Thrombosis Research 135 (2015) 423–433

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Review Article

Spatial aspects of blood coagulation: Two decades of research on the self-sustained traveling wave of thrombin

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ARTICLE INFO

Article history:
Received 4 October 2014
Received in revised form 10 December 2014
Accepted 13 December 2014
Available online 19 December 2014

Keywords:
Blood coagulation
Spatial thrombus formation
Thrombin travelling waves

ABSTRACT

In a number of experimental studies, it has been demonstrated that the forefront of blood coagulation can propagate in the manner of a signal relay. These data strongly support the concept that the formation of a blood clot is governed by a self-sustained traveling wave of thrombin. The present review critically appraises the experimental data obtained in recent decades concerning the self-sustained spatial propagation of thrombin. Open questions regarding the experimental detection of the self-sustained propagation of thrombin are discussed.

Contents

Introduction

It is well-known that blood under certain conditions can change its aggregative state. This particular property of blood normally prevents excessive bleeding in case of injury [1,2]. An unfading interest in the issues of blood coagulation is largely accounted for by the fact that disorders in the regulation of the blood aggregative state cause a range of severe pathologies that pose a threat to human health and life [3,4]. These include not only bleeding disorders but also a number

Abbreviations: PFP, platelet-free plasma; PPP, platelet-poor plasma; PRP, platelet-rich plasma; UFP, ultra-free plasma; TAFI, thrombin-activatable fibrinolysis inhibitor; TF, tissue factor

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http://dx.doi.org/10.1016/j.thromres.2014.12.014
0049-3848/

of thrombotic diseases such as myocardial infarction, stroke, pulmonary embolism, disseminated intravascular coagulation, etc. [5–7].

Coagulation mechanisms responsible for the velocity of thrombus formation sufficient for the termination of bleeding are a matter of great interest. These mechanisms appear all the more vital because the rapidity of the blood coagulation processes determines not only the efficiency of bleeding termination but also the precipitated threat with which some of thrombotic complications may emerge [8,9].

The most significant step in the understanding of the kinetic aspects of blood coagulation was the suggestion that the network of enzymatic reactions participating in the generation of thrombin acts as a type of biochemical amplifier [10,11]. This network of reactions is now known as the coagulation cascade, and its major components and reactions have been thoroughly studied [1,12]. The network of reactions leading to the generation of thrombin is traditionally divided into the so-called intrinsic and extrinsic pathways [12,13]. The former is presumed to be initiated by a negatively charged surface, whereas the latter is activated by the exposure of tissue factor on phospholipid membranes. The diagram illustrating the current view of the network of biochemical reactions that participate in thrombin generation is shown in Fig. 1.

It is remarkable that this reaction network contains a few positive feedback loops that provide up to 10^5 – 10^7-fold catalytic amplification of the generation of thrombin as soon as even a small amount of thrombin appears in the blood [10,14]. Such autocatalytic amplification provides explosive (exponential) thrombin generation in response to a single stimulus [15].

It is noteworthy that this explosive generation of thrombin only takes place in response to a stimulation exceeding a certain threshold value [16,17]. Therefore, the blood coagulation system can be considered to be a trigger-type kinetic system acting in accordance with the "all-or-nothing" principle. The rapidity of its trigger is determined by a powerful autocatalytic biochemical cascade mechanism that provides explosive self-accelerating generation of thrombin.

Spatial Aspects of Blood Coagulation

During the 1960–80s, numerous details about the *temporal* aspects of the physiological and biochemical mechanisms regulating the development of the coagulation processes were discovered [12,18–20]. Nonetheless, in the early 1990s, it was still unclear how sufficient rapidity of the *spatial* formation of the thrombus could be achieved. This issue was particularly unclear in the case of non-homogeneous initial distribution of the coagulation activator throughout the blood. For instance, consider the case of an injury to the internal surface of a vessel wall where the stimulation of the coagulation occurs only at the periphery of the blood volume.

At first, it may seem that the mere diffusion of coagulation factors from the site of injury to the inner areas of the blood volume can account for the spatial formation of the clot. However, basic estimates show that even in the case of explosive generation of coagulation factors at the border of the blood volume, the diffusional mass-transfer of the factors deep into the vessel can hardly ever provide a rate of clot growth sufficient for the formation of a macroscopic thrombus comparable with the size of the vessel lumen [21,22]. However, it is known that the formation of macroscopic occlusive thrombi blocking the vessel lumen entirely is not uncommon in clinical practice [23,24].

Thus, although the kinetic mechanisms providing the *temporal* rapidity of blood coagulation have already been researched extensively, in the early 1990s, the mechanisms that provide sufficient *spatial* rapidity of formation of blood clots remained poorly understood. Two conceptual frameworks soon appeared that suggested possible

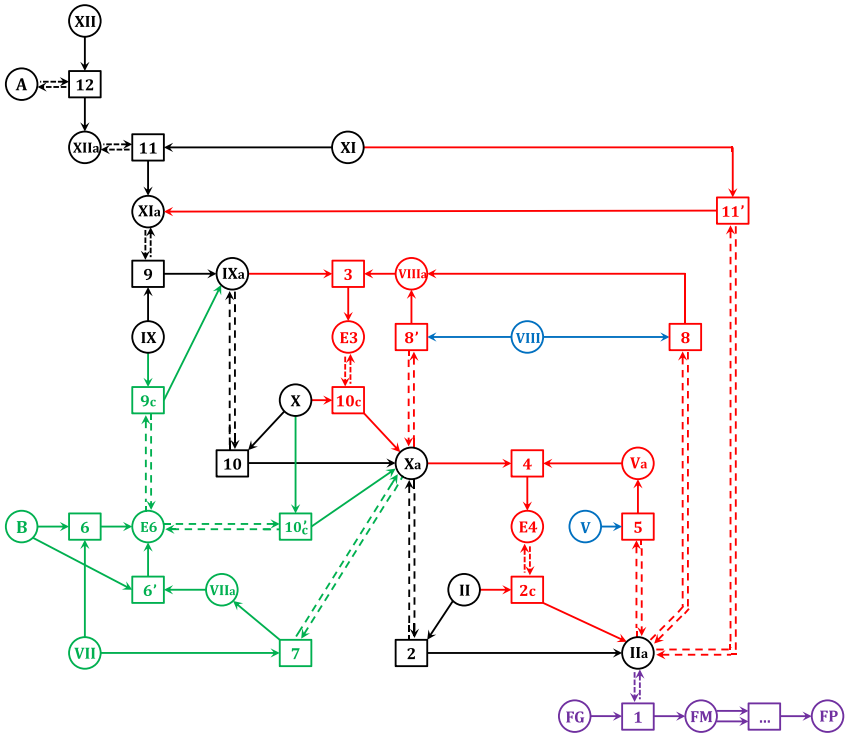

Fig. 1. Simplified diagram of the blood coagulation cascade. The circles represent the coagulation factors and the rectangles the biochemical reactions. Solid arrows from a factor to a reaction indicate participation in the reaction, and arrows from a reaction to a factor signify that the factor is a product of the reaction. Dotted arrows represent the catalytic activity of the substance. The number given in each rectangle corresponds to the number of the product formed in the course of the reaction. If some substance can be formed as a result of several different reactions apostrophes and subscripts are used to distinguish these reactions. Subscript 'c' is used to denote the reactions catalyzed by enzymatic complexes. 'E3' represents the enzymatic complex of the active forms of factors IXa and VIIIa, 'E4' the complex of factors Xa and Va, and 'E6' the complex of tissue factors and factor VIIa. 'A' and 'B' represent the initiating events of the intrinsic and extrinsic pathways, respectively. Other terms used are: II, prothrombin; IIa, thrombin; FG, fibrinogen; FM, fibrin monomer; FP, fibrin polymer.

explanations for the mechanisms of spatial propagation of coagulation far from the initial activator surface.

One framework was the concept of blood-borne tissue factor (TF), developed by Y. Nemerson and co-authors [25–27]. It suggested that a certain amount of an initial coagulation activator can exist in the blood itself. According to the current views, such blood-borne TF may be present in the blood both on the surface of microparticles released by different cell types, and in a free soluble form [28].

The second concept that appeared in the early 1990s was developed on the basis of the approaches suggested earlier for the description of self-sustained traveling waves in various active media [29–32]. According to this concept, the concentration fronts of thrombin could emerge and propagate steadily in the blood as a result of the interplay of thrombin autocatalytic generation and spatial diffusion processes. In other words, in the framework of this concept, the spatial propagation of thrombin generation could take place in accordance with the relay mechanism [21,33–35].

Basic Notions of the Active Media Theory

The following section is intended to provide a comprehensive description of major aspects of the active media theory, essential for the critical interpretation of the experimental results discussed in this review.

Before the mechanisms of spatial coupling of the reaction-diffusion processes in blood coagulation became the subject of research, considerable progress had been achieved in the study of different reaction-diffusion systems of various natures [29–32]. In fact, starting from the well-known work by Luther [36], the propagation of various concentration fronts over large distances in reaction-diffusion systems kept attracting scientific attention. The interest in the study of signal relay transmission over large distances increased significantly after the publication of the work by Zel'dovich and Frank-Kamenetskii on the propagation of slow flames [37]. The authors showed that combustion fronts are able to propagate at a constant stationary speed indefinitely far from the site of their initiation in reaction-diffusion systems containing autocatalytic reactions capable of accelerating their own progression. The value of that stationary speed turned out to be proportional to the square root of the product of the diffusion coefficient multiplied by a value representing the autocatalytic generation of the key reagent [38].

If we leave aside the chemical peculiarities of the flame propagation and combustion theory, shifting to an analogy with flames spreading across the prairies, it turns out that many aspects of flame propagation are quite easy to understand without any mathematical tools. Indeed, the combustion of dry grass at a certain location implies a local increase of its temperature above some threshold level. The value of that level, the "combustion threshold" generally speaking, should depend on grass dampness and some other properties [39]. After the fire has ignited, heat will begin to spread to the areas adjacent to the site of origin. That will lead to the increase of temperature in those areas, and as soon as it exceeds the *combustion threshold*, a local combustion will take place in the adjacent area. Thus, the flame front will begin to move, travelling from one part of the prairie to another. It is essential to stress that the propagation of flames across the prairie will be determined by the properties of the grass itself (its dryness, density, etc.) and *not* by the manner of its initial ignition. No matter how the fire was started, whether by matches, lightning or tinderbox, the propagation of fire over the prairie away from the initial combustion point will spread in a similar way – at the same speed, other factors being equal.

Similar modes of front propagation can occur in a wide variety of biological and chemical systems where a threshold activation of autocatalytic processes is possible. In the contemporary theory of self-organization of non-equilibrium systems, all media in which fronts of triggering from an inactive state to an active one may propagate are generally referred to as an *active media* [38,40]. The spatial propagation of the triggering front in an active media is commonly called a *self-sustained traveling wave* [31]. Sometimes, the term *autowave* is used as well [41].

The concepts that had been initially formulated regarding the problems of flame propagation were later quite effectively transferred to several other branches of science. In particular, these ideas, with minor modifications, formed the basis of the current concepts of propagation of nerve impulse [29,42], excitation waves in cardiac tissue [32] as well as chemical concentration waves in the Belousov-Zhabotinsky reaction-diffusion system [30,31].

Keeping that in mind, the emergence of the idea that the spatial coupling of autocatalytic processes in the kinetics of thrombin generation with diffusional processes may, in principle, lead to the formation of self-sustained travelling waves of thrombin in the former laboratory of A.M. Zhabotinsky is not surprising. The original concept was formulated in reference [21]. Later, a phenomenological model of the propagation of blood coagulation autowaves was constructed [33], and a range of possible patterns of self-sustained waves interplay under nonflow conditions was predicted. Eventually, a number of experimental evidence confirming the basic elements of the theory was obtained [34].[1]

For various reasons, the initial workgroup divided. Since March of 1996, a group led by G. Th. Guria formed a separate scientific laboratory. It should be said that after the studies of 1994-1995 [21,33,34], experimental research on the self-sustained travelling waves of blood coagulation in nonflow systems was not performed in the laboratory. Research was mainly focused on the theoretical and experimental study of the blood coagulation processes under intense flow conditions [43–45]. However, the publications concerning the research of autowave processes taking place in the blood under non-convective conditions remained within our purview.

Below, we offer a detailed overview of experimental works related to the self-sustained spatial propagation of blood coagulation conducted within the last 20 years by various groups of researchers. The aim of this article is to summarize these results and outline the scope of problems that remain unsolved.

A Review of Experimental Research Works

Over the past years, numerous experimental data confirming the existence of self-sustained modes of spatial propagation of blood coagulation were obtained (see, for instance, [35,46–55]). It has been demonstrated that under certain conditions the fronts of blood coagulation can indeed propagate at a stationary speed [35,46,54]. It turned out that the velocity does not depend on the conditions of the initiation of the travelling wave but is completely determined by the inherent properties of blood itself [35,46,49]. The dependence of the velocity of the propagation of the coagulation front on the concentrations of various coagulation factors has been investigated in a number of studies [46–51,56]. The mechanisms of termination of the travelling waves of thrombin remain considerably less studied [51,53,54,56].

The majority of the work on self-sustained travelling waves of coagulation was performed by two research groups. The first one is the group of Prof. Ataullakhanov that after the initial publications [21, 33–35] continued to explore the processes of clot growth in non-convective systems *in vitro* [46–51,56–64]. The second one is the group of Prof. Ismagilov that carried out a series of significant research works using the microfluidics technique [52–54,65–71].

Propagation of Coagulation Fronts at a Constant Speed

The propagation of self-sustained traveling waves in various homogeneous active media progresses at a constant stationary speed that

[1] A major portion of the results in references [21,33,34], initially published in Russian, was later summarized in English (in reverse chronological order) [35].

does not depend on the manner of the wave initiation [31]. As mentioned above, the speed of a front propagation in active media is determined by diffusion and by the rate of generation of an autocatalytic reactant (in the case of blood coagulation, thrombin is such a reactant). Therefore, to establish the existence of self-sustained travelling waves of coagulation, the experimental determination of the propagation speed of the clotting front was the first obvious step.

The propagation speed of coagulation fronts was measured in an experimental system without convective mass-transfer [35]. In that study, the local initiation of coagulation was achieved by placing small glass beads into a thin layer of blood plasma. It was shown that the size of the clot increases linearly with time. The speed of clot growth turned out to be approximately 40-50 μm/min.

To determine the position of the coagulation front, two methods were used: optical detection of fibrin using reflected light and detection of thrombin generation by means of a fluorogenic substrate [35]. It was established that the front of polymerized fibrin moves behind the propagating autowave of thrombin, following the former with a small lag. Moreover, both fronts turned out to be quite steep. While in the case of propagation of coagulation solely by means of diffusion, the border between clotted and liquid blood would have to be not sharp but "diffusionally" fuzzy. This can be considered as additional evidence of the self-sustained propagation of blood coagulation in the system.

More precise measurements of the speed of propagation of coagulation were later performed [46,54]. The speed of propagation of the fibrin polymerization front in healthy donor plasma was measured and found to be 39-43 μm/min at 37 ^{0}C [46]. Subsequently, the speed of the thrombin generation front was measured by means of a fluorogenic substrate [54]. It was found to be 20 μm/min at 25 ^{0}C and 41 μm/min at 37 ^{0}C. Moreover, the data provided [54] showed that the spatial profile of the thrombin generation, moving at a constant speed, retains a practically unchanging form (see Fig. 2).

The fact that, within the same experiment, the speed of propagation of the coagulation front in healthy donor plasma at 37 ^{0}C stays unchanged over time has been confirmed in a number of publications. The value of the speed measured for different plasma samples in different studies varied within the range of 35 to 50 μm/min [46–48,50,51,54,56]. Hence, it would take approximately 20-25 min for a self-sustained wave of coagulation to form a thrombus with a characteristic size of 1 mm. At the same time, the simplest estimations show that passive diffusional spread of thrombin over that distance would take approximately 16 hours [22]. In other words, the rate of formation of a thrombus of 1 mm appears to be 50 times faster in the case of self-sustained spread of blood coagulation as compared to solely diffusional propagation.

It should, however, be kept in mind that self-sustained propagation of blood coagulation can occur only in non-flow conditions or under rather slow blood flow. More specifically, it can occur only when the contribution of diffusion into the mass-transfer processes exceeds the contribution of convection [72,53,54]. Thus, the self-sustained spatial propagation of blood coagulation demonstrated experimentally *in vitro* [34,35,46–55] can possibly occur in its pure form *in vivo* only in a limited range of situations. Apparently, it may play some role in minor vessels (arterioles and venules) with slow blood flow, and also in the case of blood stasis in particular compartments of the blood circulatory system.

Independence of the Front Speed from the Manner of Initiation of Coagulation

As mentioned above, the speed of the self-sustained traveling wave in a wide variety of active media does not depend on the circumstances of its initial activation. In other words, the speed of the autowave is an intrinsic property of any active media. Similarly, the speed of the

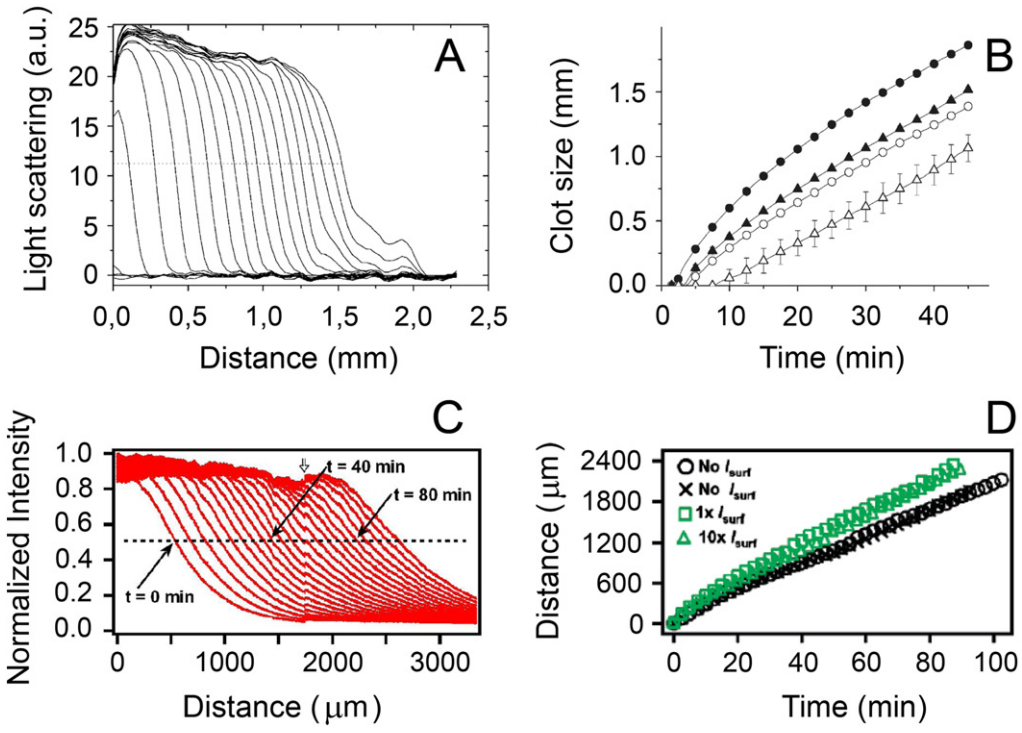

Fig. 2. A – Time-lapse light scattering profiles of clot formation measured at 2 min intervals (reproduced from reference [46]); B – Plots of clot size versus time. The coagulation was initiated using different cell types expressing various levels of TF: (●) – lung fibroblasts, (○) – macrophages, (▲) – smooth muscle cells, (△) – endothelial cells (reproduced from reference [49]); C – Time-lapse fluorescence intensity profiles acquired at 2.5 min intervals for the propagation of clotting in a microfluidic channel in the absence of flow (reproduced from reference [54]); D – Plots of clot size versus time: circles (○) and crosses (×) correspond to the results of the experiments carried out in the absence of TM, squares (□) and triangles (△) in the presence of TM (reproduced from reference [54]).

propagation of fire in the prairie depends on the dryness of grass and its density, but does not depend on the method of ignition.

In early experimental works in 1994 and 1998, coagulation was initially activated by means of either small glass beads, collagen fibers or thrombin powder [34,35]. It was stated that the process of clot formation developed almost identically in all three cases – the speed of clot growth did not depend on the form and size of the activator. Unfortunately, quantitative data about direct measurements of the speed of the coagulation fronts related to the activator type were not provided in those papers.

The independence of the speed of the coagulation front from the initial conditions was investigated most thoroughly in reference [46].[2] In this work, the speed of clot growth in the blood plasma of healthy donors and patients with hemophilia A and B was studied. The coagulation processes were measured optically in a thin (1 mm) layer of blood plasma. Coagulation was initiated on one side of the experimental system using a flat plate covered with different types of coagulation activators (glass, polyethylene terephthalate film or fibroblasts cultured on a polyethylene terephthalate film). In the experiments with healthy donor plasma, no significant difference in the speed of propagation of the coagulation fronts was observed in cases of activation by different surface activators.

It was also shown that varying the density of the fibroblast layer on the activating surface does not lead to changes in the speed of the coagulation front. The authors state that the amount of fibroblasts only affects the lag time period necessary for the primary initiation of coagulation along the activator surface, while further spatial propagation develops at an unchanged speed. In other words, the results [46] show that the speed of the travelling wave of coagulation in normal plasma does not depend on the activation method. This is consistent with the concept of self-sustained mechanism of spatial propagation of blood coagulation [21,35].

Data supporting the independence of the speed of coagulation wave from the surface activation intensity were also presented [49], where the coagulation was initiated by different cell types expressing various levels of TF (lung fibroblasts, smooth muscle cells, macrophages, endothelial cells). The experimental results in this study show that at least for fibroblasts, smooth muscle cells and macrophages, the speed of fibrin clot growth was practically the same, namely, within a 35–39 μm/min interval (see Fig. 2B).

Despite the fact that neither the methods nor the results provided in [46] have ever been doubted by anyone, their present scientific status remains not quite clear. The fact is, that a few papers have recently appeared where the speed of clot growth is considered to be a value dependent on the intensity of surface activation [58,59]. Particularly, in these experimental studies, it is stated that the rate of clot growth *in healthy donor plasma* depends considerably on the concentration of immobilized TF on the surface of the activator film.[3] These data come into direct contradiction both with the experimental results obtained in the same laboratory earlier [46,47], and the very concept of self-sustained traveling waves of blood coagulation [21,35] (see for more details the section "Some contradictions in the results of recent studies").

Clot Growth in the Plasma of Patients with Hemophilia A and B

It was experimentally shown that the propagation of coagulation in hemophiliac plasma is 4-5 times slower than in the plasma of healthy donors [46]. Additionally, steep coagulation fronts were not observed in the plasma of patients with hemophilia A and B, and the borders of clots during the growth process remained poorly circumscribed. Moreover, the relation between the polymerization front coordinate[4] and time for hemophiliac plasma presented in reference [46] appears not to be direct but rather a square root function. The authors mentioned that the addition of 10% of normal plasma to correct the factor deficiency in hemophiliac plasma led to the normalization of clot growth [46].

These data, in the authors' opinion,[5] indicate that in cases of hemophilia A and B, disruptions in the positive feedback loops in the blood coagulation cascade prevent the appearance of self-sustained travelling waves. In other words, in those cases the spread of coagulation occurs primarily through the passive diffusion of key reactants. No catalytic amplification of the generation of thrombin occurs in the course of its spatial propagation. From the medical point of view, this may indicate that the bleeding observed in cases of hemophilia A and B may be caused not only by the deterioration of the resistance of formed clots to fibrinolysis (for instance, due to insufficient activation of TAFI [73]). Another possible cause of bleeding may be the malfunction of the mechanisms of spatial clot formation, and, as a result, the formation of insufficiently dense clots within a reasonable time period.

This appears to be one of the most remarkable results since the autowave concept was suggested and subjected to primary analysis in the initial series of articles [21,33,34].

Influence of the Concentration of Various Coagulation Factors on the Spatial Propagation of Clotting

It should be noted that although the stationary speed of propagation of the autowave is practically independent of the manner of its initiation, it can depend considerably on the parameters of the medium in which the wave is propagating. In the case of self-sustained travelling wave of thrombin, the essential parameters could be the concentrations of non-activated coagulation factors, concentrations of cellular elements (such as platelets), phospholipid content of plasma, temperature, etc. Thus, all the parameters determining the rapidity of the autocatalytic generation of thrombin and its diffusion in the system can directly affect the speed of the coagulation front as well.

Data on the influence of the deficiency of various coagulation factors on spatial clot growth have been obtained in a number of works [46–51,56]. For instance, experimental dependencies of the rate of clot growth on the concentration of factors VIII and IX can be found in references [48,49] and [50], respectively. According to these data, it can be stated that the decrease in the concentration of coagulation factors leads to a reduction of the rate of clot growth. However, a more detailed interpretation of these experimental dependencies appears in some way problematic. According to the opinion presented [46,47][6], at low concentration of coagulation factors the self-sustained clot growth becomes impossible, and the spatial spread of coagulation is governed solely by diffusion. Therefore, the spatial formation of clot in these cases does not proceed at a constant speed, but with a considerable deceleration. Under these conditions, it appears incorrect to characterize clot growth in terms of "velocity" because that usage implies that the speed was constant during the entire process of clot formation. Using the term "velocity" when no self-sustained propagation is present not only leads to ambiguity in data interpretation, but may also cause delusions regarding the very nature of the observed modes of spatial propagation of coagulation.

In this regard, the values of the speed of clot growth at low concentration obtained in [48–50,56], might be considered to be somehow averaged values. However, during what period of time that averaging

[2] These results are detailed in the Ph.D. thesis of M.V. Ovanesov – "The influence of the factors of the intrinsic pathway of blood coagulation on the spatial dynamics of clot growth" [47] (in Russian).

[3] «Clot growth rate decreased ~2-fold for a TF density decrease from 107 to 5 pmol/m^2», p. 1818 [58]. In another publication, the change of "stationary speed" was 5-fold, from 5 to 25 μm/min; see Fig. 2C in [59].

[4] The forefront coordinate was defined as the point where the backscattering of light reaches ½ of its maximum.

[5] «According to our hypothesis, a deficiency in any of the factors involved in the feedback loops (such as factors VIII and IX) disrupts the self-sustained thrombin propagation. In hemophilic plasma, thrombin is produced only at the activator surface and spreads out solely by diffusion», p. 55 in [46].

was performed by the authors remains unclear. Consequently, it appears more accurate to quantify clot growth in cases with a small concentration of coagulation factors according to the size of the clotted area measured after a pre-defined time period. Unfortunately, in this way the experimental data were presented only in reference [50], where a dependence of the clot size (measured 60 minutes after the coagulation initiation) on the concentration of factor IX is given.

The Role of Factor XI in the Self-sustained Propagation of Coagulation Fronts

It is well-known that the decrease of the concentration of factor VIII or IX in blood takes place in cases of hemophilia A and B, respectively. In both cases, the positive feedback of the coagulation cascade involving factor VIII is suppressed. This leads to insufficient thrombin generation and, as a result, to increased bleeding risk, which may correlate with the impossibility of the initiation of the coagulation autowave [46].

In the case of a more rare genetic disease, hemophilia C, there is a deficiency of factor XI [74]. Factor XI, similarly to factors VIII and IX, participates in the positive feedback loops that accelerate the generation of thrombin (see Fig. 1). Recent research studies have shown that the role of factor XIa in the regulation of blood coagulation may not be limited to the generally known activation of factor IX. In the absence of factor IX, factor XIa may also contribute to the activation of factors V and X [75,76].

The positive feedback loop involving factor XI has one distinctive characteristic significant for the spatial propagation of coagulation over considerable distances. The intensification of the generation of thrombin through this loop does not require the prerequisite presence of the active forms of other coagulation factors, except thrombin itself. The autocatalytic loops involving the activation of factors V or VIII by thrombin do not have that characteristic because their function requires the presence of FXa or FIXa, respectively (see Fig. 1). Thus, due to the loop involving factor XI, the relay propagation of thrombin becomes, in principle, possible regardless of the presence (or absence) of the active forms of other coagulation factors.

Theoretical considerations on the importance of factor XI in the process of spatial formation of blood clots have been repeatedly expressed in a number of works [21,35,46,51,77,78]. However, the experimental results directly confirming these ideas have been obtained only recently [51]. In that paper, it was shown that in the case of a decrease in FXI concentration below a critical value, self-sustained travelling waves of coagulation do not develop in the experimental system.[6]

In that context, however, an obvious ambiguity exists in results obtained in an earlier publication by the same group [49], where it was shown that the rate of clot growth was «only slightly reduced in FXI-deficient plasma compared to normal plasma».[7] Thus, for the present, the experimental data available on the influence of factor XI on the propagation of coagulation under non-convective conditions remain somewhat contradictory. Arriving at a final conclusion on the subject is also impeded by the fact that in [49], the percentage of FXI in the deficient plasma used in the experiments is not reported.

Influence of Platelets on the Self-sustained Spatial Propagation of Coagulation

In addition to the concentration of various coagulation factors, the speed and initiation conditions of self-sustained travelling waves of thrombin may, potentially, be affected by formed elements of blood, particularly by platelets. The results indicating that the coagulation front propagation speed is essentially identical for platelet-poor plasma (PPP) and platelet-rich plasma (PRP) were obtained in reference [35]. A somewhat slower propagation of coagulation was observed for platelet-free plasma (PFP). The authors concluded that self-sustained propagation of clotting *in vitro* is essentially independent of the platelet count.

The dependence of clot growth rate on the concentration of activated platelets added to ultra-free plasma (UFP)[8] was obtained in a later study [57]. The experimental curve reaches saturation at high platelet counts. The rate of clot growth, at platelet concentrations over 1000 platelets/μl, was weakly dependent on the platelet count and remained within the normal range of 40-50 μm/min. At the concentration of activated platelets below 500 – 1000 platelets/μl, the rate of clot growth measured by the authors decreased significantly.

Thus, the results of both papers [35] and [57] indicate that within the normal physiological range of platelets concentrations ($1.5 \cdot 10^5$ – $4 \cdot 10^5$ platelets/μl [1]) the speed of propagation of self-sustained coagulation wave should be essentially independent of the platelet count. However, complete elimination of platelets from the system, which takes place during the preparation of UFP, leads to a slowdown of the spatial propagation of the coagulation fronts several folds [57].

The nature of this phenomenon is not entirely clear. According to current views, platelets play a principal role in the catalysis of the generation of thrombin through the coagulation factors VIII and V, as their surface is essential for the formation of active complexes of FIXa-FVIIIa and FXa-FVa [79,80]. Presumably, the results presented above demonstrate that even a very small amount of platelets is sufficient to provide the necessary catalytic surface for the amplification of the generation of thrombin needed for the self-sustained spatial propagation of blood coagulation.

Studies of Self-sustained Coagulation Waves in Whole Blood

To date, among the research studies on self-sustained travelling waves in blood coagulation, only two papers [35,52] present some information about the spatial dynamics of coagulation not only in blood plasma but also in whole blood. This situation could be partially accounted for by the fact that the optical detection of the fibrin polymerization front is much easier in transparent blood plasma than in whole blood, which scatters light considerably.

As an alternative to the optical detection of fibrin polymerization fronts using reflected light, fluorescent methods for the visualization of the spatial propagation of thrombin can be used [35,47,51–54]. Unfortunately, only in reference [35] these methods were used in experiments not only with plasma but also with whole blood. In both cases, self-sustained travelling waves of coagulation detected in the experimental system propagated at a constant speed. The authors stated that «removal of red and white blood cells did not influence significantly the clot growth characteristics». However, reference [35] offers no comparison of the values obtained in the experiments with blood plasma and whole blood of the same donor.

Other than reference [35], the detection of self-sustained spatial propagation modes of coagulation in whole blood is mentioned only as preliminary results in reference [52]. No other data on any properties of that type of modes is presented in that paper.

This summary indicates that even after 20 years of research, the accumulated experimental data concerning the self-sustained spatial propagation of coagulation in whole blood remains quite scarce. It appears that the self-sustained travelling waves in whole blood demand further and more thorough experimental study.

[6] «To test this hypothesis experimentally, we performed studies with factor XI-deficient plasma from a hemophilia C patient (Fig. 3B). A traveling wave did not form in this plasma, but was generated when a normal concentration of factor XI was added», p. 2235 in [51].

[7] «The clot growth rate [was reduced by 7–10-fold in FVII- and FX-deficient plasmas, by 2-fold in FVIII-deficient plasma and] was only slightly reduced in FXI-deficient plasma compared to normal plasma (Fig. 4c)», p. 325 in [49].

[8] UFP was prepared by additional ultra-centrifugation of PFP at 100,000 g for 1 hour at 21 °C.

Some Contradictions in the Results of Recent Studies

Summing up all data gathered in previous sections, it can be positively stated that over the last 20 years, the concept of self-sustained traveling waves of blood coagulation has been supported by numerous evidence [35,46–55]. A few general properties characteristic of autowaves in active media were demonstrated for the propagation of coagulation fronts. It was established that under non-convective conditions, coagulation can propagate in a self-sustained manner, moving at a constant stationary speed [35,46,47,54] independent of the manner of initiation of clotting [46,47,49].

However, the conditions under which self-sustained spatial propagation of coagulation can be observed in plasma or whole blood remains not well-defined. For instance, several recent publications present experimental data which come into direct contradiction with the very concept of self-sustained travelling waves of thrombin. In particular, it was stated [58,59] that the rate of spatial clot growth depends on the density of TF immobilized on the activating surface. In other words, in these papers the speed of propagation of the coagulation fronts *in healthy donor plasma* is presented as if it depended on the intensity of the activation on the clotting initiation surface.[9] It should be stressed that these data not only contradict the results previously obtained in the same laboratory [46,47,49], but are also inconsistent with the self-sustained travelling wave concept [21,35].

The situation becomes even more controversial after the publication [51] by the same research group the very next year, containing a detailed summary of the general ideas of the concept of thrombin travelling waves. Moreover, data [51] confirmed the independence of the speed of propagation of the coagulation front from the density of TF immobilized on the activator surface.[10] Actually, even the ranges of values of surface density of TF in [58] and [51] practically coincide, being 5 – 107 pmol/m^2 and 4 – 90 pmol/m^2, respectively.

In this situation, one cannot help being surprised at the absence of any discussion of such an obvious inconsistency of the results obtained by the same research group. There is no discussion of possible explanations for these contradictions either in references [51,58,59], or in subsequent papers by the same group of authors [60–64].

Irrespective of possible explanations, this inconsistency of results implies that the experimental technique used by the authors for a considerable period of time still remains incompletely standardized. It could be assumed that the divergence of results may be caused by differences in the experimental settings used in [58,59] and [51]. For instance, in reference [51] it is stated that the self-sustained coagulation waves did not propagate steadily in platelet-free plasma (PFP) unless supplemented with additional phospholipids. At the same time, the measurements of the speed of the coagulation fronts at various values of surface density of TF reported in references [58,59] were carried out in PFP without added phospholipids. In light of this, the very detection of self-sustained spatial propagation of coagulation in [58,59] becomes questionable.

It also remains unclear whether the self-sustained propagation of coagulation really occurred in the subsequent series of works by the same group [60,61,63,64], where the authors attempted to verify the medical applicability of a test based on the *in vitro* detection of spatial clot growth. Yet, the references [61–64] also lack any mention of the addition of phospholipids to PFP.

Data of the experimental measurements only strengthen the doubts of whether the results of [58–64] can be appropriately interpreted within the framework of the concept of self-sustained travelling wave of thrombin. For instance, the relation curves of the clot size and time obtained in [58,59,61–64] show that the propagation of the coagulation front in those studies does not proceed in a stationary manner, but with a considerable slowdown (see Fig. 3). In other words, the experimental data directly indicate that the speed of propagation of the coagulation front in these works was not constant, but decreased as the front moves away from the activator surface.

The non-stationary speed of the coagulation fronts in papers [59–64] is also indicated by the fact that the authors themselves distinguish two different characteristic "velocities" of clot growth: stationary and initial. These parameters are defined as average speed values of the coagulation front over certain periods of time, chosen arbitrarily by the authors.[11] That type of quantification of clot growth rate appears rather far-fetched. Three or four averaging intervals might have been identified instead of two just as well. It should also be noted that the term "stationary", used by the authors for a speed determined during the second time period, appears absolutely incorrect, particularly, in those cases when the clot growth proceeds with an obvious slowdown.

Thus, a careful analysis of the recent experimental papers [58–64] provides us with sound reasons to doubt whether the self-sustained spatial propagation of coagulation was actually observed in those studies. As a result, the relevance of these experimental data to the characteristics of clot growth under physiological conditions is also in doubt. This point appears all the more essential because in papers [60,61,63,64] a technique based on *in vitro* measurements of spatial clot growth in blood plasma was suggested as a novel hemostasis assay.

On the Mechanisms Restraining Clot Growth

In addition to providing thrombus formation speed sufficient for timely stoppage of bleeding, the normal function of the hemostasis system implies the formation of thrombi compactly localized at sites of vascular injury. Therefore, one may consider that the concept of self-sustained propagation of coagulation is in some way self-contradictory. It may appear that the relay propagation of coagulation in blood, once initiated, could spread over the entire volume of the circulatory system. However, according to the present views, the coagulation processes in humans are mostly confined and do not lead to a total thrombosis of all vessels.

It should be noted that a similar paradox emerges from the idea that coagulation can be initiated and propagated without contact of the blood to the extravascular space proposed by Y. Nemersen with the concept of blood-borne TF [81]. These paradoxes apparently result from the inadequacy of our current knowledge of the mechanisms limiting the thrombus growth. Although a number of physiological mechanisms of coagulation inhibition are known at the present [82–85], the processes that might determine the eventual size of the clot are not completely understood and still remain the subject of active research [86–89].

In the context of the concept of self-sustained propagation of blood coagulation, the possible mechanism of limitation of spatial clot growth was discussed already [21]. In that paper, the existence of a coagulation inhibitor capable of self-sustained spatial propagation in blood was hypothesized. The authors presumed that an autowave of such an inhibitor, being initiated behind the front of the thrombin autowave, might catch up with the latter and lead to the termination of further clot growth. Two necessary conditions should be satisfied for the emergence of such a travelling wave of inhibitor. First, the inhibitor molecules must be generated as a result of blood coagulation processes. And, second, autocatalytic generation of such an inhibitor must be feasible.

It was suggested that protein C might be such an inhibitor [21]. It is well-known that protein C can be converted by thrombin into an active form. That, in turn, is capable of weakening the positive feedback loops involving factors VIII and V, thereby significantly inhibiting thrombin

[9] «The clot growth rate decreased with a decrease in TF density (Fig. 3 B). Clot growth rate decreased ~2-fold for a TF density decrease from 107 to 5 pmol/m^2», p. 1818 in [58]. In the second paper, the change of "stationary velocity" was 5-fold, from 5 to 25 μm/min; see Fig. 2C in [59].

[10] «waves obtained by using 90 pmol/m^2 or 4 pmol/m^2 of TF were very similar», p. 2235 in [51].

[11] In [59–62], the time interval of 0-10 min was chosen for $V_{initial}$, and 10-40 min for V_{st}. In [63], the interval of 2-6 min was chosen for $V_{initial}$ and 15-25 min for V_{st}.

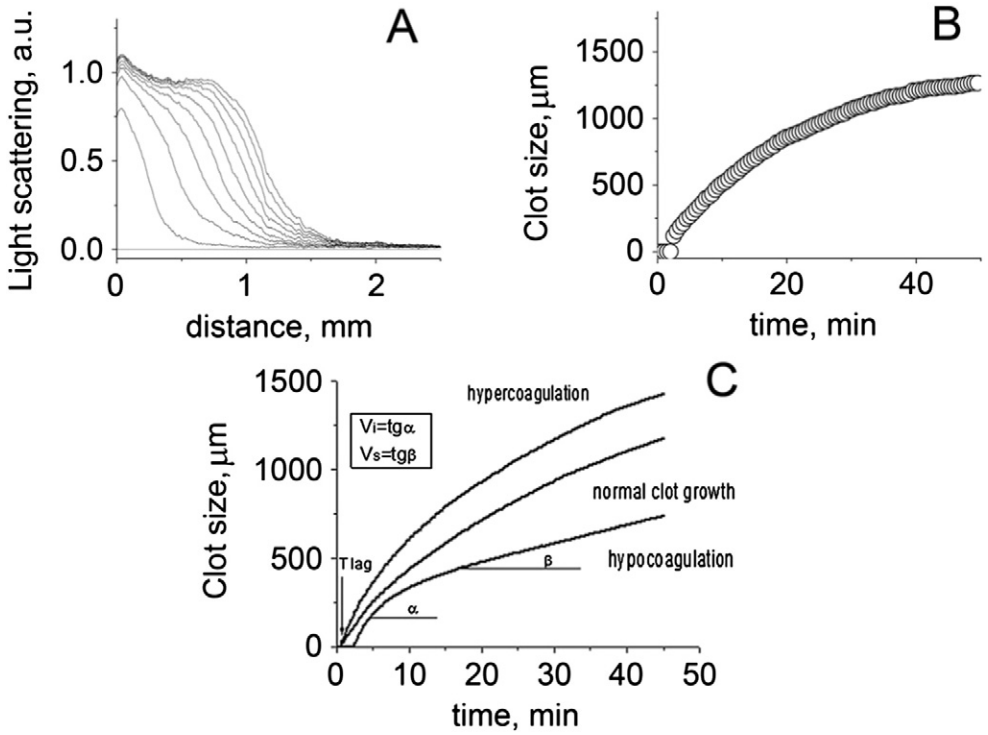

Fig. 3. A, B – Light scattering profiles of a growing clot measured at 5 min intervals and the corresponding plot of the clot size versus time reproduced from reference [58]. It can be seen that the clot growth goes on with an obvious slowdown. C – Plot of clot size versus time reproduced from reference [64]. Two characteristic "velocities" of clot growth defined by the authors of references [59–64] are presented: Vi – "initial" clot growth velocity and Vs – "stationary" clot growth velocity. Authors supposed that the values of "initial" and "stationary" clot growth velocity are mathematically equal to the tangents of the two different angles (α and β shown in the picture).

generation [90]. It should be noted, however, that any data on the possible autocatalytic generation of protein C (or any other coagulation inhibitor) are still lacking. Therefore, in contrast to the situation with thrombin autowaves, the hypothesis of the existence of inhibitor autowaves has no firm biochemical grounds.

In spite of that, a phenomenological mathematical model was formulated that described the interaction of two autowaves – an activator autowave and an inhibitor autowave [33]. It should be stressed that, being phenomenological, that model was not based on particular values of chemical rate constants, but nevertheless facilitated qualitative research of the dynamics of the system behavior at various values of its characteristic parameters. The analysis showed that within a wide range of parameters, depending on the value of the activation threshold of thrombin generation, three different patterns could occur [33]:

- stationary continuous propagation of thrombin autowave not limited in space by kinetic conditions;
- formation of a localized clot as a result of the stoppage of thrombin autowave at some distance from the initiation surface;
- formation of so-called stratified structures – alternating clotted and non-clotted areas (the result of a type of "leap-frog" interaction of two autowaves with a recurrent acceleration and amplification of the incompletely quenched thrombin autowave).

Patterns qualitatively similar to all the three patterns predicted theoretically in reference [33] were detected in subsequent experimental works of the same period [34,35]. For the adjustment of the coagulation activation threshold in these experiments, the concentration of calcium ions in blood plasma was varied (in accordance with [91]). It should also be noted that the calcium ion concentration modulates not only a number of reactions leading to the generation of thrombin but also the activation of protein C [90,92].

Unfortunately, for various reasons, the mechanisms of clot growth restraint remained outside the focus of most of subsequent experimental works. It should also be mentioned that in most of the subsequent experimental papers, there are no data on the coagulation activation threshold being intentionally adjusted. The recalcification of plasma usually was performed by the addition of a fixed amount of calcium chloride solution to the blood plasma [46–51,56–64].

Thus, to this day, we cannot state with complete certainty that all patterns observed in studies [34,35] really emerge as a result of the interactions of two autowaves, and not due to some artifactual effects.

Further Experimental Research on the Mechanisms of Clot Growth Restraining

Since the publication of [34,35], only in several experimental works any considerable attention has been paid to the question of the restraining of the self-sustained propagation of coagulation fronts [51, 56,53,54].

An attempt to explain the stoppage of coagulation fronts by the action of activated protein C was made [51,56]. A cessation of clot growth was detected in plasma in the presence of various concentrations of thrombomodulin, which is known to be a co-factor in the process of protein C activation by thrombin [90]. Stoppage of the coagulation front occurred at concentrations of thrombomodulin above or equal to 1–3 nM. It was found that the final size of a formed clot decreased as the concentration of thrombomodulin in plasma increased.

It should be noted, however, that in [51,56] thrombomodulin was homogeneously distributed within the entire blood volume. Generally speaking, this situation is inconsistent with physiological conditions because thrombomodulin is actually a membrane protein. In contrast, in the research [54] carried out by the group of R.F. Ismagilov, the detection of coagulation fronts was performed in artificial capillaries with thrombomodulin immobilized on the inner surface of their walls.

Those experiments demonstrated a significant slowdown in the front propagation in small capillaries (volume-to-surface ratio ~5 μm) and almost complete absence of any effect in larger ones (volume-to-surface ratio ~50 μm). Thus, based on the results presented in [54], it can be stated that already in the vessels with a diameter over 0.2 mm, the stoppage of coagulation fronts cannot be explained solely by the activation of protein C on the intact endothelium.

The possible influence of blood flow on the spatial localization of coagulation was investigated [53,54]. In these papers, it was very elegantly demonstrated on a microfluidic model that in the case of propagation of the coagulation front from a vessel without flow into the vessel with blood flow, the shear rate in the area of vessel junction may limit further propagation of coagulation. An increase in the shear rate above a certain threshold value (90 s^{-1} in [53]) prevented further propagation of the coagulation front into the vessel with flow.

Thus, based on the results of the research by R.F. Ismagilov's group it can be stated that in relatively small vessels thrombomodulin can contribute significantly to the process of restraining clot growth. On the other hand, in larger vessels the termination of clot growth is apparently the result of an interaction of a more complex combination of factors, including convective mass-transfer. Nevertheless, it should be noted that although microfluidic techniques allowed rather precise control of the experimental conditions, the latter were still quite remote from the physiological ones. Thus, it becomes apparent that our understanding of the mechanisms restraining the growth of real thrombi could be improved greatly by means of novel *in vivo* optical imaging techniques. For instance, most recent *in vivo* experiments have demonstrated that microfiltration and diffusion of blood components through the platelet aggregates are essential for the macroscopic clot formation [93,94].

Conclusion

In the present paper, we have provided an overview of the experimental results directly related to the self-sustained spatial propagation of blood coagulation. In this overview, we focused primarily on the analysis of experimental data, and avoid discussing the issues of computational modeling of blood coagulation. The latter area of research has been extensively developed over the past years and is beyond the scope of the current review [95–97]. Moreover, certain limitations of the applicability of mathematical modeling in blood coagulation research are now the subject of a separate discussion [98–100]. We are planning to report some of our considerations concerning those issues elsewhere soon.

Summing up the pure experimental evidence covered in this review, it could be stated that blood itself under certain conditions can be treated as an active medium. Self-sustained travelling waves of thrombin can emerge in this medium as a result of the interplay between the processes of thrombin generation and diffusion. Therefore, the generation of thrombin in this case may propagate through space in a signal relay manner. That relay mechanism is able to provide a significant increase in rate of clot growth compared to the diffusional spread of thrombin. It appears that self-sustained traveling waves of thrombin can play an important role in the maintenance of hemostasis.

However, it should be noted that a number of questions concerning the self-sustained traveling waves of thrombin still require further investigation. Our knowledge of the restraining mechanisms of spatial propagation of coagulation remains incomplete. Very scarce experimental data are available about the propagation of coagulation fronts in whole blood. Insufficiently explored are the interrelations between the reaction-diffusion processes in blood coagulation and the cellular processes of hemostasis regulation (particularly the processes of the expression and release of TF-carrying microparticles into blood flow).

Despite the considerable amount of experimental results obtained over the last 20 years, the conditions under which self-sustained spatial propagation of blood coagulation can occur remain not entirely well-defined. A number of contradictions in the results of recent experimental papers [51,58,59] indicates that the self-sustained propagation detected in these studies are insufficiently reproducible.

It is worth mentioning that, with regard to insufficient reproducibility, we do not mean some quantitative discrepancies in the numerical values presented in various papers but contradictions in the results that appear to be essential from a qualitative point of view. In the framework of the concept, the independence of the speed of propagation of the coagulation fronts from the initiation conditions is a fundamental property of self-sustained traveling waves of thrombin. In this context, publication of successive papers [58] and [51] with directly opposite results on the dependence of the speed of propagation of coagulation fronts from the surface density of TF, inevitably gives rise to definite questions concerning the reliability of the experimental techniques and methods used in those research studies.

The very fact of the publication of directly opposite data on an essential question in successive papers by the same group of authors, without any discussion of these contradictions, is quite confusing. We sincerely hope that further experimental work in this area will eliminate these contradictions and clarify the conditions under which self-sustained travelling waves of blood coagulation can take place.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The present work was partially supported by the RFBR grant 14-04-01193 and the Federal Program "5top100".

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