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Paper

Untangling the complexity of blood coagulation network: use of computational modelling in pharmacology and diagnostics

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Abstract

Blood coagulation is a complex biochemical network that plays critical roles in haemostasis (a physiological process that stops bleeding on injury) and thrombosis (pathological vessel occlusion). Both up- and down-regulation of coagulation remain a major challenge for modern medicine, with the ultimate goal to correct haemostasis without causing thrombosis and vice versa. Mathematical/computational modelling is potentially an important tool for understanding blood coagulation disorders and their treatment. It can save a huge amount of time and resources, and provide a valuable alternative or supplement when clinical studies are limited, or not ethical, or technically impossible. This article reviews contemporary state of the art in the modelling of blood coagulation for practical purposes: to reveal the molecular basis of a disease, to understand mechanisms of drug action, to predict pharmacodynamics and drug–drug interactions, to suggest potential drug targets or to improve quality of diagnostics. Different model types and designs used for this are discussed. Functional mechanisms of procoagulant bypassing agents and investigations of coagulation inhibitors were the two particularly popular applications of computational modelling that gave non-trivial results. Yet, like any other tool, modelling has its limitations, mainly determined by insufficient knowledge of the system, uncertainty and unreliability of complex models. We show how to some extent this can be overcome and discuss what can be expected from the mathematical modelling of coagulation in not-so-far future.

Key words: computational systems biology; blood coagulation; thrombin generation; bleeding; thrombosis; drug development

Introduction

Invention of computers in the middle of the 20th century heralded a new stage in the efficacy of thought experiments. It was the time when a huge bundle of descriptive knowledge in biology begat numerous attempts to synthesize the utter understanding of the nature. By describing chemical reactions or populations with mathematical equations, scientists tried to explain observed phenomena, to predict possible outcomes, to find new regimes of the system functioning.

The main flaw of this approach, if applied literally, was that to obtain a relevant result, one would need to put in the model just everything, like when Stanislaw Lem's Trurl was creating a machine that was able to write poems, he had to model the whole history of the Universe [1]. A more reasonable way is to make proper simplifications, which can be complicated for models of biological systems, where parameters are poorly defined, and where it is not obvious what is important and what is not. The balance between complexity and simplicity, between reliability and practicality, the choice of the model development strategy remain the main issues for people in the field. This is particularly important when models are used not only for basic research, but rather for practical biomedical

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problems of drug development and diagnostics. The issues with their reliability suddenly become much more important.

Here we show how one could overcome (some of) the difficulties of biomathematical modelling in blood coagulation—a typical complex biochemical network that has been an object of increasingly intense theoretical research over the past two decades [2–6]. The examples from papers published over the past years confirm that a relevant and transparent model can not only describe/predict the outcome of some *in vitro* experiment, but is even able to suggest plan of drug dosing and efficacy of a novel drug in a patient. These 'practical applications' of computational methods will be the subject of the present review.

Blood coagulation at a glance

Blood coagulation or clotting is a process of blood plasma jellification that serves to isolate sites of vascular damage and stop bleeding of both external kind (threatening with blood loss) and internal one (threatening with tissue damage, particularly dangerous for intracranial haemorrhage). The immediate cause of plasma transition from liquid to solid state is formation of a three-dimensional network of fibrin molecules owing to their polymerization (Figure 1A). However, the process of fibrin gel formation is controlled by an extremely intricate system of biochemical reactions with numerous enzymes and cofactors activating each other (a simple approximation of this system is shown in Figure 1B) that has been puzzling doctors and biochemists since 1960-ies. Importantly, the phenomenon of coagulation is spatially heterogeneous (Figure 1C). It is multiphasic, and different reactions of the coagulation cascade occur on different cell types, with diffusion as a necessary link between them [7]. Moreover, it must form a three-dimensional solid fibrin clot to plug the site of damage and not to spread beyond it. In other words, the task of coagulation is necessarily three-dimensional, similar to that of morphogenesis (with the only difference that it is not a long-term tissue that is formed but rather a short-time patch), and its biochemistry cannot be understood without considering diffusion and flow.

Finally, it should be kept in mind that coagulation cascade does not usually work by itself, and it is a part of a much larger haemostatic system that fights bleeding using an arsenal of tools. Thus, vascular haemostasis aims to limit blood loss by vasoconstriction, a controlled narrowing of the damaged vessels. Platelet-dependent haemostasis is mediated by special blood cells, called platelets, that adhere to the site of damage and form aggregates, thus plugging the wound. Platelets are known to interact with coagulation via a number of mechanisms, the most important of them being secretion of alpha granule contents and exposure of phosphatidylserine for the assembly of the membrane complexes. Platelets work best at high flow velocities, while fibrin solidifies the clot [8], which works better in slow blood flow [9, 10]. Coagulation also interacts with many other systems, the most important of them being immunity, angiogenesis and fibrin clot lysis.

Basic and practical problems in the field of blood coagulation

The concise and coherent picture displayed in the previous section might mislead the reader by suggesting that all is well in coagulation: all components and reactions are known, the sequence of events on vascular injury is clear, and there is nothing to look for. Paradoxically, nothing can be further from the truth.

It is correct that no major component of coagulation has been discovered during the past 20 years, and although some new reaction tend to appear [11], the overall biochemical network is well-established. However, there are major problems with understanding how all these reaction work *in vivo*. As one example, a heated debate over the role of factor XI (FXI) activation by thrombin [12] discovered in 1991 continues until this time: there are several hypotheses on the role of this reaction [13, 14], and the rates reported by different groups differ by orders of magnitude [15, 16] unless we take into account those who do not believe that this reaction exists in real blood [17].

From the practical point of view, challenges in the field are even more formidable, although the past decades witnessed a whole new generation of innovative approaches in diagnostics and treatment of coagulation disorders. In diagnostics, a new field of global/integral assays arose, which included methods aiming at better mimicking clotting *in vivo*, the most widely used among them being thrombin generation (TG) and thrombelastography [18]. However, problems with their sensitivity, specificity, reproducibility, standardization and interpretation are numerous (which is in part described in the next section). It is easy to obtain different results using different assays, and all the discussions leave open the question of what occurs *in vivo*.

In therapy, novel anticoagulants (rivaroxaban, dabigatran and others) appeared to provide alternative to traditional heparin and warfarin; on the other hand, novel pro-coagulants like FVIIa helped to improve coagulation. However, many problems remained. Despite better reproducibility and less need for control, all novel anticoagulants had a similar efficiency to the old ones in preventing thrombosis, with a similar risk of bleeding (though a different pattern of preferred haemorrhage types). This led to the need of developing numerous alternatives and seeking new drugs, mostly among FIXa and FXIa antagonists [19]. On the other hand, procoagulants like FVIIa became a subject of huge debate not completely resolved until now: despite wide clinical use, their mechanism of action is unclear.

Completeness of our knowledge so turned out to be misleading, and a need of additional understanding became obvious. Over the past two decades, computational modelling has been increasingly used in blood coagulation, first for basic research purposes and then for pharmacology and diagnostics. This use is the subject of the present review.

Experimental and mathematical models of blood coagulation

Blood coagulation *in vivo* can occur under different conditions. It turns out that this process is probably too complex for its modelling to have applied value at the present stage. It is intriguing that there are many models of thrombus formation *in vivo* that mostly aim at basic understanding of this process that is currently far from clear. In contrast, almost all pharmacological and diagnostic studies focused on modelling simpler (though not too simple) *in vitro* coagulation.

Historically, the first standardized methods of *in vitro* research in coagulation were clotting assays, where activation was induced by high concentrations of agonists acting via one or another pathway, and clot formation time was determined. Although some computational models of these assays were suggested [20, 21], they were not used for further research. The leading approach used for this purpose is without doubt TG assay (Figure 2A). Although formation of fibrin clot is the ultimate step of plasma coagulation, the main participant of it is considered to

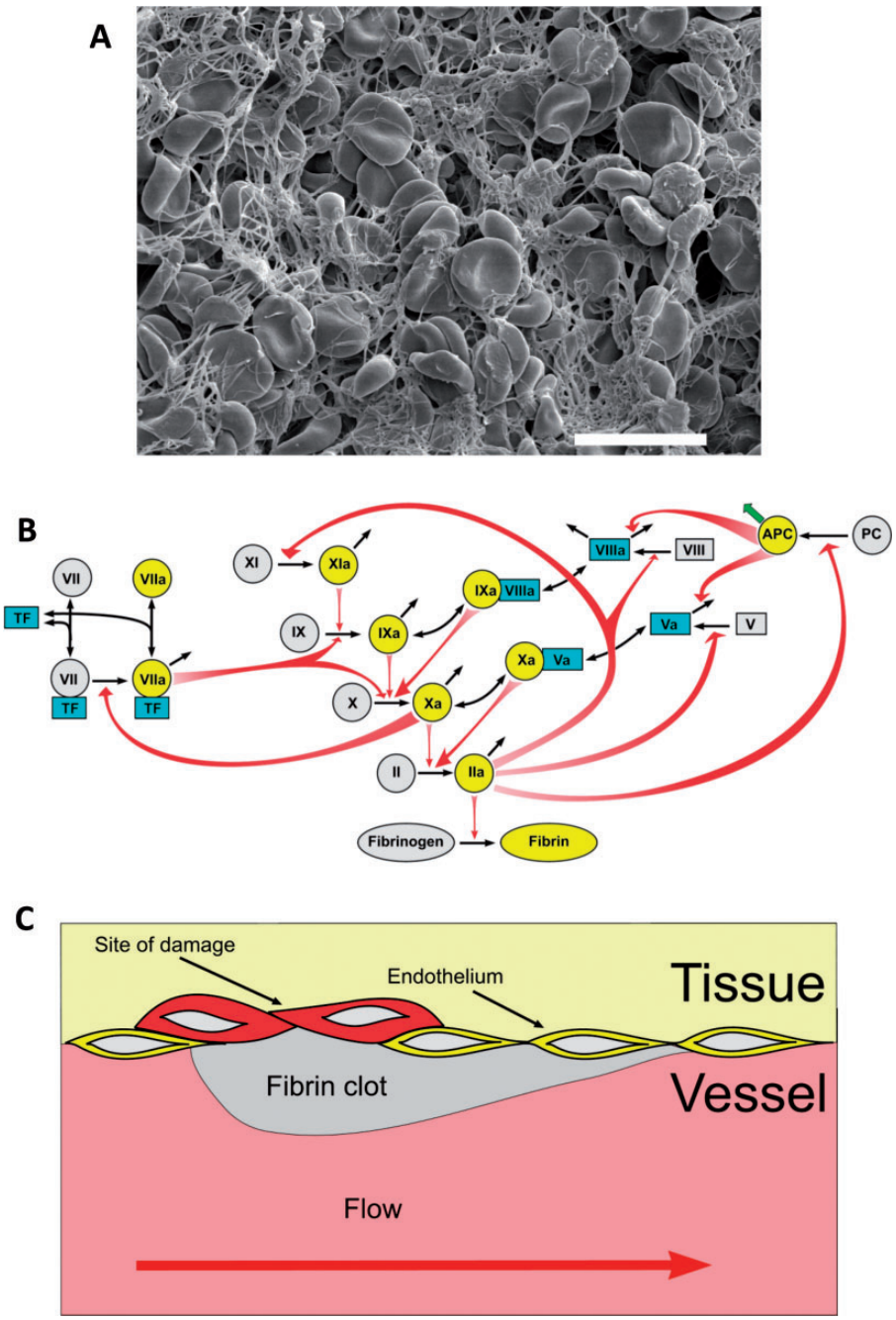

Figure 1. Blood coagulation. (**A**) Scanning electron microscopy image of a fibrin clot with entangled blood cells. Scale bar is 10 μm. The image is kindly provided by Sergey Obydennyi. (**B**) Biochemistry of blood coagulation. The basis of this cascade is formed by proteolytic enzymes called serine proteases that are capable of activating and inactivating other proteins (enzyme predecessors and cofactors) by cleaving off some portions of them. The most important proteins involved in clot formation and dissolution (factors II, VII, IX, X, XI, XII, protein C, plasminogen and others) are thus predecessors of serine proteases. Other proteins (factors V and VIII, proteins S and Z, kininogen) are cofactors that can affect enzyme-catalysed reactions; some of them can also be proteolytically activated, while others cannot. Many important reactions such as FII (prothrombin) and FX activation are mediated not by single enzymes but by multicomponent complexes of enzymes and cofactors assembled on the PL membranes provided by activated platelets; other reactions require endothelial surface (protein C pathway) or other cell type to proceed. (**C**) Blood coagulation during venous thrombus formation is propagation of the process in space and time. Note that different reactions occur at different locations, while diffusion of active factors and their convection by flow play critical roles in the regulation of clotting.

be thrombin. As it can be seen from Figure 1, it not only cleaves fibrinogen causing fibrin formation but also regulates many other reaction of coagulation cascade. Measuring thrombin concentration time course (TG test) is one of the most widely used global haemostasis assays available in the market. A mathematical model describing this experimental set-up, developed by the Mann group, can be asserted as a gold standard in modelling of blood coagulation [22, 23]. Many groups [24–26] based their models of blood coagulation on the backbone of this model. Being relatively simple, lacking diffusion, convection, platelet and endothelial interaction, it helped to obtain a lot of information about the functioning of blood coagulation.

A relatively recent global assay of coagulation discussed here is spatial reaction-diffusion model or thrombodynamics

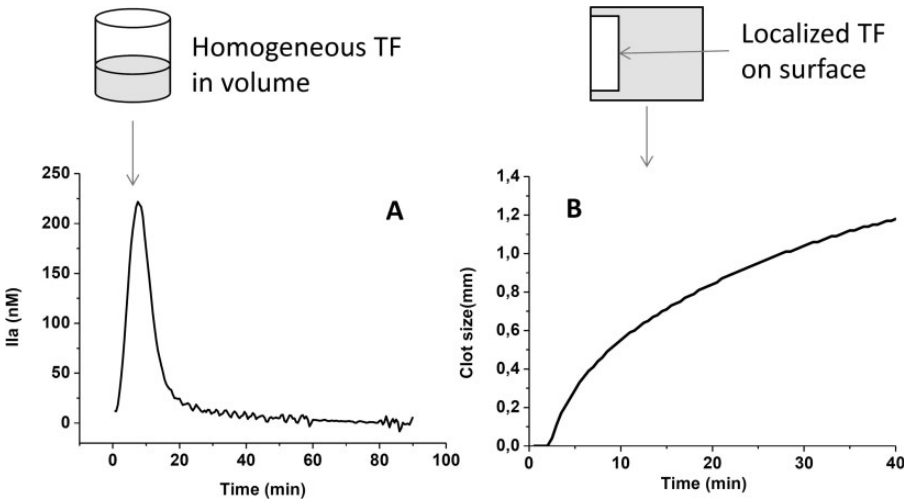

Figure 2. TG and spatial clot growth. (**A**) In TG assay, tissue factor is distributed in all reaction volume. The outcome of assay is a thrombin kinetics curve, from which all parameters of TG assay are determined. (**B**) In spatial clot growth assay, tissue factor is localized on surface. The outcome of assay is a clot size dependence over time. All assay parameters are determined form this curve.

assay [27], where clotting is activated by immobilized TF (Figure 2B). Monitoring in this assay is focused on fibrin formation, though a version involving thrombin also exists [14]. This approach helped to distinguish different phases in clot formation and explained the impaired clotting in haemophilia, the threshold in clotting activation, modular construction of the coagulation network [28].

Thrombelastography, waveform analysis, free oscillation rheometry and other global assays have not been subjects of computational approach. Although there were many other computational models of coagulation *in vivo* (as reviewed in [2, 4, 5]), it is noticeable that they were not actively used for applied research but rather for basic studies.

How the models are designed

A typical equation describing kinetics of a coagulation factor generation and inhibition usually looks like:

$$\frac{\partial[IIa]}{\partial t} = k_1 \cdot [Xa] \cdot [II] + \frac{k_2 \cdot [Xa - Va] \cdot [II]}{K_M/k_3 \cdot PL} - \left(k_1^i \cdot [AT - III] + k_2^i \cdot [\alpha_2 M]\right) \cdot [IIa] \quad (1)$$

On the left, the rate of thrombin (FIIa) concentration change with time is shown. According to the equation, it is determined by two processes given on the right: activation of prothrombin by FXa (the first term) or prothmobinase complex (the second term), and inhibition of thrombin by antithrombin and α_2-macroglobulin (the third member). Based on the known concentrations of all participants of these reactions at some time-point and on the kinetic constants, one can determine further dynamics of coagulation using a set of equations like Equation (1).

An example of how such models are designed is shown in Tables 1 and 2 that contain reactions/parameters and initial concentrations, respectively, for a simple model of blood coagulation [29] with minor modifications (there was no autoactivation of FVII:TF by FVIIa:TF complex). The set of ordinary differential equations is obtained from Table 1 using the law of mass action, while Table 2 provides the initial conditions for this set. This specific model is relatively simple and does not describe reactions beyond the initial critical step of FXa generation. More detailed and expansive models can include up to a 100 differential equations or more [22, 23].

These equations are solved using either universal mathematical software like MatLab (MathWorks, Natick, Massachusetts, USA) or professional biochemical software that is often freely available like Copasi (Copasi Project, http://www.copasi.org). The former are more flexible and can be combined with other software and macros for high-throughput simulations; the latter have the advantage of convenient representation of chemical reactions for biochemists as well as for mathematicians, as they contain built-in modules for stochastic solvers and chemical sensitivity analysis. To simplify transition of models between the platforms and programs, universal languages like SBML (Systems Biology Markup Language, http://www.sbml.org) are recommended; most of the solvers including MatLab and Copasi can export and import models, and freely exchange them, using this tool. In-house software is used increasingly rarely these days.

The output of model simulations is often represented as a plot showing how different coagulation factors change their concentration over time. For example, Figure 3 shows kinetics of FXa generation for different cases. Additional parameters of total coagulation factor profuction (that correlated to the area under the factor generation curve), maximal achieved concentration, time required to achieve the maximal concentration and others [28] are used.

For spatially heterogeneous systems or those in the presence of flow, the principles remain the same, but the mass transport equations are employed in more general forms. The equations are partial differential equations, and the rate of a concentration change is determined not only by chemical reactions, but also by diffusion and/or convection. Accordingly, a typical equation for a spatial thrombus growth model like [27] or a model describing thrombosis in a flow chamber like [10] the overall equation for each of the variables is:

$$[\text{Rate}] = [\text{Diffusion term}] + [\text{Convection term}] + [\text{Reaction term}] \quad (2)$$

Solution of these equations is more complicated. Commercial multipurpose solvers like Comsol Multiphysics (COMSOL Inc, Burlington, MA, USA) and biochemical freeware

Table 1. Reactions and parameters for a typical model of blood coagulation (from [29])

Reaction	k_1	k_{-1}	Reference
FVII+TF↔FVII:TF	$0.052\,nM^{-1}min^{-1}$	$0.0138\,min^{-1}$	[30]
FVII:TF+FXa→FVIIa:TF+FXa	$0.056\,nM^{-1}min^{-1}$		[31]
FVIIa+TF↔FVIIa:TF	$0.156\,nM^{-1}min^{-1}$	$0.0138\,min^{-1}$	[30]
FVII+FVIIa:TF→FVIIa+ FVIIa:TF	$0.026\,nM^{-1}min^{-1}$		[32]
FVII+FXa→FVIIa+FXa	$0.0009\,nM^{-1}min^{-1}$		[31]
FX+FVIIa:TF↔FX:FVIIa:TF	$1.5\,nM^{-1}min^{-1}$	$60\,min^{-1}$	[33]
FX:FVIIa:TF→ FXa:FVIIa:TF	$360\,min^{-1}$		[33]
FXa+FVIIa:TF↔FXa:FVIIa:TF	$1.32\,nM^{-1}min^{-1}$	$1140\,min^{-1}$	[33]
FXa+TFPI↔FXa:TFPI	$0.054\,nM^{-1}min^{-1}$	$0.0216\,min^{-1}$	[34]
FX+FVIIa+PL→FXa+FVIIa+PL	$5\cdot10^{-6}nM^{-1}\,min^{-1}$		[35]
FXa+ATIII→FXa:ATIII	$0.000124\,nM^{-1}min^{-1}$		[36]
FVIIa:TF+FXa:TFPI↔FVIIa:TF:Xa:TFPI	$0.23\,nM^{-1}min^{-1}$	$0.022\,min^{-1}$	[37]

Table 2. Initial values of the variables for the model described in Table 1

Factor	Value
TF	1 pM
VII	10 nM
VIIa	0.1–50 nM
X	170 nM
Xa	0
ATIII	3400 nM
TFPI	0.7 nM
PL	4000 nM

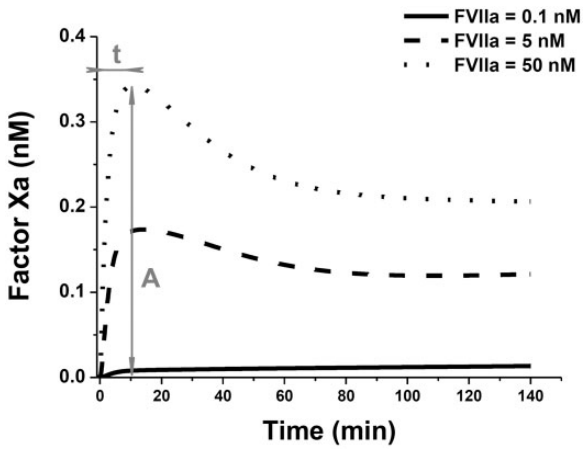

Figure 3. A typical model output. Kinetics of FXa generation depending on the initial concentration of FVIIa in a simple model of blood coagulation [29]. Increase of FVIIa concentration from physiologic (0.1 nM) up to therapeutic (50 nM) causes increase in FXa generation. As shown in [29], the total amount of FXa generated within the first 40 min of simulation correlated with the *in vitro* thrombin peak height. Additional parameters indicated in the figure include time required to reach the maximal FXa concentration (*t*) and the amplitude of this maximum (*A*); in other models the same parameters are used to characterize TG.

like VCell (http://www.nrcam.uchc.edu/) are also available for such problems, but the need to include specific features or some specific geometry/hydrodynamics not supported by these programs often necessitates development of in-house software for each case.

The mystery of the FVIIa mechanism of action

We shall begin with the problem of developing and investigating procoagulant molecules, i.e. those that should improve coagulation. The model disease where such need occurs is haemophilia. Haemophilia A or B is the name of FVIII or FIX deficiency, respectively. Its main manifestation is haemorrhage with the risk of further complications like haemophilic arthropathy. It is usually treated with infusions of the concentrates of lacking factors. Yet, high concentrations of exogenous proteins may lead to development of immune response, when inhibitory antibodies against infusing factors are produced. In this case, the common treatment does not work, and some drugs acting via other ways ('bypassing' ones) are used. One of them is recombinant activated factor VII (FVIIa).

The recommended dosing schedule is a supraphysiological dose of 90 μg/kg every 2–3 h until haemostasis is achieved, producing approximately a 250-fold increase above basal plasma concentrations of FVIIa (0.1–25 nM) [38]. FVIIa activates FX, bypassing the FIXa:FVIIIa intrinsic tenase complex formation that is impaired in haemophilia. It is licensed for haemophilia with inhibitors treatment only, but it is effective for haemorrhage cease and has no side effects, and its only limitation is a very small target group. An obstruction to widen the use of FVIIa was the lack of understanding of how it worked.

There were two possible explanations of how FVIIa restored haemostasis and why high doses of it were required to achieve the effect. One group [39] showed that FVIIa and FVII competed for TF and high doses of FVIIa were required to overcome zymogen inhibition effect. Another group [40] showed that FVIIa worked on the phospholipid (PL) or activated platelet surface by directly activating FX at very high concentrations.

Several studies investigated the mechanisms of FVIIa action. In [25], authors using Hockin–Mann model of TG demonstrated that FVIIa accelerated thrombin formation, decreasing clotting time 7-fold, thrombin peak time 3-fold, with a 400-fold FVIIa concentration increase. Thrombi peak and area under thrombin curve were much less sensitive: less that 2-fold increase for peak height and no changes for the area under curve. Authors showed that coagulation potential of blood was saturated at 15 nM of FVIIa, which correlated with whole blood model of haemophilia [39], but not with other *in vitro* studies [41, 42]. The authors proposed that the discrepancy in results appeared because the source and concentration of TF in these works were significantly different from those used by Butenas *et al.* Authors concluded that their mathematical interpretation of TG test was almost insensitive to FVIIa concentration, which can be

supported by some data [43] that clinical effectiveness of bypass therapy in haemophilia cannot be assessed by TG test.

A more detailed investigation of FVIIa modes of action was made with the help of a simple kinetic model [29], which included only FXa generation and avoided many extraneous parts of the coagulation model such as prothrombinase and intrinsic tenase reactions. It predicted that zymogen inhibition depended on only two reactions: (i) competition between FVII and FVIIa for TF; and (ii) the activation of FVII to FVIIa. In the absence of TF, only high doses of FVIIa (above 50 nM) were able to provide PL-dependent TG. This model showed that reasons why previous models were unable to agree on the relative contributions of each FVIIa mechanism were the following: (i) high level of TF masked zymogen inhibition in the cell-based model; (2) TF-initiated clot time that was used to study PL action [39] was not sensitive enough to hypercoagulation state. A sketch of FVIIa action mechanisms is presented on Figure 4.

A set of novel FVIIa molecules with increased activity on PL and/or TF and increased half-life were set in development to improve dosing strategies and even to widen target group. The only way to prove their efficacy and/or superiority over the wild type FVIIa is to pass a set of clinical trials that owing to limited target group will require a lot of time, will not provide high confidence and will face the dosing problem owing to changed activity. To facilitate the process of novel molecules development, mathematical simulation can be used for prediction of efficacy and dosing strategies of new variants of FVIIa. As is known, *in vitro* TG can be used for the estimation of the haemostasis state and thus can be helpful for evaluation of FVIIa efficacy. The above-described model of FXa generation, which showed good correlation with TG, was supplemented with FVIIa pharmacokinetic part, which described *in vivo* time course of FVIIa after infusion [44]. This PK/PD model of FVIIa action was validated with *ex vivo* TG in the patients received FVIIa or FVIIa mutant BAY 86-6150. The model helped to explain the similar efficacies of a single dose of 270 μg/kg and repeated doses of 90 μg/kg of FVIIa; it showed that the duration of the simulated haemostatic effect after a single dose was prolonged for FVIIa variants with increased TF affinity or extended half-lives, but not for those with modulated PL activity, which means that not all modifications of the FVIIa molecule may translate into a prolonged haemostatic effect. Simulations of the highly PL-active variants suggested a minor advantage in dose effect and dose prolongation, which was confirmed with the development history for the NN1731 variant. It had 10–100-fold higher activity on platelets *in vitro*, but comparable efficacy to FVIIa at FVIIa-like doses and administration intervals in phase 2 safety and dose-finding studies [45].

Mechanism of action and drug–drug interaction of tissue factor pathway inhibitor antagonists

Such bypassing agents as prothrombin complex concentrate and FVIIa turned out to be efficient clinically and successful commercially; yet, the problem of inhibitory haemophilia was not solved, as these drugs were expensive, short-lived and intravenous.

That is why pharmacologists began to look for alternatives, and one of them was an elegant idea of developing an antagonist of a coagulation inhibitor. In other words, if a bleeding disorder means insufficient coagulation factor activation, and if the most direct way to correct this (by increasing activation, like with the FVIIa) is not always satisfactory, a nice alternative is to design a molecule that would decrease coagulation inhibition. Although there are some attempts to target antithrombin-III [46], the efforts were generally focused on tissue factor pathway inhibitor (TFPI), an antagonist of the VIIa-TF complex. This is understandable: haemophilia is a disorder of insufficient FXa production by the

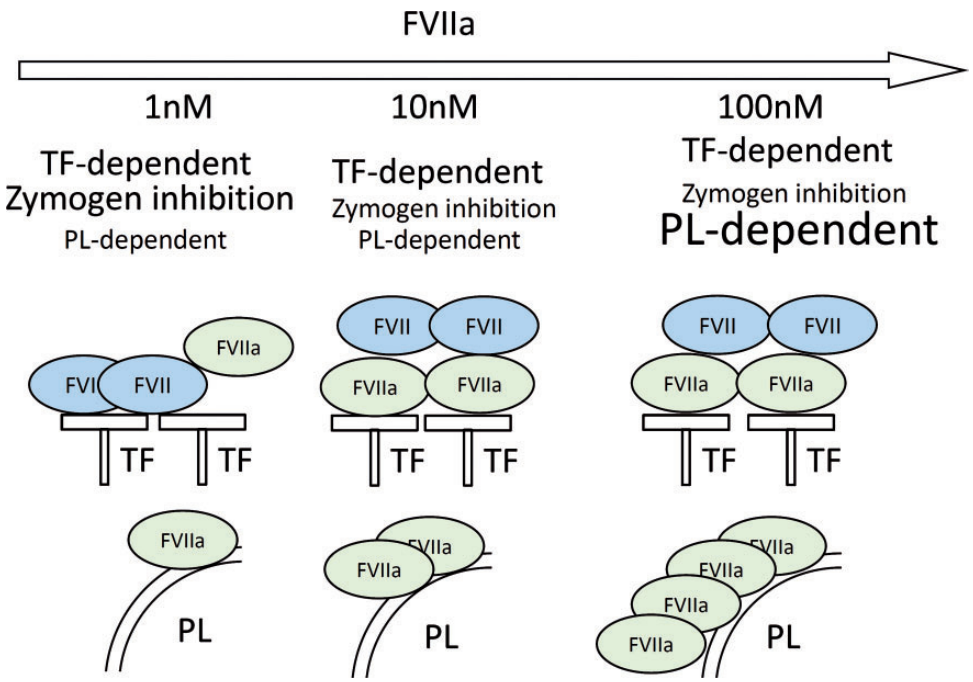

Figure 4. Mechanisms of FVIIa action. Two mechanisms of FVIIa action, TF-dependent and PL-dependent, are presented at all FVIIa concentrations; yet, at (**A**) low FVIIa concentration (<1 nM) TF-dependent pathway is prevailing with pronounced zymogen inhibition (which depends on the ratio of FVIIa/FVII). At (**B**) medium FVIIa concentrations (<50 nM) zymogen inhibition is overwhelmed, while PL-dependent pathway is still not functioning. At (**C**) high FVIIa concentrations (>50 nM) PL-dependent pathway starts to prevail over the TF-dependent pathway. The different font size in the mechanisms of action indicate relative contributions of the mechanism.

IXa–VIIIa complex, so it is natural to correct bleeding by promoting FXa formation via the another pathway by down-regulating its inhibitor. This is not the correct place to describe the whole story behind these developments, and those interested can find it in special reviews [47]. To put it briefly, preliminary experiments using antibodies against TFPI confirmed that such approach might work as a procoagulant treatment, and an aptamer ARC19499 (later termed BAX499) able to inactivate TFPI with high affinity and specificity was produced [48].

It is essential to point out that such a drug candidate is particularly interesting as an object of computational research because of three reasons. First, it has a non-trivial mechanism of action (correcting an impaired reaction by improving completely another reaction that proceeds on different cells and in different place), and as such it is not obvious how it would affect coagulation: prior experience with FVIIa demonstrated that such cases can be so complicated that discussion on their mechanisms of action can last for decade. Second, as an aptamer, it is incredibly highly specific for a human TFPI and thus cannot be adequately tested in animals. Finally, as a procoagulant molecule, it cannot be tested in healthy volunteers either, because of thrombosis risk. All this makes it an attractive target for theoretical research because a number of issues should be solved before a clinical study with patients: for example, one needs to understand potential drug–drug interactions in case of need for additional therapy.

That is why in the two of our *in vitro* studies of this molecule (one aiming at the mechanism of action [49], another focused on drug–drug interaction with FVIII [50]), we relied heavily on computer simulations. We used a well-characterized and thoroughly validated mathematical model of blood coagulation in a reaction-diffusion system developed earlier [51].

The first result obtained with the model was prediction that spatial clot formation would be affected by the BAX499 only at low TF densities. This unusual prediction turned out to be correct and helped greatly in the design of experimental research, as special low-density activators had to be developed first [52]. Another interesting aspect was that the model correctly predicted a large effect of BAX499 on fibrin clot initiation parameters, but not on the spatial propagation stage that is defective in haemophilia. The mechanism behind this (revealed only by modelling) was low diffusion efficiency of FXa that is rapidly inhibited by plasma inhibitors.

A third prediction (in a way, related to the second one) was independence of absolute effects of TFPI antagonism and FVIII supplementation in drug–drug interaction. This is critically important, as it gives some estimation on how a patient receiving the aptamer should be treated with FVIII in case of some urgent bleeding. Interestingly, experiments confirmed this last prediction in the vast majority of patients, but not all of them, indicating important limitations of computational models in that it is difficult to grasp individual patient features with them.

In the end, this drug candidate did not make it into a drug because it turned out to greatly prolong circulation of TFPI, thus increasing the bleeding risk [53]. This is indicative as an illustration of the limits of both *in silico* and *in vitro* studies. However, the obtained results on the mechanisms of action and drug–drug interactions would remain valid for any TFPI antagonists that are now under development [53].

Developing and investigating anticoagulants

As the goal of correcting hypocoagulation is the restoration of a not-functioning system, it might be rather tricky and not obvious, as we described before. Unlike that, the treatment of hypercoagulation is aimed to suppress clotting, and it is much easier to achieve. Many anticoagulants targeting different parts of coagulation cascade are under development. Still, the numerous ways that can be used to inhibit coagulation mean that there can be many solutions that are not equal. A number of studies therefore used computer simulations to find out 'critical points' in the coagulation network as potential drug targets, to compare inhibitors of various enzymes, or to compare drugs with the same target but different mechanisms as detailed in Table 3. It is interesting that the question was never the identification of the mechanism of action (in contrast to the bypassing drugs).

The first attempt of this kind [54] was undertaken only 1 year after development of the first comprehensive mathematical model of extrinsic pathway [22]. They attempted to find optimal drug targets (with a conclusion that the most potent inhibitor should be aimed simultaneously at factors VIIa and Xa and thrombin, but not IXa), and also tested a panel of real inhibitors both *in silico* and *in vivo*. Computer simulations predicted relative efficiencies of drug candidates in preventing experimental arterial thrombosis in rats with great accuracy. A later effort focused on comparing inhibitors of FXa and thrombin [55] and introduced an important distinction: these inhibitors differed not only quantitatively, but also qualitatively. FXa inactivation mainly affected initial stages of clotting, while that of thrombin decreased the amplification phase of TG as well.

A new type of problem [56] was comparison of inhibitors of the same enzyme but acting differently: direct reversible fXa antagonist rivaroxaban and fondaparinux, a cofactor for an irreversible natural antagonist antithrombin. This was combined with an attempt to mimic two regimens, chronic or acute injury, simulated as addition of the drug before or during TG. The conclusion was that rivaroxaban is better in the acute case, but comparison is not clear, as effects of fondaparinux are not specific towards Xa alone. The same group later expanded this work to include warfarin, a third type of anticoagulants that does not inhibit clotting proteases but rather prevents correct post-translational modification of their zymogens [57]. Warfarin was also compared with heparin in other works [63].

Another group used computer modelling as a tool to plan dosing and investigate efficacy/safety of a drug, the same rivaroxaban [58]. This was later expanded to include warfarin and simulate drug–drug interaction to create schedules for switching between anticoagulants [59]. These two works are an interesting exception among all those mentioned here because they simulate not TG *in vitro* but rather make an attempt to simulate clotting *in vivo* including flow.

Finally, the most abstract form of this type of research is determination of the 'sensitive targets' in a complex system. There were several studies aiming at this [28, 60–62]. They gave different results, probably because different modules of coagulation affect different outputs, and sensitivity strongly depends on the experimental conditions [28].

Modelling TG for better diagnostics: a new dimension of personalized medicine?

It might seem intuitively not obvious how computer simulations might assist in diagnostics: after all, diagnostics is just measuring something in an individual, is not it? Still, there is at least one series of works to demonstrate how this can be done. The Vermont group suggested that a combination of

Table 3. Development and investigation of anticoagulants using computational methods: what for?

Aim	Reference
1. To compare inhibitors of different enzymes	[54]
2. To predict efficiency of the drug candidates	
3. To compare effects of inhibitors of fXa and fIIa on different stages of clotting	[55]
4. To compare direct reversible and indirect irreversible inhibitors of fXa	[56]
5. To compare effects of inhibitors in different regimens (acute or chronic)	
6. To compare three radically different mechanisms of anticoagulant action	[57]
7. To assess efficacy, safety and dosing of a drug (rivaroxaban)	[58]
8. Dose scheduling for a shift from warfarin to rivaroxaban	[59]
9. Different types of sensitivity analysis	[28, 60–62]

variations in the concentrations of different pro-and anticoagulants can give abnormal coagulation dynamics even if each of the concentrations themselves is in the normal range. To test this, they measured coagulation factor levels, simulated TG and also measured it experimentally [64–67]. In some cases, FXa generation was simulated as an additional integral parameter [68]. This approach demonstrated that such combinations can indeed explain bleeding and prothrombotic phenotypes.

The natural expansion of this approach could lead to development of larger models incorporating information from genomic, proteomic and/or metabolomic studies in addition to functional biochemical methods. Computational models based on genome-wide analysis have been increasingly employed in metabolic studies [69] and have been recently expanded to protein transcription analysis [70]. Addition of proteomics expanded their capabilities [71], and there are examples of applying such techniques in the field of haemostasis, e.g. in platelet metabolism and analysis of aspirin resistance [72]. Proteomic data (though not of a personalized kind) were employed in the development of platelet signalling models [73]. As of the present moment, we are not aware of the attempts to expand such methods to blood coagulation, but the present vector of the development in the field suggests that signalling and defence networks like coagulation are the probable next target for such methods.

In addition to personalized results, general conclusions can be also achieved. In [74], the authors analysed the quantitative effects of blood plasma dilution on TG in the context of intersubject variability. They used the data from LETS study [75], where concentration of coagulation factors was measured in 472 healthy subjects and with the help of Mann's model of coagulation [62] calculated TG in undiluted, 2-, 3- and 5-fold diluted plasma. Dilution caused decrease of thrombin peak height, area under curve and maximum slope of TG curve. The effect of reduced temperature on TG was investigated in another work [76]. The Hockin–Mann model of blood coagulation was modified to describe the effects of changing temperature. For this procedure, authors generated a number of random temperature coefficients for any given temperature below 37°C for each of 44 kinetic constants of the model. Groups of TG curves computed for all temperature coefficients defined the predicted kinetic curve ranges to which the true hypothermic thrombin curves were expected to belong. Based on this approach, the authors showed that hypothermia in the temperature interval of 31° to 36°C slowed down TG, decreased maximal slope, increased area under curve and almost did not affect the peak height. Thus, authors demonstrated that simulation of TG can predict coagulopathy in human population induced by hypothermia or blood dilution.

These studies show how mathematical modelling can move from very academic, scientific research towards the in-patient study, from simulating coagulation in general to considering personal peculiarities. The review [77] asks the question of the feasibility of modelling in general medical practice, and we can say that if not now yet, but in very close future, when more mechanisms of blood coagulation regulation become clear, modelling will have to face the interpersonal variability to predict the patient's status, and there is all reason to think that it can overwhelm all difficulties, and become a valuable tool for clinical routine. There are several ways to do this even when not everything is known: although not strictly a coagulation model example, one can remember prediction of patient platelet-dependent haemostatic phenotypes after a neural network model was trained on each donor's pairwise agonist scanning experiment [78].

Conclusions

Mathematical modelling can contribute in such fields of applied blood coagulation research as development of drugs for orphan diseases, when a large-scale clinical trial is not possible either owing to low amount of patients or owing to ethical concerns when you cannot treat the control group with a drug. The same issue is valid for developing dosing strategies. Either dangerously inefficient (too low) or dangerously efficient (too high) doses of the drug can be excluded from the *in vivo* trial based on the results of the mathematical simulation.

It is interesting that almost all contemporary stories of successful use of mathematical modelling to retrieve practically relevant results in terms of drug discovery, research or diagnostics employed rather simple experimental models of blood coagulation *in vitro* as bases for their modelling. In other words, there are 20 computer models of TG per 1 model of thrombus formation in flow (although such models are abundant in the basic research papers and although it might seem natural to look for a drug mechanism of action using a more physiological model). Probably, this means that a model can provide some readily interpretable data only if we understand the system we are modelling, otherwise the noise of uncertainty would cover all possibly fruitful results. After all, there should be a reason that experimental people also widely use simple *in vitro* approaches for drug discovery and research, and only rarely (usually as a final confirmation) use complex, expensive, poorly reproducible and poorly interpretable *in vivo* methods.

We hope that further investigations of blood coagulation system, its mechanisms, feedback, effects of physical parameters like spatial distribution of reacting proteins, diffusion and flow-induced convection, all that will improve our understanding and will lead to development of better models, more

complex and more correct. Development of new clinical grade assays that are able to provide relevant scope of the current haemostatic state of a patient would be also greatly important, as modelling such assays is still far simpler than modelling *in vivo* processes. Until then, it would be difficult to create a drug that would inhibit thrombosis without affecting haemostasis using the models that have been used so far, because they do not simulate thrombosis and haemostasis, but just TG.

Key Points

- Computational modelling of blood coagulation network becomes increasingly used for drug development, therapy planning and even diagnostics.
- Modelling is particularly advantageous for drugs with complex mechanisms and when thorough human/animal studies are impossible.
- Although there are many complex models describing thrombus formation *in vivo*, almost all pharmacological and diagnostical studies use simple and reliable models of *in vitro* coagulation.
- Overcoming this limitation (by getting better knowledge of the haemostasis system and better computational capabilities) is the key to a new step in the field.

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