

Interazione tra Biomateriali e Tessuti

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**MATHEMATICAL MODEL OF
STATIC PLATELET ADHESION
ON A SOLID SURFACE**

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Introduction

Platelet adhesion on a foreign surface is one of the initial events taking place in the blood/biomaterial interface and influencing **hemocompatibility of medical devices**.

Because of the key role of platelets in **thrombogenesis** and **hemostasis**, a number of methods based on quantification of adhered platelets are currently widespread for **evaluation of blood compatible properties of materials for medical use**.

However, platelet interaction with the material surface proceeds in certain time ranges like any **dynamic process**, and is defined by:

- **interaction conditions**
- **surface properties**
- **platelet functional properties.**

Usually, adhered platelets are found on the surface **after 2–3 min of blood contact** with polymer materials.

It has been reported that **platelet adhesion does not take place until the adsorbed protein film is 100–200 Å thick**, about 60 s after the first blood contact.

According to other data, spreading and shape changes are most pronounced after 4–5 min of contact of **platelet rich plasma (PRP)** with the surface.

Maximum spreading of platelets on a pyrolytic carbon surface is observed after 20–30 min.

Changes in distribution of intracellular components are reported to take place on a 1–15 min scale after platelet attachment to the surface.

All these data prove that kinetics of the adhesion process should be studied (in a 5–30 min time interval) to obtain adequate and reliable information about its mechanisms.

It has been found that the type of adhesion kinetic curves depends on the value of **shear stress** in the vicinity of the surface, experimental **technique of adhered cell number determination**, experimental **device design**, suspending **medium**, observation **time**, etc.

Furukawa et al. reported different platelet accumulation profiles on several polymer surfaces depending on applied shear stress.

As shear stress values decreased from 5 to 0.1 dyn/cm² (quasistatic conditions), adhesion kinetic curves turned from exponential into sigmoid.

Ruckenstein et al. suggested the model for platelet sedimentation and adhesion from suspension to the horizontal surface: this model allows for **cell sedimentation velocity** in liquid medium and **overcoming of double layer potential barrier near the surface.**

The value of this double layer depends on surface electrostatic potential and may alter as adhered cells partially cover the surface right up to the change of the surface potential sign.

On the contrary, platelet adhesion is defined, on the opinion of Neumann et al., mostly by the value of **free energy of adhesion** and **other energetic parameters of the surface, cells, and suspending medium.**

In the discussion that followed, **Ruckenstein et al. and Neumann et al. had not come to an agreement about whether the saturation on kinetic curves of adhesion is of kinetic or thermodynamic origin.**

Thus, **the suggested models do not let us judge unequivocally about mechanisms of platelet/surface interaction.**

The mathematical model of Strong et al. considers **cell transport to the surface and cell– cell interactions both in flow and static conditions.**

Numerical solutions of their equations enabled the authors to assess **effective diffusion coefficient D_e in static and flow situations** as well as **surface reaction rate constant k** for platelet attachment to the surface.

The analysis of the model shows that platelet adhesion is not diffusion limited either in static or flow conditions.

Strong et al. used the experimental data of other authors to confirm their theoretical model.

In all familiar to us attempts to describe mathematically the process taking place in platelet contact with the material surface, **adhesion is considered as the only way of cell activation.**

However, **blood contact with a foreign surface results both in platelet adhesion and appearance of activated platelets in the liquid phase.**

Haycox and Ratner have demonstrated **a complex interrelation between platelet adhesion and their activation in the bulk.**

Some polymer materials, such as poly-(vinylalcohol), do not provoke considerable platelet adhesion on their surfaces, but lead to cell activation in the bulk of the liquid phase.

Other materials, such as polyethylene (PE), on the contrary, demonstrate a substantial level of platelet adhesion and spreading, followed by formation of a “passivating” layer, which excludes further platelet activation.

The accumulation of activated cells occurs under static conditions (no flow, no stirring), which may affect the character of platelet adhesion on the surface.

Thus, the study of platelet adhesion kinetics under static conditions and the development of a model of platelet/surface interaction, allowing for the bulk platelet activation, may explain complicated shapes of experimental adhesion curves.

The purpose of the present study was to work out a kinetic approach to **investigation of platelet adhesion on a solid surface** and **to suggest a platelet/surface interaction model, taking into account cell activation in the bulk of the liquid phase.**

Materials and methods

Glass plates were used as **hydrophilic substrata** for platelet adhesion.

Glasses, chemically modified with a self-assembled coating of hexadecyltrichlorosilane monolayers, and glasses with silicone coating were chosen as **hydrophobic substrata**.

Silicone coating is used to treat the glassware for manipulation with platelets.

Low-density PE for medical use was also included in the study.

The experiments were performed with the blood from healthy adult male volunteers 20–45 years old.

The study was performed with informed consent of the donors.

Nine milliliters of blood was anticoagulated 9:1 with 3.8% sodium citrate.

The blood was centrifugated at 100g for 20 min to get PRP.

PRP was collected with a plastic pipette tip and used in the experiments immediately.

PRP drops (50 μ L) were placed onto sample surfaces and **incubated in a humid atmosphere for different periods (5, 10, 15, and 20 min)** (humid atmosphere prevents drop evaporation).

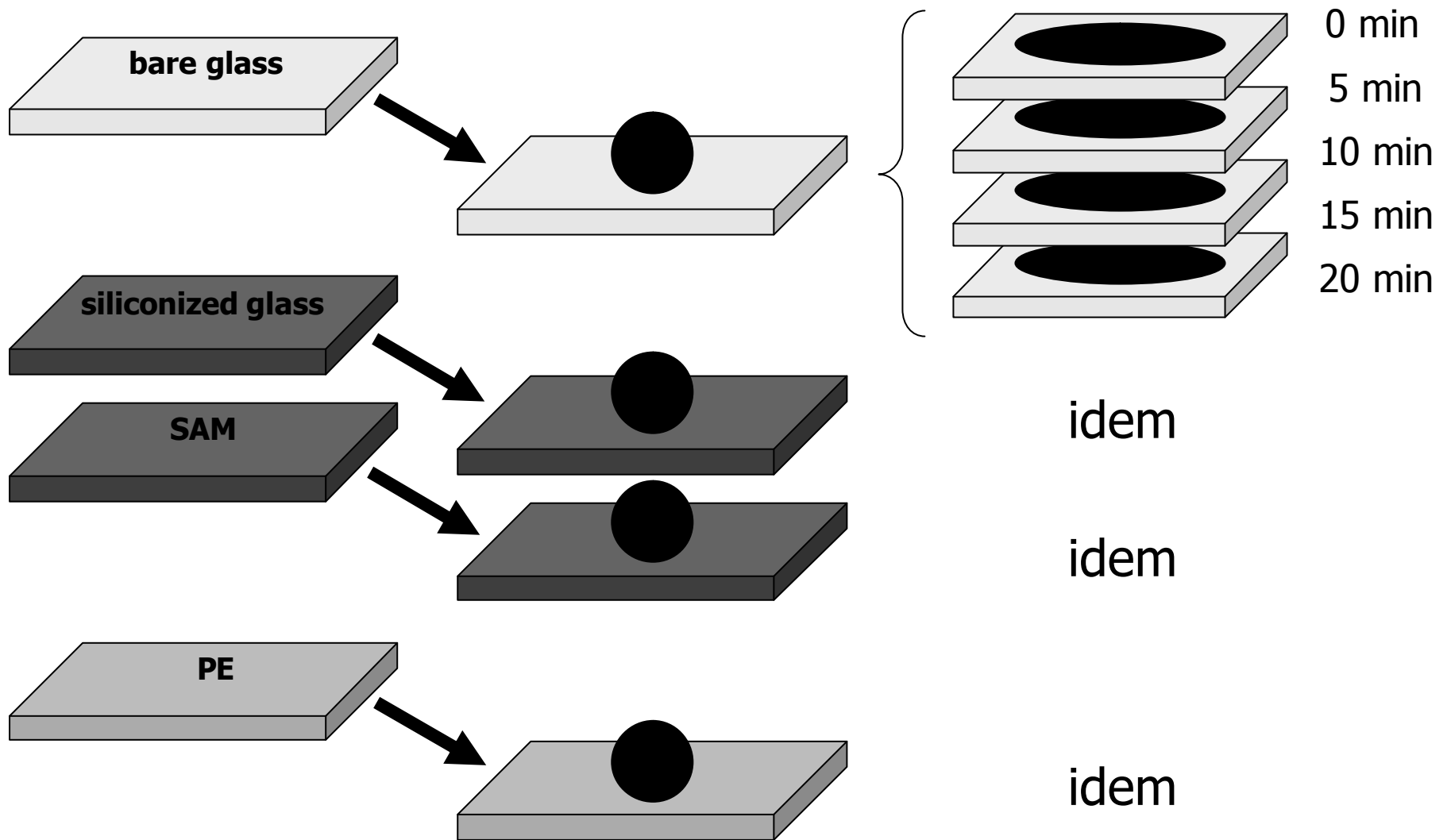
The incubation was followed by sample rinsing in normal saline and fixation in 2.5% glutaraldehyde for 30 min.

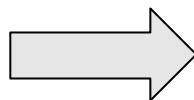
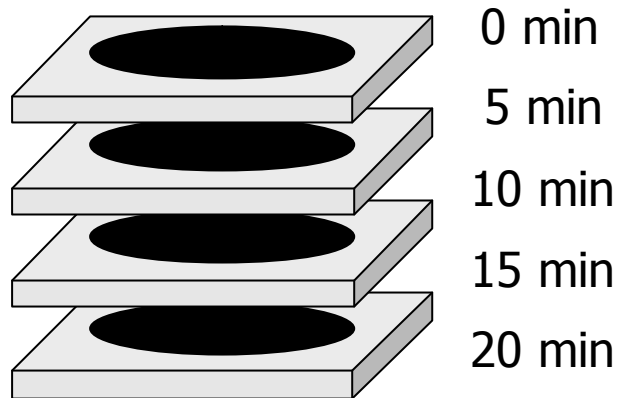
Then the samples were rinsed in distilled water, dehydrated in a series of ascending ethanols, and air-dried for 30–60 min by the standard technique.

All samples were ion sputtered with 30 nm of copper at 1.2 kV, 10 mA for 7 min (JFC-1100; JEOL, Japan).

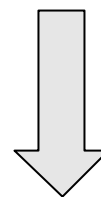
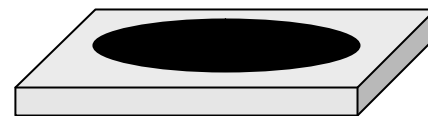
Sample surface investigations were performed by scanning electron microscope (SEM).

Then **the total amount of adhered platelets at every incubation time was determined.**

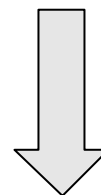
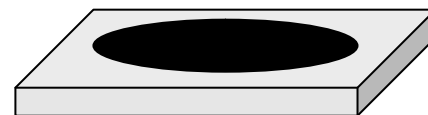




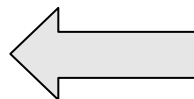
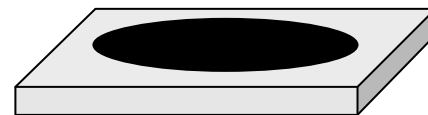
rinsing + glutaraldehyde



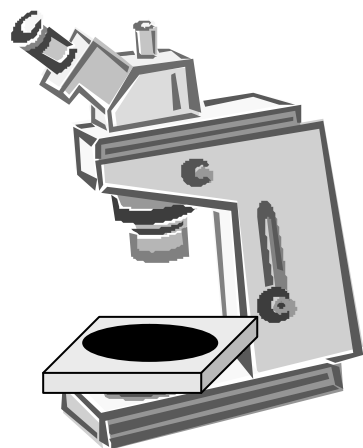
rinsing + ethanols + air-dried



sputtering



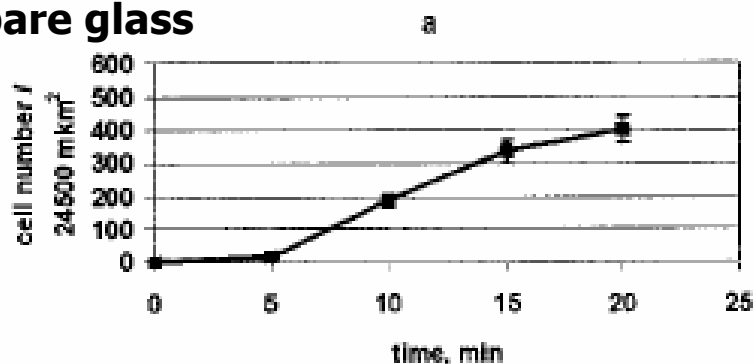
SEM



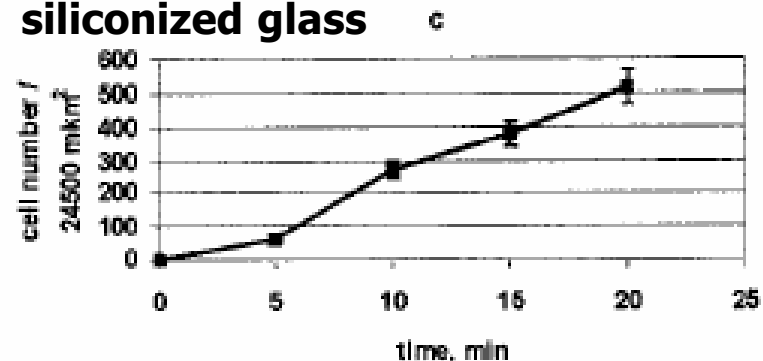
Results and Discussion

Typical kinetic curves of platelet adhesion on different surfaces are shown:

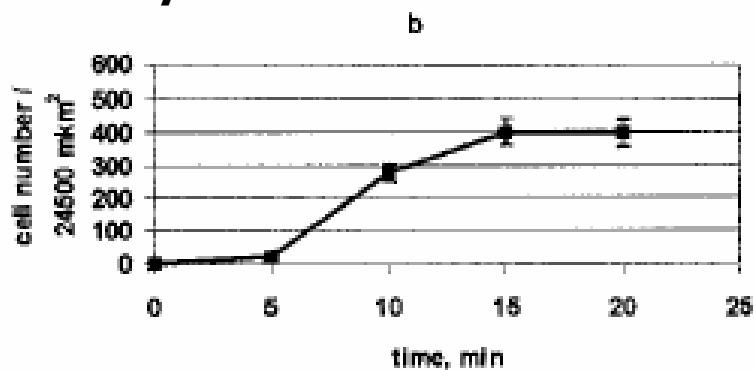
bare glass



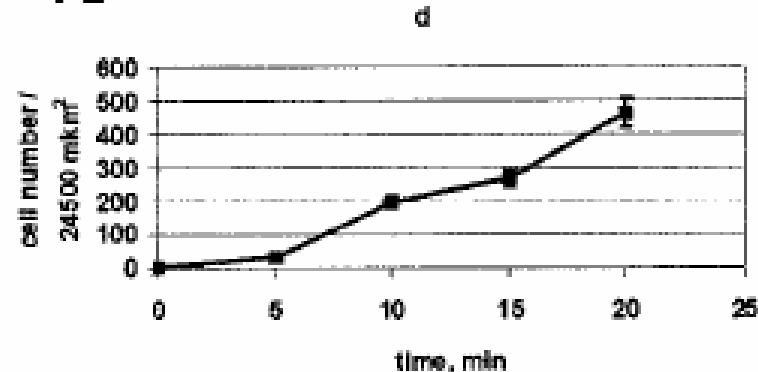
siliconized glass



hexadecyltrichlorosilane SAM



PE



The growth of adhered platelet number on bare glass [Fig. (a)] and hexadecyltrichlorosilane SAM [Fig. (b)] demonstrates s-shaped curves with saturation.

The kinetic curves of platelet adhesion on siliconized glass [Fig. (c)] and PE [Fig. (d)] have no saturation.

It is noteworthy that both saturated and nonsaturated curves are concave down in their initial parts (0–10 min).

Complex forms of the kinetic curves may be caused either by the kinetics of platelet/surface interaction or by the character of mass transfer to the surface.

Diffusion is often considered as a factor that limits adhesion (adsorption) rate and determines eventually the shape of the kinetic curve.

In the experimental scheme, however, diffusion is unlikely to have a significant role in the adhesion process.

If one imagines a layer in the vicinity of the surface where cells are transported solely by diffusion, then its assessed rate is:

$$V_{\text{diff}} = D (C_0/d)$$

where:

$D = 109 \text{ cm}^2/\text{s}$, diffusion coefficient for platelets

$C_0 = 300,000 \text{ mm}^{-3}$, bulk cell concentration

$d \sim 100 \text{ }\mu\text{m}$, effective thickness of diffusion layer.

(d is usually assumed to be much larger than the size of diffusing particles—thrombocytes with average radius $r \sim 1 \text{ }\mu\text{m}$.)

Thus, diffusion flux of cells to the surface should be:

$$V_{\text{diff}} = 0.3 \text{ [1/mm}^2 \text{ sec]}$$

However, the presented data show that the maximum experimentally observed rate of platelet accumulation on the surface is:

$$V_{\text{exp}} = 30 \text{ [1/mm}^2 \text{ sec]}$$

As $V_{\text{exp}} \gg V_{\text{diff}}$, mass transfer in the given experimental conditions is realized by mechanisms different from simple diffusion.

This conclusion is in accordance with the results of Strong, who showed that platelet adhesion is not a diffusion-limited process.

One of the probable mechanisms of cell transport in platelet adhesion experiments is **cell sedimentation in the gravitational field.**

Whereas the diffusion rate depends on particle concentration gradient at every moment, the rate of sedimentation is constant and does not depend on time and cell concentration in the near surface layer.

Consequently, **diffusion does not influence platelet adhesion kinetics under the chosen experimental conditions.**

Therefore, an adequate model of platelet/surface interaction is required, which could explain the character of processes on the surface and the shapes of the experimental curves.

Model of platelet/surface interaction

To describe and characterize mathematically the platelet adhesion process on a material surface, several assumptions should be introduced.

Consider the next scheme of platelet/surface interaction:

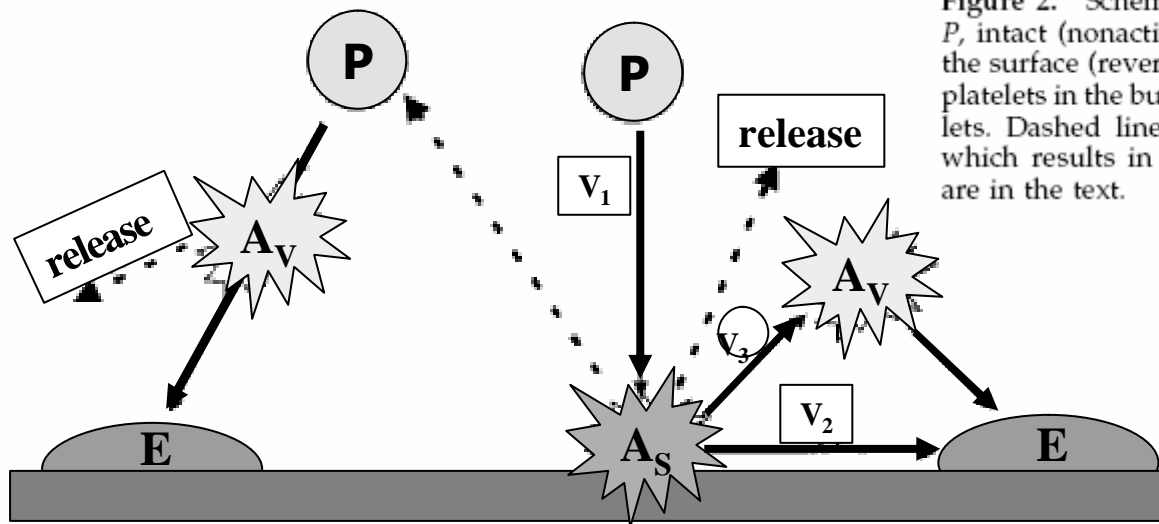


Figure 2. Scheme of platelet/material surface interaction. P , intact (nonactivated) platelets; A_S , activated platelets on the surface (reversibly adhered platelets); A_V , free activated platelets in the bulk phase; and E , irreversibly adhered platelets. Dashed lines denote release of bioactive substances, which results in intensified platelet activation. Comments are in the text.

In this scheme, cells P from the liquid phase come to the surface and interact with vacant centers N on the surface with some effective rate V_1 .

As a result, a **reversibly adhered activated form of platelet A_s appears.**

This activated platelet may further fix on the surface as irreversibly adhered form E with effective rate V_2 .

Or this activated platelet may leave the surface as free activated form A_v with effective rate V_3 .

Such free activated platelets can also adhere to the surface irreversibly.

Besides, an increase in the number of free activated forms occurs because of **the release of biologically active substances from previously activated cells.**

Thus, the number of irreversibly bound platelets E grows because of:

- reversibly bound forms on the surface A_S , in the way $A_S \rightarrow E$,
- free activated forms in the bulk A_V , in the way $A_V \rightarrow E$.

Index "S" shows that activated platelet A is on material surface and index "V" designates the same activated platelet in the liquid phase.

The rates of all processes are finite.

The number of **irreversibly adhered platelets**, E , which is observed experimentally, **increases with time right up to occupation of all accessible centers N .**

To describe this scheme mathematically we will use **fractions of adhesion centers** q_i .

Balance equation in this case is:

$$\theta_0 + \theta_{\text{rev}} + \theta_{\text{irr}} = 1$$

where:

θ_0 , vacant center fraction;

$\theta_{\text{rev}} = A_S/N$, fraction of centers, occupied by reversibly bound platelets;

$\theta_{\text{irr}} = E/N$, fraction of centers, occupied by irreversibly bound platelets;

N , constant number of accessible centers.

The next assumptions should be taken based on experimental data:

a) when given material interacts with given platelets, there is **finite number of binding centers N** (per area of platelet/material contact).
N depends both on surface properties of the material and functional properties of used platelets.

b) When calculating the number of adhered platelets on the surface, **we do not distinguish between single cells and cell aggregates.**

In other words, **cell–cell interactions in the bulk of the liquid phase or on the surface are not taken into account.**

c) The rate of accumulation of reversibly bound activated platelets V_1 is proportional to the amount of vacant binding sites on the surface:

$$V_1 = k_1 \theta_0 = k_1(1 - \theta_{\text{rev}} - \theta_{\text{irr}})$$

where k_1 [1/sec] is the effective rate constant of reversible platelet adhesion on the surface.

The physical meaning of parameter k_1 is the rate of this process at the initial moment when all binding sites are vacant.

The decrease in the number of reversibly bound platelets in the way $A_S \rightarrow E$ and in the way $A_S \rightarrow A_V$ with effective rate constants k_2 (s^{-1}) and k_3 (s^{-1}), respectively, is proportional to θ_{rev} and does not depend on time explicitly:

$$V_2 + V_3 = (k_2 + k_3)\theta_{rev}$$

Then the rate of change of reversibly adhered platelet number will be given by:

$$\begin{aligned} \frac{d\theta_{rev}}{dt} &= V_1 - V_2 - V_3 \\ &= k_1(1 - \theta_{rev} - \theta_{irr}) - k_2\theta_{rev} - k_3\theta_{rev} \end{aligned}$$

d) Generally, the rate of irreversibly bound platelet growth is determined by **the rates of change of activated forms both on the surface and in the liquid phase:**

$$\frac{dE}{dt} = - \frac{dA_S}{dt} - \frac{dA_V}{dt}$$

Reversibly and irreversibly bound platelets on the surface A_S and E are described mathematically by q_{rev} and q_{irr} .

However, there is one more variable **A_V** in this equation corresponding to **free activated platelets in the liquid phase**; to circumvent the introduction of an additional kinetic equation for A_V , we used the next approach in the model.

The rate of irreversibly adhered platelet E accumulation is determined by transitions of activated forms into irreversibly bound forms.

So, this rate is proportional at any moment to the total activated platelet concentration:

$$\frac{dE}{dt} \sim A = A_s + A_v.$$

The amount of activated platelets A in the system depends on the quantity of cells, which interacted with the surface reversibly, that is θ_{rev} .

Such reversibly bound platelets are activated by the contact with the surface and **they are able to release biologically active substances.**

This process may provoke additional platelet activation in the bulk and a substantial increase in the activated cells' generation with time.

To describe the **accumulation of activated platelets** we suggest power function of time:

$$A \sim \theta_{\text{rev}} t^m,$$

where m is a parameter that characterizes the production of free activated forms in the bulk A_V .

It reflects the contribution of processes A_V ? E into irreversible platelet adhesion.

Then the rate of irreversibly adhered platelet accumulation is:

$$\frac{d\theta_{\text{irr}}}{dt} = k_4\theta_{\text{rev}}t^m \quad (1)$$

where k_4 is a parameter characterizing irreversible binding of activated platelets A.

When there is no generation of free activated cells in the liquid phase ($m = 0$), the irreversibly adhered platelets are produced only in the way $A_S \rightarrow E$.

Equation (1) describes the total rate of irreversible binding of activated platelets both on the surface A_S and in the liquid phase A_V instead of considering reactions $A_V \rightarrow E$ and $A_S \rightarrow E$ separately: this approach significantly simplifies the mathematical formulations of the model.

These assumptions bring us to the next system of kinetic equations:

$$\begin{cases} \frac{d\theta_{rev}}{dt} = k_1(1 - \theta_{rev} - \theta_{irr}) - k_2\theta_{rev} - k_3\theta_{rev} \\ \frac{d\theta_{irr}}{dt} = k_4\theta_{rev}t^m \end{cases} \quad (2)$$

For further simplification of the model, the next assumption can be made:

$$\frac{d\theta_{rev}}{dt} \cong 0 \quad \text{or} \quad k_1(1 - \theta_{rev} - \theta_{irr}) = k_2\theta_{rev} + k_3\theta_{rev} \quad (3)$$

This condition allows solution of the ordinary differential equation (ODE) system (2) in analytical form.

This analytical solution of the system will describe irreversibly bound platelet deposition, provided the condition (3) is fulfilled in the experimental setup.

For example, the experimental procedure involves washing of the samples with removal of reversibly bound platelets, so that $A_S(t_{\text{wash}}) = 0$.

The validity of the assumption (3) in the particular experimental conditions will be discussed further.

Solving ODE system (2) with conditions (3) and, $\theta_{\text{irr}}|_{t=0} = 0$, we get:

$$\theta_{\text{irr}}(t) = 1 - \exp\left(-\frac{q}{m+1} t^{m+1}\right) \quad (4)$$

$q = k_4 k_1 / (k_1 + k_2 + k_3)$, m – positive parameters.

To avoid any uncertainty in dimension of parameter $[q] = [t]^{-(m+1)}$, **formulate Equation (4) with dimensionless variables.**

For this purpose, we assume in all previous equations

$$t = t_{\text{exp}}/T$$

where t_{exp} = experimental time in minutes,
 $T = 1 \text{ min} = \text{unit time}$.

Then t is measured in relative units, q and m are dimensionless parameters.

Furthermore, we pass on from $\theta_{irr}(t)$ to the absolute number of cells $E(t)$, observed in the experiment:

$$E(t) = E_{\max} \left[1 - \exp\left(-\frac{q}{m+1} t^{m+1}\right) \right] \quad (5)$$

The parameter E_{\max} defines the amount of adhered platelets on plateau, and the parameters q and m determine saturation time and steepness of the kinetic curve.

The values of E_{\max} , q , and m are calculated from the approximation of experimental data by Equation (5).

Therefore, **the model that includes the ODE system (2) and the condition (3) describes irreversible bound platelet accumulation $E(t)$, directly observed in the experiment.**

Unlike the original ODE system (2) without restriction (3), **our model serves only to depict irreversibly adhered cells $E(t)$, not $A_S(t)$.**

Experimentally, we can register only $E(t)$, obtained under washing conditions $A_S(t_{\text{wash}}) = 0$, which suits our model.

To study $A_S(t)$ dynamics, some additional method of detecting reversibly bound platelets is required, and the original ODE system (2) should be used to get the expression for $A_S(t)$.

But this is not necessary, because **the parameters of obtained Equation (5) provide enough information about platelet interaction with the surface.**

It is interesting to make detailed analysis of these parameters.

The parameter m , as marked above, characterizes the accumulation of free activated cells in the liquid phase: theoretical curves, corresponding to Equation (4), are:

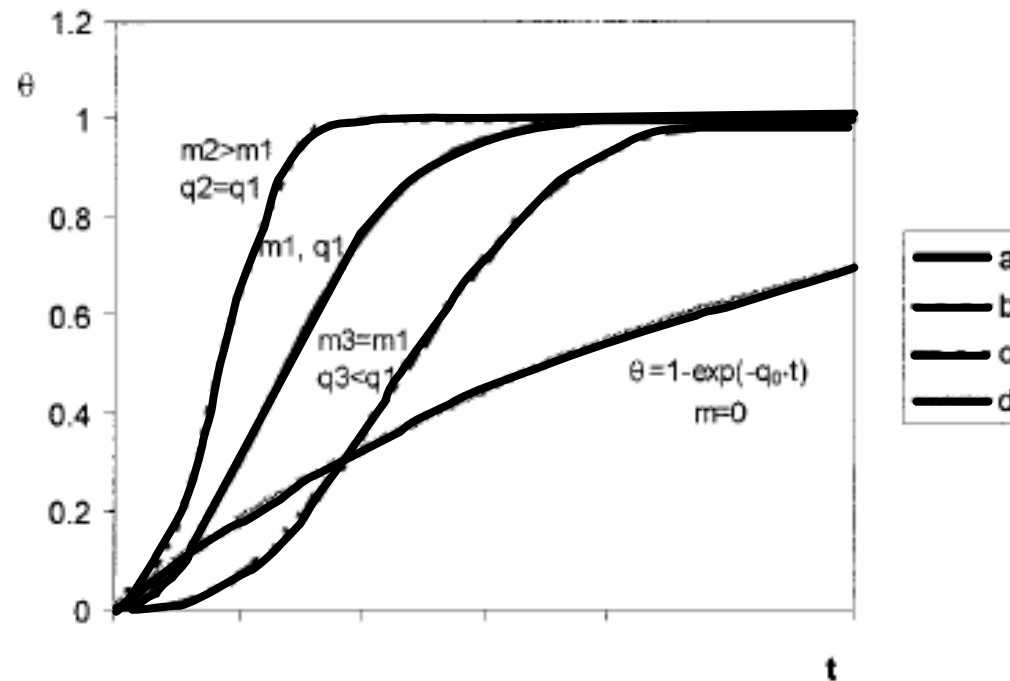


Figure 3. Theoretical curves for description of platelet adhesion on solid surfaces. Curves (a)–(c) show the effect of Equation (4) parameters on the form of the curves. When $m = 0$, curve (d) degenerates from sigmoid to exponential according to Equation (6).

In this case, an exponential dependence of E from time takes place [see Fig. 3, curve (d)]:

$$\theta_{\text{irr}}(t) = 1 - \exp(-q_0 t) \quad (6)$$

Parameter q_0 characterizes platelet adhesion in the way A_S ? E, when free activated cells A_V are absent in the bulk.

Such situation may be realized in two ways:
the conditions $k_3 = 0$ and $m = 0$ are valid, or A_V are removed by the flow in the experiment.

If the first case is realized, then every surface contacting platelet will adhere irreversibly and flow conditions would not change the type of kinetic curve.

However, in the second case, the rate of adhered platelet accumulation must decrease when we pass from static experimental conditions to flow conditions.

This result is proved by experiments by Furukawa et al. with platelet adhesion on silicone sheets.

At shear stress 5 dyn/cm², free activated cells are removed by the flow, platelets adhere only in the way A_S ? E, and the kinetic curve has exponential form.

When shear stress decreases to 0.1 dyn/cm^2 (**quasistatic conditions**), free activated cells are accumulated in the surface vicinity and the adhesion kinetic curve is s-shaped.

The analysis of Equation (4) shows that **parameter q characterizes delay time**, which is needed before intensive growth of adhered platelets on the surface begins.

The less q value, the larger time, after which the kinetic curve goes steeply upward [see Fig. 3, curves (a) and (c)].

Consequently, **the probability of activated cell accumulation in the bulk is higher, and the probability of irreversible platelet adhesion is lower.**

Indeed, a small q value may be obtained physically when k_3 value is high and the k_4 value is low, which, in turn, corresponds to activated platelet accumulation in the liquid phase.

In other words, parameter q characterizes the probability of irreversible platelet adhesion.

The parameter E_{\max} defines the total amount of binding sites on the surface for the platelets with given functionality.

The E_{\max} value in every experiment, in turn, **is determined by surface physicochemical properties and donor-dependent platelet reactivity.**

Kinetic curves may not reach plateau during the observation period for some materials.

This may happen for several reasons:

- when the values of V_2 and V_3 rates are low, the growth of irreversible platelet number E is insignificant and saturation is not reached at experimental time;

and

- intensive accumulation of strongly activated platelets in the bulk takes place because of the high m value. Such severe activation results in appearance of new binding sites on the surface.

The former case is characterized by a low amount of adhered platelets E : this situation is possible when cell/surface interaction is extremely weak.

The experimental observation time should be increased under these circumstances.

In the latter case, there are very many platelets on the surface after the longest incubation period and the rate of platelet number growth on the surface is high.

The appearance of new binding sites on the surface may be assumed as a result of strong contact platelet activation and release.

At short incubation times, when the number of binding sites is constant and determined by surface and platelet properties, the kinetic curve of adhesion is described by Equation (5).

Then, new binding sites appear because of strong platelet activation; **consequently, the kinetic curve does not reach plateau and the growth of irreversibly adhered cell number continues.**

One may suppose this situation for platelet adhesion on a PE surface (Fig. 1, d).

To allow for this process, E_{\max} **should be assumed to depend on time in Equation (5).**

Particular data determine the possibility of parameter q and m calculation from the initial part of the kinetic curve without saturation.

Thus, the proposed model explains three types of kinetic curves, observed in the experiments in vitro:

- a) sigmoid curve with saturation**
- b) nonsaturated curve**
- c) exponential curve with saturation.**

It is worth mentioning that the models that have been proposed earlier can explain only one or another type of kinetic curve.

One of the reasons of limited applicability of those models may consist in not considering in the models activated platelet accumulation in the bulk of the liquid.

Because our approach includes this effect, it gives one the opportunity to generalize the experimental data, obtained by different authors.

The kinetic data of platelet adhesion from plasma on various surfaces are described by the suggested mathematical model (Figs. 4 and 5).

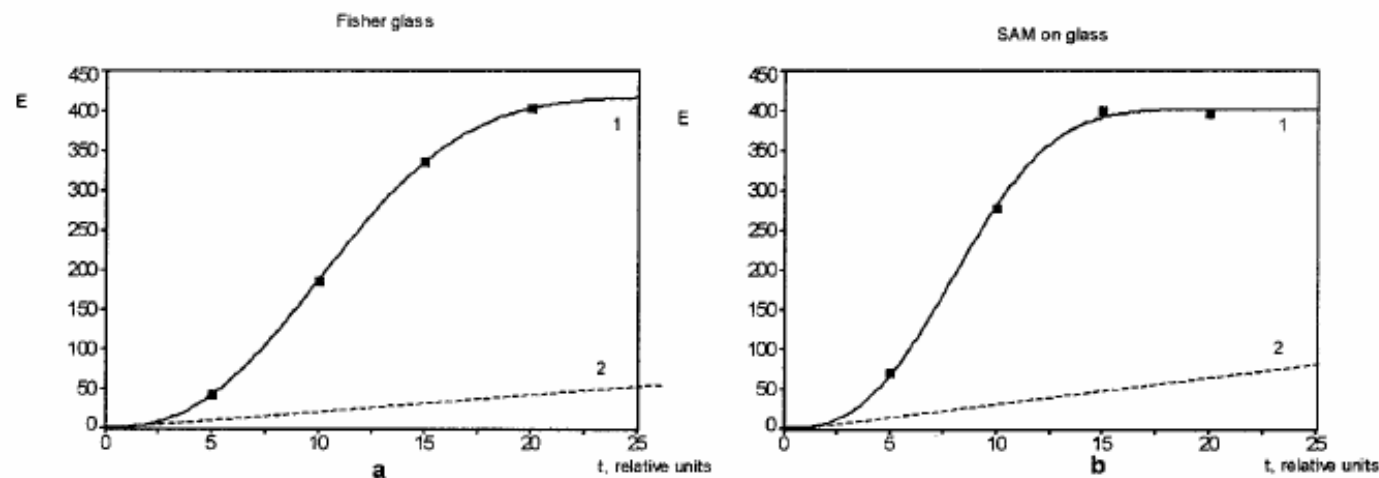


Figure 4. Description of kinetic curves of adhesion by the mathematical model. The kinetic curves with saturation: (a) bare glass; (b) hydrophobic SAM on glass. Curve 1, approximation of experimental data points by the model. Curve 2 (dashed line), theoretical adhesion curve for the situation when there are no activated cells in the bulk phase (Eq. 6).

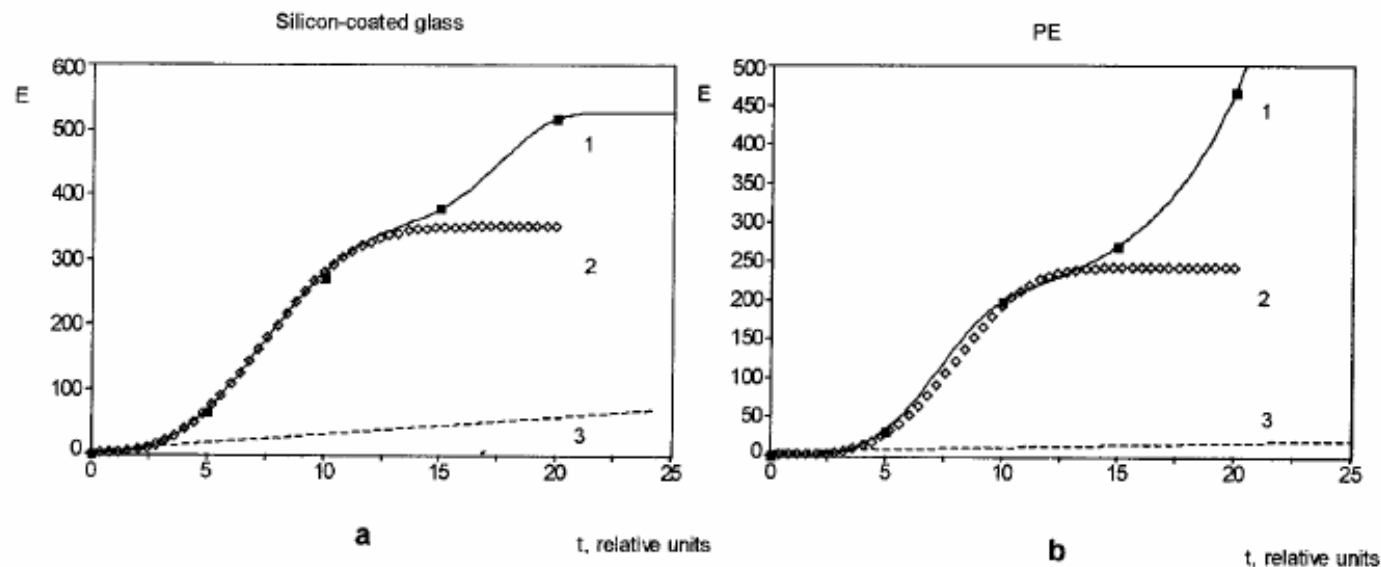


Figure 5. Description of kinetic curves of adhesion by the mathematical model. The kinetic curves without saturation: (a) siliconized glass; (b) low-density PE. Curve 1, description of all experimental data by the model with the assumption of variable number of binding sites on the surface (see comments in the text). Curve 2, model parameter assessment at the initial parts of kinetic curves. Curve 3 (dashed line), theoretical adhesion curve for the situation when there are no activated cells in the bulk phase (Eq. 6).

The curve defined by Equation (5) is drawn through experimental data points by the least-squares method.

Then the numerical parameters of the model (E_{\max} , q , m) are calculated.

The parameters for nonsaturated curves are evaluated using the initial sections of the curves (Fig. 5, curves 1).

The dashed lines in Figures 4 and 5 (curves 3) give theoretical kinetic curves, computed for the situation when $m = 0$, that is, activated platelets are absent in the bulk.

To plot these curves, calculated values q are substituted for q_0 in Equation (6).

These theoretical curves reveal the contribution of the way A ? E in irreversible cell adhesion.

The comparison of these curves with real kinetic curves proves that activated platelet accumulation in plasma is a decisive factor for platelet adhesion on all studied samples under static conditions.

To describe nonsaturated kinetic curves typical for PE and siliconized glass, one should assume that the number of binding sites on the surface N increases with time.

Presumably at first, the amount of binding sites is determined by surface properties and intact platelet functionality.

So N does not change considerably in short periods of incubation.

Furthermore, as a result of severe platelet activation, the N value sharply increases.

Let us take, for instance, the next dependence of N from time:

$$N = E_{max}^0 + C_1[1 - \exp(-C_2 t^{C_3})]$$

Where

$$E_{max}^0, C_1, C_2, C_3 = \text{some parameters.}$$

Then the kinetic curves of platelet adhesion on PE and siliconized glass are described properly by the suggested model (Fig. 5, curves 2).

The model parameter values determined by the least-squares procedure for all studied samples are presented in Table I.

TABLE I
Calculated Parameters of Platelet Adhesion
on Various Surfaces

Sample	E_{\max}	$q, \times 10^{-3}$	m
Fisher glass	416 ± 40	4.5 ± 0.5	1.51 ± 0.04
SAM	400 ± 40	6.0 ± 0.7	1.73 ± 0.09
Silicon-coated glass ^a	350	5.8	1.9
PE ^a	200	1.2	3.0

^aNote: estimates of the parameters are obtained through approximation of initial sections of the curves by the mathematical model.

Although both siliconized glass and PE samples demonstrate nonsaturated curves, **numerical parameters for these samples are different.**

This fact points at significant differences in platelet interaction with given materials.

The values q , m at the initial sections of the kinetic curve for siliconized glass are close to the corresponding parameters q , m for the glass sample.

On the contrary, the parameter estimates for PE are substantially different from those for the curves with saturation.

The absence of saturation on the kinetic curve and high m value for PE indicates **a high probability of new binding sites formation on the surface because of strong platelet activation by PE.**

For more detailed characterization of platelet/material interaction, one may apply, for example, morphological analysis of adhered platelets by SEM.

This method would provide additional information about the material's impact on the platelets.

It should be emphasized that the model for irreversibly adhered platelets neither uses nor gives any information about A_S .

Also, we do not have to describe it mathematically because reversibly adhered platelets could not be registered in the experiments.

Occasionally, one can consider (but not necessarily) the condition (3) as “steady-state” approximation for A_S .

But without experimental data for A_S , we cannot prove that “steady state” really takes place.

However, the assumption (3) is really crucial, so one should give more attention to proving its validity under given experimental conditions.

For this purpose, **the ODE system (2) without the condition (3) has been solved numerically.**

The parameters in the system k_1, k_2, k_3, k_4, m are chosen by least square method for the best fitting of experimental data.

For example, for platelet adhesion on the hexadecyltrichlorosilane SAM sample [Fig. 1(b)], the values of the parameters are assessed:

$$k_1 = 1.8, (k_2 + k_3) = 1.8, k_4 = 0.012, m = 1.8, E_{\max} = 400.$$

Calculated in this way, parameters:

$$E_{\max} = 400$$

$$m = 1.8$$

$$q = k_4 k_1 / (k_1 + k_2 + k_3) = 0.006$$

agree well with the corresponding values, obtained from approximation of experimental data by Equation (5):

$$E_{\max} = 400 \pm 40,$$

$$m = 1.73 \pm 0.09,$$

$$q = (6.0 \pm 0.7) * 10^{-3}$$

(Table I).

Equation (5) is derived from the ODE system (2) using the assumption (3), and yields the same results as the ODE system itself without this restriction.

Consequently, the condition (3) is met in our experiment.

The physical meaning of introduced assumption (3) may be explained, for example, from considering it as a “steadystate” approximation for the reactive intermediate A_S .

However, some additional measurements are required to prove a “steady state” unequivocally.

All experimental kinetic curves of static platelet adhesion (both with plateau and without plateau) are concave down in their initial parts.

Such shape of kinetic curves is explained by our model as a result of **platelet activation and accumulation in the liquid phase.**

Maximum effect of activated platelet accumulation is realized experimentally through high cell concentration in the PRP, no stirring or cell removal from initial contact site (no shear flows).

All these factors lead to activated platelet accumulation near the surface; this, in turn, increases the probability of activated platelet adhesion on the surface.

Thus, chosen experimental conditions are stricter than conditions of intended material use as a part of blood contacting medical device.

When the material operates in physiological surroundings, platelet adhesion and material thrombogenic properties may be less pronounced than in the static in vitro experiments.

An experimental scheme providing maximum impact of material surface on platelets is necessary to emphasize the effect of material on platelet activation in the bulk.

Conditions of medical device use are crucial for the appearance of activated platelets in the liquid phase.

For example, **small-diameter vascular grafts have high area/volume ratio and low blood flow rate, which increases the risk of platelet adhesion through the bulk platelet activation mechanism.**

Simultaneously, the same materials are suitable for large-diameter prosthetic vascular graft production.

So, these differences may be accounted for within the framework of the proposed model.

Conclusion

The scheme of platelet/surface interaction and the kinetic model for static platelet adhesion on a solid surface are suggested.

The elaborated approach takes into consideration not only irreversibly adhered platelets, but free activated platelet accumulation in the bulk of the liquid phase.

The proposed model is shown to describe the experimental kinetic curves of irreversible platelet adhesion obtained by SEM.

Unfortunately, in the used experimental scheme, only irreversibly surface-bound cells may be registered by SEM.

Some additional methods are required to measure activated platelets in the bulk.

For example, the method to measure adenosine diphosphate (ADP) release from platelets upon their contact with different surfaces would prove the role of release reactions in the platelet/surface interaction.

Besides, laser diffraction spectroscopy may be used to control platelet parameters in the bulk phase (size, refraction index, etc.) and thus to quantify free activated platelets in plasma.

Then, **the model will be extended to include the kinetics of free activated cells.**

Thus, measurements of platelet activation in the bulk and its mathematical description are the object of further study.

The suggested model explains three types of kinetic curves of adhesion, obtained by various authors: sigmoid curves with or without saturation and an exponential curve with saturation.

According to the model, the shape of the curve is determined by material surface properties and experimental conditions of cell/surface interactions.

Experimental conditions providing activated platelet accumulation in the liquid phase are worked out to verify the main suppositions of the model.

Sigmoid kinetic curves of platelet adhesion obtained in static conditions are described mathematically with the help of the proposed model.

Calculated parameters of the model quantitatively characterize platelet adhesion on the solid surface.