Extracellular Vesicle Propagation in Acidic Tumor Microenvironment

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ABSTRACT

A complex and dynamic tumor microenvironment (TME) plays a crucial role in angiogenesis, proliferation, and invasion of tumors. The acidity of the TME has a key impact on the release of extracellular vesicles (EVs) which, in turn, provide novel opportunities to combat tumors. This observation opens up the development of efficient cancer treatment strategies where knowing the EV distribution becomes essential. However, an analytical model of the EV biodistribution which accounts for the effects of extracellular pH (pH_e) has been missing. Here we propose a simple model for an EV-mediated molecular communications (MC) system which comprises tumor cells as transceivers and the extracellular matrix of the TME as the EV propagating medium. We account for the effect of the acidity considering the pH_e-modulated EV release from tumor cells, and analytically describe the EV propagation dynamics using a partial differential equation that we solve analytically and verify its solution using the numerical method based on finite element analysis. The obtained results show that the TME acidity characterized with pH values largely affects the EV biodistribution in the TME.

CCS CONCEPTS

• **Applied computing → Life and medical sciences**; *Computational biology*; Telecommunications; Health care information systems.

KEYWORDS

Cancer, Tumor Microenvironment, Molecular Communications, Extracellular Vesicles, Extracellular pH.

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1 INTRODUCTION

The combat against cancer has been heavily influenced by the principle of Paul Ehrlich's 'magic bullet' where the drug should be injected into the body and transported to the correct target [7]. The idea was originally introduced more than 100 years ago, and validated by the discovery of antibiotics 50 years later. With cancer still prevailing, the scientific community is urged to create and develop novel therapeutic solutions. Naturally occurring nanoparticles — extracellular vesicles (EVs) — have recently emerged as potential vehicles to deliver therapeutics targeting sick cells [9].

EVs are nanosized cellular vesicles released from various types of normal and neoplastic cells, which perform various biological functions (for example, disposal of cellular waste products, release of foreign invaders, control of gene expression, and activation of the immune system) [11]. EVs act as mediators of intercellular communication in the body by transferring active biomolecules to adjacent and distant cells [9]. They also modulate the tumor microenvironment (TME) into a tumor-promoting system [19], by transferring biomolecules [3] which promote tumor proliferation, evasion of cell death, angiogenesis, modified metabolism, invasion, and metastasis [19]. For this reason, EV-mediated signaling may hold promising cancer treatment strategies and be an effective platform for 'magic bullet'-based drug delivery [2, 10, 13, 20]. However, diverse biological factors affect EV production and their release and include cellular differentiation, stress, activation, senescence stimulation with cytokines, shear force exposure to ATP, apoptotic cellular breakdown and, particularly in the TME, microenvironmental acidity [1]. Moreover, administration of EVs may reach and accumulate in other sites beyond the tissues of therapeutic interest. Therefore, analysis of the EV biodistribution is a prerequisite for the development of EV-based therapeutics.

In this paper, we propose using the molecular communications (MC) paradigm [4–6], which combines analytical approaches from information and communication theory and biology, to establish an analytical framework which could be used to estimate the EV biodistribution in the TME. The MC paradigm has already been applied for the analyses of EV-based signaling systems [15], complementing some of the existing frameworks, for example, developed for the analyses of nanoparticle distribution in the brain [17]. However,

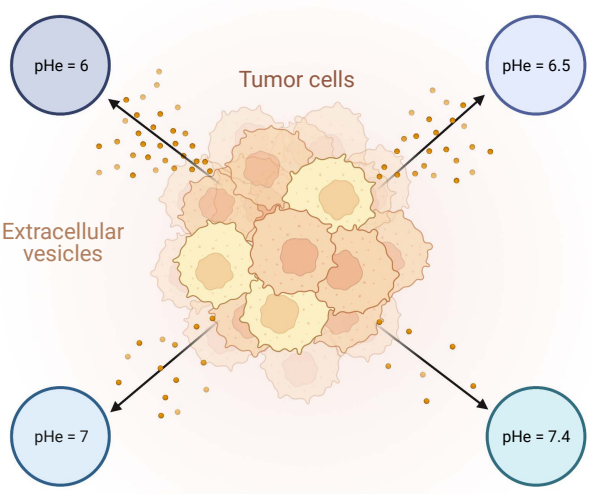


Figure 1: The extracellular pH (pH_e) influences the release of extracellular vesicles from tumor cells into the tumor microenvironment. The illustration was created using BioRender.com.

the developed frameworks do not consider the TME as the propagating medium, and thus could not be readily applied for the EV biodistribution analysis in the TME. Thereby, we present here the first attempt that investigates the TME from the MC engineering perspective.

Our contribution is to examine the influence of the TME acidity, which we characterize by extracellular pH, i.e. pH_e, on the EV release from tumor cells and, consequently, the EV biodistribution in the TME. In comparison with healthy tissues, many tumors have ~0.3-0.5 more acidic pH_e than tumor intracellular pH, i.e. pH_i [16], which is a cause of deregulated metabolism and poor vascularization [8]. The so-called reversed pH gradient ($pH_e - pH_i < 0$) promotes tumor cell growth and invasion [8], and reduces the viability and function of most normal cells, including cytotoxic T cells that ordinarily mediate the immune response to tumor antigens [7]. Hence, manipulating the TME acidity could serve as a selective strategy to increase tumor cell death and/or to reduce proliferation and invasion by increasing pH_e. Given this, we analytically model the pH_e-modulated EV release from tumor cells and incorporate the effect of pH_e into the EV-mediated MC channel model for the tumor tissue (Fig. 1). Our developed model presents the first step towards establishing a more realistic channel model that reflects the TME acidity property. Inspecting the EV distribution could potentially serve as a marker of the TME acidity level change for creating an MC-based approach for cancer treatment and diagnosis.

We organize the remainder of this paper as follows. Section 2 presents the developed channel model for the EV propagation under pH_e influence in the TME. Section 3 provides analytical solution to the proposed EV-based MC channel model. Section 4 presents the numerical and analytical results that stem from the proposed model and its analytical solution. Finally, Section 5 concludes the paper and indicates future research ideas.

2 EV-BASED MOLECULAR COMMUNICATION CHANNEL MODEL FORMULATION

TME is a space where numerous molecules diffuse transporting metabolic information among cells. Even though this transport of molecules is complex, it has been successfully described using relatively simple models. Here, we propose a channel model for EV propagation in the TME incorporating tortuosity (λ) and volume fraction (α) [14] as relevant characteristics of the medium, and the influence of the TME acidity on the release of molecules from tumor cells. More precisely, we model and analyze the impact of the TME's pH_e on the EV propagation considering the isotropic EV diffusion. As an application scenario for numerical results, we consider the brain extracellular matrix (ECM), while the proposed model is applicable to other ECMs with tumor problem. Of note, we limit the paper's content to the aforementioned properties of the EV propagation in the TME with awareness of many other possible factors which may affect the system model — few of which we are planing to incorporate in the future work. In other words, this work presents an initial model which will be further developed and advanced for creating plausible biological equivalent.

Tortuosity represents the degree to which molecular transport is slowed down by the porous medium and incorporates: (a) the additional distance a molecule must travel to move around obstacles in the medium, and (b) how a molecule is affected by the interaction with the walls and extracellular matrix, or exclusion from pathways due to molecular size [14]. Tortuosity is defined as [17]:

$$\lambda = \sqrt{\frac{D}{D^*}},$$

where D and D* are the diffusion coefficient and the effective diffusion coefficient of EVs in the (brain) extracellular matrix, respectively.

Volume fraction defines a percentage of the total tissue volume accessible to the propagating EVs and is defined as $\alpha = V_{ECM}/V_{Tissue}$, where V_{ECM} denotes the volume of the extracellular matrix and V_{Tissue} denotes the volume of the whole tissue measured in a small region of the brain [18]. Tortuosity of approximately ≈ 1.6 (for small molecules) and volume fraction of about 20% should be consistent across brain regions [14].

We consider a tumor cell as the EV source which is influenced by the TME's pH_e — the more acidic the TME (lower pH_e), the higher the EV release from a tumor cell [9]. The EV transport between cells is driven by diffusion and advection. Initially, we only consider diffusion as it primarily defines molecular dynamics in the extracellular space [5]. Diffusion-based molecular transport is when molecules move from a region of higher concentration to a region of lower concentration as a result of thermal motion. We also consider an isotropic TME where tortuosity λ takes a scalar value, and the radial symmetry of the EV concentration where the angular coordinates are not considered. We denote $C(r,t)$ as the EV concentration in the TME around a tumor cell as a function of both time t and radial distance r, and describe its dynamics with the following diffusion partial differential equation (PDE):

$$\frac{\partial C(r,t)}{\partial t} = D^* \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial C(r,t)}{\partial r}\right) + \frac{s(r,t)}{\alpha}, \text{ in } \Omega \times [t_0, t_{\text{end}}], \quad (1)$$

where $[t_0, t_{\text{end}}]$ interval and Ω denote simulation time and space intervals, respectively. The first term on the right side of (1) is the 'diffusion' term, and represents the EV propagation in the TME, where the effective diffusion coefficient is defined by tortuosity. The second term on the right side of (1) is the 'source' term, $s(r,t)$, and we define it as

$$s(r,t) = f(t, pH_E) \exp\left(\frac{-(r-r_S)^2}{2\sigma^2}\right), \tag{2}$$

where $f(t, pH_E)$ represents the EV release in time 'modulated' by pH_e, and pH_E is a variable that describes the pH_e value in the TME. The second product defines the spherical source where r_S defines the source position and σ defines the cell size. We model function $f(t, pH_E)$ using the experimental data [12], and assume that the same nature of the EV release applies to all tumor cells:

$$f(t, pH_E) = A \exp\left(\frac{pH_0 - pH_E}{B} t\right), \tag{3}$$

where A and B are fitting parameters, $pH_0 = 7.4$ is a referent pH value of an alkaline TME when the EV release can be approximated as constant. We assume that pH_E is constant in time and space. In reality, pH_e changes in time and space distancing from a tumor cell, i.e., tumor source.

We set zero initial and boundary conditions, defined in (4) and (5), respectively, which would ease the analysis of the EV dynamics:

$$\text{Initial Condition :} \quad C(r, t_0) = 0, \text{ in } \Omega, \tag{4}$$

$$\text{Boundary Condition :} \quad \frac{\partial C(\infty, t)}{\partial r} = 0, \text{ for } t \in [t_0, t_{\text{end}}]. \tag{5}$$

In the following section, we present the analytical solution to the defined analytical EV propagation model (1). After that, we describe the applied methodology to obtain results for (1) and (10), and provide results in Section 4.

3 ANALYTICAL SOLUTION

We propose a closed form solution for the partial differential equation (PDE) (1), by rewriting it to contain coefficients independent of the space. This can be done using the following auxiliary function:

$$U(r,t) = rC(r,t). \tag{6}$$

After applying (6) to (1) and performing simple manipulations, we yield:

$$\frac{\partial U(r,t)}{\partial t} = D^* \frac{\partial^2 U(r,t)}{\partial r^2} + S(r,t), \text{ in } \Omega \times [t_0, t_{\text{end}}], \tag{7}$$

where

$$S(r,t) = r\frac{s(r,t)}{\alpha}. \tag{8}$$

Eq. (7) can be solved using the Green's function theorem. In this way, the solution is expressed as the convolution of the channel impulse response $G(r,t)$ (the problem Green's function) and the source function $S(r,t)$. A closed form representation of $G(r,t)$ is then given by:

$$G(r,t) = \frac{1}{\sqrt{4\pi D^* t}} e^{\frac{-r^2}{4D^* t}}, \tag{9}$$

and, finally:

$$U(r,t) = \int_0^\infty \int_0^\infty S(r-r', t-t') G(r', t') dt' dr'. \tag{10}$$

The convolution integral in (10) can be easily calculated using the 2D fast Fourier transform. This approach could also be exploited for an arbitrary source function. Furthermore, it could be adapted to a bounded medium with a symmetric boundary condition by using appropriate Green's function.

4 RESULTS

In this section, we present numerical results to verify the solution given in (10). Here, we adopt a finite element method by employing MATLAB's PDE tools for verification. First, we state in the following paragraph the specific limitations imposed by (1) with respect to the whole (brain) physiological organization and model simplification.

The tortuosity value is assumed as constant. The observed pH_e range is from 7.4 to 6, since this range presents a transient region from an alkaline to acidic TME, which includes both states. The pH_e values lower than 6 are considered as purely acidic, and values greater than 7.4 are considered as purely alkaline. The time period for the numerical and analytical simulations is set to 4 days to contain enough time to observe the effect of the acidification process. We conduct simulations for four scenarios with different pH_e values: 6, 6.5, 7, 7.4. We consider a large distance radius within the TME which imposes an unbounded EV propagation medium. As a source, we consider a mass of tumor cells centered at $r_S = 0$ and spatially extended following the Gaussian function given by (2). We numerically compute the channel model using (1) and analytically using (10). Other TME parameters and their values for the EV propagation model are specified in Table 1 and taken from [18].

Fig. 2 displays the EV release quantified in picograms per cell ([pgr/cell]) from a tumor cell defined by (3) for four TME acidity levels — pH_e: 6, 6.5, 7, 7.4. The figure shows that lower pH_e values lead to the higher EV release from a tumor cell. This source consequently has impact on the EV concentration in the TME, which is further indicated in Figs. 3a – 3d. The corresponding color scale

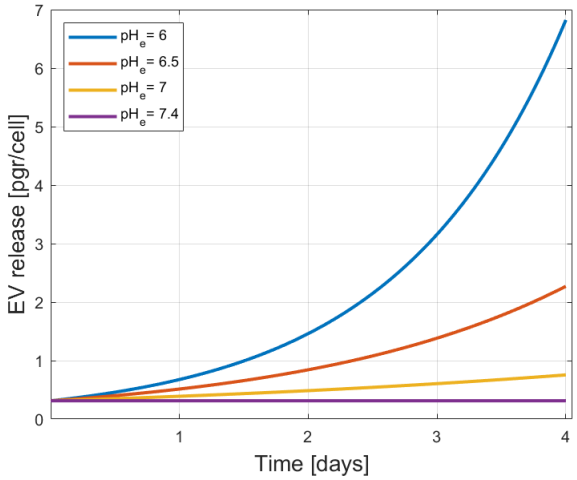
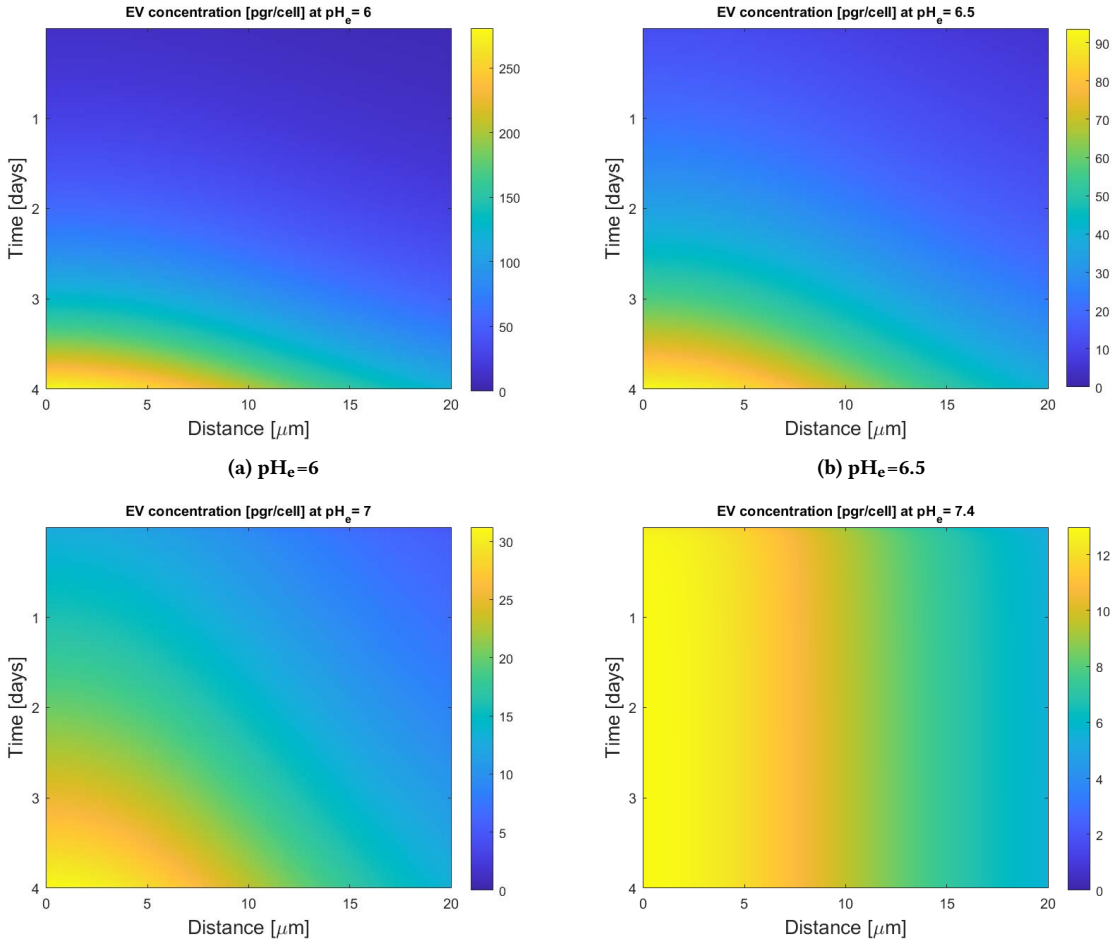


Figure 2: The effect of the tumor microenvironment acidity level (pH_e) on the temporal evaluation of the EV release from a tumor cell evaluated using (3). The data for the EV release dynamics are taken from the experiments [12].



(a) pH_e=6 **(b) pH_e=6.5**

(c) pH_e=7 **(d) pH_e=7.4**

Figure 3: Temporal and space dynamics of the EV concentration for four different pH_e scenarios based on (1).

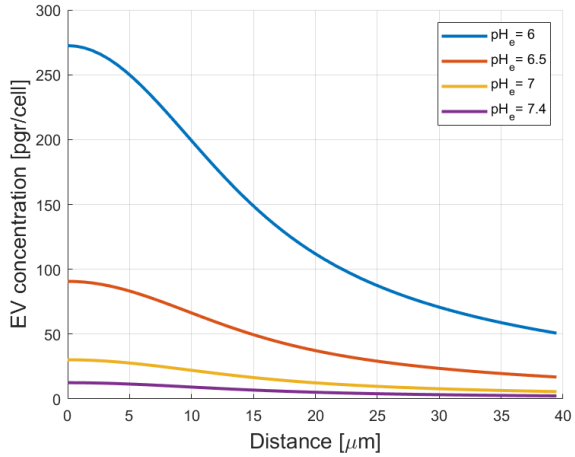


Figure 4: Distance evaluation of the EV concentration based on (1) given for $t = 4$ days for four different tumor microenvironment acidity level (pH_e) values.

Table 1: The values of simulation parameters for EV propagation in the TME.

D [18]	α [18]	λ [18]	pH_0	A	B	σ
$15\ \frac{\mu m^2}{s}$	0.2	1.6	7.4	0.3132	$1.57 \cdot 10^5$	$7 \cdot 10^{-6}$

bars in Figs. 3a – 3d show that the EV concentration is generally higher for the lower pH_e values.

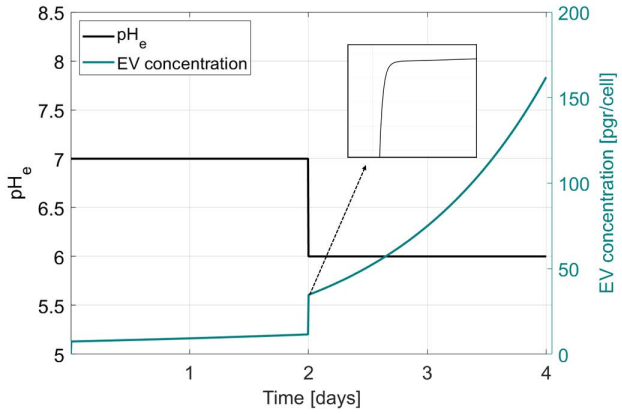
Fig. 4 illustrates the distance evaluation of the EV concentration given for $t = 4$ days for all four simulated scenarios. Please note that in the scenario depicted in Fig. 3d, the EV release is constant in time as the model suggests according to the experimental data.

We also conduct numerical simulations by considering time varying pH_e. In the first simulated scenario, pH_e decreases from 7 to 6 after 2 days in a step manner. The pH_e time-varying function and the simulation result are depicted in Fig. 5a. This scenario shows that a lower pH_e value has higher impact on the EV concentration.

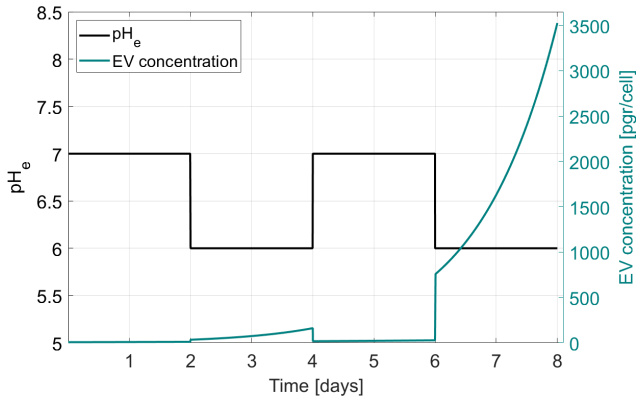
Step change in pH_e results in a very quick transition in the EV concentration (the transition period is zoomed in and shown in the inset). In the second simulated scenario, pH_e is defined as a periodic function with a 2-day-time period and is simulated in an 8-day-time frame. The pH_e time-periodic function and the simulation result are depicted in Fig. 5b. This scenario shows that as pH_e changes to 6, higher EV accumulation is observed by comparing the levels in the first and the third time period. In both scenarios (Fig. 5a and Fig. 5b), we notice very rapid changes in the results upon the pH_e changes.

Finally, we present the comparative representation of analytical and numerical results for four different scenarios with different pH_e values. We calculate analytical results using (10) for a simulation time period of 4 days. The comparative analytical and numerical results for four scenarios at $t = 4$ days are shown in Fig. 6a and Fig. 6b, considering a distance evaluation of the EV concentration in the last simulated time point. The analytical solution approaches numerical solution after 20 μm. Of note, we only observe the spatial evaluation approximately out of a tumor cell, whose size is parameterized by σ.

The current simplified model presented in (1) and (10) grounds on biological experimental results, where we have used the corresponding dataset [12] for model formulation and parameterization. The model correctness has been evaluated using both the numerical and analytical approaches. Nonetheless, the model has not been verified using the available dataset. Unfortunately, there exists no other datasets on EV biodistribution which could have potentially been used for the pressented model verification. The presented results are also limited by the computer memory since the analytical

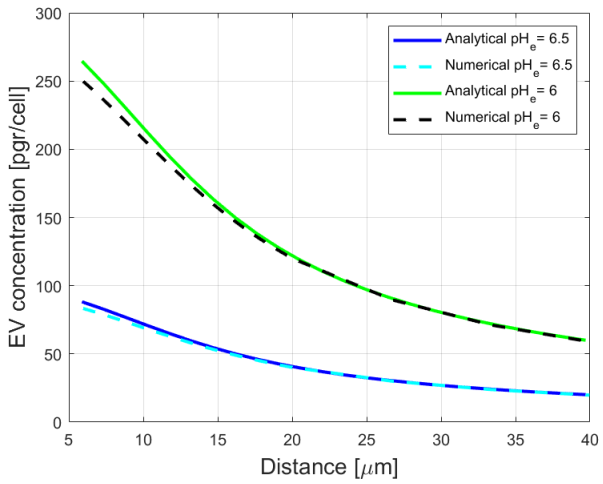


(a) Time-step decreasing pH_e scenario.

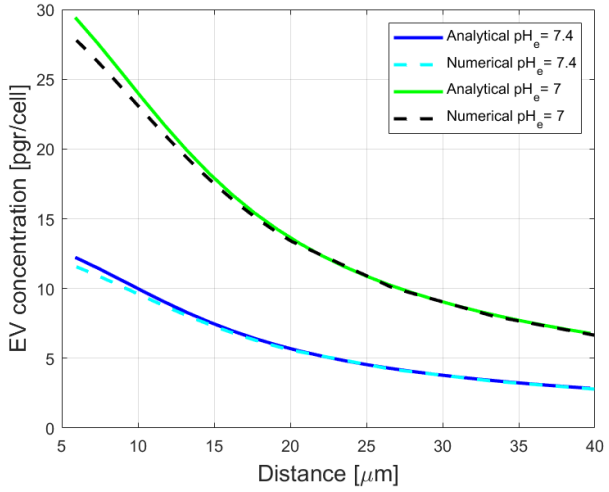


(b) Time-periodic pH_e scenario.

Figure 5: Temporal evaluation of the EV concentration for two pH_e scenarios based on (1).



(a) pH_e 6 and 6.5.



(b) pH_e 7 and 7.4.

Figure 6: The comparison between analytical (solid line; based on (10)) and numerical (dashed line; based on (1)) distance evaluation of the EV concentration in the TME. The comparison plots are shown at $t = 4$ days for four pH_e scenarios.

solution depends on the time-space grid size to perfectly match the numerical solution.

5 CONCLUSION

In this paper, we have developed a model of the extracellular vesicle (EV) propagation in the tumor microenvironment, which is modulated by the extracellular acidity (pH). We have first formulated a partial differential equation in one-dimensional space and solved it analytically and numerically applying the finite element method. The source term, which imposes the input boundary condition, presents the EV release from a tumor cell which is modulated by the extracellular pH value.

From the communication perspective, we have observed that the more acidic TME increases the EV release from tumor cells which, in essence, amplifies the transmitter's signal power. This idea shows that it is possible to modulate the tumor-cell-originating EV release and tumor operation by manipulating the acidic TME property. This natural phenomenon is well-known and has been studied in the past from medical and chemical perspectives, but still imposes new challenges and ideas. Our proposed model can further be attributed for the control and detection of tumor progression and existence. Moreover, it can be used for the optimization and development of drug delivery systems.

We aim to improve this model further by considering a time and space distribution of hydrogen (H^+) ions in the tumor microenvironment, advection and degradation of EVs.

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