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

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1

1. General Introduction

1.1 Cardiovascular Diseases

Cardiovascular diseases (CVD) still remain the most common cause of deaths in Europe. Recent statistics show that the number of death casualties due to CVD was over 4 million (47% of all deaths) in Europe and around 1.9 million (40% of all deaths) in the European Union (EU) area [1]. Despite the fact that CVD takes millions of lives every year, it also imposes an enormous economic burden that is estimated to cost almost 196 billion euros to the EU economy [1]. CVD is associated with heart and blood vessels and can take several forms, but the most common types of CVD are stroke and coronary artery disease (sometimes also referred as coronary heart disease).

1.2 Coronary artery disease

There are three main layers in the coronary arteries wall. The first and innermost layer is known as tunica intima which is composed of a single layer of endothelial cells (EC) and is further supported by a thin layer of elastic tissue known as internal elastic lamina (IEL). IEL acts as a flexible barrier between the endothelium and the smooth muscle cells (SMC). The IEL is a fenestrated membrane and a major proportion of these fenestrae do not have cellular contents. Moreover, the functional significance of these fenestrae is not known but one of the possible roles for these holes is to act as low-resistance pathways for the diffusion of substances between intima and media [2]. The second (middle) layer of the vessel wall is known as tunica media which mainly

consists of SMCs and extracellular matrix (ECM) components including elastin and collagen. A thin layer of external elastic lamina (EEL) separates the tunica media from the tunica adventitia (the outermost layer). The adventitia is mainly composed of fibroblasts and thick bundles of collagen fibrils [3].

Coronary artery disease (CAD) is the foremost type of CVD that accounts for almost 1.8 million deaths in Europe each year [1]. CAD occurs mainly due to the development of atherosclerosis (a specific form of arteriosclerosis) where a plaque or stenosis composed of fatty material builds up within the tunica intima and tunica media of the coronary arteries and restricts blood supply to the heart. The growth of plaque ultimately leads to the occlusion of the artery, resulting in a decrease in blood supply, and causes stroke or myocardial infarction.

CAD is also linked to smoking, alcohol consumption, diabetes, hypertension, nutrition and physical activities that are mostly associated with our daily life style [4]. Once the disease develops and is diagnosed, the patient needs to be treated with a surgical interventional procedure.

1.3 Coronary artery treatment

Percutaneous transluminal coronary angioplasty (PTCA) was first introduced in 1977 [5]. At that time, the treatment procedure involved insertion of a balloon, guided through a catheter, at the site of blockage in the coronary artery where it is inflated at a certain pressure to cause forced expansion of the blocked artery. This mechanical expansion of the plaque and artery is also known as balloon angioplasty. Once the procedure is completed, the balloon is deflated and withdrawn from the artery. After its invention, it has been practiced as the most frequent treatment for patients suffering from CAD. Initially, PTCA was only limited to balloon angioplasty and despite its high procedural success, this procedure was limited by the development of restenosis in which arteries blocked again and patients required re-treatment. With the use of Balloon angioplasty, 30 - 50% of the patients tend to suffer from restenosis [6]. Another major drawback of balloon angioplasty was negative remodelling [7] in which the area of the vessel decreases and leads to collapsing of the vessel.

In order to avoid restenosis and unexpected vessel collapsing, stents were introduced and the first coronary stent implantation results were available in 1987 [8]. A stent is a tiny metallic mesh crimped over the angioplasty balloon catheter and moved to the area of stenosis. The stent is deployed by inflating the balloon at a certain pressure. Once deployed, the stent acts as a scaffold

and prevents the vessel from collapsing. The balloon catheter is then deflated and removed from the patient. Balloon angioplasty alone and balloon angioplasty with stent placement are minimal invasive treatments that can only be used on patients whose coronary arteries are not completely blocked. Another invasive treatment to treat the patient suffering from severe CAD is to perform open-heart surgery where a vascular graft is used to bypass the blocked artery. The new-bypassed path allows oxygen-rich blood to flow through the heart muscle. The treatments options available to patients suffering from atherosclerosis depend heavily on the condition of the artery occlusion.

1.4 Coronary stents and in-stent restenosis

In-stent restenosis (ISR) is a regrowth of tissue within the vicinity of a stent that occurs in response to the endovascular treatment (figure 1.1). Tremendous efforts have been done in the field of endovascular stents in terms of their design, flexibility, material, and coatings but ISR still remain a serious complication [9,10,11].

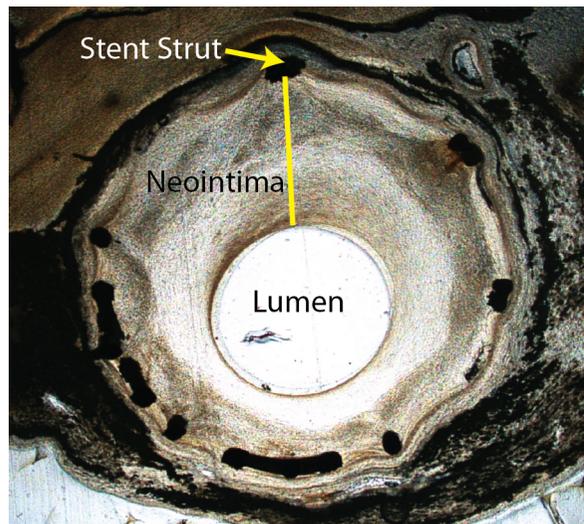


Figure 1.1: A histological image of a stented porcine coronary artery showing neointimal growth inside the vessel. The histological section was taken at 28 days post stenting.

Bare metal stents (BMS) were the first stents, which were often made from 316L stainless steel or cobalt chromium alloy. BMS coated with polymers that elute drugs such as sirolimus or paclitaxel are known as drug eluting stents (DES) [12]. Sirolimus or Paclitaxel eluting DES were the 1st generation DES. Newer DES coated with antiproliferative drugs such as everolimus or

zotarolimus are considered as 2nd generation DES [13]. The current research in the field of stents focuses on new drugs and materials that can be used for improving stent design and to provide better efficacy and optimum flexibility along with required strength. In recent years, stents with different coatings that can target specific cell types, such as endothelial progenitor cells in the vascular wall, have been developed with the aim of capturing those cells as a prime target for faster vascular healing in order to avoid post stenting complications [14].

Initially, the stents were deployed into the vessel wall along with the balloon angioplasty where a stent is crimped on a deflated balloon and inserted into the vessel. Once arrived at the location of stenosis, the balloon is inflated pushing the stent to open. The pressure exerted by the balloon forces the stenosed artery to open along with the stent itself. Once the stent is deployed, the balloon is deflated and withdrawn from the vessel. However, the stent remains there at the blockage site and acts as a scaffold and keeps the vessel open. As an improvement in the stent delivery process, self-expandable stents have been developed and are also currently in use in the clinical practice [15]. Despite this dramatic progress in the stent design, ISR still remains a big problem, although the chances of developing an ISR have been dropped significantly from 30 % (with BMS) to 5-10 % with the use of DES [16]. Although DES have now been widely used due to reduction in the risks of ISR, DES have also been associated with a late stent thrombosis (LST), very late stent thrombosis (VLST) and re-infarction [17,18]. Additionally, delayed re-endothelialisation and vessel wall toxicity in the form of medial necrosis have been reported [17,19,20,21,22]. The antiproliferative drugs coated on DES inhibit neointimal hyperplasia but at the same time, these drugs also hinder the regrowth of endothelium. The sites of incomplete endothelialisation far beyond 30 days remain a potent thrombogenic stimulus and may lead to develop (late or very late) thrombosis [23]. Restenosis rates using endothelial progenitor capturing stents are still not known and results are conflicting so far. There are controversies where some studies have reported a decrease in the restenosis thickness whereas some others observed no reduction in the growth of ISR when using endothelial capturing stents [14,24].

1.5 Pathophysiology of in-stent restenosis

Enormous efforts in the past decades have been done to understand the pathophysiology of ISR and to discover treatment options that can target specific cells to avoid the neointimal development. However, the mystery behind this mechanism still remains an open question [16,25]. The process of ISR can be

categorized into four phases: thrombus formation, inflammation, SMC migration and proliferation and arterial remodelling [16,26,27]. However not fully elucidated, it is now widely accepted that the trauma originated due to the balloon angioplasty and stent deployment damages the vascular intima and produces a stretch on the medial layer. Subsequent to this injury to the intimal and medial layers of the vascular wall [28,29], endothelial cell loss due to stent deployment results in an exposure of thrombogenic sub-endothelial molecules which activate platelets, causing them to aggregate. The healing process later includes the involvement of circulating inflammatory cells, such as neutrophil, lymphocytes, monocytes etc. that adhere at the site of injury [26,30]. These cells further migrate into the thrombus and release growth factors such as platelet-derived growth factor that promote the activation of the underlying SMCs to change their phenotype from a physiological quiescent state to a more pathological synthetic state [31]. This phenotypic change facilitates SMCs to migrate from the medial layer into the lumen where they proliferate. The process further continues to extracellular matrix formation and finally arterial remodelling [16].

The critical determinants associated with the severity of ISR development are related to the design of the deployed stent such as cross-sectional strut shape and arrangement, strut thickness, inter-strut spacing, stent length, the stretch and injury within the stented segment. These factors seem to have profound effect on the initiation and progression of ISR by influencing the biological events occurring in the vessel [32]. In addition to the above-mentioned factors, changes in the hemodynamic parameters such as shear stress and cyclic stretch (due to the pulsatile nature of blood flow) have been shown to influence both normal and pathologically affected vessels. Arteries adjust their luminal area in response to alterations in flow to maintain basal levels of shear and tensile stresses [33,34,35].

1.6 2D Multi-scale model of ISR

In the past few years, *in silico* experimentation has become a very attractive method to understand complex biological processes, allowing to make predictions and to formulate new hypotheses that can be further tested using *in vivo* or *in vitro* trials [36]. Biomedical systems usually are very complex and their inherent complexity originate due to their multi-scale multi-science nature [37,38]. Such systems are organized into a hierarchy of scales that span from the molecular to the organ level (spatial scales) where each process also occurs at different temporal scales. There is a complex interplay between all the

scales from top level down to the grass root and because of this interplay, any change at one scale affect tremendously the behaviour of the whole system. The computational complexity involved in modelling a biological system such as ISR, requires a decomposition of the problem into small single scale models [39,40,41,42,43]. The basic formulation and design of the model was developed in the COAST project (www.complex-automata.org). The aim of that project was to develop a generic framework for modelling and simulating multi-scale multi-science complex systems, based on a hierarchical aggregation of single scale sub models. A MULTIscale Coupling Library and Environment (MUSCLE) was first developed within COAST [44] to glue all single scale models together and this modelling framework is further improved during the MAPPER project (www.mapper-project.eu) [45,46]. The basic idea behind COAST was that a complex multi-scale system could be disintegrated into N single scale models that mutually interact across the scales [40,41,43].

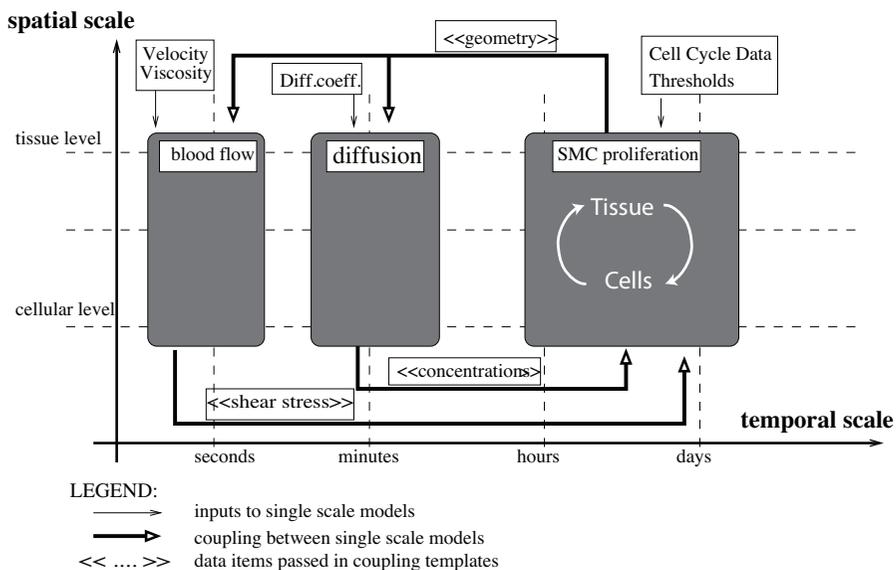


Figure 1.2: Simplified scale separation map (SSM). Adapted from Caiazzo et al. [47]

A detailed scale separation map (SSM) of ISR, where all relevant single scale processes were identified and represented according to their temporal and spatial scales, was built [39]. A simplified and more practical version was further considered to implement in the ISR model [47]. This simplified version of the SSM including blood flow (BF), a tissue growth model (SMC) and drug diffusion (DD) from the struts, is shown in figure 1.2. The single scale models (BF, SMC and DD) run independently and they are coupled through conduits in such a way that the output of one

becomes the input of another. From figure 1.2, it is clear that scale separation between the single scale models is confined to temporal scale. However, there is a scale separation on the spatial scale that exists within the SMC model, which includes processes that occur at the cellular level and those occurring at the tissue level. The blood flow is the fastest process in this multiscale model which is dictated by the length of one cardiac cycle (1 sec), whereas the SMC proliferation is the slowest process which is based on the cell cycle of SMCs (32 hours for porcine SMC [48]). The temporal scale of the drug diffusion resides between the blood flow and SMC scales due to the spatial dimensions of the arterial tissue in combination with the diffusion coefficients of the considered drugs [47].

In this thesis mainly results of our two dimensional ISR model will be presented. This model was first formulated and developed within COAST [39,47,49], and was further developed and modified as a part of this thesis research. The major modifications were in the tissue model (SMC), which was further explored by tuning its parameters and where additional biological processes were considered by adding the effect of re-endothelialisation and their subsequent effect on the inhibition of SMC proliferation. This was a step-by-step process. Therefore, details relevant to specific results will be presented in separate chapters of this thesis. The more general details about single scale models and their mutual couplings are explained in the next sections of this chapter. These details have previously been published by Caiazzo et al. [47].

1.6.1 Single Scale Models

Blood Flow solver (BF): Alterations in the Wall shear stress (WSS) distribution within the stented vessel segment is a central ingredient that stimulate neointimal hyperplasia [50]. Therefore, modelling ISR requires computing the WSS distribution within the stented vessel. The blood flow in the stented vessel is modelled as a steady state incompressible Newtonian fluid using the Lattice Boltzmann Method [51,52,53]. Periodic boundary conditions are applied at the inlet and outlet whereas bounce back boundary conditions are applied at the flow interfaces. The vessel domain was discretised into regular lattice grids with a grid size of 10 μm . In 2D, we used the most common D2Q9 L-BGK formulation of the Lattice Boltzmann method..

SMC Model: The dynamics of cells in the SMC model are simulated using an Agent Based Model where each cell is represented as an agent. These agents are identified by a set of state variables such as position, cell size, biological state and drug concentration (in case of drug eluting stent). Each time step of

the tissue model involves two types of solvers:

Physical Solver: This solver simulates the structural dynamics of the cells by computing inter-cell forces between 2D cells. For two overlapping cells, it is assumed that there are two forces between the agents. One representing the repulsive force as the cells are pressed into each other and the other representing an attractive force associated with the interactions at the surfaces (separation). The degree of attraction and repulsion between cells is based on the cells overlap and separation respectively. The repulsive force is computed as a function of the separation between the centres of two cylindrical bodies with parallel axes based on Hertzian contact [54,55].

Assuming Hertzian contact, the contact force per unit length is:

$$F_H = \frac{a^2}{4} \cdot E^* \cdot \pi \cdot \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \dots\dots\dots (1.1)$$

Where R_1 and R_2 are the radius of both cylinders, $E^* = \frac{E}{1-\nu^2}$ with E the elastic Young modulus and ν the Poisson ratio.

Additionally, we may define a as the radius of the contact area:

$$a = \frac{\sqrt{R_1^2 \cdot R_2^2 - 0.25 \cdot (s^2 - (R_1^2 + R_2^2))^2}}{s} \dots\dots (1.2)$$

where s is the distance between the centres of both cylinders.

If it is assumed that there is an attractive force per unit length that is proportional to the contact length, with constant of proportionality K :

$$F_A = 2aK \dots\dots\dots (1.3)$$

Then the total force per unit length between two cylindrical bodies is:

$$F = 2aK - \frac{a^2}{4} \cdot E^* \cdot \pi \cdot \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \dots\dots\dots (1.4)$$

Every iteration of the physical solver involves computing the new equilibrium positions of the SMCs using an over-damped version of Newton's second law of motion. Neglecting inertial terms, the model is described by the equation:

$$C \frac{dx}{dt} = F(t, x, r) \quad \dots\dots\dots (1.5)$$

where x is the vector of cell displacements, r is the vector of cell radii and C is a matrix of friction coefficients. The equation of motion is currently solved by a simple first order explicit Euler method. A measure of stress is also computed by adding the absolute magnitudes of all the forces applied to the cell in each of the radial and axial directions.

Biological Solver: The second solver is a biological solver that simulates the cell cycle based on the biological rule-set. SMC growth and division is described as different phases of the cell cycle: quiescent state G0, a growth state G1 and a mitotic stage S/G2/M where a mother cell divides into two daughter cells. Progression through the cell cycle takes place at a fixed rate and finally results in mitosis. Cell can enter or leave the G0 (inactive phase) depending on certain rules. The rules deciding whether a cell enters or remain in G0 include contact inhibition (based on the neighbour count), WSS (for SMCs in contact with the flow) and local drug concentration (in case of DES). The 2nd chapter of this thesis includes the contribution of WSS during the SMC cell cycle progression. However, this rule is modified in other chapters where WSS sensed by functional endothelial cells is used to produce nitric oxide (NO). Therefore those chapters include an NO rule in the SMC biological solver instead of WSS rule.

Drug Diffusion (DD):

In case of DES, antiproliferative drugs coated on the stent struts have proved to be an effective way of inhibiting neointima formation after the injury caused by the stent and balloon angioplasty. The stent struts act as a source that elute drug into the vessel. This drug is then diffused into the cellular tissue. Thus the spatial domain for drug diffusion model is equivalent to that of the SMC model.

The process of drug diffusion is modelled with a generic anisotropic diffusion equation, solved numerically by a Finite difference method. The computational domain is discretised and mesh points are classified into three categories. The portion of the space, which was occupied by SMCs, is considered as tissue. Stent struts act as a source of drug. The lumen is considered as a sink where drugs eluted from the struts are immediately and continuously flushed away by the blood. Since, the drug concentration on each cell is required as an input to the SMC model at every time step (Figure 1.2), therefore steady state drug concentrations were obtained and passed to the SMC model.

1.6.2 ISR Connection Scheme and Conduits

The above mentioned three single scale models (also called kernels) are combined using MUSCLE [44,46]. The coupling of these kernels requires a connection scheme which specifies the communication topology. A connection scheme for ISR2D is shown in figure 1.3. Special agents called conduits were implemented to couple the single scale models. These conduits were also used to perform filtering operations as well as data conversion where the output data from one single scale model is converted in such a way that it becomes an appropriate input for another single scale model.

Conduit: SMC -> BF: This conduit is used to convert the SMC domain (lattice free) into a lattice based domain. The BF solver needs a new domain configuration after each iteration of the SMC solver. This conduit generates the domain configuration (computational mesh) from the current list of cell positions and their radii. The computational mesh is further decomposed into fluid and solid nodes.

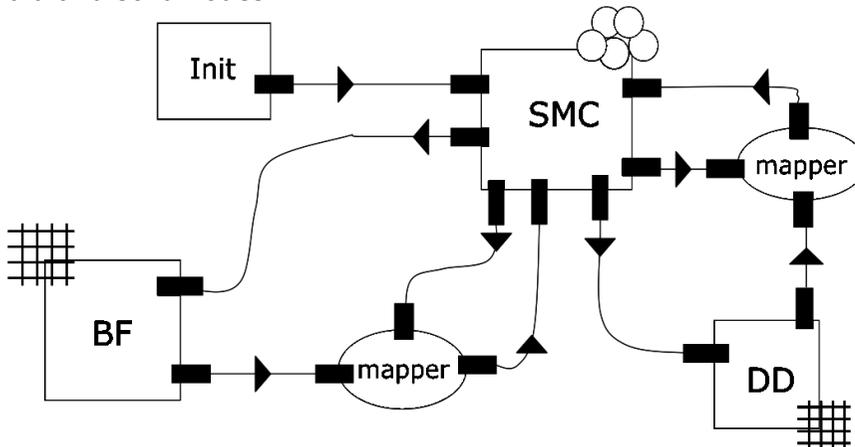


Figure 1.3: ISR2D connection scheme (taken from Caiazzo et al. [47]). Single scale models (BF, SMC and DD) are coupled together through using mapper agents and the conduits.

Conduit: SMC -> DD: Similar to the SMC -> BF conduit, this conduit also converts the array of cell position and their radii into a computational mesh for the drug diffusion solver. Since the drug is eluted from the stent struts and diffuses into the cellular tissue and it is also flushed away into the lumen due to the blood flow. Therefore, the computational mesh is marked with three different types of nodes: tissue, solid (source) and fluid (sink).

When an input to the SMC model is required from other single scale models, the coupling between single scale models becomes slightly more complex. This is due to the fact that multiple inputs

are required to generate one output that can further be fed to a single scale model as an input. For that purpose, Mapper agents were introduced which work like a conduit but can handle multiple simultaneous inputs.

Mapper: BF -> SMC: After computing the flow field using the BF solver, the observable that needs to be send to the SMC model is the shear stress which is required by the biological solver in the SMC model. This mapper reads from a conduit the boundary nodes of the fluid grid and the corresponding shear stress values. Additionally, it receives the current list of cells from the SMC model. The mapper then further maps the shear stress values from the fluid grid to each cell. Depending on the flow grid resolution, shear stress on a cell is calculated by averaging the values of the closest nodes.

Mapper: DD -> SMC: Similar to the shear stress mapping from grid to the cells, this mapper maps the current drug concentration on each cell. This mapper receives grid nodes belonging to the tissue along with the corresponding drug concentrations from the DD model. It also receives the current list of cells and their positions. Depending on the DD grid resolution, drug concentrations are approximated in a similar fashion, as done in the BF -> SMC mapper.

Figure 1.3 shows an additional single scale model (Init), which is executed only once at the start of every simulation to generate the vessel geometry and to simulate the stent deployment into the cellular tissue. The vessel geometry is achieved by generating an array of SMCs and IEL* cells occupying the region of the artery wall with a given packing density. The process of generating vessel with densely packed cells includes a random assignation of the cells sizes (within a fixed range) and cell positions. Therefore, based on the cell-cell interaction rules, the generated cells may not be in a state of equilibrium with their neighbours. The SMC physical solver is operated with no external forces to obtain the equilibrium states of the cells. The injury in the tissue is simulated by pushing stent struts into the vascular tissue.

A stent is deployed by pushing a surface into the artery. The surface of the stent is described as a barrier, the position of which is updated as the stent is deployed. Stent deployment is modelled

* A monolayer of IEL agents is created to represent a lining of the IEL. The equilibrium positions between the IEL agents are computed in a similar fashion as done for SMCs using the potential function (based on attractive and repulsive intercellular forces).

by computing the forces on the cells that come into contact with the stent during the deployment procedure. The direction of the force is normal to the surface of the stent and the magnitude is determined by the overlap of the stent with the agent in the same way as any other circular agent. Due to the force exerted by the struts on the tissue, IEL cells are removed based on the longitudinal and hoop (circumferential) stress. This removal of the IEL is considered as an injury to the vessel. The removal of IEL is directly proportional to the deployment depth of the struts, meaning that higher deployment depth damages a bigger portion of the IEL layer, hence greater injury.

For further details about the single scale models, their implementation and mutual couplings please refer to [39,47,49].

1.7 Research Motivation and thesis outline

The research presented in this thesis is highly motivated by the desire to better understand the dynamics of the ISR process using computer models. There is an increasing appreciation of a role for computer modelling to aid our understanding of complex biological problems. Such understanding is crucial in order to develop new devices and therapeutic treatments. However, we are a long way away from having such models that are sufficiently robust to answer the most fundamental questions. 'Why does ISR start' and 'why does it stop' are the most fundamental questions within the context of ISR modelling. The current set of models and simulations presented in the next chapters of this thesis allow us to do hypothesis testing which might help to find explanations for the above-mentioned primary questions. One hypothesis that has been tested might be that the re-establishment of the contiguous, functional endothelium stops SMCs proliferation and hence stops ISR. The other main questions being addressed in this thesis are: How does the ISR progress after the injury? How does endothelium growth rate influence the neointimal growth process? The neointimal growth in response to different endothelium growth rates might suggest that why do some patients develop ISR whereas some others don't? Does the migration of SMCs from the medial layer influence the overall development of the ISR tissue? The thesis also addresses the challenges involved in modelling such a biological system and describes how to proceed further in modelling ISR for a better understandability of the process in real three dimensional environment, either idealized or patient specific.

Given the need to understand the dynamics of the complex process of ISR, the process is modelled by dissecting the overall system from the top level into single scales models. These single

scale models are glued together in such a way that the overall system represents the behaviour that can be matched with the trends seen in the histology. Although the current status of the model is rather simplistic and does not include all the processes involved in neointimal hyperplasia, it can still sufficiently reproduce results that match well with the available histological and *in-vivo* experiments in a qualitative fashion.

Another important point in modelling ISR is the availability of the data that can be fed into the models. The availability of such data in case of humans is relatively sparse. Pigs overall physiology exhibits close similarities with the humans where the most common key organ systems being comparable in anatomy and function [56,57] especially the coronary anatomic structure and vessel sizes and, most importantly, in terms of the response to arterial injury caused by the penetration of a stent into the vessel wall [58]. Moreover, the rationale for choosing to simulating ISR in a porcine artery is based on the fact that an extensive literature from clinical and experimental studies performed on pigs is available to us, whereas there is no similar time-series data available for human implants. There is one main difference in simulating ISR in pig arteries from the human situations - the pig arteries are healthy whereas the ISR response studied in the humans is always on diseased arteries. So these porcine models are not able to reflect the influence of the arterial disease.

The main road map of the thesis is as follows:

Chapter 2 presents first results of our multi-scale model of ISR. The development of the simulated restenosis as a function of stent deployment depth is compared to an *in vivo* porcine data set. Moreover, the influence of strut size and shape is investigated and the effect of a drug released at the site of injury, by means of a drug-eluting stent, is also examined. A strong correlation between strut thickness and the rate of SMC proliferation has been observed.

Chapter 3 shows further improvements to the previous model from chapter 2. The effects of re-endothelialisation and NO release on neointimal growth are investigated *in-silico* using a two dimensional multi-scale model of ISR. The effect of stent deployment depths on the development of ISR is studied as a function of time after stenting. Shear stress distribution on endothelial cells, obtained by blood flow simulations, was translated into NO production that keeps the SMCs in a quiescent state. The cellular growth trends were plotted as a function of time to investigate the correlation between neointimal growth and

strut deployment depths in the presence of a functional endothelium.

Chapter 4 describes the effects of the origin of endothelium regrowth on ISR development where both histology (*in vivo*) and computational simulations (*in silico*) are used to evaluate neointimal growth patterns within coronary arteries along the axial direction of the stent. Comparison of the growth configurations *in vivo* and *in silico* was undertaken to identify candidate mechanisms for endothelial repair. Two re-endothelialisation scenarios (endothelial cell (EC) random seeding and EC growth from proximal and distal ends) were implemented *in silico* to evaluate their influence on the morphology of the simulated lesions.

Chapter 5 presents a stand alone vascular tissue model to simulate the process of ISR using the Cellular Potts Model (CPM) where the focus was on the initial migration of SMCs after vascular injury. The mechanism tested using this model is to evaluate the number of initial SMCs migrated from the medial layer into the lumen where they start to proliferate. At the moment, this model does not include the presence of blood flow inside the domain. The relationship between the initial migrated SMCs and injury score is highlighted. Moreover, a link between the initial migrated SMCs with the speed of the initial neointima development was identified.

Chapter 6 presents the first results obtained from our state of the art three dimensional multi-scale model of ISR, by coupling a blood flow and tissue model including the effect of re-endothelialisation.