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Quantitative Modeling of Fibrinogen Adsorption on Different Biomaterials

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Abstract—Adsorption processes of biological macromolecules on solid surfaces, e.g. on implant materials, are of scientific, medical and technological interest. The adsorption process of the blood plasma protein fibrinogen from aqueous solutions on biomaterial surfaces is simulated by solving the diffusion equation in one dimension in combination with a two dimensional cellular automaton model. The adsorption/desorption kinetics of fibrinogen on different metallic biomaterials is tracked until a dynamic equilibrium is reached. In addition to mass transfer towards the surface, adsorption of fibrinogen molecules and surface diffusion of single fibrinogen molecules and clusters the algorithm also accounts for the desorption of single molecules. By varying the desorption behavior in the simulation calculation, the equilibrium surface coverage changes in the range of 0.2–0.85, implying more flexible and higher surface coverage than previous models. The influences of surface properties of the biomaterials and of the initial concentration of fibrinogen on the adsorption process are quantified. Experimental results from the literature on fibrinogen adsorption kinetics are compared with our simulation results and illustrate good quantitative agreement that is only achievable using desorption as essential model component.

Keywords—Adsorption, Biomaterials, Computer simulation, Fibrinogen, Kinetics.

INTRODUCTION

Adsorption processes of biological macromolecules on solid surfaces occur under a variety of circumstances, e.g., on the materials of bone or dental implants in the human body or on packing materials in the food industry.

The adsorption of blood plasma proteins on biomaterials is a key process that governs the response of the human body to the implant material. Consequences of protein adsorption include blood coagulation, platelet adhesion, plaque formation or fouling.[6] For the integration of artificial biomaterials in the human body, one of the most abundant blood plasma proteins, fibrinogen, plays a crucial role. Since fibrinogen includes both hydrophobic and hydrophilic regions, it possesses amphiphilic properties. This entails that fibrinogen can adsorb on nearly all kinds of surfaces.[11] Cell binding sites in hydrophobic and hydrophilic regions of the molecule facilitate the cell aggregation ability. In combination with its function as a precursor of the blood coagulation cascade, fibrinogen is assumed to be one of the major factors causing thrombogenesis on biomaterials.[21] Additionally, the fibrinogen adsorption process is of utmost interest owing to the significant role of fibrinogen during the formation of the conditioning film on implant surfaces. This formation is the precondition to the medically highly relevant and problematic attachment of bacteria to implants.[15,19]

Although protein adsorption has been studied experimentally in numerous instances, the underlying physical principles that are leading to function, structure or the development of active sites of adsorbed proteins are not fully understood and require further studies.[14] Insight from experimental studies that is substantiated by theoretical analysis is a prerequisite to better control of adsorption processes and their consequences.

Since the discovery of the Vroman effect,[38] i.e. a characteristic temporal adsorption order of blood plasma proteins, the kinetics of fibrinogen adsorption processes on solid surfaces is continuously being investigated in biomaterials research. Thus, a wide variety of experimental studies dealing with the adsorption kinetics on different surfaces is documented

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in the literature.[9,12,13,18,25,32] In these studies it is shown that an adsorption–desorption equilibrium is reached with a specific equilibrium surface coverage during the fibrinogen adsorption process.

Numerical studies on atomistic length scales, e.g., molecular dynamics simulations, can in principle give detailed insight into the structure and properties of adsorbing proteins. However, considering the necessary statistics and time scale, at present a realistic study of the protein adsorption kinetics requires macroscopic simulation approaches.[23] Frequently used approaches for simulating protein adsorption kinetics include the random sequential adsorption (RSA) model based on Monte-Carlo type simulations, and the ballistic deposition (BD) method.[31] The RSA algorithms that were introduced in the 1960s describe the deposition (irreversible adsorption) of particles on surfaces on randomly chosen adsorption sites. Recent progress in RSA modeling includes the description of protein adsorption with diverse protein shapes and the incorporation of the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory.[1–3,28,39]

In contrast to the RSA models, BD models employ a more particle based approach. Single proteins are treated as independent particles that do not influence each other in particular in the solution prior to adsorption. Thus the transport of proteins to the adsorbing surface is modeled using, e.g., a particle based Brownian motion approach combined with external force fields like gravity.[31] One of the first studies using a BD model for describing the deposition of globular proteins introduces interaction energies between proteins and proteins and proteins and surface calculated according to the DLVO theory.[26]

An approach simulating the adsorption process of different globular proteins (lysozyme, albumin) with a conformational change after adsorption has recently been presented.[5]

The models using BD type methods describe the transport process of the proteins accurately. The chosen adsorption sites result from of the trajectories of the particle in the solution and are not randomly chosen like in RSA approaches. With a hybrid finite difference/cellular automaton (CA) approach the relevant modeling/computational effort is directed on the processes that are involved in the response of proteins to biomaterials, rather than on the transport processes from the solution to the interface. Our previously published hybrid FD/CA model includes mass transfer toward the surface by diffusion, adsorption of protein molecules, surface diffusion of both protein molecules and clusters as well as inner-cluster diffusion.[33]

However, despite the progress in modeling the adsorption kinetics of proteins with the above-mentioned approaches, ample open questions remain. It has been shown experimentally that achievable equilibrium surface coverage for fibrinogen adsorption ranges from ≈0.2 to ≈0.8.[4,9] This wide range is, to our best knowledge, so far not covered by previous models.

In the present work, we propose an essential extension to our previously published hybrid FD/CA) model[33] by incorporating desorption processes. Surface induced phenomena as, e.g., changes in the proteins' conformation on the surface after the adsorption process are macroscopically incorporated. By implementing the desorption process, the time scale of the adsorption kinetics is treated adequately.

In the extended model, we focus on the dynamics of the fibrinogen adsorption and desorption processes for various degrees of surface coverage.

In a parameter study, a large range of initial fibrinogen concentrations in the solution and different materials surfaces are treated. In addition, experimental results from the literature on fibrinogen adsorption kinetics are compared with our simulation results.

MODEL DESCRIPTION

Basic Assumptions

An adsorbing biomaterial surface submerged in an aqueous solution containing fibrinogen is considered. Adsorption is modeled until a single monolayer is formed, which is realistic for initial fibrinogen concentrations in solution up to 1 mg mL^{-1}.

Details of the numerical procedure are given elsewhere.[33] Here we outline the principle of the model and specify its extensions with respect to previously published work. For the representation of the geometry of the surface we use a square grid with a constant spacing of 15 nm. The morphology of a fibrinogen molecule is approximated by considering its experimentally determined length and width (45 and 15 nm, respectively). Thus, an aspect ratio of 3:1 is attributed to the molecules. The alignment of the fibrinogen molecules on the grid is horizontal or vertical. Other orientations are not allowed, resulting in a limited achievable cluster packing density. However, the main features of the simulation remain unaffected (see Discussion in Siegismund *et al.*[33] and below). All lattice sites adopt one of two possible cell states. The first indicates an uncovered surface, the second a coverage by a part (a third) of a fibrinogen molecule. Adsorption, desorption or surface diffusion events cause a change of state of the involved lattice sites.

The adsorption of fibrinogen molecules from a homogeneous solution leads to a concentration gradient in the solution and to diffusion of fibrinogen

toward the surface. To determine the quantity of fibrinogen molecules arriving at the solid/liquid interface, Fick's second law is applied.

Fibrinogen molecules close to the material's surface that are not absorbed remain in the solution and change the local concentration, the diffusion process in the water and the subsequent adsorption processes.

All processes influenced by the surface (adsorption, movement on the surface, and desorption) are modeled by introducing the interaction energy E_i, which is a strongly surface dependent parameter. For E_i the value determined by atomistic simulations[30] is used. The absolute value reflects the energy that is necessary to detach a single adsorbed fibrinogen molecule. In the present study it is attributed a negative value because the adsorbed protein is favorable, leading to an energetic gain.

The magnitude of E_i can be derived from simulation calculations or from measured surface properties. E_i is available for a few materials from molecular dynamics calculations.[30] For hydrophobic surfaces like graphite E_i is ca. -1200 kJ mol^{-1}.[30] For hydrophilic surfaces like mica E_i is one order of magnitude higher.[30] Such values for E_i are plausible, since the 20 proteinogenic amino acids interacting with a gold surface achieve interaction energies between -18 and -44 kJ mol^{-1}.[17] For fibrinogen several hundred residues per molecule are in contact with the surface[8] (due to steric effects and the hindered rotation of the peptide bond between the amino acids within fibrinogen, the actual interaction of one residue is expected to be weaker than for single amino acids as determined in Hoefling *et al.*[17]).

The adaptation of E_i in the present work to each surface is in accordance with the simulated values as well as the ones that are to be expected due to the variations of contact angles of water on the surfaces of different materials.

After the diffusion of fibrinogen molecules toward the surface, the probability of molecule adsorption is determined by:

$$P_A = 1 - \exp\left(\frac{E_i}{Z}\right) \quad (1)$$

P_A is the probability of adsorption of a fibrinogen molecule, E_i the surface dependent interaction energy and Z the virtual lattice temperature in R units. A random number between 0 and 1 is generated. If the number is below the calculated P_A, the molecule adsorbs.

The adsorbed fibrinogen molecules undergo translative movements on the surface, possibly forming clusters. Clusters are two or more fibrinogen molecules that are neighboring each other in the square grid. Movements of molecules inside the clusters are possible and may change the molecule density within the clusters.

For the diffusion of fibrinogen molecules within a cluster, the movement probability decreases with an increase of neighboring occupied lattice points. Due to a lack of more precise information we assume a linear dependency, and thus implicitly assume that the binding sites of a molecule are energetically equal. Further details of the algorithm can be found elsewhere.[33]

Desorption

In addition to the model features as described in Siegismund *et al.*[33] desorption is considered, allowing the detachment of fibrinogen molecules that are adsorbed as single molecules or as part of fibrinogen clusters.

Two different types of desorption events of fibrinogen molecules are implemented. The first is the desorption of a single molecule that is not in contact with other molecules, the second is the desorption of a single molecule from a cluster. The desorption of entire clusters is assumed to be improbable and not considered.

The desorption probability of a fibrinogen molecule is influenced by the neighboring molecules and, in analogy to the adsorption process (Eq. (1)) by the surface dependent term E_i. E_i reflects the affinity of fibrinogen to the given biomaterial surface and is strongly dependent on the surface characteristics, e.g., hydrophobicity.

A surface dependent desorption parameter D_P is introduced that incorporates surface dependent processes occurring after protein adsorption (which differ for different materials) on a macroscopic length scale. D_P reflects conformational rearrangements in the secondary structure of the fibrinogen molecules (e.g., the ratio between α-helix and β-sheet structures) and other processes that lead to an energetic gain after adsorption, thus, lowering the probability of desorption.

The sheer fact that fibrinogen molecules cluster on a surface indicates that the more neighboring cells of a fibrinogen molecule are occupied, the lower is the desorption probability. This reasoning is confirmed by recent observations of the formation of fibrinogen clusters on solid surfaces.[20] Due to a lack of more detailed information, a linear dependence of desorption probability on the number of neighboring molecule parts is used, assuming implicitly that the binding sites of a molecule feature similar binding energies with the neighboring molecules.

In analogy to the adsorption probability, the surface dependent desorption probability P_D of a single fibrinogen molecules is defined by:

$$P_D = D_P \exp\left(\frac{E_i}{Z}\right) \quad (2)$$

E_i is again the interaction energy, adopting the same value as for the adsorption process.

D_P correlates with parameters characterizing the surface properties (e.g., hydrophobicity or surface charge) for a material. Since for the materials used in the present work, such values were not available in the literature therefore D_P is adjusted to fit the experimental results.

Due to the dynamics of the model, the desorption process is not a simple reduction of the number of adsorbed proteins in the adsorption calculation. In fact, all model parts leading to protein movement and cluster formation change the surface configuration. Thus the starting point for desorption relies on the newly achieved coverage scheme at each point in time.

In this study we do not aim for the tracking of surface induced phenomena, e.g., the change of the secondary structure for every single protein, but rather on an overall macroscopic description using D_P and E_i.

The model thus contains five successively executed parts: (1) mass transfer toward the surface (diffusion); (2) adsorption of a defined fraction of the protein molecules; (3) surface diffusion; (4) inner-cluster diffusion; (5) desorption. It is implemented in the numerical computing environment MATLAB (The MathWorks, Inc., Natick, MA, USA). A schematic overview of the five different mechanisms included in the model is presented in Fig. 1.

RESULTS

Equilibrium Surface Coverage

Simulation of fibrinogen adsorption as documented in the literature has so far mostly been limited to cases with low surface coverage.[2,26,28] The present model deals with fibrinogen adsorption processes for a broad equilibrium surface coverage range from 0.2 to 0.85.

The two most important parameters influencing the kinetics of attaining the equilibrium surface coverage are the surface dependent desorption parameter D_P and the initial fibrinogen concentration in solution c_0.

In Fig. 2 it is shown how a variation of the desorption parameter D_P changes the calculated surface coverage as a function of time and the asymptotic value of the equilibrium surface coverage. Each of the curves represents the average of five independent simulation runs (as in all results presented in this paper). For the lowest surface coverage of ~0.2, the asymptotic value is reached after about 1000 s, for intermediate coverage of ~0.48 after 4000 s and for high surface coverage of ~0.8 after 6000 s. As expected, during the *initial* stages of the adsorption process up to ~100 s, desorption does not play a significant role, and the curves for the different desorption parameters coincide.

Figure 3 shows the fibrinogen adsorption kinetics for different initial fibrinogen concentrations in solution c_0. Unlike in Fig. 2, the initial fibrinogen concentration influences the values and consequentially the complete shape of the curve from the beginning.

Comparison with Experimental Results

Simulation results obtained with the present model are compared quantitatively with experimental studies of different authors (see Figs. 4 and 5 and Table 1) focusing on fibrinogen adsorption kinetics on different solid surfaces.[4,12,13,25]

The initial concentration of fibrinogen in solution has been shown to drastically change the equilibrium surface coverage on stainless steel (316 LVM).[9] In Fig. 4, experimental results of Desroches and Omanovic[9] and our simulation results for the initial fibrinogen concentrations $c_0 = 36\ \mu g\ mL^{-1}$ and $c_0 = 71\ \mu g\ mL^{-1}$ are compared.

For the simulation results shown in Fig. 4, all simulation parameters and conditions (e.g., physical constants and temperature) were identical, except the initial fibrinogen concentration c_0 that was set to the experimental values as given in Desroches and Omanovic.[9]

Adsorption thermodynamics and kinetics of fibrinogen on three different biomaterial surfaces were subject of a study in Bai *et al.*[4] In Fig. 5, the results of the study concerning the fibrinogen adsorption kinetics on NiTi are compared with our simulation results. Bai *et al.*[4] also presents adsorption results on stainless steel and on Ti. The simulation results for stainless steel correlate similarly well as for NiTi (not shown here). On Ti, a multilayer adsorption process is described in Bai *et al.*[4] that is not implemented in the present model.

In addition to D_P, the value for the interaction energy E_i is a crucial parameter for the simulations, as it includes the characteristics of the adsorbent material. Regarding the kinetics of the fibrinogen adsorption process, E_i influences the value of the equilibrium surface coverage without changing the time scaling behavior (curve shape). The applied values for stainless steel and NiTi are -1400 and $-1500\ kJ\ mol^{-1}$, respectively, thus being in the plausible order of magnitude for materials with this degree of hydrophobicity. D_P is fitted to 0.04.

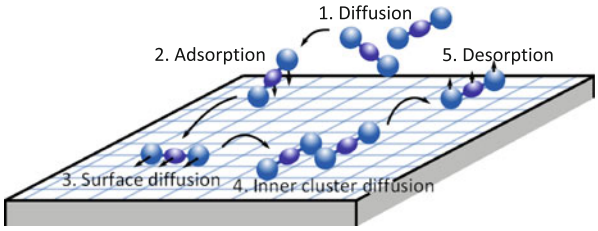

FIGURE 1. Illustration of the processes simulated by the present model.

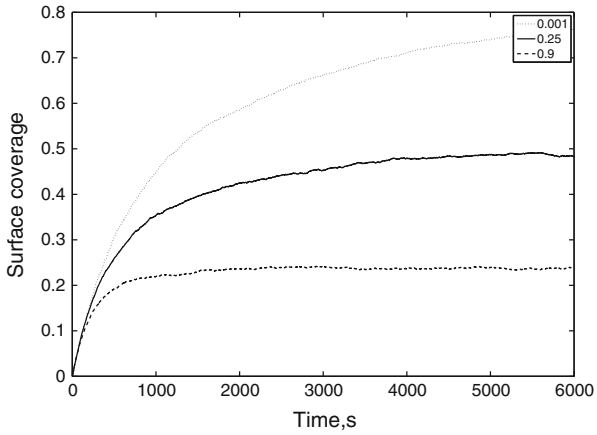

FIGURE 2. Surface coverage as a function of time for different desorption parameters D_P, the asymptotic final value illustrates the range of equilibrium surface coverage that can be reproduced by the present model; $E_i = -2500$ kJ mol^{-1}; $c_0 = 36$ μg mL^{-1}.

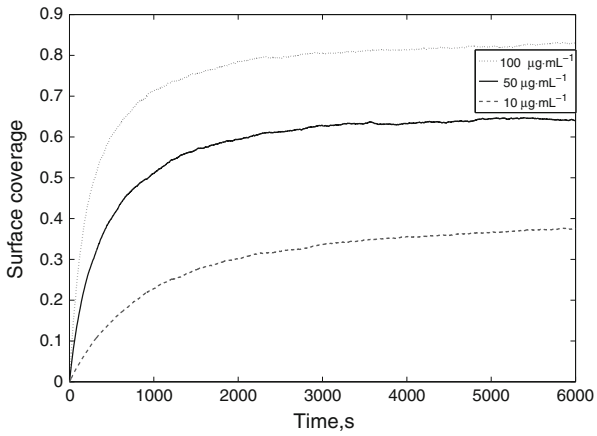

FIGURE 3. Influence of different initial fibrinogen concentrations c_0 in solution on the fibrinogen adsorption kinetics; $D_P = 0.04$; $E_i = -1400$ kJ mol^{-1}.

A comparison of the present model, the model presented in Siegismund *et al.*[33] (without desorption) with the experimental results of the fibrinogen adsorption process on stainless steel (Desroches and Omanovic[9]) is shown in Fig. 6.

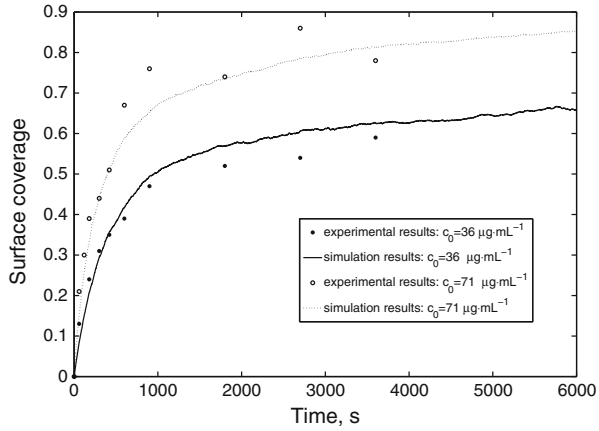

FIGURE 4. Fibrinogen adsorption on stainless steel, comparison of simulated and experimental[9] results for two initial fibrinogen concentrations; $D_P = 0.04$; $E_i = -1400$ kJ mol^{-1}.

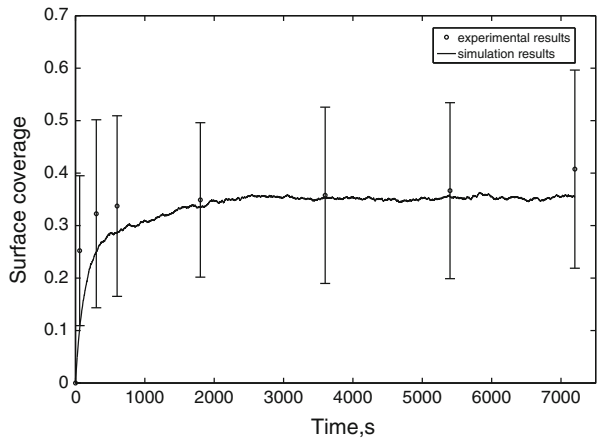

FIGURE 5. Fibrinogen adsorption on NiTi, comparison of simulated and experimental[4] results; $c_0 = 50$ μg/mL; $D_P = 0.38$; $E_i = -1500$ kJ mol^{-1}.

For low to medium initial fibrinogen concentrations in solution up to an initial concentration of 1 mg mL^{-1}, fibrinogen adsorbs according to a Langmuir type adsorption process.[4,9] Such simple conditions allow to compare our model to analytical equations. The kinetics of the Langmuir adsorption can be mathematically described by a pseudo-first-order kinetic model[9]:

$$\theta(t) = \frac{k_a c_0}{k_a c_0 + k_d}(1 - \exp(-k_a c_0 + k_d)t) \qquad (3)$$

where $\theta(t)$ is the surface fibrinogen coverage at a given time, c_0 the initial fibrinogen concentration in solution, k_a the adsorption rate constant, k_d the desorption rate constant and t the time.

Equation (3) is used by several authors[9,12,25] for fitting experimental results on fibrinogen adsorption

and determining the rate constants k_a and k_d. In Table 1, an overview is given over different experimentally determined rate constants for fibrinogen adsorption and those determined from our simulation results by fitting with Eq. (3).

Clustering Behavior of Fibrinogen on Surfaces

In Fig. 7 an overview over fibrinogen clustering in dependency on the desorption parameter D_P is presented. The upper part (Fig. 7a) shows the kinetics of the numbers of clusters for $D_P = 0.04$ and 0.9. In the lower part (Fig. 7b) snapshots of the surfaces at three different time points (250, 1000, and 2000 s) are presented.

For the low value of D_P ($D_P = 0.04$), the number of clusters rapidly increases up to ≈140 after 250 s. This peak is followed by a decrease of the number of clusters to one after 750 s. For the large value of D_P ($D_P = 0.9$), the clustering process is decelerated in comparison with $D_P = 0.04$. The number of clusters reaches ≈75 after 250 s, followed by a slow decrease to ≈15 after 2000 s. The snapshots in Fig. 7b support this observation showing inherently different clustering behavior. For $D_P = 0.04$ the formation of large clusters is fast and leads to a dense network after 1000 s. For $D_P = 0.9$ the clusters are significantly smaller at early stages of the adsorption process. Numerous single molecules remain after 250 s and evolve to only a few larger clusters (1000 s). Eventually, one big aggregate has formed after 2000 s.

DISCUSSION

In the present hybrid FD/CA model we incorporated desorption processes to reproduce equilibrium surface coverage which has been shown experimentally to fall in a range between ≈0.2 and ≈0.8. The desorption parameter D_P sets the probability of a desorption event of a single fibrinogen molecule. Surface dependent processes occurring after protein adsorption, e.g., conformational rearrangements, are adopted into D_P macroscopically. It has been shown that hydrophobicity determines the prevailing secondary structure of fibrinogen.[35] Thus, D_P is dependent on the material's surface. Its values range between 0.001 and 0.9. The strong influence of D_P on the simulation results is illustrated in Fig. 2. If the desorption constant D_P is varied, the simulation results follow the expected behavior. Particularly, at early stages of adsorption when the surface coverage is low, the curves for different initial concentrations coincide due to the excess of fibrinogen molecules in solution at the interface, and due to the small quantity of adsorbed fibrinogen molecules that originated from the previous time steps, hence D_P has a minor effect.

Thus, at this stage essentially only adsorption events occur, and different desorption probabilities have no effect. Later on, different values of the desorption parameter D_P have a drastic impact on the equilibrium surface coverage equilibrium.

In Fig. 7 the impact of D_P on the clustering behavior is shown, which is inherently different despite the kinetic aspects discussed above. Not only the interaction energy E_i influences the clustering behavior (as shown in our previous study[33]), but also the desorption characteristics play a significant role. Adsorbed fibrinogen molecules are the precondition for a thrombin-mediated fibrin network formation and subsequently lead to different inflammatory and thrombogenic responses.[10] Due to different binding sites of the fibrinogen molecules to platelet integrins (e.g., GPIIb/IIIa), the spatial distribution of fibrinogen on surfaces is a key feature.[16] Low values of D_P lead rapidly to a very dense fibrinogen network owing to the low desorption probability of fibrinogen molecules, which implies a higher overall resident time of the adsorbed molecules. As a consequence, surface diffusion which is faster for monomers and small clusters thus facilitates the faster formation of a fibrinogen network.[20]

For $D_P = 0.9$ the resident times of fibrinogen molecules are lower, and subsequent clustering is slower. Despite the surface chemistry, desorption is an important factor influencing the dynamics of fibrinogen network formation and hence the dynamics of inflammatoric behavior of biomaterials. Especially the exposure of cryptic, naturally hidden binding sites is crucial which is affected by the fact how and how fast the fibrinogen network is developed.[22]

Conformational changes of the fibrinogen secondary structure caused by the interaction with the material's surface are incorporated in the model by changing D_P. Especially the ratio between α-helices and β-sheet structures is different for different surfaces.[36] This affects the number of binding sites with the surface and yields a change of the value of D_P.

Certainly there are also changes in the conformation of adsorbed fibrinogen molecules depending on the residence time of fibrinogen on the surface and the initial fibrinogen concentration in the solution.[7,34] These processes may be implemented in the model as soon as reliable values with statistical validation are available. For the initial concentrations employed in the present study, the equilibrium surface coverage values are well represented by the model. This underlines that the simplifying assumption for the arrangement of fibrinogen molecules either horizontally or vertically does not deteriorate the simulation

TABLE 1. Different kinetic adsorption parameters determined in experimental studies in comparison with our simulation results: initial fibrinogen concentration in solution (c_0); adsorption rate constant (k_a); desorption rate constant (k_d); contact angle with water (θ_c).

Surface	c_0 (μg mL^{-1})	k_a (mL μg^{-1} s^{-1})	k_d (s^{-1})	Θ_c (°)	References
Stainless steel	36	4.26×10^{-5}	1.39×10^{-3}	61.1	Desroches and Omanovic[9]
		2.93×10^{-5}	7.30×10^{-4}		This work (simulated data $D_P = 0.04$; $E_i = -1400$ kJ mol^{-1})
Stainless steel	71	3.46×10^{-5}	6.70×10^{-4}	61.1	Desroches and Omanovic[9]
		2.57×10^{-5}	6.72×10^{-4}		This work (simulated data $D_P = 0.04$; $E_i = -1400$ kJ mol^{-1})
NiTi	50	1.57×10^{-4}	1.25×10^{-2}	68.8	Bai *et al.*[4]
		2.35×10^{-5}	2.44×10^{-3}		This work (simulated data $D_P = 0.38$; $E_i = -1500$ kJ mol^{-1})
Hydrophilic silica	100	4.06×10^{-5}	3.50×10^{-4}–2.5×10^{-6}	28.0	Nygren *et al.*[25]
		2.33×10^{-5}	5.99×10^{-4}		This work (simulated data $D_P = 0.01$; $E_i = -1000$ kJ mol^{-1})

results significantly. A surface coverage of 0.85 can still be simulated with the present model, while earlier approaches based on RSA reach the jamming limit at significantly lower surface coverage.

The initial concentration of fibrinogen molecules in the solution has a drastic effect on the overall simulation results. Due to the interrelation of the five different processes accounted for in the present model, all aspects of the adsorption kinetics are influenced strongly by a variation of the initial concentration (see Fig. 3).

It has been shown in different experimental and theoretical studies that a higher amount of blood plasma proteins is adsorbed on uncharged hydrophobic surfaces, as compared to hydrophilic surfaces.[24,30,37] The reason is that there is a higher probability for water molecules to stay closer to the hydrophilic surface as compared to hydrophobic ones, as demonstrated by molecular dynamics studies.[29] Consequently, the protein detachment process and surface re-hydration is facilitated for hydrophilic surfaces,[37] leading to a lower interaction energy E_i. With the present model these correlations have been validated with interaction energies for the simulation runs of -1400 kJ mol^{-1} for stainless steel and -1500 kJ mol^{-1} for NiTi. The contact angle with water on the two materials has been determined to be 61.1° and 68.8° respectively.[4,9] In our previous study we applied a higher value for the interaction energy of -1630 kJ mol^{-1} for a more hydrophobic silicon surface with a contact angle with water of 97.6°.[33] Thus, hydrophobicity appears to be one of the main influencing parameter that governs the effect of the surface on fibrinogen adsorption. A decrease of the surface hydrophobicity leads to a decrease of the interaction energy E_i.

Exemplary simulation runs without desorption on stainless steel with a $c_0 = 36$ μg mL^{-1} demonstrate the pronounced influence of desorption on the simulation results (see Fig. 6). Acceptable agreement between experimental and simulated results can only

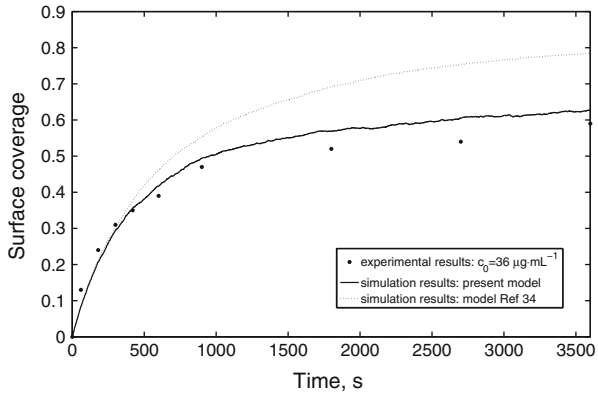

FIGURE 6. Comparison of the present model with the model used in Siegismund *et al.*[33] (no desorption); experimental results of fibrinogen adsorption on stainless steel taken from Desroches and Omanovic[9]; $D_P = 0.04$; $E_i = -1400$ kJ mol^{-1}.

be achieve if desorption is included. Especially at the later stages of the adsorption process after 500 s, there is significant deviation between the curves, since the surface coverage originating from the preceding time steps leads to a larger contribution of desorption.

The time scale of the CA model matches the real time scale for fibrinogen adsorption kinetics surprisingly well (Figs. 2 and 3) without any fitting. CA models are constructed with a virtual time scale (“CA steps”) that can *per se* not be correlated with the real time scale. However, in the present model a realistic time scale is introduced *via* the Finite Difference diffusion calculation which sets the initial conditions for the CA part.

An increase of the number of surface diffusion steps (up to three) per time increment for diffusion did not show a significant effect on the adsorption kinetics (not shown here). The surface coverage slightly increases because of the faster formation of clusters on the surface. This, in turn, leads to a decrease of the average desorption probability of fibrinogen molecules because desorption within clusters occurs with a lower desorption probability. The number of CA steps per time increment in the Finite Difference calculation

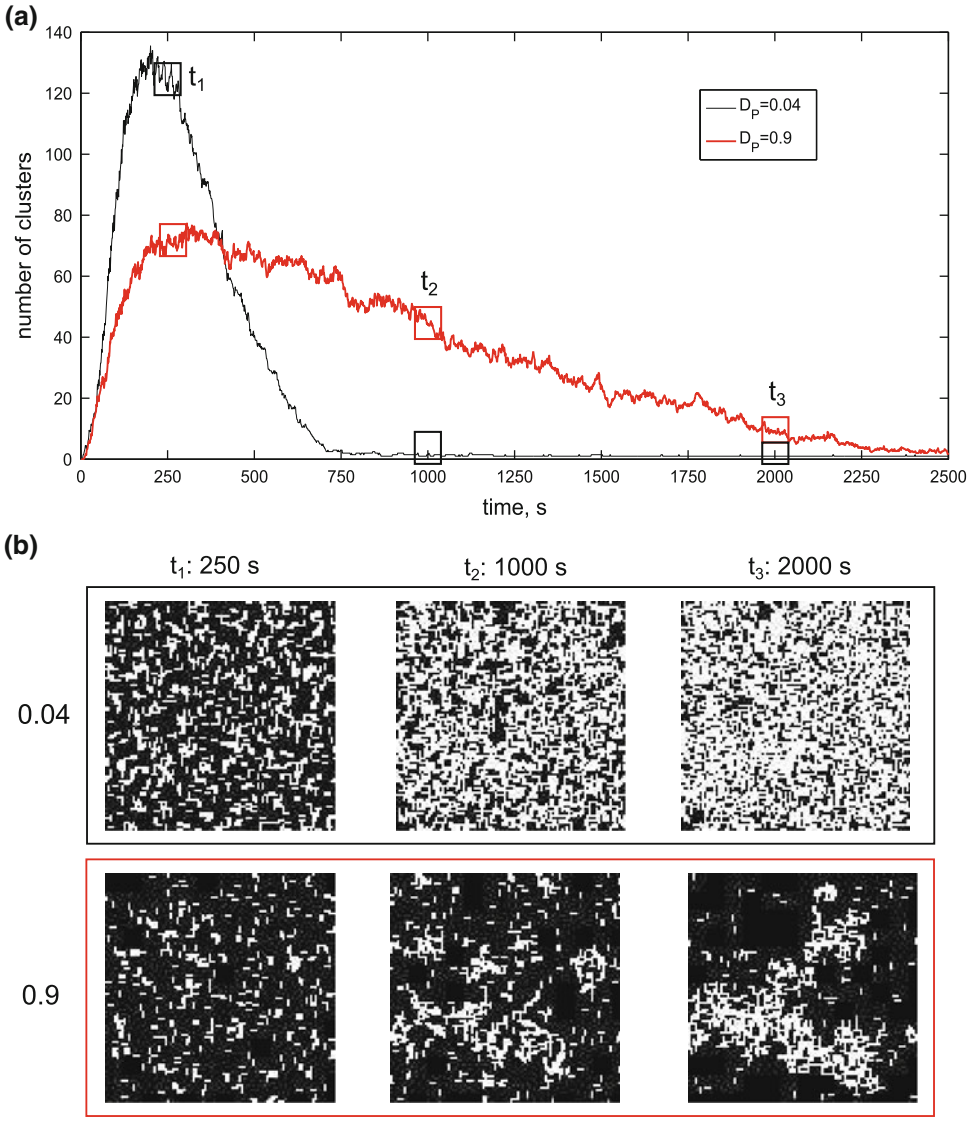

FIGURE 7. Clustering behavior of fibrinogen on surfaces with different desorption parameters (D_P = 0.04 and D_P = 0.9); E_i = −1400 kJ mol^{-1}; c_0 = 36 μg/mL; (a) kinetics of the number of clusters and (b) snapshots of the fibrinogen clusters at different time points (250, 1000, and 2000 s)—white: surface site occupied by fibrinogen molecule; black: unoccupied surface; snapshot size: 1.5 × 1.5 μm^2.

could thus be used to adjust adsorption kinetics and final surface coverage. At present it appears to be impossible to obtain reliable experimental results with some statistics that quantify the movement of proteins on a material's surface. In the present study, the CA stepping has thus not been further elaborated.

From the experimentally determined and simulated rate constants describing the fibrinogen adsorption process, it can clearly be seen that the desorption process should not be neglected. The experimentally confirmed adsorption rate constant k_a is in the same order of magnitude (between 2.0×10^{-4} and 4.0×10^{-5} mL μg^{-1} s^{-1}) for all materials investigated in the present work. This implies that the adsorption rate is relatively weakly dependent on the specific material's surface. The desorption rate constant k_d varies over a wider range, particularly from ca. 1×10^{-2} to 7×10^{-4} s^{-1}. Thus, the desorption rate of fibrinogen is obviously more strongly dependent on the surface and on the initial fibrinogen concentration in solution. The surface dependent desorption probability P_D is suitable to obtain good agreement of simulation results and experimental values.

The rate constants determined by the simulations are generally in good qualitative agreement with the experimentally identified ones. For the NiTi surface, there is a difference of about one order of magnitude for k_a and k_d, respectively.

In comparison the experimentally determined adsorption curve for NiTi shows a steeper slope in the first 300 s approaching the equilibrium concentration faster at a higher level of ≈0.35 (see Fig. 5). The divergence of the simulation result is within the error bars of the corresponding experimental results. The calculation of the equilibrium adsorption constant $K = k_a/k_d$ only shows a difference of ca. 30%, indicating that the process is well described by the simulation.

In the present model the desorption process is influenced by surface induced phenomena, e.g., cluster formation or surface/cluster diffusion. An incorporation of desorption by solely reducing the number of adsorbed fibrinogen molecules is not adequate. The analysis of the influence of D_P on k_a and k_d confirms that a separation of adsorption and desorption is meaningful. The dependency of the two reaction rate constants on D_P is highly different. Comparison of simulation runs with different D_P and otherwise constant conditions shows that there is no change of k_a with varying D_P. Contrarily, D_P has a strong impact on k_d with a linear correlation: the higher D_P, the higher is k_d (not shown). The same behavior could be observed in experiments where solely k_d changes with the change of the respective biomaterial surface.

Parameter study and comparison with the experimental data from the literature show that the main model parameters are the surface dependent desorption parameter D_P, the initial fibrinogen concentration in solution c_0 and the surface dependent interaction energy E_i. The interaction energy is an input parameter for the present model and needs to be determined in molecular dynamics simulations or from measurements. If the two input parameters E_i and D_P are defined, the adsorption kinetics for macroscopic surface is modeled realistically.

CONCLUSION

By including the description of desorption processes in the simulation of fibrinogen adsorption, a large variation in the equilibrium surface coverage as found in different experimental work can be described.

In the parameter range modeled here, good quantitative agreement of the simulated adsorption kinetics with experimental data has been found when applying the coefficients E_i and D_P. The agreement concerning the adsorption kinetics is due to an intrinsically set realistic time scale which stems from the diffusion algorithm. With the present state of our model, adsorption of non-globular molecules on different material surfaces can be described quantitatively, as demonstrated by the comparison of the adsorption and desorption constants and by the direct comparison of measured and calculated surface coverage.

We thus consider the model as a step towards a general mesoscopic adsorption model that can simulate adsorption for a variety of biomaterial surfaces with different surface hydrophobicities, initial concentrations and eventually number of layers of proteins. At present it is limited to the formation of a monolayer.

It appears that in the current era of Systems Biology, the interplay between experiments and modeling needs to be exploited for practically directed research. Models of the type we propose here have the potential to support extensive experimental studies by investigating large parameter ranges numerically and thus support and accelerate the further development of implant materials.

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