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PREDICTING CELL ADHESION PROBABILITY VIA THE BIOCHEMICAL STOCHASTIC PI-CALCULUS

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This paper presents a stochastic model of the lymphocytes recruitment in inflamed brain microvessels. The framework used is based on stochastic process algebras for mobile systems. The automatic tool used in the simulation is the biochemical stochastic π-calculus. The biochemical stochastic π-calculus is an efficient tool for describing the concurrency of the different interactions driving the phases of lymphocytes recruitment. It models a biochemical systems as a set of concurrent processes selected according to a suitable probability distribution in order to quantitatively describe the rates and the times at which the reactions occur. We use here this tool to model and simulate the molecular mechanisms involved in encephalitogenic lymphocytes recruitment. In particular, we show that the model predicts the adhesion probability of the lymphocytes as a function of contact time of the cells with endothelium. The results of the model reproduce, within the experimental errors, the behavior of the data obtained either from laboratory measurements or from a classical deterministic treatment of the mechanics of cell adhesion.

1. Introduction

With recent progress in cell and molecular biology, the field of cell mechanics has grown rapidly over the last few years. The focus of these recent developments is on three interconnected areas: (a) the responses of the cell to mechanical forces, (b) the mechanics of cell adhesion and (c) the deformation of biomolecules. In particular the biophysics of cell adhesion is the most intensively investigated area of cell mechanics. These studies have been driven by the strong interest in many biological processes of which cell adhesion is an important element, leading to the development of a number of mathematical models. Cell attachment to and detachment from a surface, such as, for example, endothelial surface that lines the blood vessel wall is a central aspect in the inflammatory processes. The lymphocytes are the cells whose mechanical properties are most deeply studied, because of their central role in the tissues response to inflammation.

Lymphocytes roll along the walls of vessels to survey the endothelial surface for chemotactic signals, which stimulate the lymphocyte to stop rolling and migrate through the endothelium and its supporting basement membrane. Lymphocyte adhesion to the endothelial wall is mediated by

binding between cell surface receptors and complementary ligands expressed by the endothelium. The dynamic of adhesion is regulated by the bond association and dissociation rates: different values of these rates give rise to different dynamical behaviors of the cell adhesion.

The most common approach to the simulation of rolling process of lymphocyte is based on hydrodynamical models of the particle motion under normal or stressed flow [1,15,18]. At a macroscopic scale, the process is generally modeled with the typical equations of mass continuity, momentum transport and interfacial dynamic. At a microscopic scale, the cell rolling is simulated as a sequence of elastic jumps on the endothelial surface, that result from sequential breaking and formation of molecular bonds between ligands and receptors [15,6,8]. This kind of model is able to simulate the time-evolution of bond density. In general, these models are highly complex and with many parameters, yet they are still idealized and rely on a number of nontrivial assumptions, that often are not supported by sufficient quantitative experimental observations.

Moreover, an other difficulty for a mechanical approach is to treat the disparate scales between the cell (typically of the order of micrometers) and the bonds (of the order of nanometers). In fact, rolling involves either dynamical interaction between cell and surrounding fluid or microscopic elastic deformations of the bonds with the substrate cells. Moreover recent studies have revealed that the process leading to lymphocyte extravasation is a sequence of dynamical states (contact with endothelium, rolling and firm adhesion), mediated by partially overlapped interactions of different adhesion molecules and activation factors. The classical mechanical models are inefficent tools to describe in an easy and nimble way the concurrency of the molecular interactions; furthermore, also if they treat the physical system at the scale of intermolecular bonds with appreciable detail, they are not able to reproduce the sensitivity to the small pertubations in the reagent concentrations or in reaction rates typical of microscopic stochastic systems governed by complex and concurrent contributions of many different molecular reactions. The probabilistic nature of a biological system at the molecular scale requires new languages able to describe and predict the fluctuations in the population levels. We rely on a stochastic extension [22,23] of the π-calculus [17], a calculus of mobile processes based on the notion of naming. The basic idea of this biochemical stochastic π-calculus is to model a system as a set of concurrent processes selected according to a suitable probability distribution in order to quantitatively accommodate the rates and the times at which the reactions occur.

We use here this framework to model and simulate the molecular mechanism involved in encephalitogenic lymphocyte recruitment in inflamed brain microvessels. In particular we show that the biochemical stochastic π-calculus model reproduces, within the estimated measurement errors, the same functional behaviour of the cells adhesion probability versus the contact time, as it was found in laboratory experiments [26].

The paper is organized as follows. In the next section we report a very brief survey of the physiology of the lymphocytes interactions with endothelial surface. Section 3 briefly recalls the basics of the biochemical stochastic π-calculus. Then it shows our specification of the lymphocyte recruitment, and finally, it discusses the results of the stochastic simulation and compares them with the experimental observations. In the last section we show some conclusions.

2. Molecular mechanism of autoreactive lymphocyte recruitment in brain venules

A critical event in the pathogenesis of multiple sclerosis, an autoimmune disease of the central nervous system, is the migration of the lymphocytes from the brain vessels into the brain parenchima. The extravasation of lymphocytes is mediated by highly specialized groups of cell adhesion molecules and activation factors. The process leading to lymphocytes migration, illustrated in Fig. 1 , is divided into four main kinetic phases: 1) initial contact with the endothelial membrane (tethering) and rolling along the vessel wall; 2) activation of a G-protein, induced by a chemokine exposed by the inflamed endothelium and subsequent activation of integrins 3) firm arrest and 4) crossing of the endothelium (diapedesis). For this study, we have used a model of early inflammation in which brain venules express E- and P-selectin, ICAM-1 and VCAM-1 [20]. The leukocyte is represented by encephalitogenic $CD4^+$ T lymphocytes specific for PLP139-151, cells that are able to induce experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis.

Tethering and rolling steps are mediated by binding between cell surface receptors and complementary ligands expressed on the surface of the endothelium. The principal adhesion molecules involved in these phases are the selectins: the P-selectin glyco-protein ligand-1 (PSGL-1) on the autoreactive lymphocytes and the E- and P-selectin on the endothelial cells. The action of integrins is partially overlapped to the action of selectins/mucins: α_4 integrins and LFA-1 are also involved in the rolling phase, but they have

a less relevant role.

Chemokines have been shown to trigger rapid integrin-dependent lymphocyte adhesion in vivo through a receptor coupled with G_i proteins. Integrin-dependent firm arrest in brain microcirculation is blocked by pertussis toxin (PTX), a molecule able to ADP ribosylate G_i proteins and block their function. Thus, as previously shown in studies on naïve lymphocytes homing to Peyer's patches and lymph nodes, encephalitogenic lymphocytes also require an in situ activation by an adhesion-triggering agonist which exerts its effect via G_i-coupled surface receptor.

The firm adhesion/arrest is mediated by lymphocyte integrins and their ligands from the immunoglobulin superfamily expressed by the endothelium. The main adhesion molecules involved in cell arrest is integrin LFA-1 on lymphocyte and its counterligand ICAM-1 on the endothelium. The action of α_4 integrins is partially overlapped to the action of LFA-1: α_4 integrins are involved in the arrest but they have a less relevant role [20].

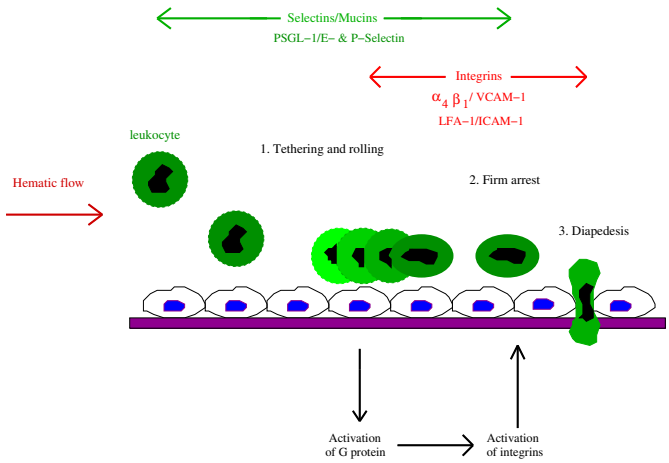

Figure 1. The process leading to lymphocyte extravasation is a finely regulated sequence of steps controlled by both adhesion molecules and activating factors. It involves: 1. initial contact (tethering) and rolling along the vessel wall mediated by selectins (PSGL-1/E- and P-selectin) and integrins($\alpha_4\beta_1$/VCAM-1) and (LFA-1/ICAM-1); 2. chemoattractant-induced heterotrimeric G protein-dependent intracellular biochemical changes leading to integrins activation; 3. integrin-dependent firm arrest, due pricipally to LFA-1/ICAM-1 interaction: and 4. diapedesis.

3. The BioSpi model implementation and results

We first recall the syntax and the intuitive semantics of the stochastic π-calculus [23]. We then describe our specification of the lymphocyte recruitment process, and eventually we discuss the simulation results.

Biomolecular processes are carried out by networks of interacting protein molecules, each composed of several distinct independent structural parts, called *domains*. The interaction between proteins causes biochemical modification of domains (e.g. covalent changes). These modifications affect the potential of the modified protein to interact with other proteins. Since protein interactions directly affect cell function, these modifications are the main mechanism underlying many cellular functions, making the stochastic π-calculus particularly suited for their modeling as mobile communicating systems. The syntax of the calculus follows

$$P ::= \mathbf{0} \mid X \mid (\pi, r).P \mid (\nu x)P \mid [x = y]P \mid P|P \mid P + P \mid A(y_1, \ldots, y_n)$$

where π may be either $x(y)$ for *input*, or $\overline{x}y$ for *output* (where x is the *subject* and y is the *object*) or τ_i for *silent* moves. The parameter r corresponds to the basal rate of a biochemical reaction and it is an exponential distribution associated to the channel occurring in π. The order of *precedence* among the operators is the order (from left to right) listed above. Hereafter, the trailing $\mathbf{0}$ will be omitted.

The prefix π is the first atomic action that the process $(\pi, r).P$ can perform. The parameter r is the unique parameter of an exponential distribution. The input prefix binds the name y in the prefixed process. Intuitively, some name y is received along the link named x. The output prefix does not bind the name y which is sent along x. The silent prefix τ denotes an action which is invisible to an external observer of the system. Summation denotes nondeterministic choice. The operator | describes parallel composition of processes. The operator (νx) acts as a static binder for the name x in the process P that it prefixes. In other words, x is a unique name in P which is different from all the external names. $A(y_1, \ldots, y_n)$ is the definition of constants (hereafter, $\tilde{y}$ denotes $y_1, \ldots, y_n$). Each agent identifier A has a unique defining equation of the form $A(y_1, \ldots, y_n) = P$, where the y_i are distinct and $fn(P) \subseteq \{y_1, \ldots, y_n\}$ (see below for the definition of free names fn).

We recall the notion of free names $fn(\mu)$, bound names $bn(\mu)$, and names

$n(\mu) = fn(\mu) \cup bn(\mu)$ of a label μ.

μ	$Kind$	$fn(\mu)$	$bn(\mu)$
τ	Silent	$\emptyset$	$\emptyset$
$\overline{x}y$	Output	$\{x, y\}$	$\emptyset$
$x(y)$	Input	$\{x\}$	$\{y\}$

Functions fn, bn and n are extended to processes in the obvious way. Below we assume that the *structural congruence* $\equiv$ on processes is defined as the least congruence satisfying the following clauses:

- P and Q α-equivalent (they only differ in the choice of bound names) implies $P \equiv Q$,
- $(\mathcal{P}/_{\equiv}, +, \mathbf{0})$ is a commutative monoid,
- $(\mathcal{P}/_{\equiv}, |, \mathbf{0})$ is a commutative monoid,
- $(\nu x)(\nu y)P \equiv (\nu y)(\nu x)P, (\nu x)(R \mid S) \equiv (\nu x)R \mid S$ if $x \notin fn(S)$, $(\nu x)(R \mid S) \equiv R \mid (\nu x)S$ if $x \notin fn(R)$, and $(\nu x)P \equiv P$ if $x \notin fn(P)$.
- $A(\tilde{y}) \equiv P\{\tilde{y}/\tilde{x}\}$, if $A(\tilde{x}) ::= P$ is the unique defining equation of constant A

The biological interpretation is as follows. Processes model molecules and domains. Global channel names and co-names represent complementary domains and newly declared private channels define complexes and cellular compartments. Communication and channel transmission model chemical interaction and subsequent modifications. The actual rate of a reaction between two proteins is determined according to a constant *basal rate* empirically-determined and the concentrations or quantities of the reactants . Two different reactant molecules, P and Q, are involved, and the reaction rate is given by $Brate \times |P| \times |Q|$, where $Brate$ is the reaction's basal rate, and $|P|$ and $|Q|$ are the concentrations of P and Q in the chemical solution computed via the two auxiliary functions, In_x, Out_x that inductively count the number of receive and send operations on a channel x enabled in a process.

The semantics of the calculus thereby defines the dynamic behaviour of the modeled system driven by a *race condition*, yielding a probabilistic model of computation. All the activities enabled in a state compete and the fastest one succeeds. The continuity of exponential distributions ensures that the probability that two activities end simultaneously is zero.

The reduction semantics of the biochemical stochastic π-calculus is

$$(\ldots + (\overline{x}\langle z\rangle, r).Q)|((x(y), r).P + \ldots) \xrightarrow{x, r_b \cdot 1 \cdot 1} Q|P\{z/y\}$$

$$\frac{P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} P'}{P|Q \xrightarrow{x, r_b \cdot r_0' \cdot r_1'} P'|Q}, \begin{cases} r_0' = r_0 + In_x(Q) \\ r_1' = r_1 + Out_x(Q) \end{cases}$$

$$\frac{P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} P'}{(\nu\, x)P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} (\nu\, x)P'} \quad \frac{Q \equiv P, P \xrightarrow{x, r_b \cdot r_0 \cdot r_1} P', P' \equiv Q'}{Q \xrightarrow{x, r_b \cdot r_0 \cdot r_1} Q'}$$

A reaction is implemented by the three parameters r_b, r_0 and r_1, where r_b represents the basal rate, and r_0 and r_1 denote the quantities of interacting molecules, and are computed compositionally by In_x and Out_x.

3.1. *Specification*

The system of interacting adhesion molecules that regulate the lymphocytes recruitment on endothelial surface illustrated in Fig. 1 has been implemented in the biochemical stochastic π-calculus. The system is composed by eight concurrent processes, corresponding to the eight species of adhesion molecules, that regulate the cell rolling and arrest: PSGL1, PSELECTIN, CHEMOKIN, CHEMOREC, ALPHA4, VCAM1, LFA1 and ICAM1. The code implements the four phases of the lymphocyte recruitment: the interaction between PSGL1 and PSELECTIN, the ALPHA4 and LFA1 activation by chemokines and the firm arrest mainly caused by the interaction between the active form of LFA1, LFA1_ACTIVE, and ICAM1 and in part also due to the interaction of the active form of ALPHA4, ALPHA4_ACTIVE, with VCAM1. Its specification is

We simulated the role and the contribution of the different interactions as bi-molecular binding processes occuring at different rates. The selectins interaction PSGL1/PSELECTIN plays a crucial role in guaranting an efficient rolling, therefore the channels rates for the communication in the binding process between PSGL1 and PSLECTIN have been calculated from the deterministic rates of the Bell model, that reproduce the tethering and rolling motion. Analogously, for the ALPHA4_ACTIVE/VCAM1 interaction, that contributes to rolling and, in part, also to cell arrest, the channels rate have been calculated from the Bell model rates that recreate the rolling motion. The interaction LFA1_ACTIVE/ICAM1 is the main responsible of

$SYSTEM ::= PSGL1|PSELECTIN|CHEMOKIN|CHEMOREC|ALPHA4$
$|VCAM1|LFA1|ICAM1$

$PSGL1 ::= (\nu\ backbone)BINDING\_PSITE1$
$BINDING\_PSITE ::= (\overline{bind}\langle backbone\rangle, RA).PSGL1\_BOUND(backbone)$
$PSGL1\_BOUND(bb) ::= (\overline{bb}, RD_0).PSGL1$
$PSELECTIN ::=$
$(bind(cross\_backbone), RA).PSELECTIN\_BOUND(cross\_backbone)$
$PSELECTIN\_BOUND(cbb) ::= (\overline{cbb}, RD_0).PSELECTIN$

$CHEMOKIN ::= (\nu\ chemobb)BINDING\_CSITE$
$BINDING\_CSITE ::= (\overline{lig}\langle chemobb\rangle, RA\_C).CHEMOCHIN\_BOUND(chemobb)$
$CHEMOCHIN\_BOUND(chemobb) ::= ACT1|ACT2|ACT3(cbb)$
$ACT1 ::= (\overline{alpha\_act}\langle sign1\rangle, A).ACT1$
$ACT2 ::= (\overline{lfa\_act}\langle sign2\rangle, A).ACT2$
$ACT3(chb) ::= (\overline{chb}, RD\_C).CHEMOKIN$
$CHEMOREC ::=$
$(lig(cross\_chemobb), RA\_C).CHEMOREC\_BOUND(cross\_chemobb)$
$CHEMOREC\_BOUND(ccr) ::= (ccr, A).CHEMOREC$
$ALPHA4 ::= (alpha\_act(act\_a), A).ALPHA4\_ACTIVE$
$LFA1 ::= (lfa\_act(act\_l), A).LFA1\_ACTIVE$

$ALPHA4\_ACTIVE ::= (\nu\ backbone2)BINDING\_ASITE$
$BINDING\_ASITE ::= (\overline{bind2}\langle backbone2\rangle, RA).ALPHA4\_BOUND(backbone2)$
$ALPHA4\_BOUND(bb2) ::= (\overline{bb2}, RD_1).ALPHA4$
$VCAM1 ::= (bind2(cross\_backbone2), RA).VCAM1\_BOUND(cross\_backbone2)$
$VCAM1\_BOUND(cbb2) ::= (\overline{cbb2}, RD_1).VCAM1$

$LFA1\_ACTIVE ::= (\nu\ backbone3)BINDING\_SITE3$
$BINDING\_SITE3 ::= (\overline{bind3}\langle backbone3\rangle, RA).LFA1\_BOUND(backbone3)$
$LFA1\_BOUND(bb3) ::= (\overline{bb3}, RD_2).LFA1\_BOUND$
$ICAM1 ::= (bind3(cross\_backbone3), RA).ICAM1\_BOUND(cross\_backbone3)$
$ICAM1\_BOUND(cbb3) ::= (\overline{cbb3}, RD_2).ICAM1\_BOUND$

$RA = 6.500 \quad RA\_C = RD_0 = 0.051 \quad RD_1 = 5.100$
$RD_2 = 1.000 \quad RD\_C = 3.800 \quad A = infinite$

$Radius\ of\ vessel = 25\ micromenters \quad Length\ of\ vessel = 100\ micromenters$
$Volume\ of\ vessel = 1.96 \times 10^5\ cubic\ micrometers \quad Radius\ of\ lymphocyte = 5\mu m$

firm arrest of the cell on the endothelium and thus the rates of communication between LFA1_ACTIVE and ICAM1_ACTIVE have been calculated from those reproducing the firm adhesion in Bell model simulations.

The activation of ALPHA4 and LFA1 integrins by the chemokines is implemented in two steps: firstly a chemokine CHEMOKIN binds to its receptors CHEMOREC and changes to a "bound" state CHEMOKIN_BOUND. Then the complex CHEMOKIN_BOUND sends two names *sign1 and* sign2 on the channels *act_alpha and* act_lfa, on which the processes ALPHA4 and LFA1 are ready to receive them as inputs. After ALPHA4 and LFA1 have received the signals from CHEMOKIN_BOUND, they change to the active

form ALPHA4_ACTIVE and LFA1_ACTIVE.

In our model, the whole process of lymphocyte recruitment occur in a space of $V = 1.96 \times 10^5 \mu\text{m}^3$, corresponding to a volume of a vessel of 25μm of radius and 100μm of length, and in a simulated time of 15 sec. In the considered volume V, the number of mulecules is of the order of 10^6. In our simulations the values of the volume and of the molecules number have been proportionally re-scaled by this factor, in order to make the code computationally faster.

The stochastic reaction rates for bimolecular binding/unbinding reaction are inversely proportial to the volume of space in that the reactions occur [9], in particular for the stochastic association rate we have that $RA = k_{on}/V$ and for the stochastic dissociation rate we have $RD = 2k_{off}/V$, where the k_i's are the deterministic rates with values shown in the following table.

Process	k_{on} (sec $^{-1}$)	k_{off} (sec $^{-1}$)
Tethering	84	1
Rolling	84	100
Chemokines activation	0.5	75
Firm adesion	84	20

The output of simulation is the time-evolution of number of bonds (shown in Fig. 2) assuming the following densities expressed in μm^{-2}: PSGL-1 [19] and P-SELECTIN 5600, ALPHA4 [5] and VCAM-1 85, CHEMOREC and CHEMOKINES 15000, LFA-1 [10] and ICAM-1 5500. The characterization of the steps and the adhesion molecules implicated in lymphocyte recruitment in brain venules was performed by using intravital microscopy, a potent technique allowing the visualization and analysis of the adhesive interactions directly through the skull in live animal.

The BioSpi simulations reproduce the hyperbolic behavior ($-1/x + const$) predicted by the classical mechanical hydrodynamical models presented in the literature [6,7,8,11,15,18].

Only lymphocytes that express high levels of PSGL-1, high levels of LFA-1 and or α_4 integrins and the corresponding receptors for the activating factors presented by the endothelium will be able to efficiently be recruited in inflamed venules. Starting from this experimental evidence [20], the mathematical model of the cell adhesion probability is

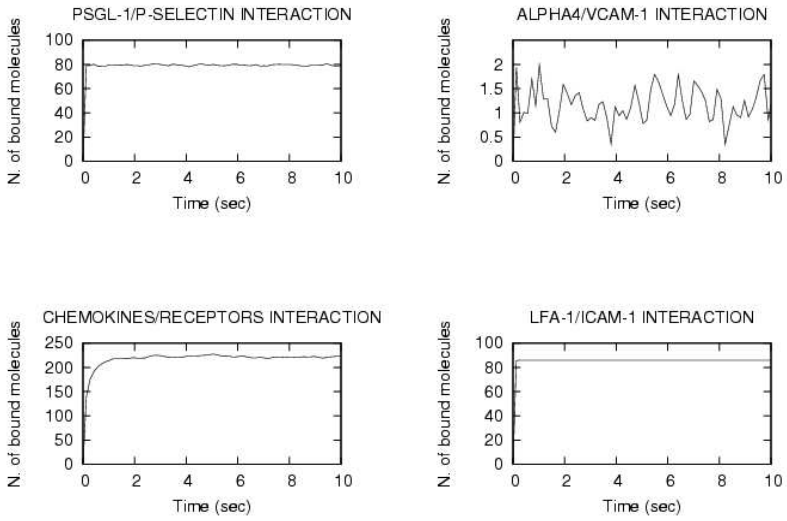

Figure 2. BioSpi simulation of 4-phases model of lymphocyte recruitment.

$$Pr(adhesion) = \frac{1}{N_l} \sum_i w_i N_i \tag{1}$$

where $N_l = S_{endothelium}/S_{contact}$ is the total number of lymphocytes on the laminar flux in contact with endothelium given by the ratio between endothelial surface ($\sim 15700 \mu m^2$) and cell contact area ($\sim 200 \mu m^2$), N_i indicate the number of bound molecules for the i-th molecular interaction, and the w's are the weights of the linear model, that quantify the statistical influence of the different molecular interactions in the cell adhesion mechanism. In our model the weights can take values in the range between 0 and 1. Because of the lack of experimental quantifications for the statistical influence of the different molecular interactions, we assume that $w_i = 1/8 = 0.125$ for all the considered interactions of the eigth molecular species.

The model given in (1) is plotted in Fig. 3. It is in agreement, within the errors ranges, with the theoretical and experimental results in, one of

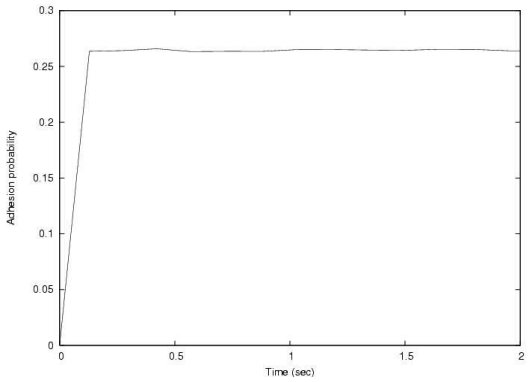

Figure 3. BioSpi model of cell adhesion probability versus contact time (eq. (1).

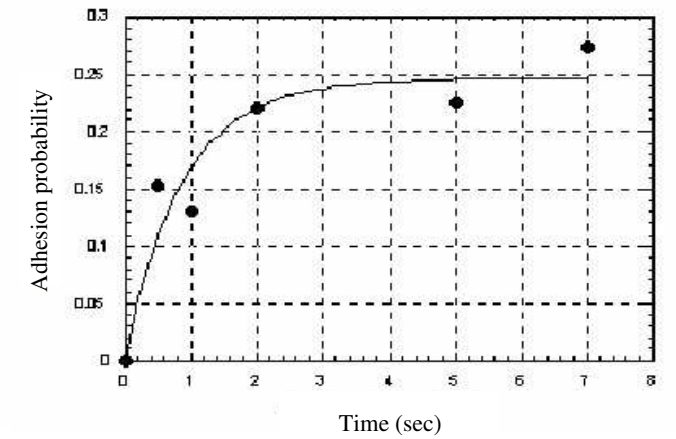

Figure 4. PSGL-1/P-SELECTIN binding curve.

which illustrated in Fig.4[a] [16]. A more complete presentation of the recent experimental observations and of the physical treatment of the adhesion mechanics, in agreement with our BioSpi model, is given in [16,21,26].

[a]At the present the most studied molecular interaction of the cell adhesion machanics if the PSGL-1/P-SELECTIN interaction.

4. Conclusion

The usage of new languages such as stochastic π calculus to describe and simulate the migration of autorective lymphocytes in the target organ will help us better understand the complex dynamics of lymphocyte recruitment during autoimmune inflammation in live animal. Furthermore, our approach may represent an important step toward future predictive studies on lymphocyte behavior in inflamed brain venules. The stochastic calculus may, thus, open new perspectives for the simulation of key phenomena in the pathogenesis of autoimmune diseases, implicating not only better knowledge, but also better future control of the autoimmune attack. Finally, the obtained results show the efficiency of biochemical stochastic π-calculus to simulate experimental data, offering the possibility to model and predict data of biological observations on a computer (*in silico* experiments). This new opportunity provided by the computer science may allow the biologists and medical researchers to save time by reducing the number of needed experiments in the case in which the computer simulation can exclude inadequate hypothesis.

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