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

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6. Three Dimensional In-Stent Restenosis Model

The work described in the previous chapters of this thesis was restricted to two dimensional (2D) models. This has led to a better understanding of the pathophysiology of in-stent restenosis (ISR), and a hypothesis as to why the neointima formation starts, why the rate of formation correlates with injury score, and why it stops. However, a better representation of the vessel in three dimensions (3D) is necessary to accurately compare the \textit{in silico} growth trends with histology and to be able to take into account more complex flow features in three dimensions, e.g. due to curvature of the vessel. Despite the importance of the work done with the two dimensional model, which is relatively easier to implement and much faster to simulate the dynamics of ISR, a 3D multiscale model is expected to be much more predictive and accurate in terms of computing the flow in realistic geometries and resembles closely to the \textit{in vivo} stented vessels. The predictive power of a 3D ISR model can be used to determine the effects of commercially available stent designs towards the growth of ISR and may further be extended to incorporate animal or patient specific geometries as an initial condition to simulate ISR. Simulating ISR in animal specific or patient specific geometries will allow us to better capture the hemodynamics of blood flow in curved vessels. However, this has not been done yet and there is still a long way to go. To date, an idealized 3D multiscale ISR model has already been developed [59,91]. This 3D model includes all the improvements and modifications that have
already been tested and published using the ISR2D model. This chapter will provide a short introduction to the 3D ISR model and will present some of the dynamical growth curves obtained in the presence of a functional endothelium. A much more detailed set of publications on the 3D model and results obtained with it are in preparation.

6.1 3D ISR Model
A 3D multi-scale model of ISR including blood flow and a tissue model (SMC) has been developed. A simplified geometry of a BiodivYsio stent was deployed in a cylindrical 3D vessel. The vessel wall is composed of three layers: internal elastic lamina (IEL), smooth muscle cells (SMC) and external elastic lamina (EEL). The stent deployment creates stretch on the IEL or causes a rupture of the IEL layer depending upon the deployment depth of the stent. This stretched or ruptured IEL then allows the underneath SMCs to become synthetic and proliferate. For the results presented in this chapter, a cylindrical vessel with a length of 4.5 mm is created and then a 3.5 mm long stent is deployed in the middle of the vessel, leaving 0.5 mm un-stented segment on both sides (proximal and distal) of the vessel. The lumen diameter prior to stenting and vessel wall thickness is set at 1.8 mm and 0.2 mm respectively. Figure 6.1 shows the benchmark 3D stented vessel. The MUSCLE library [45,46] was used to couple the blood flow and SMC model. During stent deployment, a complete endothelium denudation is assumed. The condition of the IEL (stretched or ruptured) is varied between different simulations.

6.1.1 Blood Flow
The steady state blood flow inside an idealised stented 3D cylinder is modelled using a standard BGK Lattice Boltzmann Method. The public domain Lattice Boltzmann Library PALABOS (www.palabos.org) was used to simulate the blood flow dynamics inside the vessel considering blood as a homogenous, Newtonian, incompressible fluid. The vessel domain was discretised into a regular lattice grid with a grid size of 15 µm. We used the most common D3Q19 model to simulate the flow along with the periodic boundary conditions at the inlet and outlet. A bounce back boundary condition was employed at the flow boundaries. The wall shear stress (WSS) distribution on each cell is computed and this information is then passed to the SMC model where cells can grow and proliferate based on the shear stress levels. In case of presence of a regenerated endothelial layer, wall shear stress magnitudes are used to produce nitric oxide (NO), which will eventually allow or inhibit cell growth and proliferation, using the model as described in chapter 3.
6.1.2 SMC Model

The 3D tissue model is based on the agent based method similar to the one applied in the ISR2D, however the potential function used in the 3D model to obtain the intercellular forces is modified to represent a more realistic hyperelastic arterial tissue. An agent, which is identified by a set of state-variables such as position, radius, biological state, and structural stress, represents each single cell. Each SMC agent evolves in time according to its current state, and to the states of neighbouring cells. Each time step involves a physical solver, simulating the structural dynamics of the cells, and a biological solver, which simulates the cell cycle, according to a biological rule set.

**Physical Solver:** From a structural point of view, 3D cells are represented by an off-lattice model. The model characterises these cells as spheres and the structural state of the cell ensemble is entirely determined by the position and radii of the cells. Interactions between neighbouring cells give rise to forces, which alter the cells' positions, thus deforming the tissue. If $r_k$ is the position of the $k$-th cell centre and $F_{ij}$ denotes the force on cell $i$ due to cell $j$, the force is assumed on the direction of the vector connecting the cells, which means:

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*Figure 6.1: A 3D stented vessel. Stent (brown), SMC (blue), IEL (sky blue) and EEL (red) are shown.*
The total force on cell $i$ is given by $\vec{F}_i = \sum_j \vec{F}_{ij}$, where cell $i$ is affected by force contributions from all its neighbouring cells $j$. Two cells are neighbours if they either overlap or are separated by less than a given distance. Cells that overlap are assumed to be in contact, and as such they experience a repulsive force. On the other hand, cells that become separated experience an attractive force. The degree of repulsion or attraction is governed by the amount of overlap or separation. Then the movement of cells is solved by means of an over-damped version of Newton’s second law of motion,

$$\gamma \frac{d\vec{r}_i}{dt} = \vec{F}_i$$

Where $\gamma$ is a damping coefficient. This over-damped formulation prevents cells from oscillation following a repulsive force when they come close together and an attractive one when they separate from each other. Instead, they slowly go to their equilibrium position.

A few off-lattice models are compared in [161], where the qualitative large-scale behaviour of the cell ensemble does not change on varying the exact form of the interaction law, although there can be problems with stability if the repulsion is not strong enough as cells approach each other, leading to an “implosion”. One could then think that the choice of the interaction force is not relevant, as long as it shows a repulsive profile if cells are too close and an attractive profile for detached but neighbouring cells and parameters are chosen with enough care in order not to allow the aforementioned “implosion”. However, all of the models considered in [161] are only suited for small strains. If we take for example Hertz's theory of elastic contact, which assumes linear elasticity and infinitesimal strains, we can see in [162] that a systematic underestimation of the normal force by the Hertz model is observed on the normal contact of soft elastic spheres if finite strains are considered. The error arising was found to increase quadratic with increase in the normal deflection. Therefore, as arterial tissue exhibits large strains under stent deployment, we will concentrate our efforts in improving the repulsive force in our model.

Considering the results in [161], we will use the simplest attractive term possible, a linear term. Whereas for the repulsive term we will devise an extension to Hertz’s theory of elastic
contact with the aim to model more faithfully the processes of deployment and growth. The reason why we are more careful in modelling the repulsive term, is because it plays a much more important role in the simulations due to both the compressive effect caused by stent deployment and cell division and to the overdamped formulation of Newton’s second law of motion, which ensures that there will be no oscillation and therefore the attractive term will not become active in cells that are initially compressed. Hertz’s theory of contact mechanics provides an expression for the contact between two spheres:

$$F = \frac{4}{3}E^*R^{3/2}d^{3/2}$$  \hspace{1cm} (6.1)

Where R is the effective radius defined by $1/R = 1/R1 + 1/R2$, where R1 and R2 are the radius of each cell, d is the depth of the overlap between the spheres, $E^* = \frac{E}{1-\nu^2}$ with E the elastic Young modulus and $\nu$ the Poisson ratio. Additionally, we may define $a = \sqrt{Rd}$ as the radius of the contact area between the two spheres.

If we take a definition of stress and linear elastic strain as:

$$\sigma = \frac{F}{\pi a^2}, \hspace{1cm} \epsilon_e = \frac{\sigma}{E^*},$$

where the subscript denotes that we are using a linear elastic strain, then

$$\epsilon_e = \frac{4}{3\pi} \frac{a}{R}$$

However, as we have stated previously, arterial tissue under stent deployment undergoes large strains and is often modelled as a hyperelastic material [163]. That is, it exhibits a nonlinear relationship between stress and strain without exhibiting viscous effects. The simplest hyperelastic model is the Neo-Hookean model which, under uniaxial tension and incompressibility assumption behaves like:

$$\sigma = 2C(\lambda - \lambda^{-2})$$  \hspace{1cm} (6.2)

Where $\lambda = \epsilon + 1$ and we have used a standard engineering notation. In engineering notation, stresses and strains are positive in tension and negative in compression. However, as we are modelling exclusively compression now, we will take $\sigma$ and $\epsilon$ to be positive in compression. We therefore redefine the stretch
ratio as $\lambda = 1 - \epsilon$. If we then substitute (6.2) in (6.1) we find that our Hertzian NeoHookean force is

$$F = \frac{8a^3C(16a^2 - 36\pi aR + 27\pi^2R^2)}{3R(4a - 3\pi R)^2}$$

To determine the value of the constant $C$, we use that we must recover our definition of linear elastic strain under small strains. If we take the limit we obtain that $C=E^*/6$.

Expressions for other kind of hyperelastic corrections to Hertz’s theory approximated from different hyperelastic materials such as Ogden, Fung or Mooney-Rivlin can be easily obtained following the same procedure. Even though a more comprehensive study is left for future work, where different hyperelastic materials should be compared and finite element simulations should be performed to test whether the approximation $a = \sqrt{Rd}$ is still valid under the strains considered. Overall performance of the model, which assumes uniaxial tension between pairs of cells, should also be assessed. Despite the present simplifications, the model also tries to capture anisotropy by multiplying by a factor of 2 all the projections of the forces along the axis of the artery, making it more difficult to compress or stretch the artery along its axis, a well-known feature of vessels [164].

![Figure 6.2: Comparison between different models. Hertz model (Black dashed), Neo-Hookean model (Black solid) and Hertz Neo-Hookean extension (Grey solid).](image)

It can be seen in the figure 6.2 that the neo-hookean extension of the hertzian model yields a higher force than the linear elastic hertzian model for the same overlap of the cells. The difference
between the two models increases as the overlap increases. Not only this, but if we add a quadratic term to the hertzian model, we observe that it fits quite well with the neo-hookean extension, being the coefficient of this term dependent on the radius of the cells and the overall young modulus of the system. This is in line with the conclusions drawn in [162] and leads us to believe in hyperelastic extensions of the hertzian model as good candidates to model the structural behaviour of an off-lattice model for in stent restenosis.

A Taylor series expansion of the devised force reveals that

\[
F = -\frac{4}{3}E^*R^{1/2}d^{3/2} + O(d^2)
\]

As expected, we recover the force predicted by hertz for small displacements. Then, if we deploy our stent by expanding it in a series of steps in which we increment its radius by a quantity \(dr\), if \(dr\) is sufficiently small the results obtained with the Hertz force should tend to the ones obtained with the neo-hookean expansion. This is shown in figure 6.3. Ideally, we would take an infinitely small step size that means an infinite number of steps. As we cannot do that we look for a method which provides us good results in a reasonable amount of time. In this regard, the neo-hookean approach provides us a very similar result to the one that the hertzian approach provides, but with (roughly) 10 times less computational effort (figure 6.3C and 6.3F).

One might wonder if such a model (neo-hookean expansion) could be of use for other kind of off-lattice simulations of cells, even when finite strains are not that predominant. As stated in [165,166], accurate models of cell-cell interactions might not show a significantly different bulk behaviour (as in [161]), but they might be of great interest at the cell scale. Different amounts of overlap correspond to different degrees of cell deformation, and this may become important if cell mechanotransduction (the influence of cell deformation on intracellular signalling) is taken into account, as this could play a key role in the mitotic rate of a particular cell. Thus, a different interaction law may affect the overall growth rate of a growing cell colony, even if the bulk mechanical response remains largely unaffected. In this regard, we must state that the current model does not include any adhesion force between cells, which is for example present in the JKR model (Johnson, Kendal, Roberts) [167,168].
Figure 6.3: Comparison of cell distribution between neohookean extension and hertz approach during stent deployment. (A) slice showing stent and artery before deployment. Stent is shown in yellow, IEL in pink, SMCs in blue and EEL in black. (B) slice showing cell distribution using neohookean extension and 100 deployment steps. (C) neohookean extension and 1000 deployment steps. (D) slice showing cell distribution using hertz force and 100 deployment steps. (E) hertz using 1000 steps. (F) hertz using 10000 steps. The amount of IEL that we see in the slicing and its location is a good tool to compare both approaches during the deployment. It is clear from figure C & F that neohookean extension results (IEL) compare better with the hertzian counterparts that were simulated with a deployment step 10 times smaller. That supports the idea of neohookean approach recovering the hertzian theory of contact mechanics for small displacements. IEL is never broken in the slices shown in this figure. The reason why we don’t see full IEL in the slices
(except slice A) is because the cells get displaced in the axis direction due to the stenting.

The JKR model is based on the Hertz model as well, but it includes an extra term to model adhesion. It should therefore be feasible to devise a hyperelastic extension to the JKR model as well if needed. However, if mechanotransduction is to be modelled in detail one could also start wondering about the validity of approximating cells as spheres.

This whole discussion of the influence of cell deformation on the biological behaviour of the cell sets the ground for the explanation of the biological solver in our model, which does not directly include mechanotransductive effects, even though it takes them into account in an indirect manner.

**Biological solver:** The cell cycle model consists of a discrete set of states. A quiescent state G0, a growth state G1 and a mitotic state S/G2/M. Progression through the cell cycle takes place at a fixed rate, depending on the time step, culminating in mitosis (cell division; a mother cell divides into two new daughter cells).

We model the process of growth and mitosis in the following way. First a cell grows until it doubles its volume. Then the mother cell returns immediately to its original volume and a daughter cell appears. The centre of the mother and daughter cells are randomly displaced within a certain range and the orientation of the cells is completely random. Subsequent cell-cell interactions due to overlapping determine the final distribution of the cells. Even though this is only a simplified model for mitosis, one must note that the overlap caused by the appearance of a daughter cell will be initially big no matter what step size we use to calculate the repulsion. In this sense, the results using the hertz force or the neo-hookean extension will differ.

Cells may enter or leave an inactive phase of the cell cycle (G0) depending on certain rules based on contact inhibition (calculated by neighbour account). The contact inhibition rule set builds on the work of [169,170], starting with the observation that cells in the centre of colonies of epithelial cells (grown in a monolayer) become quiescent due to intercellular signalling which is mediated via cadherin cell–cell physical bonds. By implementing a contact inhibition rule, the authors saw a good qualitative fit between the computational epithelial growth model and the in vitro culture growth under similar conditions (with respect to calcium concentration). In the present SMC model, the contact inhibition rule was applied to SMCs within the vessel wall in, permitting the maintenance of quiescence in a densely packed, uninjured intact
vessel wall. In particular, the number of SMCs, IELs, EELs, and obstacle agents (e.g. the stent struts) within a certain range are computed. If the weighted sum exceeds a pre-determined threshold, the cell is contact inhibited.

The current 3D model also includes the process of re-endothelialisation. Once a functional endothelium is present, it senses fluid shear stresses and translates it into NO. The process of re-endothelialisation in the 3D ISR model is similar to the one explained earlier in the chapter 3 of this thesis. The re-endothelialisation time point used in the current 3D simulations is based on the scenario of 3-23 days (refer to case-2 in Table 3.1 for more details). The cell cycle progression of the SMCs is based on the presence of NO concentration. If the NO concentration for a specific SMC is higher than the predefined threshold, that SMC is forced back into the G0 quiescent stage. If the NO concentration is lower or not present at all due to the absence of functional endothelium, the SMC continues to progress in the cell cycle (given the fact if it is not contact inhibited) and enters into the G2 stage where it further divides into two daughter cells. Once SMC enters into the G2 stage, no further rules (contact inhibition or NO concentration) are applied.

Table 6.1: Injury scores details.

<table>
<thead>
<tr>
<th>Injury</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury 1</td>
<td>Stent is deployed to a certain depth and IEL layer is not allowed to break. Stent deployment stretches the IEL layer and this creates fenestrae in the IEL layer.</td>
</tr>
<tr>
<td>Injury 2</td>
<td>This implies a deeper penetration of the stent into the tissue as compared to the stent deployment depth used in injury 1. IEL in this case also remains un-broken.</td>
</tr>
<tr>
<td>Injury 3</td>
<td>Stent deployment depth in this injury is similar to the one used in injury 2. However, IEL is broken in this injury category.</td>
</tr>
<tr>
<td>Injury 4</td>
<td>This injury includes a broken IEL but the stent deployment depth is bigger than the one used in injury 3.</td>
</tr>
</tbody>
</table>

6.1.3 Injury Scores
In order to observe the neointimal growth inside the vessel, we deployed a stent at different deployment depths to invoke different injury levels. These injury levels are slightly different than the ones specified by Gunn et al. [69]. The details about the injury scores used in the current chapter are listed in Table 6.1
6.1.4 Distributed Multiscale Computing
From a computational point of view, every submodel of the ISR3D multiscale model has different hardware requirements. The blood flow solver is a highly-parallel fluid dynamics flow solver, requiring a cluster with Infiniband interconnects; while the tissue model is an agent based model parallelized with OpenMP, performing best on a large SMP machine. This situation is exacerbated when we take into account that the multiscale model is tightly coupled, that is, it requires frequent communication between submodels. Such a case demands distributed multiscale computing, as was recognized by the scientific communities behind the MAPPER project (http://www.mapper-project.eu/) [45,171,172]. Simulating ISR until 72 days in the vessel shown in figure 6.4 takes approximately 72 hours (3 days) where 32 processors were assigned to compute blood flow. The SMC model was also run in parallel using 8 processors.

![Figure 6.4: Neointimal growth inside the stented vessel at a follow-up time point (28 days post stenting). The injury score 2 was used here as an initial condition.](image)

6.2 Results
Simulations were run using the initial conditions created through injury scores details provided in Table 6.1. Figure 6.4 shows a
snapshot of the neointimal growth inside the stented vessel at a follow-up time point (28 days post stenting). It is quite clear from Fig 6.4 that a huge amount of neointima is present inside the stented area of the vessel.

In order to observe the neointimal growth response in each injury category, we calculated the average neointimal thickness as a function of time after stenting, see figure 6.5. It is clear from figure 6.5 that a bigger injury results in a faster and higher neointimal growth which resembles well with the results obtained using the ISR2D model (chapter 2 & chapter 3) as well as the observed ISR growth in the animal model (see chapter 2). Moreover figure 6.5 shows that a higher neointimal growth was observed in the case where stent deployment depth was similar but the difference in the initial conditions was only based on the IEL layer (un-broken - injury 2 or broken - injury 3).

![Figure 6.5: Neointimal thickness over the whole vessel as a function of time.](image)

The 3D model is now in a production mode and we are currently producing results in order to obtain standard deviations in the results. We also plan to perform simulations using the actual vessel and stent dimensions (will be taken from *in vivo* data) and to compare the neointimal growth trends in the comparable geometries. This will require huge simulation runs, and we are currently preparing distributed multiscale computing runs coupling the SuperMUC system in Germany with Cartesius in Amsterdam.
This is still in progress and full detailed results will be submitted soon for publication.