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MODELLING OF THE BLOOD COAGULATION CASCADE IN AN IN VITRO FLOW SYSTEM

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ABSTRACT. We derive a mathematical model of a part of the blood coagulation cascade set up in a perfusion experiment. Our purpose is to simulate the influence of blood flow and diffusion on the blood coagulation pathway. The resulting model consists of a system of partial differential equations taking into account the spatial distribution of the biochemical species. An important issue is inclusion of a dynamic boundary condition describing adhesion of activated platelets on a collagen coated top lid in the perfusion chamber. The validity of the model is established through criteria on the reaction diffusion and flow equations, which guarantee non negative concentrations at all times. The criteria is applied to the model of the blood coagulation cascade.

1. INTRODUCTION

The development of a new medicament is a long term enterprise and a typical time span from the first ideas to a product on the market is of the order 10 years and the development costs can easily exceed one billion Euro. Substantial expenditures are on testing for effectiveness to cure and on testing for adverse effects. Since the mid nineties drug development has increased in costs by about a factor of two, and at the same time the number of new products have been reduced by half. A further pressure on drug development comes from governments seeking cheaper medical treatment in order to avoid exploding health care expenses. The pharmaceutical companies are seeking ways to make the drug development more predictable and financially safe. We believe that mathematical modelling of biosystems can be used as one of more scientific tools to advance the development of medicine. In particular when it comes to structuring large data sets and understanding the dynamics of biochemical reactions, cell and organ functioning and other processes. Due to the complexity of bio systems this is far from an easy task and progress is difficult.

In this article we shall illustrate the use of mathematical modelling in the pharmaceutical industry by an example from the development of a blood coagulation treatment with the coagulation factor VII [1, 2]. More specific we derive a mathematical model for a blood coagulation cascade set up in a perfusion experiment conducted at Novo Nordisk A/S. Cleaned blood with thrombocytes are used and coagulation factors are added in a controlled

fashion. We investigate the influence of blood flow and diffusion on the blood coagulation pathway by deriving a model consisting of a system of partial differential equations taking into account the spatial distribution of the bio chemical species. In the experiment activated blood platelets adhere to a collagen coated top lid of the perfusion chamber. The amount of adhered platelets can be measured and the production of platelets as function of various parameters can be investigated experimentally. In the mathematical model the adhesion is described by a dynamical boundary condition for the activated platelets. The validity of the model is established by a mathematical criteria, which states conditions on the reaction diffusion advection equations, guaranteeing non-negative concentrations at all times. While some sufficient conditions for positive invariance of diffusion-reaction equations are known in the literature, e.g. in [3], [4] and [5], we present here a criterion that is also necessary. The criteria is applied to the model of the blood coagulation cascade.

2. THE BLOOD COAGULATION CASCADE AND THE MATHEMATICAL MODEL

The blood coagulation cascade is an important bio chemical reaction pathway for preventing leakage in the vascular system in cases of vein or artery injuries. The zymogen with the name factor VII flows in the blood, while its co-factor is buried in the blood vessel walls. In the event of an injury the co-factor becomes exposed to factor VII and activates this zymogen to the enzyme factor VIIa, which in turn starts the coagulation cascade. The cascade involves a number of biochemical species, including blood platelets and thrombin. Thrombin activates formation of fibrin, which polymerizes into actin fibers, and the platelets turns sticky and seals off the leakage. The formed clog of platelets is mechanically stabilized by the actin fibers [6].

Recombinant factor VIIa has proven to be an effective treatment of a number of disorders in the blood coagulation cascade [1, 2] as it enhances thrombin production at the site of an injury. In order to investigate the coagulation cascade, perfusion experiments have been conducted [1, 7]. Washed platelets and red cells were combined to obtain plasma-free blood. The reconstituted blood was perfused over a collagen-coated surface in the presence of a thrombin generating system consisting of purified coagulation factors rFVIIa, Factor FX and prothrombin. Platelets adhere to the surface, become activated, and expose procoagulant phospholipids. Subsequently, rFVIIa binds to these phosphatidylserine-exposing platelets, on which it activates FX independently of TF. The resulting FXa combines with Factor V released from platelets α -granules, and the resulting prothrombinase complex converts prothrombin to thrombin. Platelet adhesion was expressed as the percentage of the surface covered with platelets [1].

In this way measurements of the amount of adhered platelets provides information on the production of activated platelets and its dependence on

various species or factors and parameters in the reaction pathway. Non-activated thrombocytes is denoted by T. The activated platelets play an important role in forming binding sites for the bio chemical reactions for the formation of thrombin, factor IIa. Thrombin catalyzes transformation from inactivated to activated platelets, thereby enhancing the activation of platelets.

In the case where we incorporate blood flow and diffusion of say k biochemical species, the mathematical models of the blood coagulation cascade has the general form

$$(1) \quad \frac{\partial u_i}{\partial t} = f(u_1, \dots, u_k) + \nabla(D_i \nabla u_i) - \nabla(v u_i) .$$

Here k is the number of species and $i=1, 2, \dots, k$. The concentration of species i we denote $[i]=u_i(r, t)$ and it depends on the spatial variables $r = (x, y, z)$ and time t . The function f describes the dynamics of the bio chemical reactions and ∇ is the operator $(\partial_x, \partial_y, \partial_z)$. The solution of the above concentrations is sought in a given space Ω with boundary $\Gamma=\partial\Omega$. Unique solutions u_i are determined from specified boundary and initial conditions. The function f determines the reaction dynamics, the blood flow velocity is $v=v(r, t)$ and D_i is the diffusion constant matrix, which here is diagonal. Anisotropic diffusion is modelled by using different values of the diagonal constants. The presence of erythrocytes (red blood cells) in blood vessels tend to push the much smaller platelets from the center to the vessel walls, a phenomenon which can be taken into account by invoking anisotropic diffusion. The same is observed in perfusion channels when erythrocytes is present. A number of studies present various models of the blood coagulation cascade or parts of the cascade [8, 9, 10, 11]. Furthermore, numerous suggestions for simplified models comprising just a few coupled equations are presented in the literature [12, 13, 14, 15].

We have developed a model for a perfusion experiment [1] consisting of 17 biochemical factors or species. These include thrombocytes T, Ta, factors II (prothrombin) and IIa (thrombin), factors X and Xa, factors VII and VIIa, factors V and Va, the tenase complex Xa-Va-Ta, where factor Xa and Va is bound to the surface of an activated platelet Ta. Finally, we have included binding sites on the platelets specific for the factors X, VII and II [16]. Here we shall not present the entire system of coupled equations for the coagulation cascade studied, but discuss one equation to illustrate how the complete model is constructed. In the perfusion experiment thrombin activates thrombocytes according to the reaction



In the above reaction scheme k denotes the reaction rate. For Ta the model equation becomes [16]

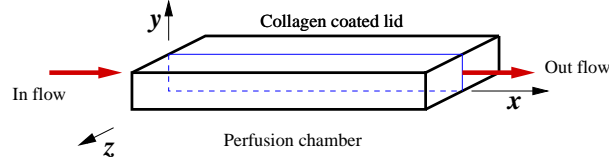


FIGURE 1. Sketch of the perfusion chamber.

$$(3) \quad \frac{\partial [Ta]}{\partial t} = -k [T] \frac{[IIa]}{c + [IIa]} + D_{Ta} \Delta [Ta] - \nabla(v[Ta]) .$$

The variable $[Ta]$ is the concentration of thrombocytes and Δ is the Laplacian. We have conducted simulations of the blood coagulation cascade as it appears in a perfusion experiment. The perfusion chamber is shaped as a rectangular box of length ℓ , width w and height h , and constructed so that $h \ll w \ll \ell$. A coordinate system is inserted where the x -axis is parallel to the box length ($0 \leq x \leq \ell$), the y -axis is parallel to the height edge ($0 \leq y \leq h$) and the z -axis is parallel to the width edge of the box ($-w/2 \leq z \leq w/2$). The boundary conditions used are: influx, with uniform concentration of the reacting species, at $x = 0$, outflow at $x = \ell$ and isolation at all other surfaces with one exception. The top lid of the perfusion chamber is coated by collagen and activated sticky platelets will adhere to the collagen. In mathematical terms this leads to a dynamical boundary condition where the concentration of the bounded platelets TaB becomes a time and space dependent variable $[TaB]$ which satisfy a differential equation defined on the boundary and coupled to the full system (1) defined in Ω . In the experiment presented in reference [1] activated blood platelets Ta attach to the collagen coated top lid Γ_1 leading to the reaction



with k_1 being the associated binding rate. Here θ is the concentration of free binding sites on the lid. TaB is an activated platelet bounded to site θ . The total number of binding sites θ_0 is assumed conserved and hence the concentrations of binding sites and activated bounded platelets obey $[\theta] + [TaB] = \theta_0$. The boundary condition now becomes dynamic for Ta and reads [9, 17]

$$(5) \quad \frac{\partial [TaB]}{\partial t} = k_1 [Ta] (\theta_0 - [TaB]) \quad \text{on } \Gamma_1 .$$

Note that in our perfusion system all other species than Ta satisfy insulating boundary conditions on the top lid. The dynamic boundary condition in (5) provides the concentration $[TaB]$ from which we can calculate the total

amount of adhered platelets, which in turn provides information on the production of activated platelets.

3. VERIFYING MODELS CONTAINING DIFFUSION, TRANSPORT AND INTERACTION OF SPECIES

For models of bio chemical reactions we must demand that the concentration of species are non-negative at all times. In deriving a model like (1) it is not beforehand guaranteed that this property is satisfied, and numerical simulations cannot answer the question if the model in this sense is valid or not. Even though numerical simulations can provide empirical evidence we can never be sure. In order to validate reaction diffusion convection equations, we have proved the below theorem 1, which provides criteria for the model guarantying non-negative concentrations for all times [18]. The theorem is proved for a slightly generalized version of Eq. (1) in the form

$$(6) \quad \frac{\partial u}{\partial t} = a\Delta u - \gamma \cdot Du + f(u),$$

with initial conditions $u(r, 0) = u_0(r)$, and the boundary conditions are of Dirichlet type $u(r, t) = 0$ on Γ . The matrix a is $(k \times k)$ with constant coefficients such that $a + a^* > 0$ (a is positive definite), and $f \in C^1(R^k, R^k)$. Here $\gamma \cdot Du = \sum_{i=1}^k \gamma_i \partial_{x_i} u$, with γ_i a $(k \times k)$ -matrix with constant coefficients. We assume that solutions u to (6) with initial data $u(0, \cdot) = u_0$ exist under appropriate compatibility conditions. We establish a criterion for positive invariance of the positive cone $K^+ = \{u_1 \geq 0, \dots, u_k \geq 0\}$, that is if u is a solution originating from initial data u_0 then

$$(7) \quad u_0 \in K^+ \implies u(t) \in K^+.$$

We have proved the following theorem

Theorem 1: *Let a , γ_i , $i = 1, \dots, k$, be $(k \times k)$ -matrices with constant coefficients, such that $a + a^* > 0$ and $f \in C^1(R^k, R^k)$. Let $u_0 \in L^2(\Omega, R^k)$ and the compatibility conditions on the data of (6) hold. Then in order to preserve the non-negative cone for (6) necessary and sufficient conditions are that the matrices a and γ_i , $i = 1, \dots, k$ are diagonal and*

$$f_i(u_1, \dots, 0, \dots, u_k) \geq 0, \\ \text{for } u_1 \geq 0, \dots, u_k \geq 0.$$

The proof of the theorem 1 is not provided here but is presented in reference [18]. We should remark that the result of the Theorem 1 can be extended to the cases a) Robin boundary condition (both homogeneous and non homogeneous) and b) Dynamic boundary condition (both homogeneous and

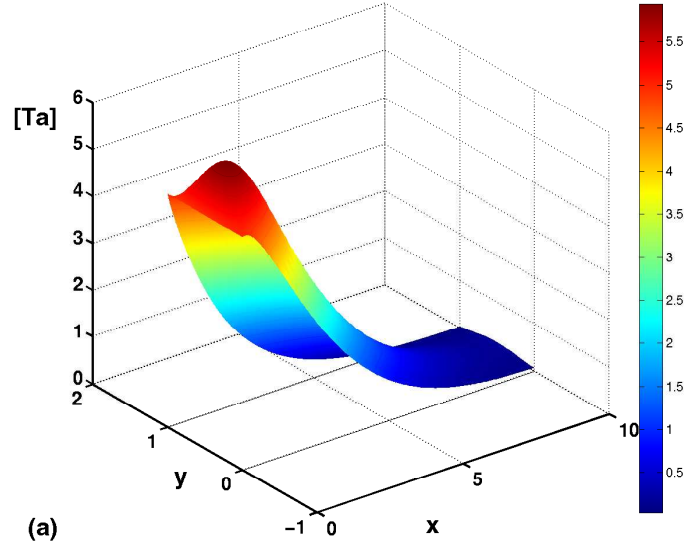


FIGURE 2. The concentration $[Ta]$ of activated platelets Ta in the perfusion chamber at time $t = 10$. Parameters: $k = 1$, $c = 1$, $\theta_0 = 1$, $k_1 = 0.04$. The diffusion matrix is $D_T = \begin{bmatrix} D & 0 \\ 0 & 100 \cdot D \end{bmatrix}$ where $D = 4.2703 \cdot 10^{-5}$. Concentration at inlet: $[Ta] = 5$. The Poiseuille flow velocity is $v = a_c y(1 - y)$, where $a_c = 1.7297$.

non homogeneous) with suitable assumptions on the boundary data (for example, in the case $u|_{\Gamma} = g_1(x')$ we have to assume $g_1 \geq 0$ or in the case $\partial u / \partial n|_{\Gamma} = g_2(x')$ accordingly $g_2 \geq 0$ e.t.c. By inspection of our full system of equations for the perfusion experiment we observe that each component of the vector f satisfies the condition in theorem 1. Hence the model is valid with respect to providing non-negative concentrations at all times.

4. SIMULATIONS OF THE BLOOD COAGULATION MODEL

In order to illustrate the solution of the model of the blood coagulation cascade in the perfusion experiment, we have implemented a scaled version of the model in the finite element program Comsol [19]. The scaling is chosen to provide variables and constants without units in the model equations. This is done by multiplying the concentration variables, the space variables and the time by suitably chosen constants. We have conducted simulations of a two dimensional slice of the perfusion chamber (xy -plane) placed at the center of the chamber ($z = 0$). The blood flow through the

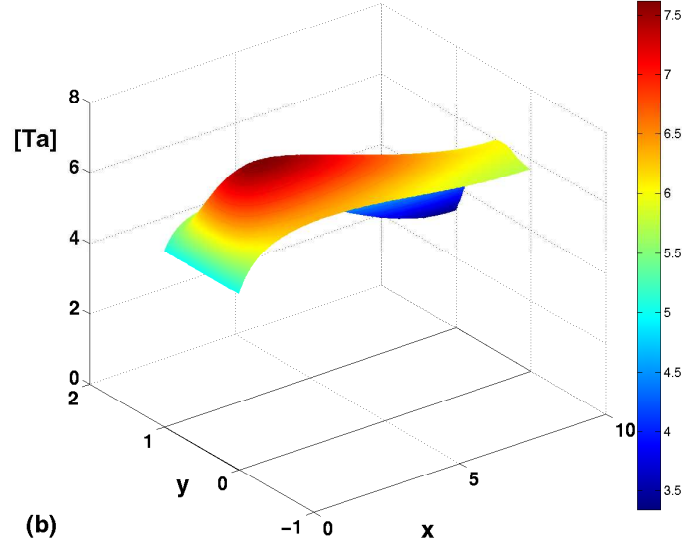


FIGURE 3. The concentration of activated platelets in the perfusion chamber at time $t = 40$. Parameters as in Fig. 1.

perfusion chamber is assumed to be a Poiseuille flow depending only on y through the relation for the flow velocity $v(y) = a_c y(1 - y)$, where the parameter a_c is a constant determining the flow volume. The simulation results are presented in figures 2 and 3, showing the concentration $[Ta](x, y, t)$ as function of space at times $t = 10$ and $t = 40$. In accordance with theorem 1 we observe that the concentration of Ta is non-negative throughout the solution space. The influence of the diffusion is relatively large and leads to nearly uniform concentration down stream at times larger than about $t = 50$.

The dynamic boundary condition (5) influences the concentration of the active platelets at the top boundary ($y=h$). Initially the platelet adhesion decreases the concentration of platelets close to $y = h$, see Fig. 2. At later times the free binding sites on the top lid are all filled and the concentration of bound platelets TaB saturates. The saturation leads to no adhesion of the platelets at the top boundary and eventually the boundary acts as an insulating boundary for Ta, resulting in increased concentration of Ta close to $y = h$, see Fig. 3.

In experiments the total amount of platelets sticking by adhesion to the top lid is measured. The time evolution of the concentration of the bound platelets TaB is presented in Fig. 4 showing that first platelets close to the inlet at $x = 0$ binds to the surface of the top lid. Downstream less platelets are available and the concentration of bound platelets are low. Eventually, the bound platelets will fill in the empty sites downstream and

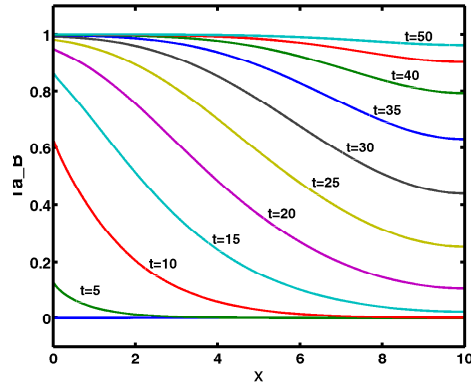


FIGURE 4. The concentration $[TaB]$ as function of x of activated platelets bounded to the top lid of the perfusion chamber at $y = 1$ for the times indicated. Parameters as in Fig. 1.

the entire lid saturates. A more realistic model of the adhesion of platelets at the boundary should allow for layers of platelets to build up and hence the boundary becomes a moving boundary, a complexity we have chosen to neglect here.

In summary we have developed a mathematical criteria for judging the validity of reaction diffusion convective type differential equation models. The criteria guaranties non negative values of the concentrations of the species for all times. We have used the criteria on a model of the blood coagulation cascade as investigated in a perfusion experiment with a reduced number of coagulation factors. The model is solved using a finite element code in order to illustrate the use of the criteria and to illustrate the influence of diffusion and convection on the coagulation cascade with a dynamic boundary condition modelling adhesion of blood platelets to a collagen coated surface. Models of the blood coagulation cascade, or other bio chemical reaction pathways, we believe can guide experiments or serve as a tool where it is possible to see processes otherwise not visible in experiments, that could be concentrations in hidden places, or concentration gradients, etc. Together with actual experimental results, model simulations can supplement the scientific data collection for fruitful discussions and aid more reliable and fast development of new medical treatments.

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