Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2022, 13, 92-111
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Evaluation of Red Blood Cell Contribution to Platelet Activation in the Bulk Applying Red Blood Cell—Platelet Thrombus as a Point Source Model

Taha M. Alkhamis*, Bahieh M. Alma'atah

Department of Chemical Engineering, Mutah University, Mutah, Jordan
Email: *alkhamis@mutah.edu.jo

How to cite this paper: Alkhamis, T.M. and Alma'atah, B.M. (2022) Evaluation of Red Blood Cell Contribution to Platelet Activation in the Bulk Applying Red Blood Cell—Platelet Thrombus as a Point Source Model. *Advances in Bioscience and Biotechnology*, **13**, 92-111.
https://doi.org/10.4236/abb.2022.132004

Received: February 9, 2022
Accepted: February 21, 2022
Published: February 24, 2022

Abstract

In this study, a point source mathematical model is proposed to describe the diffusion of adenosine di-phosphate (ADP) from either damaged red blood cell (RBC) or activated platelet. The convective diffusion equation is reduced to describe the suggested problem. The final differential equation is solved using Laplace transforms and ADP concentration profiles around the source are obtained. Thrombi of 5 to 20 μm^3 containing platelets and a range of red blood cells (RBCs) (0%, 25%, 50%, 75%, 100%) concentrations are used to apply the model. Reported ADP concentrations in the literature are used and its dynamic release from the point source is calculated. Results suggest that RBC chemical contribution to platelet aggregation in the bulk is much less than that of platelet (almost) negligible. However, the physical effect of RBCs is dominant in the bulk through augmentation of released ADP and platelets diffusivities. Moreover, the chemical contribution reported in previous studies is suggested to be as a result of interaction of RBC with the surface under the influence of shear stresses in the boundary region.

Keywords

Platelet, Red Blood Cell, Aggregation, Adhesion, Diffusion, ADP, Point Source, Chemical Contribution, Physical Contribution, Shear Stresses, Hemolysis

1. Introduction

Exposure of circulating blood cells to non-endothelialzed surfaces under the influence of shear forces potentiates platelet reduction (loss) via thrombi formation

on the site of the injury or on the artificial surfaces [1] [2] [3] [4]. Under normal circumstances, platelets do not adhere to endothelial surface that makes the lining of the interior walls of the circulatory conduits, and they usually do not adhere to other platelets (aggregate) either near the surface or in the bulk. However, for systems involving foreign surfaces or on the site of the injury or even in abnormal nature of a circulatory conduit surface (arteriosclerosis), platelets seem to have the tendency to adhere to such surface followed by aggregation to form a thrombus. About two million cardiovascular procedures and open-heart surgery are performed annually in the United States alone [5]. All such procedures require, at least, short-term blood cell contact with different biomaterials and a large portion of them involve implantation of a permanent prosthetic devices.

As a result of blood cell interaction with the artificial, surfaces several platelet agonists, such as adenosine di-phosphate (ADP), thrombin, and thromboxane A2 are either released or generated. Substantial evidence has been found to suggest that red blood cells (RBCs) exposed to shear potentiate platelet adhesiveness to other platelets and to artificial surfaces, due to shear induced release of ADP from RBCs [1] [2] [3] [4]. The mechanism is often referred to as the chemical mechanism. The other contributing mechanism to RBC potentiation of platelet aggregation (PAG) is referred to as the physical mechanism, which involves RBC augmentation of platelet convective diffusion and thereby increases the likelihood of platelet-surface interaction (adhesion) as well as platelet-platelet interaction (aggregation).

Most of past studies concentrated on experimental means to clarify the mechanism by which platelet adhesion and aggregation occur [6] [7]. Limited studies considered mathematical modeling to investigate this problem [8]. Failure to produce active non-thrombogenic materials is a result of a failure to understand the mechanism of biomaterial-associated thrombosis [9]. Platelets are known to act immediately to from thrombus at the site of the injury to stop bleeding forming what is known as a hemostatic plug [10]. Red blood cells are believed to play an essential role in platelet aggregation and adhesion under the influence of blood dynamics in artificial blood contacting devices [11]. Despite the effort of extensive research on blood constituents damage as a result of blood contact with artificial surfaces, the mechanism is still not fully understood [12]. Mechanical trauma is reported to affect blood cells and mainly causes aging of red blood cell membrane which may accelerate damage of such membrane causing their constituents such as hemoglobin and ADP to be released in the bulk [13].

Red blood cells and platelets are both responsible for thrombus formation [6]. The contribution of red blood cell contribution is not well understood [2]. Mathematical models to explain the chemical and physical contributions of RBCs are limited. Platelet adhesion rate constant was shown analytically determined as a function of shear rate, hematocrit, and average size of platelets and RBCs [14]. Physical and chemical effects of RBCs have been suggested to affect platelet aggregation and adhesion on the surface and the bulk [1] [2] [6]. Physical effect is due to the presence of the large size of RBCs which augments the diffusion of

platelets to the surface, and the chemical effect is due to the release of ADP from RBCs. Platelets aggregates (thrombi) may capture RBCs that are detached from the surface after their temporary adhesion. The release of ADP from these aggregates has not been studied as a point source in the stagnant region near the surface, where initially activation of platelets may occur and consequently thrombi formation. This study will discuss the contribution of RBCs and platelets to ADP release from formed thrombus as a stagnant point source near the surface. As a result, the level of the physical and chemical effects of RBCs on platelet adhesion may be evaluated. Therefore, this theoretical study is designed to consider the release of ADP from thrombus as a point source problem. Concentration of ADP away from the source is obtained to evaluate the effect of RBCs on platelet adhesion and aggregation.

2. Methodology

2.1. Mathematical Model Formulation

The damaged RBC, activated platelet, or a thrombus that is formed of RBCs and platelets are considered as point sources of ADP releases in to a stagnant bulk near the surface (**Figure 1**). Red blood cells and platelets concentrations are low in the surface region and high in the core. Moreover, previous studies suggest that the release of ADP, as a platelet agonist, occurs at the surface from platelets and possibly form RBCs (chemical mechanism) [1] [2] [15]. Therefore, thrombus formation starts in the boundary region where the fluid can be considered stagnant.

The dimensions of either platelet or RBC are too small compared to the dimensions of the apparatus that contain them. Diffusion from the point source is too fast that may be considered to occur in seconds. Therefore, the convective diffusion equation in spherical coordinates is reduced to:

$$\frac{\partial C_A}{\partial t} = D_{AB} \frac{\partial}{\partial r} r^2 \frac{\partial C_A}{\partial r} \tag{1}$$

Several approaches have been suggested in the literature to solve the point source diffusion problem [16], and Laplace transforms approach is adopted in this study to solve this problem for its applicability to the problem in hand. The following condition is considered to be valid in order to be able to adapt Laplace transforms to this point source problem:

$$Z = rC_A \tag{2}$$

The equation can be solved using Laplace transforms, however, we have to substitute Equation (2) to be able to transform the equation in a form that can be handled by the method of solution, so we get:

$$\frac{\partial Z}{\partial t} = D_{AB} \frac{\partial_2 Z}{\partial r^2} \tag{3}$$

To get reasonable initial and boundary condition we assume the next two valid conditions:

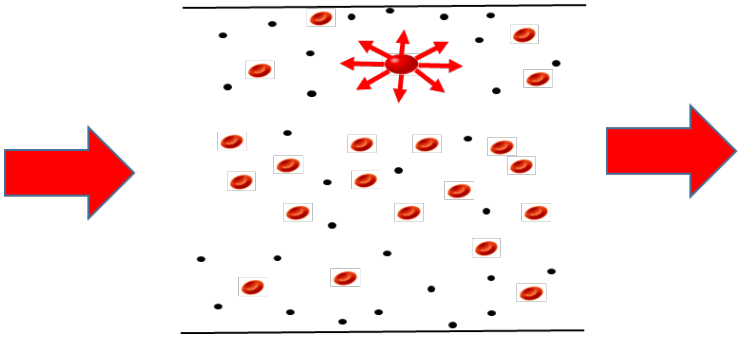

Figure 1. Point source schematic model; Red Blood Cell: , Platelet: ●, Thrombus: .

1): $Z = rC_A$ is finite

2): $\int_0^\infty 4\pi r^2 C_A \mathrm{d}r = n_0$

where: n_0 is the initial amount of ADP within the source.

Applying the above two condition we can obtain the required initial and boundary conditions as:

$$t = 0 : C_A = 0 \to Z = 0$$

Applying the following Laplace transforms to Equation (3) to obtain Equation (4) below:

$$\ell f(r,t) = \int_0^\infty \mathrm{e}^{-st} f(r,t)\mathrm{d}t = \overline{f}(r,s)$$

$$\ell f'(r,t) = s\ell f(r,t) - f(r,0)$$

$$s\overline{Z}(s,r) = D_{AB}\frac{\partial_2 \overline{Z}}{\partial r^2}$$

or:

$$\frac{\mathrm{d}^2\overline{Z}}{\mathrm{d}r^2} - \frac{s}{D_{AB}}\overline{Z} = 0 \tag{4}$$

Equation (4) has a general solution represented by:

$$\overline{Z}(s,r) = C_1 \mathrm{e}^{\sqrt{\frac{s}{d_{AB}}}r} + C_2 \mathrm{e}^{-\sqrt{\frac{s}{d_{AB}}}r} \tag{5}$$

Now, the next two steps include application of the suggested assumptions as:

1) Applying the first assumption (*i.e.*) $Z = rC_A$ is finite leads to:

$$C_1 = 0$$

Therefore, Equation (5) reduces to:

$$\overline{Z}(s,r) = C_2 \mathrm{e}^{-\sqrt{\frac{S}{D_{AB}}}r} \tag{6}$$

Next,

2) Applying the second assumption by performing Laplace transform we get Equation (7):

$$\ell \int_0^{\infty} 4\pi r Z \mathrm{d}r = \ell n_0$$

$$\int_0^{\infty} 4\pi r \bar{Z} \mathrm{d}t = \frac{n_0}{S} \tag{7}$$

Substituting for $\bar{Z}$ of Equation (6) into Equation (7) to get Equation (8) below:

$$4\pi C_2 \int_0^{\infty} r \mathrm{e}^{-\sqrt{\frac{S}{D_{AB}}}r} \mathrm{d}r = \frac{n_0}{S} \tag{8}$$

The general solution of the above integral is in the form:

$$\int_0^{\infty} x^b \mathrm{e}^{-ax} \mathrm{d}x = \frac{b!}{a^{b+1}}, \quad b = 1, 2, 3, \cdots$$

Provided that: $a > 0$, where $a = \sqrt{\frac{S}{D_{AB}}}$ …from Equation (6) above.

Taking the appropriate terms and the required rearrangements we can evaluate C_2:

From the relation: $4\pi C_2 \frac{D_{AB}}{S} = \frac{n_0}{S}$ we get:

$$\Rightarrow C_2 = \frac{n_0}{4\pi D_{AB}} \tag{9}$$

To get the concentration in time domain we apply the following Laplace inverse:

$$\ell^{-1} \mathrm{e}^{-k\sqrt{S}} = \frac{k}{2\sqrt{\pi t^3}} \exp\left(\frac{-k^2}{4t}\right)$$

Application of the above Laplace inverse on Equation (6) with k is taken as the following relation:

$$k = \frac{r}{\sqrt{D_{AB}}}$$

The time domain $Z(t,r)$ is obtained as:

$$Z(t,r) = \frac{rC_2}{\sqrt{D_{AB}\pi t^3}} \exp\left(\frac{-r^2}{4D_{AB}t}\right) \tag{10}$$

Substitution for $Z = rC_A$ from Equation (2) we get the final concentration profile $C_A(t,r)$ of the point source diffusion model proposed, with A represents ADP concentration profiles as:

$$C_A(t,r) = \frac{n_0/4\pi D_{AB}}{\sqrt{D_{AB}\pi t^3}} \exp\left(\frac{-r^2}{4D_{AB}t}\right) \tag{11}$$

2.2. Model Application

The above model was used to evaluate the concentration profiles of ADP around

RBC, platelet, and different thrombi combine both RBCs and platelets. The volume of RBC was taken as 87 μm^3 and platelet volume was 4.19 μm^3. The ADP inside the RBC was considered as 17.7×10^{-9} nM/RBC and 40×10^{-9} nM/platelet. The diffusion coefficient of ADP was considered as 1×10^{-9} cm^2/s [17]. These values were used to obtain ADP concentration profiles at distances; 2, 4, 6, 8, and 10 μm away from 5, 10, 15, 20 $(\mu m)^3$ thrombi containing; 0%, 25%, 50%, 75%, 100% RBCs.

3. Results

The ADP concentration profiles were obtained for different thrombi sizes of platelets and RBCs in the bulk or detached from the surface. Results indicate insignificant chemical contribution of red blood cells to platelet activation and further adhesion and aggregation at the surface and in the bulk, and their contribution is dominant by their physical effect. The chemical contribution seems to be less significant as compared to physical contribution reported in other studies [1] [15]. Therefore, a positive possible role of RBCs may be seen due to their existence with platelets in the different thrombi designed in this study. **Figure 2** shows that the initial ADP concentration released, from pure 5 $(\mu m)^3$ platelet thrombus, is above 10 and less than 100 μM at 2 and 4 μm distances away from the thrombus. This concentration is at a level that can cause irreversible aggregation of platelets [15] [17] [18]. Initial concentrations at distances between 6 and 10 μm are at a magnitude level of 1 μM or less which indicates a reversible effect of ADP on virgin platelets at these positions. Asymptotic ADP concentration of about 1 μM is reached at about 60 s for all distances. Therefore, thrombus of 5 $(\mu m)^3$ of pure platelets shows a formation of ADP cloud up to 5 μm away which can initiate irreversible bulk aggregation. The effect lasts for about 60 s only after which the concentration of ADP becomes ineffective, which is less than the 2 minutes' threshold needed to cause ADP induced platelet activation [2] [3] [15].

Figure 3 shows the release from 5 $(\mu m)^3$ thrombus consisting of 25% RBCs and 75% platelets. Initial concentrations at 2 and 4 μm are at a level that can still cause irreversible aggregation, but the corresponding concentrations are lower than that of pure platelet thrombus. Asymptotic value of 1 μM is also reached at 60 s time. Moreover, increasing RBCs concentration seems to reduce the ADP released levels.

Figure 4 shows the release for the same size thrombus but with composition of 50% platelets and 50% RBCs. The same trend is seen as the previous figures; however, the ADP concentrations are lower than those coming from thrombi with higher platelet composition. Moreover, the asymptotic ADP concentration falls below 1 μM in 60 s for all distances, at which reversible aggregation may occur.

Figure 5 shows the results of 5 $(\mu m)^3$ thrombus of 75% RBC concentration. Initial ADP concentrations are reduced for all distances. Ineffective ADP concentrations at 2 and 4 μm appear after 50 s. The results lead to suggest insignificant RBCs chemical contribution.

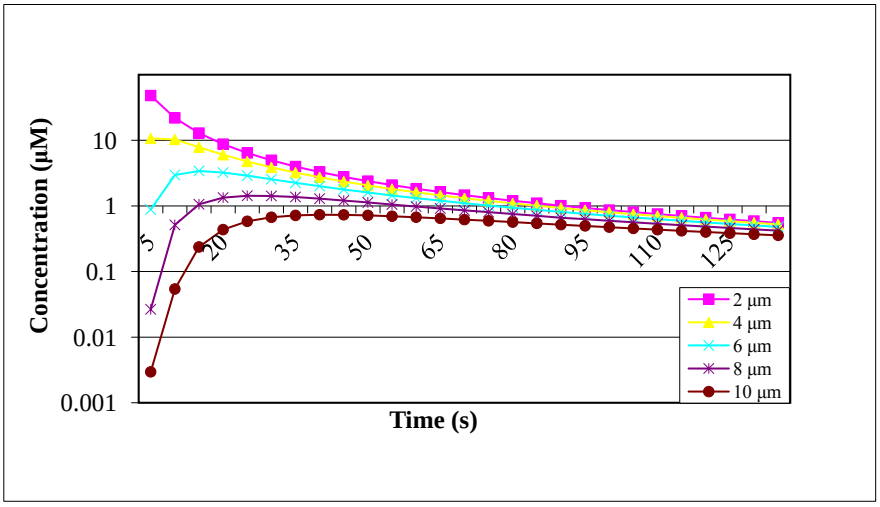

Figure 2. ADP concentration as a function of distance away from 5 μm thrombus of 0% RBC.

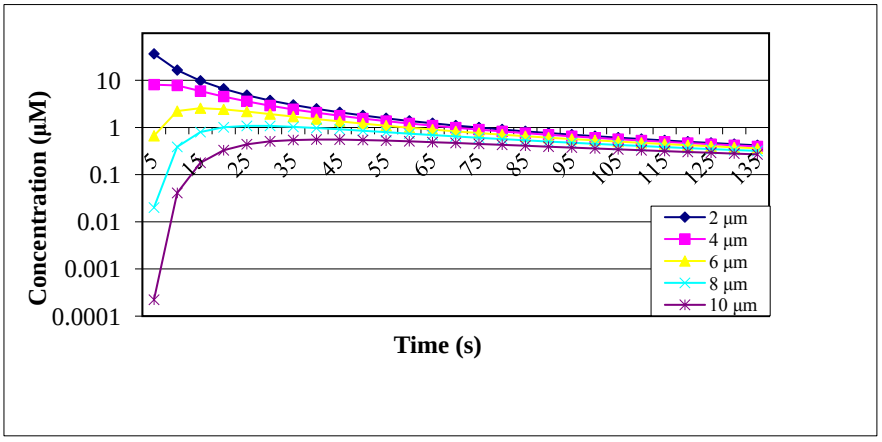

Figure 3. ADP concentration as a function of distance away from 5 μm thrombus of 25% RBC.

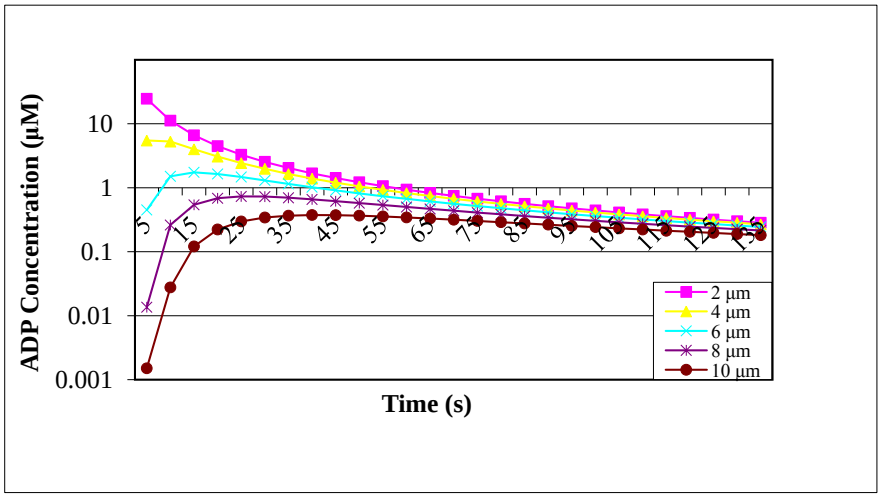

Figure 4. ADP concentration as a function of distance away from a 5 μm thrombus of 50% RBC.

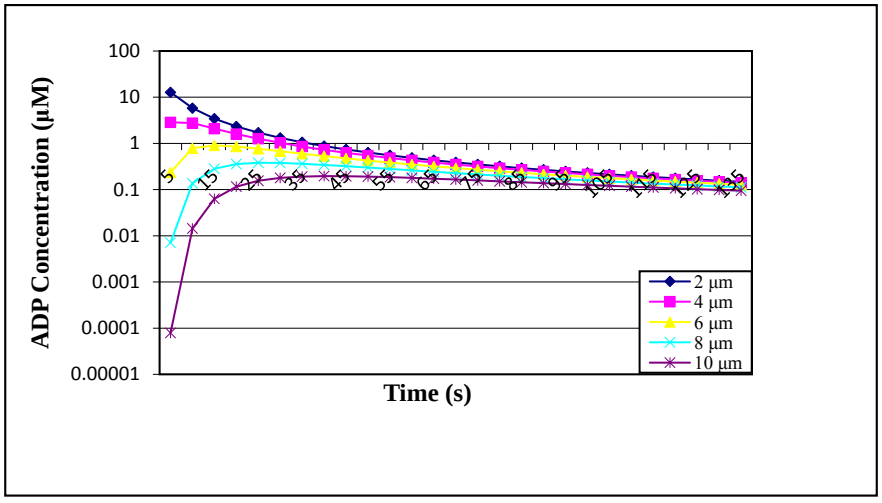

Figure 5. ADP concentration as a function of distance away from a 5 μm thrombus of 75% RBC.

Figure 6 represents ADP concentrations as a function of distance for pure 5 $(\mu m)^3$ RBC thrombus. The initial ADP release in the bulk at 2 μm is seen to be at 1 μM level, which is considered to be non-effective with respect to platelet activation. Therefore, at this size of RBCs aggregate ADP release in the bulk seems to be non-effective, which supports the above suggestion of insignificant chemical contribution of RBCs in the bulk.

Figure 7 represents ADP concentrations data related to a 10 $(\mu m)^3$ pure platelet thrombus. A significant initial increase above the 5 μm^3 thrombus and asymptotic ADP concentration is observed to be above 2 μM even after two minutes. Hence, pure platelet thrombi in the bulk seem to be extremely effective for 10 μm^3 thrombus size.

Figure 8 shows results of 10 $(\mu m)^3$ including 25% RBC concentration. It is obvious that addition of RBCs to the platelet thrombus reduces ADP released concentration. However, the overall effect of ADP release is still within extreme effective levels.

Figure 9 shows the effect of increasing RBCs concentration to 50% of the 10 $(\mu m)^3$ thrombus. The concentrations of ADP are reduced further, and the asymptotic concentrations approach the reversible effect levels after two minutes of initial release.

Figure 10 represents ADP concentration profiles associated with 75% RBCs of a 10 $(\mu m)^3$ thrombus. The reduction of ADP concentrations is obvious compared to the 50% RBCs thrombus. Moreover, the asymptotic ADP reversible value is reached in about 100 s.

ADP Concentration profiles associated with pure RBCs thrombus of 10 $(\mu m)^3$ are presented in Figure 11. Initial ADP release is only significant at 2 μm distance (*i.e.*) a thin film forming a cloud around the thrombus. Reduction of about 90% is seen in pure RBCs thrombus as compared to a 75% RBCs one. Further, the concentrations, for all distances, drop below effective ADP concentration just after 30 seconds.

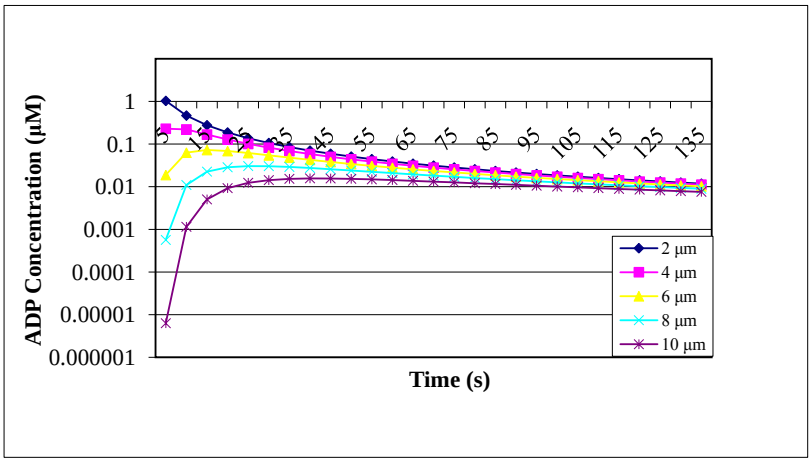

Figure 6. ADP concentration as a function of distance away from a 5 μm thrombus of 100% RBC.

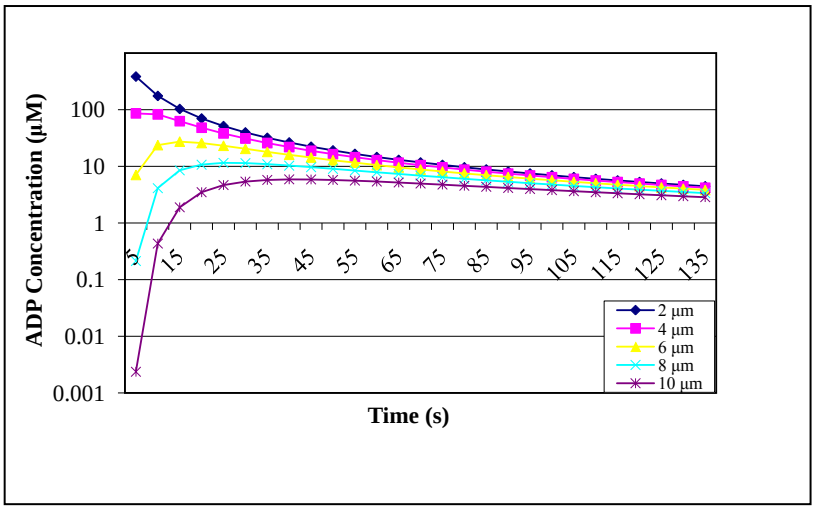

Figure 7. ADP concentration as a function of distance away from a 10 μm thrombus of 0% RBC.

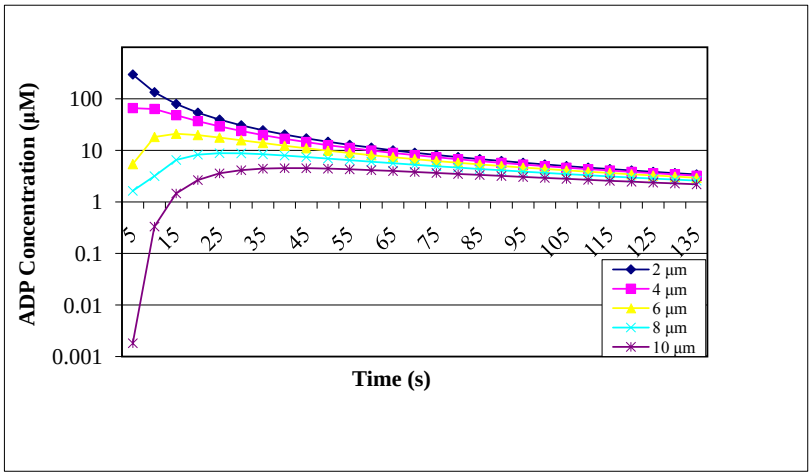

Figure 8. ADP concentration as a function of distance away from a 10 μm thrombus of 25% RBC.

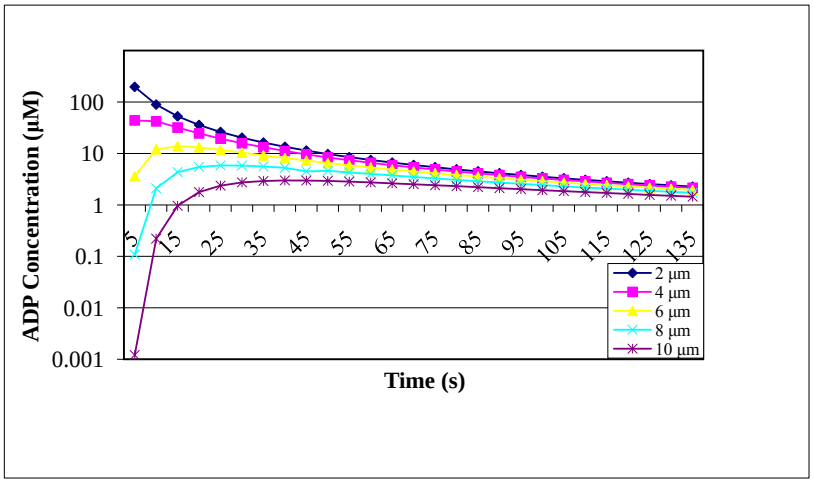

Figure 9. ADP concentration as a function of distance away from a 10 µm thrombus of 50% RBC.

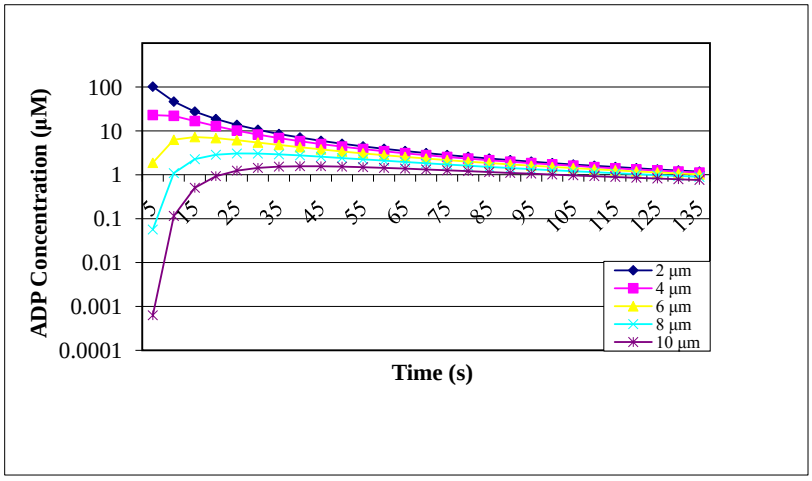

Figure 10. ADP concentration as a function of distance away from a 10 µm thrombus of 75% RBC.

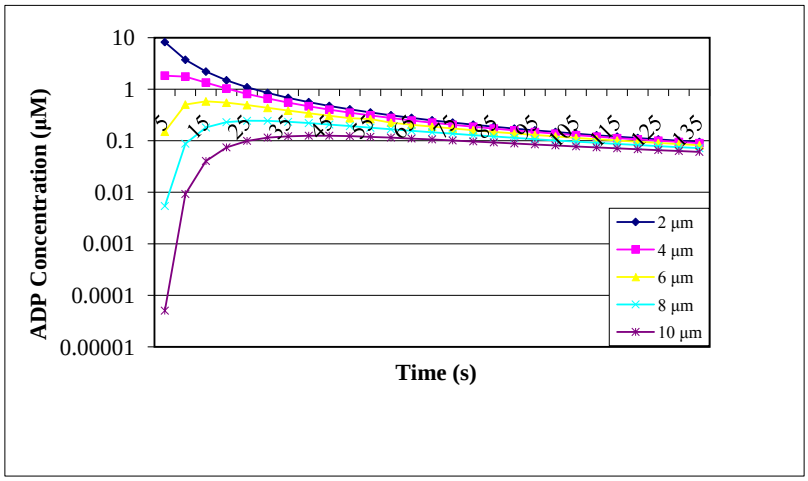

Figure 11. ADP concentration as a function of distance away from a 10 µm thrombus of 100% RBC.

Figure 12 shows the results for platelet pure thrombus of 15 $(\mu m)^3$. An initial increase of about 150% above 10-micron thrombus, of the same composition, is obvious. Also, asymptotic ADP values stay well above the minimum effective concentration. The effect of such thrombus, if it exists, will extend to far distances away from the source.

Effect of including 25% RBCs in a thrombus of 15 $(\mu m)^3$ is clearly seen in Figure 13. Overall concentrations are reduced; however, all values are still way above the minimum ADP effective concentration. As pointed above, if this type of thrombus exists in a system, then there effect will extend to a far distance from the source.

Figure 14 represents ADP concentrations obtained from a 15 $(\mu m)^3$ thrombus containing 50% RBCs. The initial concentrations as well as the asymptotic values are reduced; however, they are still in a range that consequence effects are vital with respect to platelet activation and further adhesion and aggregation. The effect of increasing the concentration of RBCs within a 15 $(\mu m)^3$ thrombus on ADP release is shown in Figure 15. The concentration profiles are reduced and the asymptotic values are seen to be slightly above the minimum effective ADP concentration. This also may suggest that a thrombus of this type may not exist within known artificial systems, especially those under the influence of low shear stresses.

ADP concentration profiles associated with pure RBCs of 15 $(\mu m)^3$ thrombus are presented in Figure 16. Initial concentrations up to 60 s after ADP release are at a level that may cause irreversible aggregation. Therefore, it may be suggested that if damaged RBCs can form aggregates; this will lead to formation of an ADP cloud around the thrombus for about 60 s. However, asymptotic values drop below the effective ADP concentration just after 65 s.

Results on ADP concentration profiles originating from pure platelet thrombus of 20 $(\mu m)^3$ size are shown in Figure 17. The high concentration values, reported in the figure, are impossible to exist in real system. Therefore, these results support the suggestion that only small size pure platelet aggregates may exist in real systems with reasonable stress levels are available and blood compatible surfaces are lining the blood flow compartments.

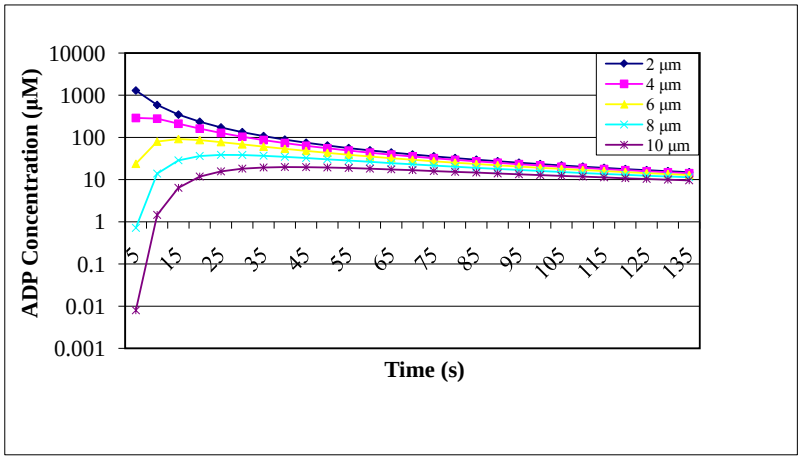

Figure 12. ADP concentration as a function of distance away from a 15 μm thrombus of 0% RBC.

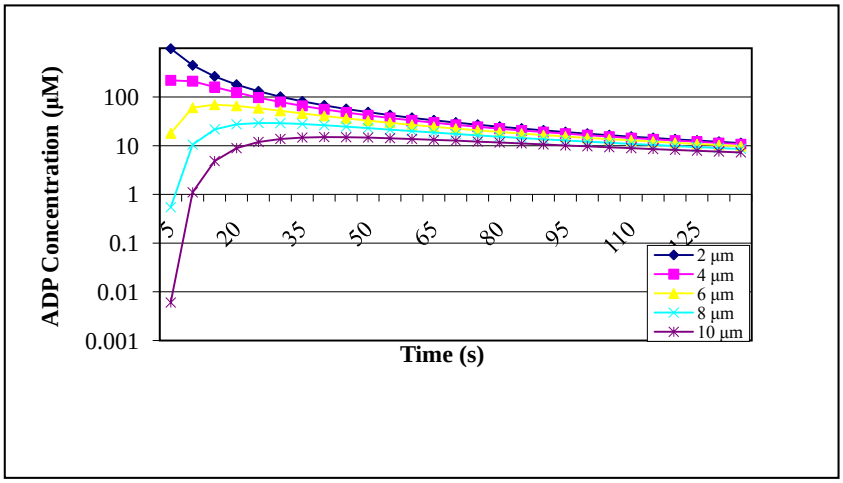

Figure 13. ADP concentration as a function of distance away from a 15 μm thrombus of 25% RBC.

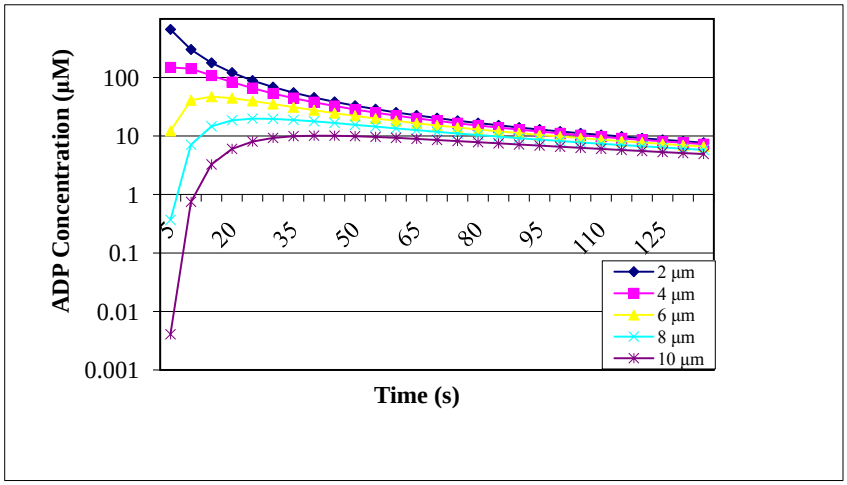

Figure 14. ADP concentration as a function of distance away from a 15 μm thrombus of 50% RBC.

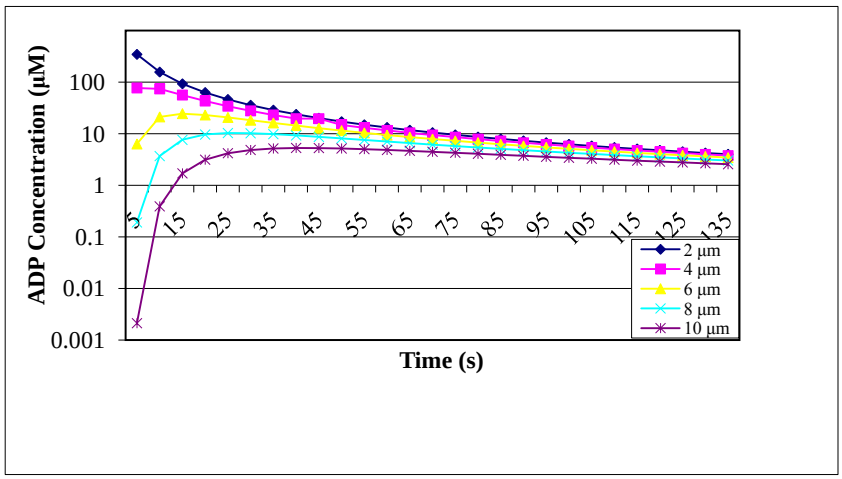

Figure 15. ADP concentration as a function of distance away from a 15 μm thrombus of 75% RBC.

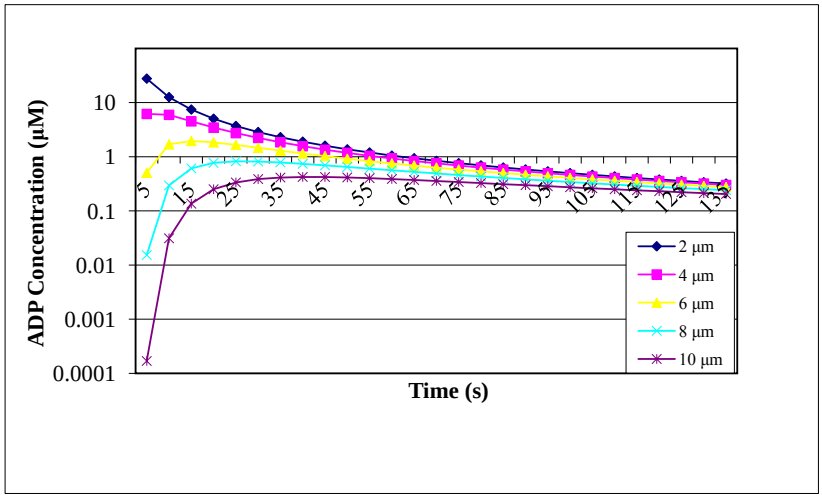

Figure 16. ADP concentration as a function of distance away from a 15 μm thrombus of 100% RBC.

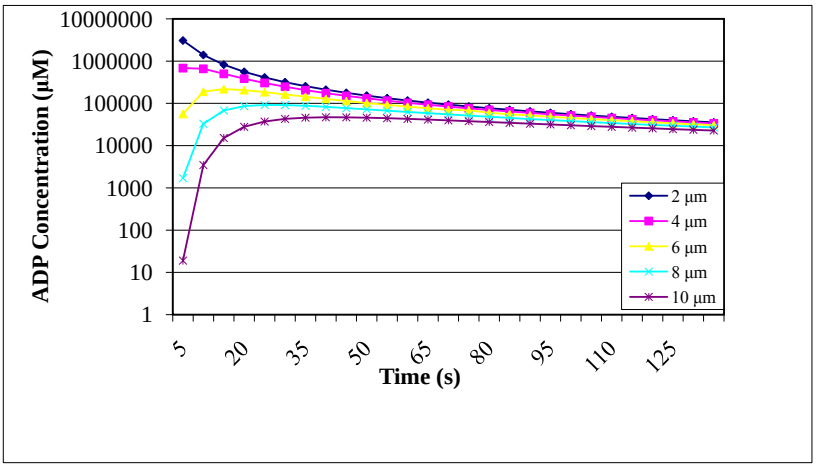

Figure 17. ADP concentration as a function of distance away from a 20 μm thrombus of 0% RBC.

Figure 18 shows the effect of introducing 25% of RBCs within the thrombus. ADP initial concentrations are reduced by at least 1000 times. However, with this reduction the asymptotic values are seen to be way above the minimum effective ADP concentration (2 μM). These results may indicate that this thrombus type may not exist also in real systems.

Increasing the RBCs concentration to 50% in a thrombus reduces the initial concentration and the asymptotic value, but not to an acceptable real systems levels. This argument is supported by the results shown in Figure 19. The results shown in the figure support the above suggestion of insignificant chemical contribution of RBCs and an obvious physical contribution.

Figure 20 shows the ADP concentration profiles for the 20 $(\mu m)^3$ of 75% RBCs. Initial concentrations are reduced compared to the previous results of lower RBCs concentrations. Despite that the ADP concentrations are high enough initially to activate platelets for further adhesion and aggregation; they drop to a

safe level (1 μM) just after 125 s as we increased RBCs concentration.

Figure 21 shows some sort of decrease in initial ADP release concentrations associated with pure RBCs within 20 $(\mu m)^3$ thrombus. Asymptotic values reach safe ADP concentration levels just after 110 s. Moreover, such thrombi of RBCs alone may not exist in real systems because RBCs are not known to adhere to each other, therefore, the results shown in the figure cannot be used to support their chemical effect.

Table 1 presents the effective distance with respect to platelet activation and consequently platelet aggregation as a result of ADP release from thrombi of various sizes. Moreover, the elapsed times of the effective ADP concentrations are also presented (Table 1). It is obvious that as the increase of RBCs concentrations reduces the effective distances as well as the elapsed time which reaches below the threshold of irreversible aggregation of two minutes in all results of 5 μm dimeter thrombi and high RBCs concentrations of other thrombi.

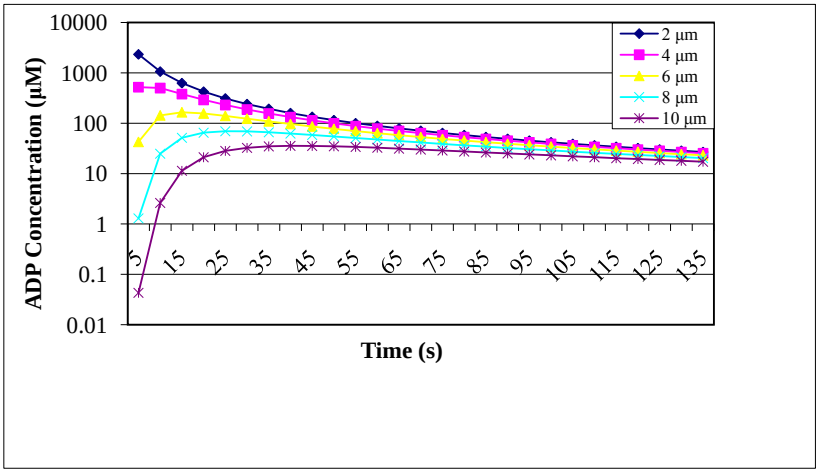

Figure 18. ADP concentration as a function of distance away from a 20 μm thrombus of 25% RBC.

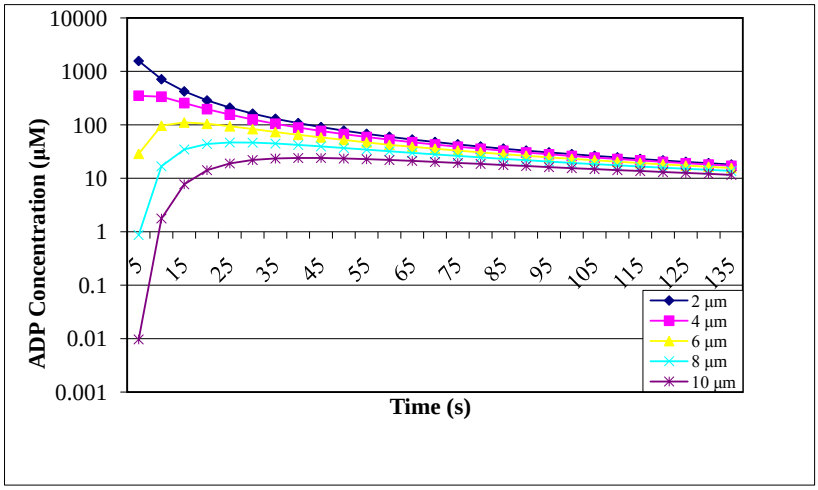

Figure 19. ADP concentration as a function of distance away from a 20 μm thrombus of 50% RBC.

Table 1. Effective ADP concentration released from different thrombi sizes and the duration at 2, 4, 6, 8, and 10 μm distance from thrombus point source.

Thrombus Size (μm)	RBCs Concentration (%)	Distance away from the source to have ADP concentration at a level to cause irreversible aggregation (μm)	Time elapsed for the released ADP to stay at a level to cause aggregation (s)
5	0	2, 4	60
	25	2, 4	60
	50	2, 4	60
	75	2, 4	50
	100	<1	0
10	0	2, 4, 6 8 after 20 s 10 after 25 s	>125
	25	2, 4, 6 8 10 after 25 s	>125
	50	2, 4, 6 8 after 15 s 10 after 25 s	>125
	75	2, 4, 6 8 after 20 s	>100
	100	2, 4	<15
15	0	2, 4, 6 8 after 15 s 10 after 20 s	>125
	25	2, 4, 6 8 after 15 s 10 after 20 s	>125
	50	2, 4, 6 8 after 15 s 10 after 20 s	>125
	75	2, 4, 6 8 after 20 s 10 after 20 s	>125
	100	2, 4 6 after 20 s	≈65
20	0	2, 4, 6, 8, 10	>125
	25	2, 4, 6 8 after 10 s 10 after 15 s	>125
	50	2, 4, 6 8 after 5 s 10 after 15 s	>125
	75	2, 4, 6 8 after 15 s 10 after 20 s	>125
	100	2, 4 6, after 10 s 8 after 20 s	≈110

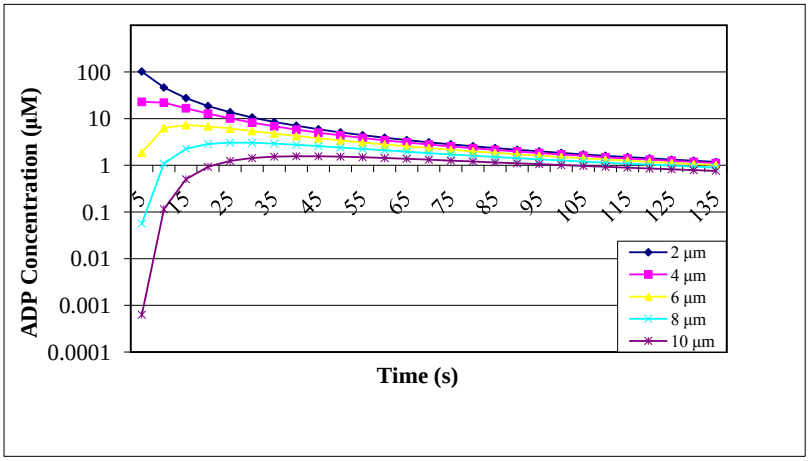

Figure 20. ADP concentration as a function of distance away from a 20 μm thrombus of 75% RBC.

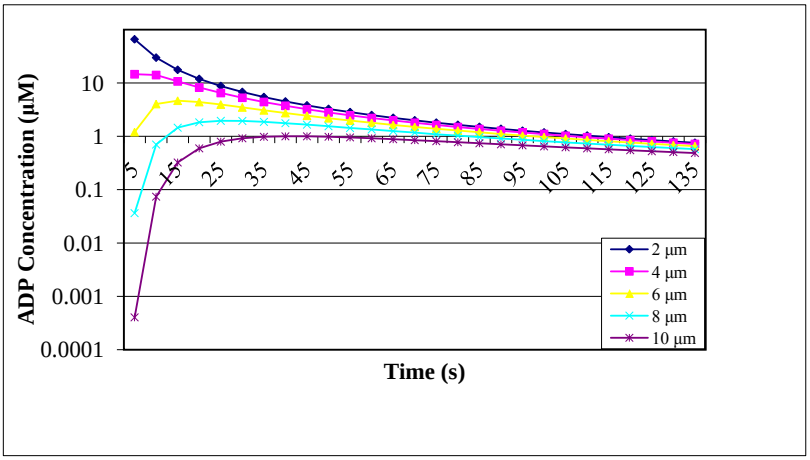

Figure 21. ADP concentration as a function of distance away from a 20 μm thrombus of 100% RBC.

To determine the chemical effect contribution of RBCs to the effective levels of ADP release from thrombi, the concentrations at 2 μm distance from 5, 10, 15, 20 μm^3 are reported in Table 2. Results of ADP release from all thrombi sizes is at a level that can cause platelet adhesion aggregation. However, the duration of effective ADP concentration is less than 2 minutes for 5 μm^3 thrombi sizes and pure RBCs thrombi (Table 1). Moreover, the increase of RBCs concentration decreases the ADP levels of all thrombi sizes. This may indicate a lower level of chemical contribution of RBCs to platelet adhesion and aggregation.

4. Discussion

Results indicate that an increase of RBCs concentrations within a thrombus reduces the ADP concentration levels and the duration time of effective concentration. Thrombi of 5 μm^3 size, the concentration of ADP is at a level that can initiate platelet activation after 2 seconds period, and All of ADP diffuses in 3 seconds. Therefore, it is clear that when ADP starts to release from this size

Table 2. ADP released concentration after 5 seconds at 2 μm distance from thrombus point source as a function of RBCs concentration.

Thrombus size (μm^3)	RBCs Thrombus Concentration (%)				
	0	25	50	75	100
	ADP Concentration after 5 seconds at 2 μm distance from the thrombus point source				
5	48.1	36.4	24.6	12.8	1.03
10	385	296	197	102	8.21
15	13×10^2	982	664	346	27.7
20	30.8×10^5	2330	1570	819	65.7

thrombus a cloud starts to build that has the potential to activate platelets during the first 5 seconds. Effective ADP concentration is observed at 2 and 4 μm only, however this level lasted only for less than 60 s. It is clear that time which is required for ADP to be at a level that can activate platelets at different diameters increases as the diameter increases. Moreover, the results may indicate that RBCs chemical contribution to platelet aggregation in the bulk is not significant, and the dominant effect is due to platelets. However, the increase of RBCs concentration within the thrombus decreases the effective release of ADP, and consequently decreases the potential of platelet aggregation within the bulk. Thrombi of 5 μm^3 in the bulk do not seem to have a significant effect on platelet activation. It is evident that duration of effective ADP concentration at this size is less than 60 seconds, which is not enough to cause irreversible platelet aggregation [1] [2] [3] [15].

Released ADP concentration from 10 μm^3 thrombus show effective levels at 2, 4, and 6 μm within 5 seconds of starting the release, while it appears after about 20 seconds at 8 μm and after about 25 seconds at 10 μm. containing 75% RBCs. Increasing the concentration of RBCs decreases the time required for ADP effective levels to be observed and the time of duration of these effects. This may indicate that increasing the concentration of RBCs increases diffusivity of ADP and decreases the release. Therefore, it may be suggested to have more physical contribution of RBCs than chemical contribution. Pure RBCs thrombi show effective level at 2 and 4 μm, however this level lasts for less than 15 seconds which support the above findings (Table 1) concerning the physical and chemical contribution of RBCs.

Large size thrombi (15 and 20 μm) show extensively high ADP released concentration at an order of magnitude of 10^2 to 10^5 μM (Table 2), which are at levels that may not be possible to exist in real system. The effect of the ADP released from these thrombi lasts for more than 2 minutes for all distances except that of pure RBCs thrombi which lasted for less than 110 seconds. This result, also, supports the suggestion of higher physical effect, of RBCs, than chemical effect within the bulk.

The results presented above indicate more chemical contribution of platelet than RBC, in the bulk, to platelet aggregation as a result of ADP release. The RBCs contribution is limited due to their sizes. Presence of RBCs within thrombus negatively affects diffusion of ADP in the bulk. The overall picture of RBCs contribution to platelet adhesion and aggregation at the surface and the bulk can be understood if diffusion of ADP from the surface is considered along with the findings of this model. Spreading of platelets and RBCs may increase RBC contribution to ADP concentration profiles due to limited RBC size effect. The physical contribution of RBC to platelet aggregation may be larger than its chemical contribution. Further development of the model will show RBC exact contribution.

Therefore, it may be suggested that as RBCs increase, in the thrombus, the effect of ADP release decreases and the duration of the effect decreases to levels below 60 s, which are not enough to cause irreversible aggregation. Small size thrombi (5 μm) do not show effective ADP levels even in the absence of RBCs. Thrombi of 10 μm^3 show an effective level at up to 10 μm and a duration time of more than 2 minutes except for pure RBCs thrombi which show effective levels at 2 and 4 μm and a duration time of 100 seconds. Large sizes thrombi show impossible levels of released ADP concentration. Therefore, it may be suggested that thrombi of 5 to 10 μm may exist in the bulk. Moreover, ADP release from pure RBCs thrombi does not show significant effective levels. This may indicate a low chemical contribution of RBCs to platelet aggregation and adhesion.

The above results may indicate that the contribution of RBCs to platelet aggregation in the bulk is dominant by physical effect through increasing the diffusivity of platelets and ADP. On the other hand, the chemical effect of RBCs which is not significant in the bulk is yet to be determined in future work at the surface. Moreover, this results imply that RBC chemical contribution reported in previous studies [1] [2] [3] [15] may be suggested to occur at the surface due to reversible adhesion of RBC at the surface which leads to stretching the membrane and consequently increasing the diameters of the pores, under the influence of shear stresses, that allows hemoglobin and other smaller molecules such as ADP to be released.

Moreover, the results of this study will contribute to the comprehensive understanding of the role of RBCs in platelet adhesion and aggregation under the influence of low shear stress conditions. This is because a much improved knowledge of ADP release and how it affects thrombosis is needed. Mechanisms of this phenomenon are close to being suggested. If the right mechanism is obtained the solution of problems concerning the use of artificial organs may be achieved.

5. Conclusions

It may be concluded that as RBCs increase, in the thrombus, the effect of ADP release decreases and the duration of the effect decreases to levels below 60 s, which are not enough to cause irreversible aggregation. Small size thrombi (5 μm)

do not show effective ADP levels even in the absence of RBCs. Thrombi of 10 μm^3 show an effective level at up to 10 μm distances and a duration time of more than 2 minutes except for pure RBCs thrombi which show effective levels at 2 and 4 μm and a duration time of 100 seconds. Large sizes thrombi show high levels of released ADP concentration which are impossible to exist. Therefore, it may be suggested that thrombi of 5 to 10 μm may only exist in the bulk. Moreover, ADP release from pure RBCs thrombi does not show significant effective levels. This may indicate a low chemical contribution of RBCs to platelet aggregation and adhesion.

Therefore, the main conclusions of this study include that the contribution of RBC to platelet activation in the bulk is mainly physical through augmentation of platelet and released ADP diffusivities as a major contribution while their chemical contribution through ADP release is almost negligible. Moreover, existence of RBCs within thrombi decreases the released levels of ADP, which makes it unlikely to have them in thrombi within the bulk. Furthermore, the chemical effect of RBCs is more likely due to temporary adhesion to the surface followed by membrane stretching and reversible deformation to allow hemolysis (hemoglobin release as well as ADP and similar compounds).

Acknowledgements

The authors wish to thank Mutah University for granting the corresponding author (Taha M. Alkhamis) a sabbatical leave for a year in which this work was partially carried out. We also wish to thank Dr. M.T. Al-Khamis for helping figures' preparation and Miss Leen Al-Khamis for proof reading the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Reimers, R.C., Sutera, S.P. and Joist, H. (1984) Potentiation by Red Blood Cells of Shear-Induced Platelet Aggregation: Relative Importance of Chemical and Physical Mechanisms. *Blood*, **64**, 1200-1206. https://doi.org/10.1182/blood.V64.6.1200.1200

[2] Alkhamis, T.M., Beissinger, R.L. and Chediak, J.R. (1990) Artificial Surface Effect on Red Blood Cells and Platelets in Laminar Shear Flow. *Blood*, **75**, 1568-1575. https://doi.org/10.1182/blood.V75.7.1568.1568

[3] Alkhamis, T.M., Beissinger, R.L. and Chediak, J.R. (1988) Red Blood Cell Effect on Platelet Adhesion and Aggregation in Low-Stress Shear Flow: Myth or Fact. *ASAIO Transactions*, **34**, 868-873.

[4] Turitto, V.T. and Weiss, H.J. (1980) Red Blood Cells: Their Dual Role in Thrombus Formation. *Science*, **207**, 541-543. https://doi.org/10.1126/science.7352265

[5] U.S. Department of Health and Human Services (1983) Guidelines for Blood Material Interactions—Report of the National Heart, Lung and Blood Institute Working Group.

[6] Shawaqfah, A.T. and Alkhamis, T.M. (2004) Effect of Artificial Surface Electric Charge on Laminar Flow Shear-Induced Single Platelet Loss. *Alexandria Engineering Journal*, **43**.

[7] Berliner, S., Ben-Ami, R., Samocha-Bonet, D., Abu-Abeid, S., Schechner, V., Beigel, Y., Shapira, I., Yedgar, S. and Barshtein, G. (2004) The Degree of Red Blood Cell Aggregation on Peripheral Blood Glass Slides Corresponds to Inter-Erythrocyte Cohesive Forces in Laminar Flow. *Thrombosis Research*, **114**, 37-44. https://doi.org/10.1016/j.thromres.2004.04.009

[8] Linderman, J.J. and Lauffenburger, D.A. (1986) Analysis of Intracellular Receptor/Ligand Sorting. *Biophysics*, **50**, 295-305. https://doi.org/10.1016/S0006-3495(86)83463-4

[9] Gorbet, M.B. and Sefton, M.V. (2004) Biomaterial-Associated Thrombosis: Roles of Coagulation Factors, Complement, Platelets and Leukocytes. *Biomaterials*, **25**, 5681-5703. https://doi.org/10.1016/j.biomaterials.2004.01.023

[10] Anselmo, A.C., Modery-Pawlowski, C.L., Menegatti, S., Kumar, S., Vogus, D.R., Tian, L.L., Chen, M., Squires, T.M., Gupta, A.S. and Mitragotri, S. (2014) Platelet-Like Nanoparticles: Mimicking Shape, Flexibility and Surface Biology of Platelets to Target Vascular Injuries. *ACS Nano*, **8**, 11243-11253. https://doi.org/10.1021/nn503732m

[11] Zhao, R. (2008) Experimental and Theoretical Investigation of Blood Flow Dynamics Associated with Blood Contacting Devices. Ph.D. Thesis, Carnegie Mellon University, Pittsburgh.

[12] Olia, S.E., Maul, T.M., Antaki, J.F. and Kameneva, M.V. (2016) Mechanical Blood Trauma in Assisted Circulation: Sub-Lethal RBC Damage Preceding Hemolysis. *The International Journal of Artificial Organs*, **39**, 150-159. https://doi.org/10.5301/ijao.5000478

[13] Buerck, J.P., Burke, D.K., Schmidtke, D.W., Snyder, T.A., Papavassiliou, D. and O'Rear, E.A. (2019) A Flow Induced Autoimmune Response and Accelerated Senescence of Red Blood Cells in Cardiovascular Devices. *Scientific Reports*, **9**, Article No. 19443. https://doi.org/10.1038/s41598-019-55924-y

[14] Tokarev, A.A., Butylin, A.A. and Ataullakhanov, F.I. (2011) Platelet Adhesion from Shear Blood Flow Is Controlled by Near-Wall Rebounding Collisions with Erythrocytes. *Biophysical Journal*, **100**, 799-808. https://doi.org/10.1016/j.bpj.2010.12.3740

[15] Alkhamis, T.M. (1988) Effect of Red Blood Cells on Platelet Adhesion and Aggregation in Low Stress Shear Fields. Ph.D. Thesis, Illinois Institute of Technology, Chicago.

[16] Kim, A.S. (2020) Complete Analytic Solutions for Convection-Diffusion-Reaction Source Equations without Using an Inverse Laplace Transform. *Scientific Reports*, **10**, Article No. 8040. https://doi.org/10.1038/s41598-020-63982-w

[17] Leggas, M. (1999) Characterizing Erythrocyte Motions in Flowing Blood. Master's Thesis, University of Tennessee, Knoxville.

[18] Born, G.V.R. (1962) Aggregation of Blood Platelets by Adenosine Diphosphate and Its Reversal. *Nature*, **194**, 927-929. https://doi.org/10.1038/194927b0