Modeling Biological Receivers: the Case of Extracellular Vesicle Fusion to the Plasma Membrane of the Target Cell

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ABSTRACT

A continuously increasing number of biomedical applications exploits the exchange of extracellular vesicles (EVs) between biological cells to develop innovative and more efficient therapeutic protocols. The challenge is to provide alternative strategies to existing low-success drug delivery technologies, for the treatment of particular diseases. An interesting contribution in this direction is provided by interdisciplinary and non-conventional approaches, interpreting biological cells as transmitting and receiving devices that communicate through extracellular vesicles, among other chemical signals. This paper addresses the processes and functionalities of the cells as receiving devices, with particular focus on the EV internalization through their fusion with the plasma membrane of the target cells. In fact, poor information is currently available regarding such processes which makes it impossible to fully exploit the potentiality of EV-based therapeutic protocols. Therefore, with aim of retrieving such a deficiency of information a mathematical methodology is derived to supply an analytical model, representing a first crucial step towards a more general and comprehensive contribution to the study of EVs communication.

CCS CONCEPTS

• **Applied computing** → **Telecommunications**; *Computational biology*; **Systems biology**; • **Mathematics of computing** → **Ordinary differential equations**.

KEYWORDS

Extracellular Vesicle Communication, Molecular Communication, Biological Membrane Fusion, Mathematical Model, EV Uptake

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1 INTRODUCTION

New opportunities of innovation are more and more often offered from interdisciplinary and non-conventional approaches, exploiting well-established conceptual and practical tools, in combination with the definition and development of new field-specific solutions. In this context, the ability of biological cells to respond to a wide range of stimuli of different nature and to produce changes, as well, in the surrounding environments that other cells can sense and react to, has been recently formalized according to information and communication theory paradigms, where the biological cells are interpreted as transmitting and receiving devices that communicate through chemical signals [1].

A particular type of cell communication, that is gaining increasing attention in the bio-medical community, is achieved through the exchange of extracellular vesicles (EVs), i.e., spherical and nanometrical particles covered of lipid membrane [7]. It has been, in fact, recently envisioned that natural or synthetic-engineered EVs can be engaged for the treatment of diseases to deliver drugs to target ill cells. As an example, in particular brain diseases where the success rate of existing drug delivery technologies in deep located cells is still low, EV-based therapeutic protocols may result to be a more efficient solution [4, 19].

In Fig. 1, the EV communication system is depicted. The EVs, once released by donor cells, diffuse in the extracellular space. When they come in proximity of the target cells, several uptake mechanisms can take place. In particular, two of them are worth of mention: receptor-mediated endocytosis and EV fusion with the plasma membrane of the target cell [10, 15]. The general process of receptor-mediated endocytosis is well documented in literature[10]. In [21], a mathematical model of the endocytosis has been presented and in [19], it has been utilized to derive the receiver transfer function, in a communication scheme focusing on endocytosis as reception mechanisms.

Concurrent to the endocytosis, the EVs fusion with the plasma membrane of the target cell seems to involve a great amount of EVs. It is reasonable to expect that differences among various EV internalization processes performed by the target cells may be related to different functionalities, even producing different effects. Therefore, a deep investigation of the possible interactions, cooperation or competition among concurrent internalization process is important in a large variety of applications. To this purpose, understanding the dynamics of EV fusion with the plasma membrane of the target cell represents a first step in the direction of building a more general picture of the cell communication mechanisms.

In this context, this paper addresses the processes and functionalities of the cells as receiving devices, with particular focus on the derivation of a mathematical model of the EVs internalization

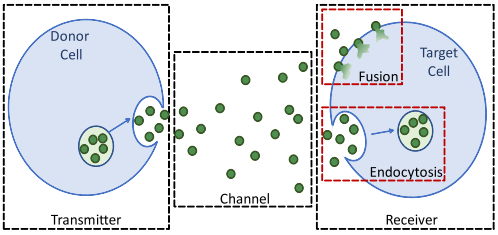

Figure 1: Extracellular Vesicle communication system

through their fusion with the plasma membrane of the target cells. To this aim, different phases of the fusion process have been identified and described through a system of ordinary differential equations. The parameters of the equations are the rates of the chemical reactions involved in EV binding and fusion, as well as in the recycling/regeneration process of the involved fusogenic proteins.

This is crucial because poor information is available in literature about the parameters regulating the processes of our interest. In fact, the EV fusion with the plasma membrane of the target cells, in contrast with the EV fusion with membrane bound compartments and organelles inside the cell, has received attention only recently and has not been deeply investigated yet. The majority of knowledge about the external surfaces fusion between EVs and cells comes mainly from the study of either the fusion between two cells or of virus with cells, which have suggested interesting hypothesis about the identity of the proteins involved in the cell surface fusion of EVs, as well as possible new line of investigation on this topic. Nevertheless, proper meaningful values for the model parameters are still not available.

In such a context, our approach is to define a mathematical model of the EV fusion process and compare the values obtained by the model to the experimental results. This, in fact, would allow to infer reasonable values for the parameters regulating the fusion process through a reverse engineering approach.

The paper is organized as follows. In Section 2, the EV fusion to the plasma membrane of the target cell is described. Then, in Section 3, a mathematical model of the fusion is derived and a mathematical methodology to supply an analytic solution of the model is provided. Numerical results are shown in 4. Finally, some conclusions are drawn in Section 5.

2 BINDING AND FUSION OF EVS WITH THE PLASMA MEMBRANE OF THE TARGET CELLS

EVs are spherical and nanometrical particles, coated with a phospholipid bilayer and insertion proteins that allow their recognition by target cells [7], and classified according to their size and their biogenesis. Once generated and released by donor cells, EVs meet different destiny. Some EVs release their cargo in the extracellular space as the result of the dissolution of their membrane. Another fraction of EVs undertakes a long-term navigation in the extracellular fluids. Other EVs remain local and establish various types of interaction with so-called target cells. In particular, two types of interactions are worth mentioning, i.e. endocytosis of various forms and fusion with the plasma membrane of the target cell [10, 15].

In this work we focus on the fusion of EVs to the plasma membrane of their target cells, as depicted in Fig. 2. Note that this process has not to be confused with the apparently analogous intracellular fusion process between vesicles and membrane-bound cell compartments and organelles, such as for example with lysosome. In fact, while the intracellular fusion has been extensively investigated and illustrated in literature [6, 8, 10], on the contrary, evidence of the surface binding and fusion of EVs with the target cells has been only recently described [15, 18] and numerous details remains yet to clarify.

Fusion is a well-known natural phenomenon taking place when two separated membrane portions come close and melt into each other. Several activating factors have been identified, such as for example the temperature, the acidity of the surrounding environment, the presence of specific lipids like cholesterol, the membrane curvature or the presence of specific proteins or receptors [16]. As far as the fusion between the membranes of EV and cells is concerned, it seems to be activated by specific fusogenic proteins. More precisely, high affinity binding of at least two pairs of surface fusogenic proteins (one protruding from the EVs and the other from the plasma membrane of the target cell) is expected to be required [15]. However, the identity of the fusogenic proteins involved in the fusion process is still under investigation. More specifically, the knowledge about the surface binding and fusion between EVs and target cells, similar to the fusion taking place between two cells, comes mainly from the study of the fusion of virus with cells, which involves four classes of proteins [13]. The presence of proteins of two of these classes, such as syncitin-1 and syncitin-2, as well as the receptor MSFD2a and the neutral amino acid transporter ASCT-2, have been discovered on the plasma membrane of several type of cells, such as placental cells [14, 17], human gametes [2], blood cells [17] and tumor cells [3, 9], among others, and their participation on the cell-to-cell fusion process has been observed [2, 14, 17]. Interestingly, these proteins were found also on placental trophoblast exosomes (a particular type of EV) destined to bind and fuse with blood cells [17] and may be present also on the EVs of other cells, which suggests their possible involvement in the binding process that precedes the fusion of EVs to target cells [15].

After the bond between the fusogenic proteins is established, their hydrophobic segments begin to deepen into the plasma membrane, followed by the molecular re-arrangement (pre-folding and post-folding) of the bound proteins and the re-organization of the closely attached membrane portion of both EV and target cell, until their dissolution at the fusion site. The EV membrane is, then, inserted in the plasma membrane, which becomes continuous.

Unlike in other simultaneous internalization processes that occur through the cell membrane, such as receptor-mediated endocytosis, where the EVs are internalized with their membrane and, only subsequently, broken down to metabolize their contents, in the fusion the vesicle content is released directly into the cytosol.

3 FUSION MODEL

In this section, a mathematical model of the EV fusion to the plasma membrane of the target cell is derived, with an approach similar to the one used in [21] to model endocytosis.

Let F be the concentration, in number of pairs per cell surface unit, of fusogenic protein pairs on the plasma membrane of the target cell, and let us denote as F_a, F_b and F_f the concentrations of fusogenic protein pairs that are available for binding, or currently bound to an EV, or in a post-fusion state, respectively. We assume that the EVs bind to a pairs of available fusogenic proteins at a rate a_b. The binding process can be described as follows:

$$F_a + V_e \xrightarrow{a_b} F_b + V_b \tag{1}$$

where V_e and V_b are the concentration of EVs in the extracellular space close to the plasma membrane and bound to the fusogenic proteins, respectively. Near the periphery of the cell, the EVs initially exhibit a surface sliding behavior until their movements drastically decrease, as an effect of the high affinity between the fusogenic proteins on the surface of both cells and EVs. The binding between those proteins is, at this point, established [15]. The duration of the surface sliding, which depends on the type of cells and EVs, determines, among other factors, the binding rate, a_b, of EVs to the plasma membrane. Soon after, the binding evolves into fusion, at a rate that we denote as b_f. The evolution of EVs from the extracellular space to the fusion EV-cell can be summarized by the following set of reactions:

$$\{extracellular\ medium\} \xrightarrow{k_V} V_e \underset{b_u+b_x}{\overset{a_b}{\rightleftarrows}} V_b \xrightarrow{b_f} V_f \tag{2}$$

where k_V is the delivery rate of EVs to the cell surface, i.e. the number of EV supplied to the cell by the extracellular medium in the time unit, and V_f is the concentration of EVs whose fusion to the plasma membrane is completed and whose cargos have been released into the cytosol. Note that, at this point, the fused EV does not exist anymore as a physical entity, since it has been broken down and decomposed into a piece of plasma membrane and its original content. However, the variable V_f is introduced to take into account the time needed for the fusion to take place, after the binding has been established, as well as the possibility that not all the bound EVs evolve into fused ones, due to the fraction of the EV-protein bounds, that may disassociate before the fusion activation.

In order to model the evolution of the fusion process, we first focus on the time-dependent concentrations of the available, bound and post-fusion fusogenic proteins on the cell surface, $F_a(t)$, $F_b(t)$ and $F_f(t)$, respectively, and consider the events that affect their temporal variation. In particular, let us note that the binding of an EV to an available fusogenic protein produces a decrease in the concentration of available fusogenic proteins, $F_a(t)$, and a corresponding increase in the concentration of bound ones, $F_b(t)$. The contribution of this event follows by the application of the law of mass action to the reaction (1). Similarly, the fusion of a bound EV to the plasma membrane, at the rate b_f, accounts for a negative contribution in the temporal variation of the bound fusogenic proteins, $F_b(t)$, and a positive contribution to the temporal variation of the post-fusion fusogenic proteins, $F_f(t)$. Besides the binding and fusion events, also the recycling processes of the fusogenic proteins affect the temporal variation of their concentration and need to be considered. However, a deep knowledge about the fusogenic proteins involved in the fusion process between EVs and target cells is not well documented yet. Nevertheless, it is reasonable to assume that a protein recycling process occurs similarly to what happens in other biological entities within the cell components and activities [5, 20]. More specifically, it is reasonably expected that after the fusion of the EVs is completed, a fraction of the post-fusion proteins becomes again available for binding new EVs, while at the same time old fusogenic proteins are degraded and new available fusogenic proteins are produced by the cell. Accordingly, a negative contribution is given to the temporal variation of the post-fusion protein concentration $F_f(t)$ by the recycled and destroyed proteins at the rates b_d and b_a, respectively. Likewise, the recycled proteins produces a positive contribution in the temporal variation of available proteins concentration $F_a(t)$. Additionally, let k_F be the production rate of new fusogenic protein, i.e. the concentration of new synthesized fusogenic protein pairs per unit time, which contributes positively to the concentration $F_a(t)$. It is worth noting that according to the dynamics implemented by the cell to regulate such a recycling process, an equilibrium between recycled, new produced and old degradated proteins may or may not be reached. Therefore to take into account the more general case, the production rate is introduced in the model as a function of time, i.e. $k_F(t)$. Finally, the possibility that a fraction of the EV-protein bounds may disassociate before the fusion process is activated, produces, at the rate b_u, an increase of the concentration of available fusogenic proteins, $F_a(t)$, and a decrease of the bound ones, $F_b(t)$. All the above considerations lead to the following equations:

$$\frac{dF_a(t)}{dt} = k_F(t) + b_d F_f(t) + b_u F_b(t) - a_b F_a(t) V_e(t) \tag{3}$$

$$\frac{dF_b(t)}{dt} = a_b F_a(t) V_e(t) - \left(b_f + b_u + b_x\right) F_b(t) \tag{4}$$

$$\frac{dF_f(t)}{dt} = b_f F_b(t) - (b_d + b_a) F_f(t) \tag{5}$$

where b_x represent the portion of proteins that are destroyed after the bound dissociation.

With similar considerations, let us now focus on the temporal evolution of the concentrations of EVs in the extracellular space close to the target cell $V_e(t)$, bound to the fusogenic proteins $V_b(t)$, and fused to the plasma membrane $V_f(t)$. By applying the law of mass action to reactions (2), we obtain the following equations:

$$\frac{dV_e(t)}{dt} = k_V(t) + (b_u + b_x) V_b(t) - a_b F_a(t) V_e(t) \tag{6}$$

$$\frac{dV_b(t)}{dt} = a_b F_a(t) V_e(t) - \left(b_f + b_u + b_x\right) V_b(t) \tag{7}$$

$$\frac{dV_f(t)}{dt} = b_f V_b(t) \tag{8}$$

Note that the concentration of EVs in the extracellular medium may not be spatially uniform. However, the fluid dynamics of EVs delivery to the cell surface [19] is out the scope of this work, since we are here interested on the dynamics of binding and fusion of the EVs to the plasma membrane. For this reason, we assume that there is a layer of fluid close to the plasma membrane of the target cell, where the concentration of the EVs can be considered spatially uniform and we define V_e as the concentration of EVs in this portion of extracellular fluid. Therefore, we can consider here the solely dependance of V_e from time. This concentration of EVs, close to the plasma membrane of the target cell, is supplied to the system from an external source at the delivery rate $k_V(t)$.

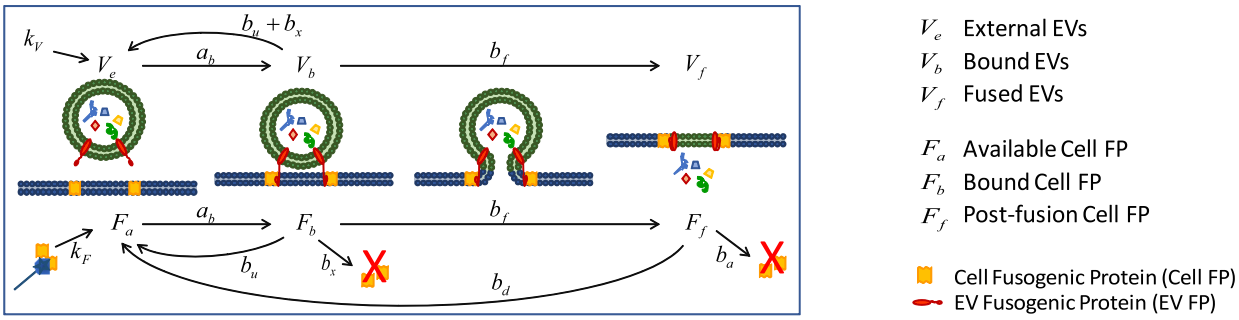

Figure 2: EV Fusion to the Plasma Membrane of the Target Cell

3.1 Model solution

The set of equations (3)-(8) constitute, in its general form, the system of ordinary differential equations (ODEs) that models the binding and fusion process of EVs to the plasma membrane of a target cell. However, note that equations (4) and (7) have the same expression. In fact, since a pair of fusogenic proteins bind with a single EV, the number of bound fusogenic protein pairs is equal to the number of bound EVs, i.e.:

$$V_b(t) = F_b(t) \tag{9}$$

Therefore we can substitute (7) with (9).

Furthermore, the variable $V_f(t)$ appears only in (8), and can be calculated by integration from $V_b(t)$, or equivalently $F_b(t)$, that is:

$$V_f(t) = c + b_f \int F_b(t)\, dt \tag{10}$$

where c is the constant of integration. Thus, let us focus on the system of ODEs (3)-(6). These equations are non-linear, due to the term with the product between $F_a(t)$ and $V_e(t)$. However, since this term appears in three of the four above equations, we can easily derive the following identities:

$$\begin{aligned} &a_b F_a V_e - b_u F_b = \\ &= \frac{dF_b}{dt} + \left(b_f + b_x\right) F_b = -\frac{dF_a}{dt} + k_F + b_d F_f = -\frac{dV_e}{dt} + k_V + b_x F_b \end{aligned} \tag{11}$$

where the dependency from time has been omitted for the sake of simplicity. Now from (5) let derive the expression of F_b as a function of F_f, i.e.:

$$F_b = \frac{1}{b_f}\frac{dF_f}{dt} + \frac{b_d + b_a}{b_f} F_f \tag{12}$$

By substituting (12) in (11) and integrating each side of (11), after some manipulation the following relations are obtained:

$$\begin{aligned} F_a = \int k_F dt - \frac{1}{b_f}\frac{dF_f}{dt} - \frac{b_d + b_a + b_f + b_x}{b_f} F_f + \\ + \left(b_d - \frac{(b_f + b_x)(b_d + b_a)}{b_f}\right) \int F_f dt \end{aligned} \tag{13}$$

$$V_e = \int k_V dt - \frac{1}{b_f}\frac{dF_f}{dt} - \frac{b_d + b_a + b_f}{b_f} F_f - (b_d + ba) \int F_f dt \tag{14}$$

In order to simplify the notation, let apply the following substitutions:

$$\int F_f dt = z, \quad F_f = z'_t, \quad \frac{dF_f}{dt} = z''_t, \quad \frac{d^2 F_f}{dt^2} = z'''_t \tag{15}$$

Table 1: Coefficients of Equation (16)

$\mathcal{A} = -\frac{a_b}{b_f}$
$\mathcal{B} = -\frac{a_b}{b_f}\left(2\left(b_d + b_a + b_f\right) + b_x\right)$
$\mathcal{C} = -a_b\left((b_d + 2b_a) + \frac{b_x}{b_f}(b_d + b_a)\right)$
$\mathcal{D}(t) = b_d + b_a + b_f + b_u + b_x + a_b \int (k_V(t) + k_F(t))\, dt$
$\mathcal{E} = -\frac{a_b}{b_f}\left(b_d + b_a + b_f\right)\left(b_d + b_a + b_f + b_x\right)$
$\mathcal{F} = -a_b\left((b_d + 2b_a)\left(b_d + b_a + b_f\right) + \frac{b_x}{b_f}(b_d + b_a)\left(b_d + ba + 2b_f\right)\right)$
$\mathcal{G}(t) = a_b\left(b_d + b_a + b_f\right)\int (k_V(t) + k_F(t))\, dt + a_b b_x \int k_V(t)\, dt +$ $+\left(b_f + b_u + b_x\right)(b_d + b_a)$
$\mathcal{H} = -a_b\left(b_f b_a (b_d + b_a) + b_x (b_d + b_a)^2\right)$
$\mathcal{I}(t) = a_b b_f\left((b_d + b_a)\int k_F(t)\, dt + \left(b_a + \frac{b_x}{b_f}(b_d + b_a)\right)\int k_V(t)\, dt\right)$
$\mathcal{J}(t) = -a_b b_f \int k_F(t)\, dt \int k_V(t)\, dt$

where the notations $z'_t = \frac{dz}{dt}$, $z''_t = \frac{d^2 z}{dt^2}$, $z'''_t = \frac{d^3 z}{dt^3}$,... are used for the total derivatives. Let now substitute (15) in (13), (14) and (12), and the resulting equations in (4). After some manipulation, the following ODE is obtained:

$$\begin{aligned} z'''_t + \mathcal{A}\left(z''_t\right)^2 + \mathcal{B} z'_t z''_t + \mathcal{C} z z''_t + \mathcal{D} z''_t + \\ + \mathcal{E}\left(z'_t\right)^2 + \mathcal{F} z z'_t + \mathcal{G} z'_t + \mathcal{H} z^2 + \mathcal{I} z + \mathcal{J} = 0 \end{aligned} \tag{16}$$

whose coefficients are calculated as in Table 1.

So far, the system of ODE (3)-(6) has been reduced to the single non-linear ODE of the third order (16), for which, unfortunately, from the best of our knowledge, a solution method is not known. Therefore, in the following, several substitutions are applied to transform the equation into a more easy to manage form. We will see, that (16) can be transformed in an Abel ODE of the second kind, in normal form, whose solution has been proposed in [11, 12].

The first substitution to be applied is:

$$\begin{aligned} z'_t &= r(z) \\ z''_t &= \frac{dr(z)}{dt} = \frac{dr}{dz}\frac{dz}{dt} = r'_z z'_t = r'_z r \\ z'''_t &= \frac{d\left(r r'_z\right)}{dt} = \frac{d\left(r r'_z\right)}{dz}\frac{dz}{dt} = r^2 r''_z + r\left(r'_z\right)^2 \end{aligned} \tag{17}$$

which reduces the third order ODE in (16) into the following second order ODE:

$$r_z'' = f_1(r)\left(r_z'\right)^2 + f_2(r,z,[t])\, r_z' + f_3(r,z,[t]) \tag{18}$$

where:

$$\begin{aligned} f_1(r) &= -\mathcal{A} - \frac{1}{r} \\ f_2(r,z,t) &= -\mathcal{B} - \frac{\mathcal{C}z + \mathcal{D}(t)}{r} \\ f_3(r,z,t) &= -\mathcal{E} - \frac{\mathcal{F}z + \mathcal{G}(t)}{r} - \frac{\mathcal{H}z^2 + \mathcal{I}(t)\, z + \mathcal{J}(t)}{r^2} \end{aligned} \tag{19}$$

The coefficients f_2 and f_3 in (19) are functions of the variables r, z, and t. However, through the substitution (17), the independent variable in (18) is now z, whereas t serves as a parameter. Therefore the notation adopted in (18) and in the following, to clearly distinguish whether in a given equation the arguments of a function have to be handled as independent variables or parameters, consist of embracing the list of parameters in square bracket. Note that, for the sake of simplicity and without loosing generality, the parameters can be sometimes omitted.

The second order ODE in (18), through the following substitution:

$$\begin{aligned} r_z' &= u(r) \\ r_z'' &= \frac{du(r)}{dz} = \frac{du}{dr}\frac{dr}{dz} = u_r' r_z' = u_r' u \end{aligned} \tag{20}$$

can be further reduced to the following first order ODE in the form of an Abel differential equation of the second kind, where the independent variable is r, while z and t act as parameters:

$$u u_r' = f_1 u^2 + f_2(r,[z,t])\, u + f_3(r,[z,t]) \tag{21}$$

The following functional transformation [22]:

$$w(r) = u(r)\, E(r), \quad E(r) = e^{-\int f_1(r)dr} = \alpha r e^{\mathcal{A}r} \tag{22}$$

where α is a constant of integration, allows to rewrite (21) as:

$$w w_r' = g_1(r,[z,t])\, w + g_0(r,[z,t]) \tag{23}$$

where:

$$\begin{aligned} g_1(r,z,t) &= f_2(r,z,t)\, E(r) \\ g_0(r,z,t) &= f_3(r,z,t)\, E^2(r) \end{aligned} \tag{24}$$

Finally, by the following substitution [22]:

$$\begin{aligned} x(r) = \int g_1(r,z,t)\, dr = R(r) \quad &\Leftrightarrow \quad r = R^{-1}(x) \\ y(x) = w\left(R^{-1}(x)\right) \quad &\Leftrightarrow \quad w(r) = y(R(r)) \end{aligned} \tag{25}$$

the Abel equation of second kind (23) can be written in the normal form:

$$y(x)\, y_x'(x) - y(x) = Q(x) \tag{26}$$

where:

$$Q(x) = \frac{g_0(x)}{g_1(x)} = \frac{g_0\left(R^{-1}(x)\right)}{g_1\left(R^{-1}(x)\right)} \tag{27}$$

More specifically, after some manipulation, (24) and (25) can be calculated, respectively as follows:

$$\begin{aligned} g_1(r,z,t) &= -\alpha\left(\mathcal{B}r + \mathcal{C}z + \mathcal{D}\right) e^{\mathcal{A}r} \\ g_0(r,z,t) &= -\alpha^2\left(\mathcal{E}r^2 + (\mathcal{F}z + \mathcal{G})\, r + \mathcal{H}z^2 + \mathcal{I}z + \mathcal{J}\right) e^{2\mathcal{A}r} \end{aligned} \tag{28}$$

and

$$\begin{aligned} x(r) &= -\alpha\left(\frac{\mathcal{B}}{\mathcal{A}}r - \frac{\mathcal{B}}{\mathcal{A}^2} + \frac{\mathcal{C}z + \mathcal{D}}{\mathcal{A}}\right) e^{\mathcal{A}r} = R(r) \\ r(x) &= \frac{1}{\mathcal{A}}\left(W\left(-\frac{\mathcal{A}^2}{\alpha\mathcal{B}} e^{-1+\frac{\mathcal{A}}{\mathcal{B}}(\mathcal{C}z+\mathcal{D})} x\right) + 1 - \frac{\mathcal{A}}{\mathcal{B}}(\mathcal{C}z + \mathcal{D})\right) \end{aligned} \tag{29}$$

where W is the Lambert W function.

A methodology to solve (26) has been presented in [11, 12].

Now, following the inverse procedure, from the solution of (26) tigether with (25), the solution of (23) is:

$$w(r,[z,t]) = y(R(r),[z,t]) \tag{30}$$

which through (22) gives the solution of (21) as:

$$u(r,[z,t]) = \frac{w(r,[z,t])}{E(r)} \tag{31}$$

Now, by solving the differential equation in the first of (20) after substituting (31) in it, $r(z,[t])$ has to be derived from the following implicit equation:

$$z = \int \frac{dr}{u(r,[z,t])} = S(r,z,[t]) \tag{32}$$

Similarly, by substituting the solution of (32) in the first of (17), $z(t)$ has to be derived from the following implicit equation:

$$t = \int \frac{dz}{r(z,[t])} = T(z,t) \tag{33}$$

Now, substituting the solution of (33) in (15), the expression of $F_f(t)$ is provided, which in turn allows to calculate $V_e(t)$, $F_a(t)$ and $F_b(t)$ from (14), (13) and (12), respectively. Finally, once the expression of $F_b(t)$ is known, also $V_f(t)$ and $V_b(t)$ can be calculated from (10) and (9), respectively.

4 NUMERICAL RESULTS

In this section we show a typical solution of the system of ordinary differential equations (3)-(8) modeling the fusion process. The temporal evolution of the concentrations of EVs and fusogenic proteins in each phase of the fusion process is drawn in Fig. 3(a) and Fig. 3(b), respectively. As expected, after an initial phase where all the available fusogenic proteins establish the binding with the EVs, the concentration of bound EVs-proteins, $V_b = F_b$, remains constant, and the concentration of EVs linearly decreases in the extracellular space, V_e, while it linearly increases inside the cell, V_f. This behavior is shown for about 15 hours, when the concentration of EVs in the extracellular space becomes lower than the concentration of available fusogenic proteins F_a. At this point the concentration of the bound EVs-proteins gradually decreases while the EVs remaining in the extracellular space are slowly internalized.

5 CONCLUSIONS AND FUTURE WORKS

In biological cell communication through exchange of EVs, several concurrent uptake mechanisms take place. It is reasonable to expect that differences among various EV internalization processes performed by the target cells, may be related to different functionalities, even producing different effects. Therefore, a deep investigation of the possible interactions, cooperation or competition among concurrent internalization processes is important in a large

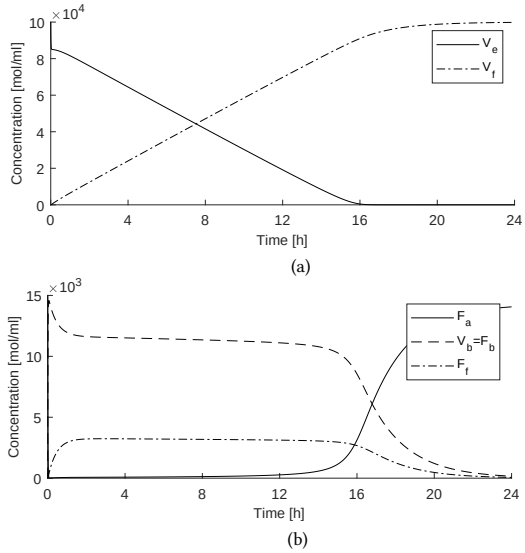

Figure 3: Concentration of EVs (a) and fusogenic proteins (b). Initial conditions: $F_a(0) = 1.5{\cdot}10^4\mathbf{mol/ml}$; $V_e(0) = 10^5\mathbf{mol/ml}$; $F_b(0) = F_f(0) = V_b(0) = V_f(0) = 0\mathbf{mol/ml}$. **Parameter values:** $a_b = 3 \cdot 10^{-7}\mathbf{mol}^{-1}\mathbf{s}^{-1}$; $b_f = 1.4 \cdot 10^{-4}\mathbf{s}^{-1}$; $b_d = 5 \cdot 10^{-4}\mathbf{s}^{-1}$; $b_u = 10^{-6}\mathbf{s}^{-1}$; $b_a = 0\mathbf{s}^{-1}$; $b_x = 10^{-6}\mathbf{s}^{-1}$; $k_F(t) = 0$; $k_V(t) = 0$.

variety of applications. To this purpose, understanding the dynamics of EV fusion with the plasma membrane of the target cell, which is the focus of this paper, represents a first step in the direction of building a more general picture of the cell communication mechanisms.

To this aim, a mathematical model of the membrane fusion between EVs and target cells has been derived. More specifically, different phases of the fusion process have been identified and described through a system of ordinary differential equations. The variables of these equations are the temporal evolution of the concentration of both EVs and fusogenic proteins on the membrane of the target cells, in different phases of the fusion process. The parameters of the equations are the rates of the chemical reactions involved in EV binding and fusion, as well as in the recycling/regeneration process of the fusogenic proteins.

Unfortunately, poor information is available in literature about those parameters, since the membrane fusion between EVs and cells has received attention only recently and has not been deeply investigated yet. Therefore, proper meaningful values for the model parameters are still not available. In order to retrieve such a deficiency of information, a possible strategy is to compare the analytic solution of the proposed model with biological experimental results. This would allow to upwards infer reasonable values for the model parameters. The needed biological experiment are currently in progress and interesting results are expected in the near future. However, one of the major challenges in the proposed strategy lies in the arduousness of providing a parametric solution of the system of non linear differential equations, modeling the surface EV-cell fusion. With that in mind, this work provides the mathematical methodology to supply an analytic solution of the model, so representing a first crucial step towards a more general and comprehensive contribution to the study of EVs communication.

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