# A multiscale model to evaluate the efficacy of anticancer therapies based on chimeric polypeptide nanoparticles

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A multiscale model for tumor growth and its chemotherapy using conjugate nanoparticles is presented, and the corresponding therapeutic outcomes are evaluated. It is found that doxorubicin assembled into chimeric polypeptide nanoparticles cannot eradicate either vascularized primary tumors or avascular micrometastasis even administrated at loads close to their maximum tolerated doses. Furthermore, an effective and safety treatment demands for conjugate nanoparticles targeted to the malignant cells with much higher specificity and affinity than those currently observed in order to leave most of the normal tissues unaffected and to ensure a fast intracellular drug accumulation. © *2011 American Institute of Physics*. [doi:10.1063/1.3551619]

Despite the progress that has been made in imaging, diagnosis, and treatment of cancer, the survival rates of patients with tumors at unresectable locations and metastatic or recurrent neoplasies are still low.[1] On the quest for alternative treatments, nanotechnology appears as a major and promising route to the development of innovative anticancer agents.[2] Nanoparticles (NPs), such as quantum dots,[3] colloidal gold,[4] magnetic carriers,[5] and polymeric nanomicelles,[6] can be used to target malignant cells and their microenvironment when conjugated with ligands specific against markers on the surface of these targets. Nowadays, several nanotherapeutic strategies are under very active experimental tests and clinical trials.[7]

Recently, Mackay *et al.*[8] reported on a chimeric polypeptide (CP) that self-assembles into NPs on doxorubicin (Dox) attachment. These CP-Dox NPs are near-monodisperse, biodegradable, exhibit low toxicity, and were able to induce nearly complete cancer regression of a murine tumor implanted in mice after a single dose.[9] The high antitumoral efficacy, assembly flexibility, and genetically encoded synthesis that provide great pharmaceutical viability make such recombinant CP-NPs uniquely attractive for the development of advanced drug carries.

Nonetheless, several fundamental issues and technical hurdles must be understood in order to enhance the efficacy of antitumor nanotherapy. The nonlinearities and complexities inherent to tumor-drug interactions claim for a mathematical approach. Quantitative models can enlarge our understanding of the parameters influencing therapeutic outcomes, guide essays by indicating relevant physiological processes for further investigation, and prevent excessive experimentation needed to develop effective treatments. In this letter, we integrate varied processes concerning tumor growth, CP-Dox pharmacokinetics, and cytotoxity into a multiscale model for tumor pathophysiology and its systemic response to chemotherapies.

The model, adapted from our work on oncolytic virotherapy,[10] consists of a square lattice (the tissue) fed by a single capillary vessel. Any site is empty or occupied by only one of the cell types (normal or tumoral). The nutrient concentration is described by a linear diffusion equation

$$\frac{\partial N}{\partial t} = \nabla^2 N - \alpha^2 N\sigma_n - \lambda\alpha^2 N\sigma_c, \tag{1}$$

with distinct uptake rates for normal and cancer cells and a characteristic length scale $\alpha$ for nutrient diffusion.[11] $\sigma_n=0$ or 1 and $\sigma_c=0,1,2,\ldots$ are the local populations of normal and cancer cells, respectively. Equation (1) obeys a periodic (Neumann) boundary condition along the horizontal axis (at the border of the tissue, $i=L$). At the capillary ($i=0$), the concentration is $N=1$ (fixed supply). This is a simplified representation of hypoxic regions within vascular tumors or avascular metastases, critical for evaluating chemotherapeutic outcomes of solid tumors.[9]

The tumor grows from a single malignant cell according to a stochastic dynamics involving cellular division, migration, and death whose probabilities depend on the local nutrient concentration and the intracellular level $C_3(t)$ of the released Dox. For cancer cells, their functional forms were chosen as

$$P^c_{\mathrm{div,die}} = 1 - \exp[-V^2(N/\sigma_c\theta^c_{\mathrm{div,die}})^2], \tag{2}$$

$$P^c_{\mathrm{mov}} = 1 - \exp[-V^2(\sigma_c/N\theta^c_{\mathrm{mov}})^2], \tag{3}$$

where $V$ is an effective cell viability affected by $C_3$. In turn, normal cells only die due to drug cytotoxity or nutrient deprivation with a probability $P^n_{\mathrm{die}}$, as in Eq. (2). $\theta^c_{\mathrm{div}}, \theta^c_{\mathrm{mov}}, \ldots, \theta^n_{\mathrm{die}}$ are model parameters. $C_3(t)$ is determined by the pharmacokinetics of CP-Dox NPs.[8]

The chemotherapy begins when the tumor attains $N_0$ cells and consists of periodic systemic administrations of CP-Dox at a dose $C_0$. In the blood, the NP concentration evolves as

$$\frac{dC_1}{dt} = -k_1 C_1 + C_0\delta(t - n\tau), \tag{4}$$

where $k_1=k_{el}+k_2$ is the drug's removal rate due to both clearance from the systemic circulation ($k_{el}$) and accumulation in the tissue ($k_2$); $n=0,1,2,\ldots$ and $\tau$ is the interval between administrations. CP-Dox leaking from the capillary into the tissue diffuses as

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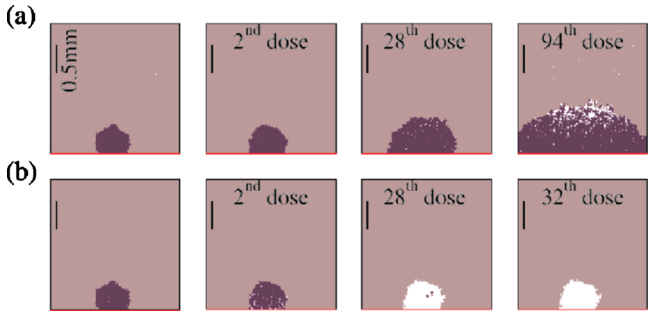


FIG. 1. (Color online) Progression of a vascular tumor treated with perfectly selective CP-Dox NPs ($\beta_n=0$). In (a) $\beta_c=5\times10^{-4}$ $h^{-1}$ is small and therapy fails. In (b) a large $\beta_c=5\times10^{-3}$ $h^{-1}$ eradicates the tumor. $C_0=500$ $\mu$M and $\tau=12$ h were used. The cancer (normal) cells are shown in darker (lighter), dead cells in white, and the capillary is the solid line at the bottom.

$$\frac{\partial C_2}{\partial t} = D\nabla^2 C_2 - \sum_{\vec{x}} [\beta_n \sigma_n(\vec{x}) + \beta_c \sigma_c(\vec{x})], \tag{5}$$

with diffusivity $D$ and endocytic rate $\beta_n$ ($\beta_c$) for normal (cancer) cells. The boundary conditions are periodic (null flux) along the horizontal axis (at the tissue border). At the capillary, the concentration is $C_2(t)=k_2C_1(t)$. Finally, at each time step a quantity $\delta_{n,c}=\beta_{n,c}C_2(\vec{x},t)$ of CP-Dox is endocytosed per cell at every occupied site. The internalized NPs degrade and release ~68% of their drug load,[9] increasing the intracellular free Dox concentration $C_3(t)$ by $0.68\delta_{n,c}$. The free drug progressively impairs cell functions in a dose-dependent way. A cell "viability" function $V=1/\{1+[C_3(t)/IC_{50}]^p\}$ was used to model the Dox effect on cell actions.

Our main simulation results[9] reveal that chemotherapies based on current CP-Dox NPs cannot eradicate either vascular solid tumors or avascular micrometastasis even assuming that NPs are perfectly selective for cancer cells ($\beta_n=0$) and doses close to the maximum tolerated dose ($C_0\sim0.6$ mM) administered at short periods ($\tau=12$ h) [see Figs. 1(a) and 2(a)]. The fail is due to the synergy of multiple factors: the fast decay of drug concentration in the blood between successive administrations that impairs a rapid accumulation of the NPs in the tissue, a high nutrient concentration nearby the capillary that supports fast tumor growth, and primarily a small endocytic rate of the NPs that elicits a slow increase of intracellular free Dox load (Fig. 3). It is worthy to notice that although the radii of CP-Dox NPs (~20 nm) are close to the size of maximum endocytic rate,[12] the rates measured by

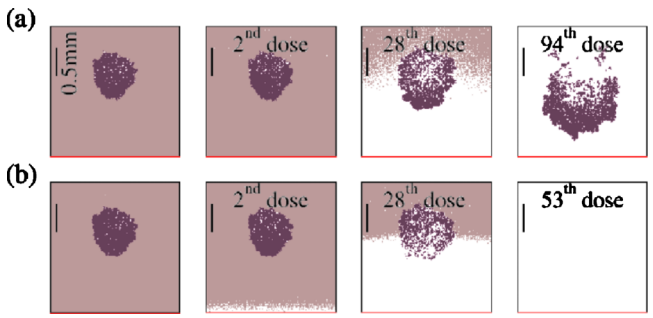


FIG. 2. (Color online) Same as in Fig. 1, but for a micrometastasis that initiated 160 lattice units from the capillary and NPs endocytosed by both normal and cancer cells at the same rate. An extensive destruction of the surrounding normal tissue is observed.

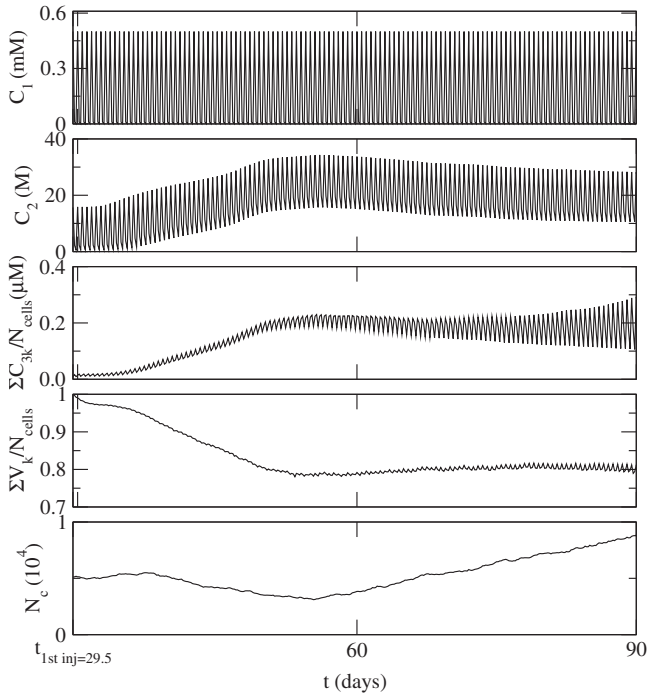


FIG. 3. Temporal evolution of the CP-Dox concentration $C_1(C_2)$ in the blood stream (tissue), mean free Dox intracellular concentration $\Sigma_{k=1}^{N_{cells}}C_{3k}/N_{cells}$, mean cell viability $\Sigma_{k=1}^{N_{cells}}V_k/N_{cells}$, and the number of cancer cells, $N_c$, in a micrometastasis. The parameters are the same as those of Fig. 2(a).

Chithrani and Chan[13] are up to 100 times smaller than the necessary ones to eradicate the cancer cells in the simulations [Figs. 1(b) and 2(b)].

As expected, the more selective are the NPs, the more effective is the therapy, because the drug internalization by normal cells decreases the available stock for cancer cell uptake. Thus, a chemotherapy using, for instance, $C_0=500$ $\mu$M and $\tau=12$ h demands 33 (18) administrations to eradicate vascular tumors (micrometastasis) fixing $\beta_n=0$ and $\beta_c=5\times10^{-3}$ $h^{-1}$ (with NPs perfectly selective), but 101 (55) administrations if the NPs are internalized at the same rate by both normal and cancer cells. Furthermore, the less selective are the NPs, the larger is the destruction of the normal tissue. Hence, unless a high selectivity is achieved, the use of CP-Dox NPs against tumors is predestined to a failure.

In summary, cancer chemotherapy using CP-NPs was evaluated through computer simulations of a multiscale model that combines diffusion equations for the nutrients, nanoparticle pharmacokinetics, and stochastic rules for the cell actions. Our results indicate that this therapy fails to eradicate solid tumors primarily due to the small CP-NP endocytic rates. Effective treatments should rely on CP-NPs exhibiting long residence time in the bloodstream, high selectivity for, and large endocytic rates by cancer cells. Such factors emerge as the main hurdles to be overcome in the search for efficient oncolytic nanotherapies.

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