phase. So, a larger stretch of the IEL due to a larger deployment depth (larger injury score) results in a higher number of migrated SMCs in the lumen and in turn results in a faster growth speed. Our hypothesis is that this mechanism could explain the observed positive correlation between injury score and neointima formation.

The current CPM model allowed us to model the phenomenon of the SMC’s migration, while they are in the G1 growth stage, as a function of injury scores. The results presented in this chapter show a direct correlation between the injury scores induced by the stent with the number of SMCs migrated through the IEL into the lumen. The initial number of migrated SMCs then further dictates how fast a neointima will grow: a higher number of migrated cells corresponds to a faster neointimal growth and this is evident from the results presented in this chapter (Figure 5.6 and 5.7).

The results also show a faster proliferative response as a function of injury score and growth trends match well with the in vivo data shown in figure 2.6 (chapter 2). This faster proliferative response is due to the higher number of initial SMCs that migrated into the lumen and continued to proliferate. However, the results presented in this chapter are limited in terms of statistical analysis and moreover, simulations were run based on a single deployment depth in each injury category which does not cover the full range in each injury score. Therefore a detailed study is in progress where a different range of deployment depths will be used in each injury score category. Using the whole range of different levels of deployment depths in each injury group will result in a bigger variation in the results and further statistical analysis will be applied to validate the hypothesized relation between injury score, migrated SMCs and rate of neointima formation.

In this chapter, the fitted growth rates at 6th day fitted time interval are quite high in the first few days after the injury. However, the fitted growth rates tend to go down with an increase in the fitted time interval. The growth rate measured in the previous study presented in the chapter 2 of this thesis show a slightly lower growth rate \((r \approx 0.30/day)\) when compared to the current results of injury score 3 \((r = 0.53/day)\) obtained with 6
day fitted time interval). The difference is due to following reasons: (i) Logistic growth fitting was applied in chapter 2 where initial number of cells \(N_0\) was fixed to 10. Fixing the \(N_0\) will result in a different growth rate. (ii) The fitting in chapter 2 was applied to neointimal cell number counted until day 40 after stenting. The figure 5.8A clearly shows that fitted growth rate decreases with an increase in the fitted time interval. The value of the fitted growth rate from 13 day fitted time interval results in a value of \(r = 0.316/\text{day}\) which is quite comparable to the one observed in our previous study (chapter 2). (iii) The effect of flow on the SMCs is not present in the current CPM model. The growth rates obtained from the injury scores 1 & 2 used in the current chapter cannot be directly compared with our previous study because these injury scores belong to a different initial condition where IEL is not broken.

To conclude, an in silico CPM model of a 2D stented vessel has been developed that allows us to investigate the role of SMC migration during ISR development. A direct relationship between the initial number of SMCs migrated through the IEL has been observed as a function of injury score. Moreover, faster growth speed was observed with a high injury and this may be due to increase in the number of initial SMCs migrated into the lumen. Based on the results reported in this chapter, we hypothesized that the development of the neointimal tissue heavily depends on the number of initial migrated SMCs immediately after stenting. A decrease or increase in the number of migrated SMCs during the early days after stent seems to have a predominant effect on the speed of neointimal growth. This hypothesis however warrants some further detailed animal experiments.