Chapter 3

In-stent Restenosis\textsuperscript{1}

3.1 In-stent Restenosis

A stenosis is a narrowing of a blood vessel lumen due to the presence of an atherosclerotic plaque. This can be corrected by balloon angioplasty, after which a stent (metal mesh) is deployed to prevent the vessel from collapsing. The injury caused by the stent can lead to a maladaptive biological response of the cellular tissue (mainly due to smooth muscle cell proliferation). The abnormal growth can produce a new stenosis (re-stenosis).

Restenosis develops under conditions of pulsatile flow and there exists an interaction between the much studied biological pathways and those of a physical nature [45, 46]. The multi-science and multi-scale nature of in-stent restenosis has been discussed in detail previously by Evans et al. [32].

The design and geometry of the stent employed influences the biological events occurring in the vessel following deployment. Strut thickness, number, cross-sectional shape and arrangement, and stent length all influence the haemodynamics and degree of injury and stretch observed within the stented segment [47]. These in turn, are critical determinants of the severity of restenosis observed. Additionally, stents may be coated with active compounds targeted at the biological processes responsible for driving the progression of restenosis which, when eluted locally at the stented site, can prevent proliferation of smooth muscle cells and neointimal growth.

The development of a multi-scale \textit{in silico} model capable of testing both the influence of stent geometry and that of drug elution is motivated by the desire for a better understanding of the dynamics regulating restenosis. Thus providing a potentially powerful tool for improved understanding of the biology, and to assist in the process of device/therapy development.

As in many other biological systems, the dynamics of in-stent restenosis span many orders of magnitude through the scales, from the smallest microscopic scales up to the largest macroscopic ones. The wealth of experimental data that is now

\textsuperscript{1}This chapter is based on A. Caiazzo, D. Evans, J.-L. Falcone, J. Hegewald, E. Lorenz, B. Stahl, D. Wang, J. Bernsdorf, B. Chopard, J. Gunn, R. Hose, M. Krafczyk, P. Lawford, R. Smallwood, D. Walker, and A. Hoekstra, \textit{A Complex Automata approach for In-stent Restenosis: two-dimensional multiscale modeling and simulations} (submitted) and extended by the section on the thrombus formation model.
available has made \emph{in silico} experimentation an attractive tool in systems biology, allowing hypothesis testing and formulation of predictions which can be further tested \emph{in vitro} or \emph{in vivo} \cite{48}. In recent years the computational biology community has developed extremely powerful methods to model and simulate fundamental processes of a natural system on a multitude of separate scales. The next challenge is to study, not only fundamental processes, on all these separate scales, but also their mutual coupling across the scales and to determine the emergent structure and function of the resulting system \cite{49}. Despite the large body of literature on multi-scale models, we have found that there is a surprising lack of (formal) methodology for multi-scale modeling (see Sec. 2 and \cite{31}). Moreover, the key feature of multi-scale modeling, the actual coupling between scales is still at a very early stage of development \cite{50}. In this context, in Sec. 2 introduced Complex Automata (CxA) have been introduced as a paradigm to simulate multi-scale systems as a collection of single scale models, interacting across the scales (see also \cite{31, 51, 30}).

Based on the conceptual description of the relevant processes and their characteristic temporal and spatial scales which has been presented in \cite{32}, we describe a simplified CxA model of the multi-scale process, coupling a \textit{lattice Boltzmann} bulk flow (BF) solver (for the blood flow), an \textit{agent based model} for smooth muscle cell (SMC) dynamics (simulating cell growth, the cell cycle, physical and biological cell-cell interaction), and a \textit{Finite Difference scheme} for the drug diffusion (DD) within the cellular tissue.

In section 3.2, following a short introduction on in-stent restenosis, we present the two dimensional multi-scale model for ISR. We describe the main characteristics of the single scale solvers, which have been developed independently from each other, and independently from the ultimate application. We also describe, in detail, the coupling of the single-scale solvers with relevance to this particular application. Preliminary simulation results are presented in section 3.3. In Sec. 3.4 an outlook towards a 3D CxA for ISR is given. As an additional single scale model the thrombus formation model is introduced in Sec. 3.4.1. Within this subsection an overview of the biological background is given and modeling approaches are discussed. Subsequently, a very simple thrombosis model is proposed and validated, and its implementation and coupling into the 3D ISR CxA is discussed. This chapter closes with a short conclusion in Sec. 3.5.

### 3.2 Multi-scale Model of In-stent Restenosis

Restenosis, can be loosely described as a 'loss of gain' - that is, a late return of the vessel lumen to a size similar to that seen before intervention (stent deployment; see figure 3.1). It has, historically, been considered as an overreaction of the general wound healing response within vascular tissue \cite{52}. From a biological standpoint, injury caused by stent deployment (during balloon inflation) is thought to trigger a cascade of inflammatory events, that ultimately results in the development of new tissue (the neointima) \cite{53, 54}.

The majority of investigations into this phenomenon consider the biological and physical processes involved independently when, in fact, there is a complex interplay between the two. Blood flow, biological events (e.g. inflammation), stent geometry, drug elution and diffusion all influence the overall response of the the artery wall to stent deployment. The aim of the CxA model is to improve our
understanding of this complex system by considering restenosis explicitly as a multi-scale multi-science system.

Following an in depth literature review, the processes key to the regulation of restenosis were identified, and their temporal and spatial scales determined. Coupling was considered in terms of the interactions between these processes. This allowed us to generate a comprehensive conceptual scale separation map [32], defining a CxA, containing the sub-models necessary to capture the behavior of the system, and depicting the coupling between them; i.e. the flow of information between models.

The first practical implementation of the CxA reported herein considers a simplified version of the model focusing on SMC behavior, and its interaction with blood flow and drug eluted from the stent. The simplified SSM is shown in figure 3.2.

Following deployment of the stent, which is modeled as a separate process to provide an initial condition (using the SMC model itself, see section 3.3.1), SMCs start to proliferate in response to the mechanical insult. The rate of smooth muscle cell proliferation is dependent on the blood flow (specifically wall shear stress (WSS) and oscillatory stress index (OSI)), the number of neighboring smooth muscle cells, and in the case of a drug eluting stent, the local concentration of drug. The blood flow, in turn, depends on the lumenal geometry (and thus changes with the proliferation of SMCs), and the concentration of drug depends on the SMC/tissue domain (and therefore also on SMC proliferation). In the current model we assume that scale separation between the single scale models is confined to the temporal scale, however it is worth noting that scale separation on a spatial scale exists within the SMC model itself. The SMC model can sub-divided into the processes which occur on the cellular level, and those occurring on the level of the tissue, resulting in a hierarchical CxA model. The SMC proliferation is the slowest process, dictated by the cell cycle, whereas flow is a fast process, dictated by the length of one cardiac cycle. Due to the specific value of the diffusion coefficients and the
Figure 3.2: The simplified SSM, depicting the three single scale models and their mutual coupling.

typical spatial dimensions of the arterial tissue, the temporal scale of the diffusion process resides between that of flow and SMC scales. In future models we will also explicitly consider spatial scale separation.

3.2.1 Single Scale Models and Coupling Templates

In this section, the technical details of the CxA model of in-stent restenosis are presented in brief. We first describe the kernels of the CxA, i.e. the algorithms used to simulate the single scale models (Bulk Flow, SMC Behavior and Drug Diffusion). The native codes of these have been constructed independently from the multi-scale application. Then, we show how these elements are connected via smart conduits using a CxA dedicated coupling library [55].

**Bulk Flow Solver (BF)**

Blood flow is modeled as a Newtonian incompressible fluid governed by incompressible Navier-Stokes equations

\[
\rho_0 \frac{T}{T} \mathbf{u} + \rho \mathbf{u} \cdot \nabla \mathbf{u} + \nabla p = \rho \nu \nabla^2 \mathbf{u}, \quad t > 0, \quad \mathbf{x} \in \Omega_{\text{flow}}(t)
\]

\[
\nabla \cdot \mathbf{u} = 0, \quad t > 0, \quad \mathbf{x} \in \Omega_{\text{flow}}(t)
\]

\[
\mathbf{u}(t, \mathbf{x}) = 0, \quad \mathbf{x} \in \Gamma_{\text{flow}}(t)
\]

where \( \rho_0 \) is the blood density, \( \nu \) is the viscosity, assumed constant in the Newtonian approximation (a commonly accepted hypothesis for large vessels). The set
Ωflow represents the lumen domain, with Γflow(t) being its interface with the tissue domain.  

To obtain a numerical solution of (3.1), we employ a Lattice Boltzmann Method (LBM), which, unlike other CFD approaches, approximates the hydrodynamics starting from a pseudo-microscopic description of the fluid. In detail, the spatial domain is discretized using a regular lattice $\mathcal{L}(h)$, of spacing $h$, and a set of discrete velocity vectors $\{h c_i, i = 1, \ldots, b\}$, connecting neighboring nodes of the lattice. At each node $x \in \mathcal{L}(h)$, and at each time step $t$, the unknowns are the distributions $f_i(t, x)$, representing the density of particles traveling in direction of $c_i$.

Given the time step $\Delta t$, and with $\Delta x = h$, the evolution in time of the variables $f_i$ reads

$$f_i(t + \Delta t, x + \Delta x c_i) = f_i(t, x) + J_i(f(t, x))$$

(3.2)

where the right hand side defines the collision operator, and depends on the viscosity $\nu$ in (3.1).

For detailed overviews of the LBM, we refer the reader to [56, 57, 1, 41].

The observable related to the BF single scale model is the wall shear stress on the vessel boundary (WSS), which is needed as input for the SMC model, after being properly mapped from the Cartesian lattice on the individual cells.

**Smooth Muscle Cells Dynamics (SMC)**

The dynamics of the smooth muscle cells are simulated using an Agent Based Model. Each single cell is represented by agent, which is identified by a set of state-variables: position, radius, biological state, drug concentration and structural stress.

Each SMC agent evolves in time according its own current state, and to the states of neighboring cells. Each time step involves a physical solver, simulating the structural dynamics of cells, and a biological solver, which simulates the cell cycle, according to a biological rule set.

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2Equation (3.1) has to be completed with appropriate inlet-outlet boundary conditions. As this is not directly related to the multi-scale model, details are omitted for simplicity.
Physical solver  From the structural point of view, 2D cells are represented by their centers, and a potential function, which determines non-linear repulsive and attractive inter-cell forces. In addition, boundary forces, viscous friction, radial elastic forces (modeling the primary fiber direction of SMCs in a physiologically relevant 3D environment) and motility forces (modeling cell migration) are taken into account.

Neglecting inertial terms, the model is described by the system of equations

\[ C \frac{dx}{dt} = F(t, x, r) = F_{\text{rep}}(t, x, r) + F_{\text{att}}(t, x, r) + F_{\text{el}}(t, x) + F_{\text{bound}}(t, x), \quad (3.3) \]

where \( x \) is the vector of cell displacements, \( r \) is the vector of cell radii, \( C \) is a matrix of friction coefficients and \( F \) is the vector of forces on cells (including cell-cell, boundary and external forces).

At each iteration step, new equilibrium positions of SMCs are computed by iterating a finite difference scheme\(^3\) for equation (3.3) until steady state is reached. A surrogate of structural stress is then calculated and provided as input to the biological solver.

Biological solver  The cell cycle model consists of a discrete set of states, a quiescent state \( \text{G0} \), a growth state \( \text{G1} \) and a mitotic state \( \text{S/G2/M} \) (see figure 3.3).

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). Cells may enter or leave an inactive phase of the cell cycle (G0) depending on certain rules based on contact inhibition (calculated by neighbor count), structural stress, and local drug concentration (input from DD). Additionally, for SMCs in contact with the fluid, rules are based on thresholds of wall shear stress (WSS) and oscillatory shear index (OSI) received from BF also apply. Low WSS, high OSI or high structural strain are individually capable of inducing agent proliferation if drug concentration and contact inhibition criteria allow.

Drug Diffusion in Cellular Tissue

Drug eluting stents represent an effective way of inhibiting neointima formation after stent-deployment. This process is captured in the present model through implementation of the Drug Diffusion (DD) kernel. Drug is eluted from the stent and diffuses into the cellular tissue. Thus the spatial domain for the DD kernel is coincident with that of the SMC. Stent struts act as a source whilst boundaries between flow and cells are considered sinks (this assumes that drug eluted into the lumen is continuously flushed away by the faster blood flow). Biological tissues are heterogeneous in nature so we assume that this process can be described using a generic anisotropic diffusion law:

\[ \partial_t c(t, x) = \nabla \cdot (D_{\text{drug}} \nabla c(t, x)) \]

\[ c(t, x) = c_0, \quad t > 0, \quad x \in \Omega_{\text{stent}} \]

\[ c(t, x) = 0, \quad t > 0, \quad x \in \Gamma_{\text{flow}}(t) \]

\(^3\)A simple Euler method is currently used, but higher order Runge-Kutta schemes could also be employed. We found that results do not depend on the applied scheme if the numerical parameters are chosen carefully, and the Euler scheme is significantly more compatible, as it can be naturally written in the collision+propagation form.
where $c(t, x)$ is the concentration of the drug in the position $x$ at time $t$, and $D_{\text{drug}}$ is the diffusion tensor.

In equation (3.4), $\Omega_{\text{stent}}$ denotes the part of a vessel occupied by the stent strut, $\Omega_{\text{tissue}}$ represents the tissue subdomain, and $\Gamma_{\text{flow}}$ corresponds to its interface with the flow domain. In the practical algorithm, after discretization of the whole model geometry, mesh points are classified as tissue, source or sink. These are treated differently during the computation.

The diffusion tensor is chosen such that diffusion along the axis of the artery (or tangential to a cross section) is at least 10 times higher than diffusion in the radial direction [58, 59].

To solve equation (3.4) numerically, we employ a Finite Difference (FD) approach which is solved using a Propagation-Collision loop\textsuperscript{4}, thus fitting with the the CxA modeling language.

According to [59], the time scale to reach the steady state is of the order of minutes (comparable with the SSM in figure 3.2). Therefore, when coupling DD and SMC, we are mainly interested in the steady drug concentration (the time step for the SMC model, which uses the drug concentration as input, is of the order of 1 day). In the context of this CxA model, this allows direct consideration of the simplified equation:

$$\nabla \cdot (D_{\text{drug}} \nabla c(t, x)) = 0$$  \hspace{1cm} (3.5)

(with appropriate boundary conditions).

### 3.2.2 The In-stent Restenosis CxA: Kernels, Connection Scheme and Conduits

In order to combine the single scale kernels described above using MUSCLE [55], we need to define a communication graph, the Connection Scheme (CS), which specifies in detail the communication topology of the CxA, defining which pairs of kernels communicate. The Connection Scheme for the CxA model of in-stent restenosis is shown in figure 3.4.

In addition to BF, DD and SMC kernels, the current CxA setup includes a kernel which generates the initial conditions (IC) by simulating stent deployment into the cellular tissue (see section 3.3.1).

Multi-scale coupling is implemented using special agents called smart conduits. Often, these perform filtering operations, converting output data from one single scale model to appropriate input for another. This is the case for geometrical couplings (through changes in the domains), when new SMC configurations (continuum based) are transformed into lattice based computational domains for BF and DD:

**Conduit: SMC to BF.** This conduit converts the array of positions and radii of cell agents, into a computational mesh for the flow solver which is decomposed into fluid and solid nodes.

**Conduit: SMC to DD.** Similarly, this conduit converts the array of positions and radii of the cells, into a computational mesh for the drug diffusion solver.

\textsuperscript{4}LB approaches for the diffusion equation could also be used. The choice of a FD scheme was dictated by two main arguments: (i) FD schemes in general need less memory than the LBM; (ii) the choice of a FD helped us to investigate and demonstrate the coupling of the different modeling approaches within the same CxA.
marking the nodes as tissue, source, or sink.

In some instances, the interaction between kernels is slightly more complex, and multiple inputs are required to compute one output. In these cases we introduce mapper agents (see figure 3.4) which, in the present CxA, are required whenever an input to the SMC model is generated:

**Mapper: BF to SMC.** The values of fluid shear stress at the boundary affect the biological evolution of the cells. Given the output of the bulk flow solver, and the current cell configuration, a mapper agent computes the shear stress on each cell. Depending on the discretization used for the flow solver, different approximation approaches can be used. If the flow grid is coarser than the spatial scale of the SMC model (the radius of the cells), an algorithm must be used in order to determine which cells are in contact with the flow, then the shear stress is extrapolated from the closest boundary fluid nodes for each cell position. On the other hand, if the flow discretization is sufficiently fine more fluid boundary nodes interact with a single cell and the shear stress on the cell surface can be calculated by averaging the values of the closest nodes.

**Mapper: DD to SMC** In this case, the drug concentration calculated in the DD has to be mapped to the SMC agents. Given the current drug concentrations and the SMC configuration, the mapper agent approximates the concentration on each cell. As for the shear stress approximation, the algorithm used depends on the grid size of the DD model. If the grid is fine enough (with many lattice nodes per SMC), the concentration on a cell can be integrated. If a coarse DD grid is used, the concentration for each cell is extrapolated using data from the closest nodes.

### 3.3 Simulation Results

#### 3.3.1 Benchmark Geometry and Initial Conditions

As a benchmark geometry for the 2D CxA model, we consider a vessel, of length 1.5 mm and width 1.24 mm, where two square struts of side length 90 µm have been deployed. The vessel wall has a thickness of 120 µm. Smooth muscle cells are generated with an average radius of 15 µm and densely packed inside the
Figure 3.5: Left: Initial condition for the CxA model, including cell configuration, equilibrated after stent deployment, and the blood flow. Fluid shear stress is color coded (red high, blue low). Right: The same domain at 28 days post-stent deployment (672 iterations of the simulation). A neointima of SMC agents has developed in the lumen. Color bars refer to the wall shear stress within the lumen in Pascals.

wall. To obtain the initial condition based on the above geometry, an initial stress configuration compatible with the initial geometry must be provided. To do this, stent deployment is simulated, iterating the structural SMC solver until a stationary state is reached. The initial cell configuration resulting from this procedure is shown on the left in figure 3.5. The struts are clearly visible, embedded in the upper and lower vessel wall. SMC agents (blue) are lined by smaller internal elastic lamina (IEL) agents which are absent from the vessel wall region where the strut has penetrated.

3.3.2 Qualitative assessment of simulation results

We have run the simulation for an equivalent of 72 days (1700 time steps with $\Delta t = 1h$ for the SMC model) for both a bare metal stent ($n=6$) and a drug eluting stent ($n=6$). In the current 2D implementation of the model, stent deployment results in laceration of the internal elastic lamina (as is observed in vivo) allowing proliferation of smooth muscle cells into the vessel lumen. These preliminary results demonstrate neointimal growth (proliferation of smooth muscle cells) in response to stent-induced injury. If we compare the output from immediately after stent deployment with that of 28 days later (Figure 3.5) it is apparent that the developing neointima causes a reduction in lumen diameter and an increase in wall shear stress. Because the SMC ruleset dictates that SMC agent proliferation is inhibited by high shear, once the neointimal growth causes shear stress to increase past a threshold, an equilibrium is reached and no more proliferation occurs. This fits nicely with biological theory which asserts that a vessel remodels in response to changes in haemodynamic forces, until those forces are normalized [46].

The proliferative response is reduced in the presence of drug; at the simulation endpoint (72 days), average neointimal thickness at the strut site in the absence of drug was $0.206 \pm 0.005 \text{ mm}$ versus $0.192 \pm 0.001 \text{ mm}$ in the presence of drug (Figure 3.6).
Figure 3.6: Left: Neointimal thickness at 72 days is reduced in the presence of anti-proliferative drug eluted from the stent strut. Right: Despite the statistical fluctuations a clear trend of delay in the number of cells proliferating can be observed. If Normalized Peak Proliferation is considered, a single peak of proliferation occurs at approximately 20 days in the absence of drug whereas in the presence of anti-proliferative drug, peak proliferation occurs at approximately 22 days.

This trend was confirmed by examining the 'Normalized Peak Absolute Growth Fraction (NPAGF)'. This is defined as:

\[ \text{NPAGF}(t) = \frac{r_{M\text{-phase}}(t)}{N_{\text{cells}}(t)} \max_{s \geq 0} r_{M\text{-phase}}(s), \]

i.e. the product of growth fraction \( r_{M\text{-phase}} \) (Percentage of cells in M Phase/100) and total cell number \( N_{\text{cells}} \), divided by the maximum value of the growth fraction across the series (Figure 3.6). The present data suggests that peak proliferation occurs at approximately 22 days in the presence of drug, and 20 days in the absence of drug. As our cell cycle dynamics and bulk flow parameters are based on porcine data, this second value agrees well with the findings of Schwartz et al. [60] who derived the NPAGF for the rat, pig and human based on their experimental data and found the peak for the porcine series to be approximately 20 days.

### 3.3.3 Sensitivity Analysis

The single kernels have been singularly validated and their sensitivity with respect to model dependent parameters has been investigated. We remark that, given the structure of the MUSCLE framework, it is inherently simple to perform further sensitivity studies, for the global CxA setup with respect to key parameters of the single kernels. As an example, we investigated the threshold of drug concentration at which SMC agents change from a proliferative to quiescent phenotype, tunable by changing a single parameter in the global CxA setup. Figure 3.7 shows the relationship between this threshold and the amount of neointima present at seventy two days. Sensitivity analyses using different spatial resolutions for individual kernels can be also easily implemented.
Figure 3.7: Sensitivity analysis: Drug Concentration effect. By varying the threshold of drug concentration at which SMC agent proliferation is inhibited, the degree of neointima formed by 72 days is modified.

3.4 3D CxA

The two dimensional CxA provides us with a tool for testing hypotheses regarding the relationship between stent geometry, the cellular response to injury and the influence of haemodynamic forces. In order to evaluate realistic stent designs, however, it is necessary to run three dimensional simulations. MUSCLE was used to couple three dimensional versions of the bulk flow, SMC and drug diffusion kernels, and additionally, a thrombus kernel.

3.4.1 Single-scale model for thrombus formation (TF)

Platelet Thrombus Formation

The process of thrombus formation is one of the early responses of the biological system to the deployment of the stent. Endothelial cell loss due to stent deployment results in exposure of thrombogenic sub-endothelial molecules which activate platelets, causing them to aggregate. As a consequence of the change in flow geometry, activation of platelets also occurs due to non-laminar flow and increased shear stresses. Platelet activation can also be induced when blood is exposed to a reactive surface, e.g. the synthetic surface of a cardiovascular device [61]. Activated platelets can interact with the vessel wall to form mural thrombi. Activated platelets serve to achieve primary haemostasis and release a plethora of molecules that are able to influence the subsequent healing response. Once adhered to the vessel wall they secret chemicals that support the invasion of fibroblasts from surrounding connective tissue into the wounded area. Leukocytes are able to interact transiently (i.e. roll) with platelets before becoming stationary and adhering firmly. Both leukocyte rolling and firm adhesion are mediated by the interaction of adhesion molecules and their respective receptors on the leukocyte and platelet surfaces. Transmigration of the leukocyte into the vessel wall occurs in response to chemotactic agents. The platelet clot is slowly dissolved by the fibrinolytic enzyme
Figure 3.8: The time course of the processes involved in human in-stent restenosis. As an immediate response to the initial injury caused by the deployment of the stent thrombus formation is triggered and lasts in decaying strength for up to 30 days. It slightly overlaps the onset of SMC hyperplasia. In the current work thrombus formation is modeled as an initial condition to SMC hyperplasia. Leukocyte accumulation is not modeled explicitly.

(plasmin) and the platelets are cleared by phagocytosis.

The presence of adequate red blood cell numbers supports the formation of a haemostatic plug in vivo. This observation can be attributed to a red blood cells' physico-chemical effect on platelet transport and platelet-surface interactions. For example, red blood cell release of adenosine-diphosphate is likely to account for the increase in platelet aggregation that is observed as the hematocrit increases from 10% to 40% [62]. It was suggested [63] that the small size of the platelet relative to a red blood cell means that platelets may be concentrated in a peripheral layer at the blood-vessel interface, therefore increasing interaction with sub-endothelial molecules in the instance of an injured artery. The composition of a thrombus (in terms of fibrin to red blood cell ratio) is critically dependent on flow [63].

**Scales** Human platelets are between 1.5-3 $\mu$m in diameter and have a range of cross sections shaped from a circle to a square (for details see references at [http://www.imm.org/Reports/Rep018.html](http://www.imm.org/Reports/Rep018.html)). Porcine platelets are similar in size and appearance to human platelets. Softeland et al. [64] highlight certain differences between porcine and human platelets. These largely relate to differences in response to various platelet activators such as adrenaline. Regarding the temporal scales, experimental evidence suggests human platelet activation by shear requires less than 10 seconds at all shear rates above threshold [65]. Activation in response to ADP or other platelet agonists is likely to occur on a similar timescale. The earliest events involved in the formation of platelet aggregates in response to adenosine diphosphate occur in the range of 3 to 10 seconds. Initial discoid shape is lost with a decay time of 2-3 seconds, followed by the extension of pseudopods with a time constant of 7-8 seconds. This exposes GPIIb/IIIa receptors allowing the formation of fibrinogen bridges between adjacent platelets, and therefore, aggregation.

A red blood cell rich thrombus can constitute anything from the aggregation of around 50 platelets up to a mass encompassing the diameter of the vessel lumen, i.e. 100$\mu$m to 4mm. A red blood cell rich thrombus represents the aggregation of at least 50 platelets. Given that aggregation of two platelets to form a doublet may
take between 3 and 10 seconds from exposure to a given stimulus, it seems sensible to postulate that red blood cell thrombus formation takes somewhere in the region of minutes to form.

**Thrombus Modeling**

Platelet aggregation and adhesion are strongly affected by various mechanical factors amongst which the shear stress plays a dominant role. Numerous studies have shown that the shear stress enhances the thrombosis process and can directly cause platelet activation (see for example [66, 67, 68, 69]). In a flow field with high shear rate, the interaction between GPIb receptors and vWF multimers can initiate the tethering of circulating platelets to the vessel wall and to already adherent platelets [70]. In general, in the process of thrombus formation platelets get activated by abnormal physical stress. Whether this is high shear stress or low shear stress is still an open question in the community and different modeling approaches exist [71, 72, 68, 73]. With the activation platelets become "sticky", expressing surface proteins that bind to surfaces and other platelets. In regions with low stress the probability of sticking without being torn away by the blood flow is increased. Eventually, this leads to a filling and smoothing of rough surfaces as they are present in case of fresh wounds. However, also the activation dynamics (activation itself, and its decay over time) plays an important role as platelets loose their stickiness after being not stimulated for a while.

The most commonly used way to model thrombus formation is to treat blood as a continuous homogeneous medium defined by the continuity equation and the Navier-Stokes equations. The distributions of platelets and proteins relevant to thrombosis are described by the diffusion-convection reaction equations [74, 75]. In those works simplifications were made such as diffusion-limited rates of platelet-surface adhesion and constant platelet-surface reactivity allowing solutions even to be obtained in an analytical closed form [76]. An LBM approach was used in [72] to model flow and platelet-induced clotting, and in [73] flow-related clotting was modeled based on the residence time of concentrations of platelets using the same method for the advection-diffusion of the concentration fields.

Although tracking additional information like, e.g. “age” of the fluid, all of these models treat blood as a homogeneous medium and do not describe the behavior of individual blood constituents. However, blood is a highly complex suspension of chemically and electrostatically active cells suspended in an electrolytic fluid with active proteins and organic substances [77]. To gain insight into the nature of platelet activation, aggregation and adhesion, it would be beneficial to track individual platelets in the process of advection and activation. In [77] such a Lagrangian approach was demonstrated. Also a dissipative particle dynamics (DPD) approach to simulate platelet-mediated thrombosis can be found [78].

Recognizing the dynamics of activation of single platelets and the resulting sticking behavior as the most important feature of the complex thrombus formation system besides the blood flow, we have set up a model of massless non-interacting point particles that are convected in a flow field. The increase of the activity of these "platelets" is modeled as a threshold function of the shear stress the platelet is exposed to, from which the activation resulted as an integrated quantity. Competing with this is the decay of the activity with time. From the activation we then derived a function for the probability to stick in case it is next to a solid
surface which models both counteracting processes, stickiness and friction with the flow, respectively. Assuming a lattice based flow solver, lattice nodes are then solidified whose volume contains a number of sticking platelets larger than a certain threshold similar to solidification modeled in [79, 72].

Although such an activation dynamics model would offer the ability to simulate thrombus formation on the right time scale in a coupled simulation together with the other models, we, however, did not finalize this model to be part of the ISR model, also resulting from the non-triviality of tuning of the parameters of such a model. Too less physiological data could be found in the literature to extract parameters and quantify the model, e.g. in respect to relaxation times of platelet activation.

The ”Blob” model

From the above estimation of the temporal scales and the time-course of the processes involved in ISR (see Fig. 3.8) we see that the separation of thrombus formation and all successive processes involved in in-stent restenosis on the time-course is, strictly speaking, not absolutely given. However, in the models for in-stent restenosis we consider the final thrombus as an initial condition for the actual dynamic simulation of hyperplasia. This decision is not only motivated by the attempt to keep the complexity of the model controllable. It is also justified by the fact that in microscopy pictures of porcine samples at early stages of ISR the thrombus had already reached its final extent while the onset of neointimal growth was not yet clearly visible. When SMCs proliferate they migrate into a by then final thrombus tissue which transforms into fibrin-rich matrix. This fact allows for a different, much more pragmatic, very simple, but still convincing approach we called the Blob.

With Blob we ignore the above discussion on the need to resolve the dynamics of single platelets. Instead, Blob makes use of the observation that typically thrombus is found in areas which, before the thrombus formed, were characterized by circulating back flow of the blood. Regions of recirculation prevent a too quick escape of reaction products [80]. Also in [81] it was concluded that platelet aggregation occurred in annular vortices because of favorable combination of long residence time and frequent inter-particle collisions. In [71] the reattachment point was identified as first place for deposition in a stenosis model. However, experimental results show that maximum deposition occurs between the tubular expansion and the reattachment point [82] which can be explained by the fact that at the reattachment point the velocity is very low and the frequency of platelet-wall collisions is small whereas slightly upstream this situation has changed. This observation and explanation is supported by theoretical [83] and experimental [84] works. Recirculation zones can be described as sites of auto-catalytic augmentation of previously activated enzymes and sites of high residence time, both constituting ideal conditions for the deposition of platelets [71].

Given a steady flow field for a given geometry and Reynolds number, the simple Blob algorithm is defined so that in one shot all fluid nodes are marked as `thrombus node’ at which the radial velocity is opposite to the main flow direction. The rule reads therefore

\[
\text{type}(\mathbf{x}_i) = \begin{cases} 
\text{thrombus} & \text{if } \mathbf{u}(\mathbf{x}_i) \cdot \mathbf{u}_0 < 0 \\
\text{fluid} & \text{else} 
\end{cases} \quad \forall \mathbf{x}_i | \text{type}(\mathbf{x}_i) = \text{fluid} \quad (3.6)
\]
Figure 3.9: Thrombii as defined by the Blob algorithm in comparison to results of other clotting models for a steady flow through a two-dimensional channel with a rectangular stenosis. a) Blob result for $Re = 100$, b) shear stress model with high shear stress threshold, also $Re = 100$ (from [85], colors improved), c) Blob result for $Re = 550$, and d) result of a shear stress model with medium threshold. b) and d) are from [85] where for other parameter settings also thrombus can be found in front and on top of the strut as seen in a) and c).

where $\mathbf{u}(\mathbf{x})$ is the flow velocity at node $\mathbf{x}$ and $\mathbf{u}_0$ is the general flow direction, e.g. $\mathbf{u}_0 = (u_x, 0, 0)$ in case of flow from left to right in a tube aligned with the x-axis.

**Validation** In Fig. 3.9 a number of results are shown for the application of (3.6) to flow fields through simple 2D test geometries. Although this method can be described as “quick and dirty” in comparison to more realistic modeling its results surprisingly compare very well in overall shape and extension with results obtained by Harrison et al. [85] for a modified solidification model based on a residence time approach where nodes of the LBM lattice were solidified whenever the residence time of the fluid is of sufficient magnitude. In the thesis by Harrison it is shown that these in turn are very similar to experimental results with clotting milk.

**Flow Solver** In order to obtain a flow field $\mathbf{u}(\mathbf{x}_i)$ on which (3.6) can be applied a lattice-Boltzmann method (LBM) has been implemented. In section Sec. 4.3.1 the LBM will be introduced for 2D. However, the extension to 3D is straightforward. We used a D3Q19 model which means the velocity distribution function $f_{i=0..N}(\mathbf{x}_i, t)$ is represented by $N = 18$ components each standing for the probability to find a fluid particle with velocity $\mathbf{e}_i/\Delta t$ in one of the 18 lattice directions.
connecting neighbors and diagonal neighbors in the planes $xy$, $xz$, and $yz$.

As it is the computationally most costly part of the TF model we aimed at a very efficient implementation of the flow solver. Since the initial surface roughness due to the struts of the employed stent is small in comparison to the lumen width and the vessel is assumed to be approximately circular, the bulk part of the flow field is very much comparable to the that of a Poiseuille flow in a perfect tube. We made use of this fact by omitting the inner bulk flow and only allocate memory and compute the evolution of the velocity distributions function for those fluid nodes that are further than $r_{\text{bulk}}$ away from the center of the tube. The thickness of the remaining cylinder hull is determined dependent on the roughness of its outer surface by

$$R_{\text{bulk}} = R_{\text{max}} - a \cdot R_{\text{min}}$$

parallelized and memory-optimized for sparse geometries similar to the implementation presented in [86]. The inner volume of the lumen was omitted and replaced by proper velocity-boundary conditions at the inner surface of the hollow cylinder whose wall contains the actually simulated fluid volume. The thickness of the cylinder wall is determined from the roughness of the inner vessel surface. The flow field is initialized with an Poiseuille profile for a pipe radius determined from the smallest lumen and a given Reynolds number. Fluid in the hollows starts from zero velocity. All parameters of the LBM simulation are automatically determined from the geometry and Reynolds number in a way that simulation time and memory allocation is minimized. After reaching a convergence criterion the Blob algorithm then determines thrombus nodes from the flow field and these are sent to the CxA.

**Leaky Interface Boundary Conditions for Hollow Cylinder Boundary**

Since nodes inside the bulk, i.e. nodes $n$ for which $|x_n - x_{\text{center}}| < R_{\text{bulk}}$, are only virtual, an appropriate boundary condition has to be applied at the adjacent fluid boundary nodes. One way to realize this would be to consider the boundary as an impermeable moving wall in which case a bounce-back condition for moving boundaries would be appropriate like that applied at the boundaries of the LBM fluid to the suspended particles described in Sec. 4.4.1. In this case, the velocity of the solid wall $u_b$ could be set assuming a parabolic flow in the maximum free lumen.

Aiming at flow fields in the fluid ring that are as close as possible to the case flow would be simulated throughout the whole lumen, another type of boundary condition was applied, however. We applied a boundary condition that weakens the impermeability of the interface between ring and bulk domain. Whenever probability densities $f_i(x_v)$ have to be propagated from a virtual $v$ inside the bulk to a fluid boundary node at $x_i$ these are obtained using

$$f_i(x_v) = (1 - c_{\text{leak}})f_i^{eq}(u(x_v), \rho_0) + c_{\text{leak}}f_i^{eq}(u(x_i), \rho(x_i))$$

constituting a linear combination of the equilibrium values for the Poiseuille velocity $u(x_v)$ and density $\rho_0$ in the bulk and the equilibrium value for $u$ and $\rho$ at the fluid boundary node at $x_i$. With that, quantities depending on orders larger 0 of the deviation from the equilibrium, e.g. stresses, will not be resolved correctly, of course. However, we know that only a few nodes further away from the boundary these are restored in sufficient accuracy [87].
Figure 3.10: Flow, indicated by flow lines with same random seeds, and detected thrombus area (red) in a 2D test geometry (black=solid) of size 92x107. Flow is from left to right. Different boundary conditions were applied at the cylinder-bulk boundary: a) none (full domain flow), b) leaky bc, and c) bb for moving walls. When the bulk is removed, leaky bc’s result in a flow field (and hence thrombus) that only slightly deviates from the full domain simulation. The application of the impermeable bounce-back bc’s for moving walls leads to recognizable deviations from the full flow field. In d) the $y$-velocity of the fluid is shown at a distance of one lattice node along the upper bulk boundary.

In Fig. 3.10 flow through a 2D test geometry is shown together with the thrombus area as from application of (3.6) for the cases that a) the full domain was simulated, b) leaky boundaries were applied at $R_{\text{bulk}} = 18$ with $c_{\text{leak}} = 0.95$, and c) bounce-back for moving walls. The velocity at the boundary was set so that the $x$-component corresponds to the mean $x$-component of the velocity along the same line in the full domain case. The $y$-component was set to 0. In Fig. 3.10d) the $y$-component is shown as measured one lattice node into the fluid. For bounce-back, modeling an impermeable wall, it is almost zero, whereas for leaky bc we obtain a profile that is similar to that of the full domain flow. This results in a flow field that is similar to the full domain field. Therefore also the thrombii are very similar. Whereas the thrombus detected from the bounce-back flow field does deviate from the full domain version in recognizable extend.

In its 3D version, Blob was extended by a prune and enrich algorithm that, run after the thrombus node detection according to (3.6), removes single thrombus nodes which result from recirculation in features of the voxel domain that are only one lattice node wide. Mapping the volumes of the SMCs and the struts
of the stent to the voxel mesh these are unwanted consequences of the lattice representation of the flow domain. In the pruning step all thrombus nodes are removed, i.e. turned back into fluid type, that have less than 2 neighbor nodes of thrombus type. This could also be interpreted as washing out of clots that are not solid enough. In a subsequent step, agglomerates that are left are enriched by marking all fluid nodes as thrombus that have more than 5 thrombus nodes in their D3Q19 neighborhood. This effectively fills possible holes in thrombus compounds and results in compact and smooth thrombii. In Fig. 3.11 the result of applying the Blob algorithm including the prune and enrich procedure are shown for the geometry also used for a 3D ISR CxA for which preliminary results are shown in Sec. 3.4.2. To realize the TF kernel, the Fortran code for the sparse and parallel implementation of the flow solver, and parallelized codes for the Blob algorithm and the prune and enrich smoothing procedure was completed with C++ and Java wrappers where entrances and exits to the framework were realized. The TF kernel functions as part of the generation of the initial conditions, more precisely, as a stage 3 after generation of the cells and the deployment of the stent. Thrombus nodes constitute solid obstacles for the bulk flow whereas SMCs are allowed to migrate into the thrombus. For the drug diffusion model the thrombus domain is indistinguishable from the SMC tissue and receives both as a combined domain.

3.4.2 3D Results

For the 3D ISR CxA, again, MUSCLE was employed to pass information between kernels using conduits and a modified generic mapper agent (in instances when
multiple inputs were required to calculate one output). A simplified geometry of the BiodivYsio stent was deployed into a three dimensional representation of the vessel wall, causing laceration of the internal elastic lamina, thus permitting underlying SMC agent proliferation. Figure 3.12 depicts preliminary output from the 3D CxA in which neointimal formation is clearly visible in areas adjacent to stent struts. The wall shear stress distribution can be visualized at the vessel surface.

3.5 Conclusions

In this work we have shown how Complex Automata methodology can be applied in a challenging multi-scale model of in-stent restenosis. In particular, we describe implementation of the coupling of three different subprocesses which operate on different time scales. The model has been realized employing a CxA-dedicated coupling library (MUSCLE), and the results presented in this chapter demonstrate that the CxA model can be successfully implemented within this framework in both two and three dimensions.

Although the individual models are at a relatively early stage and the current CxA is simple in nature, certain emergent behaviors are already apparent. For example, proliferation begins in response to injury, peaking at approximately 20 days following deployment in the absence of drugs (Figure 3.6). We are currently in the process of running additional simulation series, to validate the CxA’ against a biological data-set obtained from in vivo and in vitro experimentation using stented porcine arteries. In particular, we aim to characterize restenosis behavior as a function of injury index [88] and to investigate the positive correlation between injury and restenosis.

This first realization of the coupled CxA is an important milestone on the journey towards a full multi-scale model of in-stent restenosis. Future developments will require development of the single scale kernels. Implementation of more complex rulesets will allow inter-cellular signalling pathways and the effects of deep injury to be modeled. We also aim to achieve more realistic local hydrodynamics by integrating a full pulsatile flow model. Moreover, the current CxA can be improved further by including extra kernels to model processes such as endothelial loss and regrowth.
Figure 3.12: The 3D ISR model in which a simplified representation of the Bio-
divYsio stent is deployed. (a) Neointima is observed forming around the stent
struts. Also shown are flakes of thrombus (red). (b) Drug distribution in the
SMC tissue (blue - low, red - high). (c) Flow lines near the wall colored according
to flow velocity. SMCs are colored according to the wall shear stress (blue - low,
red - high).