Modeling the EV Concentration in Different Internalization Phases

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***Abstract*—Extracellular Vesicles (EVs) secreted by various cells interact with target cells, inducing biological changes and responses. To exploit the potential of such cell-to-cell communication, a challenging step is the study of the EV uptake mechanisms, for which we proposes an analytical model, aimed at evaluating the EV concentration in different phases of the internalization process. Examples illustrate the use of the model for therapeutic strategy design.**

***Index Terms*—Extracellular Vesicles, Cell-to-cell Communication, Mathematical Model**

I. Introduction

Extracellular Vesicles (EVs), nano-sized spherical particles secreted by various cells, can carry diverse molecules and interact with target cells, inducing biological changes [1]–[4]. Despite their potential [5]–[7], challenges arise in studying EV uptake mechanisms and their relationship with cellular responses [8], [9]. We proposes an analytical model for EV uptake, aimed at evaluating the EV concentration in different phases of the internalization. Examples illustrate the use of the model for therapeutic strategy design by analyzing cellular responses to different EV administration patterns.

II. The Model

Fig. 1 provides a schematic representation of the EV uptake process together with the model variables and parameters. The red EVs in the figure represent the administered EVs, labeled with the fluorescent dye. The administered EVs initially in the extracellular space, V_e in the model, become first bound, V_b, and then move inside the cell. The internal EVs, denoted as V_u, are the unaltered EVs inside the cell, before they are possibly either released or processed by the cell, i.e. metabolized or addressed to lysosomes for degradation. At the same time, *de novo* EVs are produced by the cell. These may be unintentionally labeled (stained) with the fluorescent dye, like the administered EVs, as a consequence of the rearrangement into the membrane of the *de novo* EVs of the dyed membrane of the administered ones. The ordinary *de novo* EVs, produced by the target cell without the redistribution of the fluorescent dye (blue in the figure) are not of interest for the model. Instead, the stained *de novo* EVs (blue with red contour in the figure), are included in the model, to account for the measurement errors due to the dye misplacement, i.e. the efflux of fluorescent dye, caused by the secretion of the *de novo* EVs outside the cell. More in dept, the portion of processed EVs whose dye is still inside the cell, is denoted as V_x. The EVs whose dye has been moved outside the cell, by the secretion of stained *de novo* EVs, is tracked through the number of external stained *de novo* EVs, denoted as W_e.

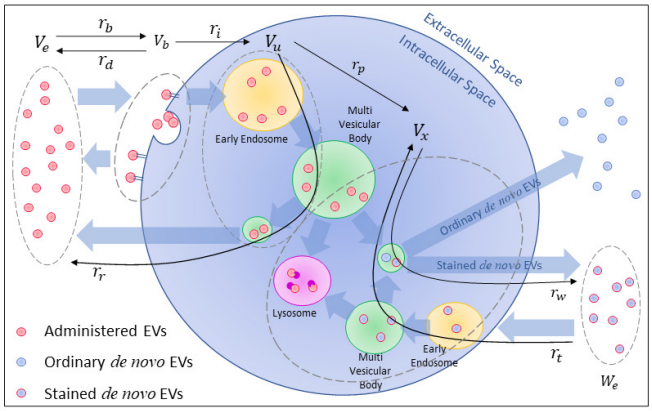

Fig. 1. Uptake model representation.

The total number V_p of processed EVs, the number V_m of EVs measured inside the cell, the number V_y of internalized EVs, and the number Z_e of EVs measured in the extracellular medium, can be calculated as:

$$\begin{aligned} V_p &= V_x + W_e, & V_m &= V_u + V_x \\ V_y &= V_m + W_e, & Z_e &= V_e + W_e \end{aligned} \tag{1}$$

The evolution of the EV uptake can be summarized by the following simplified reaction scheme:

$$\{external\ medium\} \xrightarrow{g_V} V_e \underset{r_d}{\overset{r_b}{\rightleftarrows}} V_b \xrightarrow{r_i} V_u \xrightarrow{r_p} V_x \underset{r_t}{\overset{r_w}{\rightleftarrows}} W_e \tag{2}$$

where g_V is the supply rate of EVs to the extracellular medium, i.e. the number of EV supplied to the extracellular medium by external sources in the time unit. All the above considerations lead to a system of equation modeling the EV internalization process, that can be written as follows:

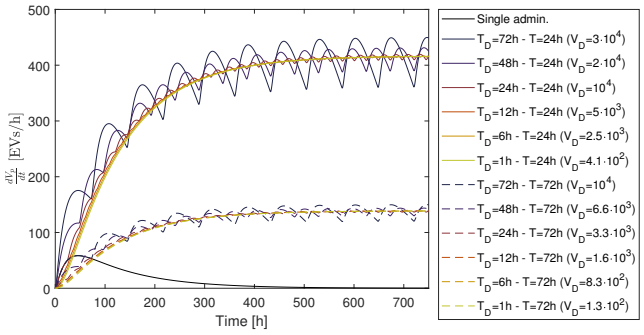

Fig. 2. Temporal behavior of $\frac{dV_p}{dt}$ for a repeated administration of EVs every T_D hours, such to maintain a dosage of 10^4 EVs in $\{24, 72\}$ hours.

$$\frac{dV_e(t)}{dt} = -r_b V_e(t) + r_d V_b(t) + r_r V_u(t) + g_V(t) \quad (3)$$

$$\frac{dV_b(t)}{dt} = r_b V_e(t) - (r_d + r_i) V_b(t) \quad (4)$$

$$\frac{dV_u(t)}{dt} = r_i V_b(t) - (r_r + r_p) V_u(t) \quad (5)$$

$$\frac{dV_x(t)}{dt} = r_p V_u(t) - r_w V_x(t) + r_t W_e(t) \quad (6)$$

$$\frac{dW_e(t)}{dt} = r_w V_x(t) - r_t W_e(t) \quad (7)$$

In order to solve and to use the above internalization model the following steps have to be computed:

Step 1 Given the temporal progression of the subset of EVs measured in a lab experiment, the analytical expression of the corresponding model variable, has to be derived.

Step 2 The best fitting of the temporal progression provided by the biological experiment has to be performed, in order to find the coefficents of the analytical expression provided by the *Step 1*.

Step 3 The model parameters for the couple EV-cell under study are found by equating the coefficients found through the best fitting analysis (*Step 2*) and their analytical expression according to *Step 1*.

In the following an example of the potential use of the model is illustrated.

III. Therapy design support

In order to maintain therapeutic effects in a target cell over time, it may be necessary to cyclically activate the pathways stimulated by the EV administration, so as to maintain a certain level of internalization over time. Testing these events in a lab experiment is challenging and costly because it requires the ability to study a large number of time points as the EVs administration evolves.

The proposed model is a good support to deal with these studies; so, in this section, we focus on the effects exerted by repeated EV administrations to a target cell. Let us suppose we are interested to administer, periodically, the same amount of EVs, and we would like to know how the evolution of the variables of interest changes with respect to the case of a single administration. The periodical administration of V_D EVs every T_D hours, for example, can be mathematically represented as an impulse train, of area V_D and period T_D, as follows:

$$g_V(t) = V_D \sum_{n=0}^{n_D} \delta(t - nT_D) \quad (8)$$

Let us focus on a time window of 750 hours, and let us consider the case where V_D EVs are administered every $T_D = \{1, 6, 12, 24, 48, 72\}$ hours. The dosage V_D is calculated such that the total amount of EVs administered in T hours remain constant and equal to $V_T = 10^4$, for $T = \{24, 72\}$ hours, i.e.:

$$V_D = V_T \frac{T_D}{T} \quad (9)$$

Fig. 2 shows the evolution of the rate of variation for the number of processed EVs, i.e. the derivative of the model variable V_p, in the above case. We can observe how the choice of T determines the overall evolution of these variables, whereas the period, and the corresponding dose according to (9), determines the variation amplitude around the overall evolution. As an example, let us assume that for some reasons that are out the scope of this papers, the desired response of the target cell is obtained when the amount of EVs processed over time is about 400 EVs/h, with variations smaller than 5%. Then the analysis of Fig. 2 allows to evaluate the administration strategy to be adopted. In particular, the best choice in this case correspond to the administration of $V_D = 5 \times 10^3$ EVs every $T_D = 12$h.

IV. Acknowledgement

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