Predicting DNA-mediated drug delivery in interior carcinoma using electromagnetically excited nanoparticles

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Abstract

Tumor-site-specific delivery of anti-cancer drugs remains one of the most prevailing problems in cancer treatment. While conventional means of chemo-delivery invariably leave different degrees of side-effects on healthy tissues, in recent times, intelligent chemical designs have been exploited to reduce the cross-consequences. In particular, the strategies involving superparamagnetic nanoparticles with surface assembled oligonucleotides as therapeutic carrier have raised affirmative promises. Process is designed in such a way that the therapeutic molecules are released preferentially at target site as the complementary oligonucleotide chains dissociate over the heat generated by the nanoparticles under the excitation of low frequency electromagnetic energy. In spite of the preliminary demonstrations, analytical comprehension of the entire process especially on the purview of non-trivial interactions between stochastic phase-transition phenomena of oligonucleotide chains and hierarchical organization of in vivo transport processes remains unknown. Here, we propose an integrated computational predictive model to interpret the efficacy of drug delivery in the aforementioned process. The basic physics of heat generation by superparamagnetic nanoparticles in presence of external electromagnetic field has been coupled with transient biological heat transfer model and the statistical mechanics based oligonucleotide denaturation dynamics. Conjunctionally, we have introduced a set of hierarchically appropriate transport processes to mimic the in vivo drug delivery system. The subsequent interstitial diffusion and convection of the various species involved in the process over time was simulated assuming a porous media model of the carcinoma. As a result, the model predictions exhibit excellent congruence with available experimental results. To delineate a broader spectrum of a priori speculations, we have investigated the effects of different tunable parameters such as magnetizing field strength, nanoparticle size, diffusion coefficients, porous media parameters and different oligonucleotide sequences on temperature rise and site-specific drug release. The proposed model, thus, provides a generic framework for the betterment of nanoparticle mediated drug delivery, which is expected to impart significant impact on cancer therapy.

1. Introduction

Targeting specific tumor site is a pivotal theme in modern medicine towards designing improved diagnosis and treatment of cancer. Pertinently, nanotechnology based drug delivery systems possess the ability of localized, controlled and targeted release of therapeutic chemicals with minimal perturbation to the neighboring normal cells [1,2]. To this purpose, of diverse types of materials being experimented over the years, magnetic elements and alloys have been most effective, predominantly due to their easily modifiable surface chemistry, visibility to non-invasive methods such as magnetic resonance imaging (MRI) and most notably, exclusive efficacy in converting weak electromagnetic waves to extremely concentrated heat energy. The heating of magnetic nanoparticles arises due to different processes of magnetization reversal in the particle system [3,4]. If the characteristics length scale of particle is reduced to few nanometers, intrinsic M–H curve becomes sigmoidal and, importantly, non-hysterratic. In this state, the magnetic moment of the particle is, as a whole, free to fluctuate in response to radiative energy, maintaining the individual atomic moments in their ordered state relative to each other. Simultaneously, the energy barrier for magnetization reversal also diminishes to such extent that thermal fluctuations can lead to relaxation phenomena. These effects, in juxtaposition, induce the heat generation process. It is
pertinent to mention that the frequency range used (350–400 kHz) to activate the magnetic nanoparticles, produces insignificant heating in healthy tissue [5].

Previously, researchers have extensively utilized magnetic neodymium-iron-bor capsules [6], magnetic microspheres [7,8], activated carbonated iron [9,10] and magnetoliposomes [11,12] as potential drug carriers. However, a recently invented strategy [13], as described in Fig. 1(a) exploiting superparamagnetic nanoparticles with surface assembled oligonucleotides (a short chain of nucleotides; \( n < 100 \)), has been perceived to possess the ability of localized release possibly to most superior extent. In this method, instead of coupling the drug directly to nanoparticle, an intermediate heat-labile linker in the form of double stranded short nucleic acid chain is introduced, which under a rise in temperature field facilitates the release process in a very confined way. While one strand of the oligonucleotide chain is covalently linked with a multivalent superparamagnetic nanoparticle, the complementary strand is coupled with the therapeutic medicine. As imaged non-invasively by MRI, these multivalent nanoparticles invade the fenestrations of the angiogenic vasculature to extravasate into the tumor stroma [13]. The antibodies carried by the nanoparticles target the epitopes either exclusive or overexpressed in cancer cells. Once the target is reached, the nanoparticles are excited with low frequency electromagnetic energy, which raises the local temperature (42–45 °C) enough to disrupt hydrogen bonding between complementary strands and facilitate site-specific release of the therapeutic agent. After the drug gets released, it is transported through the tumor interstitial space, diffuses inside the cancer cells and culminates into gross necrosis of malignant tissues.

Assimilating the aforementioned facts, it becomes readily evident that the entire process is composed of three critical thermofluidic steps namely the heat generation by nanoparticles, dissociation of oligonucleotide strands and transport of drug molecules inside the tumor site, culminating into tissue necrosis. Though these processes have been subjected to extensive experimental investigations either in isolation or in combination [14–17] there has not been much effort towards theoretical elucidation of entangled and intricate thermo-fluidic-biological processes. On the ground of largely varying experimental results, we intent to emphasize the need for extensive analysis of the governing processes, which should endow us with more rational view and optimized parametric control of this specific cancer treatment method. In the present work, we have proposed an integrated computational predictive model to interpret the efficacy of drug delivery in the process. The basic physics of heat generation by superparamagnetic nanoparticles in presence of external electromagnetic field has been coupled with transient biological heat transfer model and the statistical thermodynamics based generalized Langevin formalism [18] of complete oligonucleotide denaturation to delineate a simulation tool in order to find out the amount of drug released. With respect to the

![Fig. 1. (a) Electromagnetic field triggered drug release from a 'parent' DNA strand attached to dextran coated iron oxide nanoparticle. (b) The drug molecule entering the tumor through 1. Transvascular transport, 2. interstitial transport and 3. intracellular transport and 4. the back flow of extravasated drug due to interstitial fluid pressure gradient.](image-url)
transport of drug molecules, it is important to conceive that no uniform dogma of fluid dynamics exists as different forces prevails at different strata of physiological architecture ranging from the whole tissue to single cell. Hence, we resort to a hierarchically adaptive model approach. In this purview, first, the interstitial diffusion and convection of the various species involved in the process over time has been simulated assuming a porous media model of the tumor [19]. The transvascular migration of nanoparticle-oligo-drug assembly dictated by both hydraulic and osmotic pressure along with the drag force on the diffusing DNA strands has also been accounted to incorporate the in vivo complexities of the integrated process. We have also included the temperature dependence of diffusion coefficients, blood perfusion rate and other physical parameters. Importantly, our model retains numerous process parameters unchanged to the values quantified by previous researchers. Subsequently, the model has been validated with existing experimental results and good congruences have been obtained even in the presence of utmost uncertainties in complex biological processes.

2. Mathematical model

As mentioned above, the complete model is segregated into several thermo-fluidic modules, broadly including the process of biological heat transfer in the combined purview of blood perfusion and nanoparticle mediated heat generation, the kinetics of oligonucleotide denaturation and the transport of either nanoparticle-conjugated or free oligo-drug complexes within the interstitial space of tumor.

2.1. Bioheat transfer

We have solved the 3-dimensional transient Pennes [20] bioheat transfer equation in its classical form

\[
\rho c_p \frac{\partial T}{\partial t} + \nabla \cdot (-k \nabla T) = Q_{\text{bioheat}} + Q_{\text{metabolic}} + Q_{\text{source}}
\]

(1)

Here, \(Q_{\text{metabolic}}\) is the volumetric rate of metabolic heat generation, which has been fixed to constant and physiologically relevant magnitude. \(Q_{\text{bioheat}} = \rho_w c_b (T_b - T)\) is the heat removal rate due to blood perfusion, where \(w_b\) represents the blood perfusion rate and \(c_b\) is the specific heat of blood. We introduce the temperature and location dependence of the blood perfusion by considering, \(w_b = F(T, a, b)\), where the forms of the function \(F\) over different temperature ranges, as well as values of the parameter \(a\) and \(b\) are given in Table S-1 (see supplementary material).

The remaining magnetic source term \(Q_{\text{source}}\) has been deduced as follows. Firstly, the power generated per unit volume of nanoparticle is given by

\[
P_{\text{SPM}} = P(f, H) = \mu_0 f \chi' (f) H^2
\]

(2)

where \(f\) is the frequency, \(H\) is the strength of magnetizing field, \(\mu_0\) is the permeability of free space and \(\chi'\) is the imaginary part of the complex susceptibility \(\chi = \chi' + i\chi''\), where both \(\chi'\) and \(\chi''\) are frequency \((f)\) dependent. The out-of-phase imaginary component results in the heat generation \(P_{\text{SPM}}\). From physical perspective, as \(M\) lags behind \(H\), a positive conversion of magnetic energy into internal energy becomes possible. Subsequently, imaginary component has been modeled as

\[
\chi'(f) = \frac{Z_0 \Phi}{1 + \Phi^2}, \quad \text{where} \quad \Phi = f\tau_{N,B}
\]

(3)

Here, \(\tau_{N,B}\) is the effective relaxation time and is formulated as \(\tau_{N,B} = \tau_{N,B}^{\text{initial}} + \tau_{N,B}^{\text{influence}}\). Further, the Neel relaxation time \((\tau_N)\) is given by, \(\tau_N = \tau_0 \exp[K/V_n/k_B T]\), where \(K\) is the anisotropic energy density, \(V_n\) is the nanoparticle volume, \(k_B\) is the Boltzmann constant and \(\tau_0\) is the attempt period, which is only weakly dependent on temperature [21] and has a typical value of \(10^{-11} \text{s}\) for non interacting particles. In the context of present scenario, due to the restricted Brownian motion of molecularly large nanoparticle-oligonucleotide-drug assembly, the Brownian relaxation time \(\tau_B = 4\pi\eta r_n^3/k_B T\) becomes relatively infinite, resulting \(\tau_{N,B} = \tau_N\)

\[
\text{for } \Phi \gg 1, \quad \chi'(f) = \frac{Z_0 \Phi}{1 + \Phi^2}, \quad \text{where} \quad \Phi = f\tau_{N,B}
\]

(4)

Here, \(M_s\) is the saturation magnetization in A/m. Combining Eqs. (2)-(4), we obtain

\[
P(H) = \mu_0 M_s^2 H^2 V_n \tau_0 \exp[K/V_n/k_B T] k_B T
\]

(5)

From this expression, it is evident that the power generated per unit volume is frequency independent. Of specific significance, this phenomenon has been validated experimentally [22]. For sake of further elaboration, we express \(K\) as \(25k_0 T_B/V_n\), where \(T_B\) is the blocking temperature depending on the type and radius of the nanoparticle. Now if \(c\) is the density of the nanoparticle then combining all the previous equations we get in the final form

\[
Q_{\text{source}} = Q(H, r_n, T, \text{Type of nanoparticle}) = \frac{c^2 n_0^2 M_s^2 H^2 \sigma(T_B)^2 \pi}{M_s k_B T_0 \exp[25k_0 T_B/T]}
\]

(6)

\(\sigma\) is the saturation magnetization in emu/g. In Eq. (5), both \(M_s\) (molecular weight) and \(\sigma\) are dependent on type of nanoparticles. We have shown this source term varies with nanoparticle diameter, field of magnetization and temperature. The dependence of these parameters on the source term is shown in Figure S-2(a) and (b) (see supplementary material) for constant magnetic material density of 80 mg/cc.

2.2. Species transport

The drug vehicle travels intruding into the tumor through tumor microcirculation, and then it enters the interstitial space by transvascular transport. Subsequently, on finding the epitopes on the cancer cell, it undergoes the intracellular transport. The path of its movement is explained in the Fig. 1(b). As it is conceived, the mass transfer equation should incorporate the transient 3-dimensional diffusion equation with generation or consumption of species and convection through porous media. It is difficult to define a proper ‘pore scale’ in soft tissue and biological systems are more like a fibrilar structure consisting of proteins and proteoglycans not resembling classical porous media structure, which comprises of solid matrix with interconnected voids. For the last few decades numerous attempts have been made to evaluate the quantities like porosity, tortuosity and void fraction. Though they were not intended to model biological system as porous media but eventually porous media model have been applied to biological systems from extracellular to cellular level [23]. Though this modeling approach is not even close to complete, they give excellent congruence with experimental result and are able to explain the phenomena like cell blebbing and cytokinesis. Relevantly, approximating biological tissue as a porous media can lead to significant simplifications in implementation and analysis in vivo transport [19]. In the process, for sake of a generalized representation, we designate nanoparticle-oligo-medicine assembly as species 1, nanoparticle-oligo as species 2 and medicine molecule-oligo as species 3. The resulting generic dynamics thus yields to

\[
\frac{\partial c_i}{\partial t} + \nabla \cdot (-D_i \nabla c_i) = R_i - \nabla c_i
\]

(7)

where \(D_i\) is the effective diffusion coefficient of the species \(i (i=1, 2\ or\ 3)\) in the porous medium, given by \(D_i^{eff} = (\iota/\tau D_i)\), \(D_i\) is the
diffusivity of $i$th species in free condition, $e$ is the void fraction and $\tau$ is the tortuosity. The velocity vector is given by $\mathbf{V} = -(k/\mu)\nabla P$. Where, $k$ is the specific hydraulic permeability, $\mu$ is the viscosity of interstitial fluid and $k/\mu = K$ is the hydraulic conductivity. Empirically, the value of $K$ is related to the tissue concentration of glycosaminoglycans (GAG), in weight ratio (gm/100 gm) of tissue and is obtained [24] as

$$K = 4.6 \times 10^{-11} [\text{GAG}]^{-1.202} \left( \mu_{37}/\mu \right)$$

where $\mu_{37}$ is the viscosity of interstitial fluid at 37 °C. Here it is pertinent to mention that the interstitial transport of drug is difficult to achieve both in systemic and local delivery protocols due to the low intrinsic convective transport in the regime of elevated interstitial fluid pressure (IFP), the lack of functional lymphatic vessels and the outward gradient of IFP. Importantly, the last component enforces an adverse convective transport of extravasated drug from the interior to the periphery. Hence, diffusion is the predominant mode of transport throughout the tumor system except at the periphery where convection becomes proportionally commensurate. Given that the variation of IFP (Fig. 2(b)) along the tumor depth is an essential part of accurate determination of mass transport dynamics, we obtain this parameter by curve fitting with existing experimental results [25] and the result is given in Table S-3 (see supplementary material).

2.3. Species generation and consumption through DNA denaturation

Next, we attempt to deduce the source or sink term $R_i$ in the species equation from the prevailing biological phase transition in an increasing temperature field. It is trivial to note that the amount of species 2 or 3 generated is one mole each due to the dissociation of one mole of species 1. In order to obtain the converted fraction of species 1, exclusively through the dissociation of oligonucleotide chains, we compute the ensemble average of fraction of dissociated molecules at a given temperature ($< p >$), which is perceived to be a function of temperature $G(T)$. The dissociation or “melting” of a double stranded oligonucleotide chain into two mutually complementary single strands sequences is essentially a temperature sensitive phase-transition process. The intrinsic stochasticities of melting dynamics have recently been delineated utilizing...

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**Fig. 2.** (a) Tumor surface temperature vs. time. The dots show the experimental results. The black line shows the computational result; (b) the variation of interstitial fluid pressure with tumor depth; (c) diffusion flux at $t = 1300$ s for all the species. (d) Diffusion flux profiles for each species. The scale has the unit mole/m² s. All the figures are for $t = 1300$ s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
a generalized Langevin formalism. Due to randomly fluctuating thermal energy, comparatively weak A-T couplings between two complementary strands break, which, due to the local constraints in nucleotide motions, results in long-range cooperative effects that eventually govern the abrupt configurational entropy gradient driven chemical transition. For this reason, the oligonucleotide sequences with a stretch of A/T nucleotides in the middle forms pre-dissociation melting “bubbles”, which facilitates complete decoupling of strands. As for representative intermediating oligonucleotides, we have used three well-characterized oligonucleotide sequences namely L60B36, L42B18 and L33B9, whose denaturation kinetics and thermodynamics have been investigated extensively from both theoretical and experimental perspectives. These sequences vary on the relative length of pre-melting bubble and thus, possess different dissociation probabilities and steepness in dynamic transition kinetics at same temperature. In each step of simulation, the magnitude of $<p>$ [26] is obtained from simulation based on the modified Langevin dynamics of Peyrard–Bishop–Dauxois model, as introduced by Das and Chakraborty. It is worthwhile to note that oligonucleotide melting kinetics follows approximately a generic relationship $<p> = 0.5 \tanh ((T-T_c)/d) + 0.5$, where $T_c$ is the critical temperature at which $<p> = 0.5$ and $d$ is sequence specific fitting parameter (tabulated in supplementary material S-4). Once, $<p>$ is known, it is integrated to the mass transport equation by $R_1 = -C_1 <p>$ and $R_{2,3} = C_1 <p>$.

2.4. Transvascular generation of species

The term $R_1$ comprises one more term apart from $-C_1$ G(T) due to transvascular transport. We define $\Phi_B - \Phi_L$ as the transvascular transport term. The first term is the rate of fluid extravasation rate from blood vessels per unit tissue volume, while the second term is the rate of lymphatic drainage per unit tissue volume. There is no functional lymphatic in solid tumors. Thus, $\Phi_L = 0$. $\Phi_B$ is given by the modified Kedem–Katchalsky equation [27]

$$\Phi_B = \frac{PS}{V} \left( \frac{C_p}{K_{AV}} \right)$$

$P$ is the microvascular permeability coefficient, $S/V$ is the vascular surface area per unit tissue volume, $C_p$ is the solute concentration in plasma, $C$ is the solute concentration in the interstitial fluid and $K_{AV}$ is the available volume fraction of solute in tissues.

2.5. Diffusion coefficients

Oligonucleotides differ structurally from globular proteins and are often treated as random coils [28]. For DNA in plasma, there is significant interaction between the solvent and the molecule, and considerable solvent is associated with the macromolecule. In this case, we describe the root mean square end to end distance given by, $<d_{2,2}^2>^{1/2} = C_n n^{1/2}l$, where $C_n$ is the characteristic ratio, $n$ is the number of base pairs and $l$ is the length of each segment. In general, $C_n = l_b/l$, where $l_b$ is known as the Kuhn statistical segment length [29]. For DNA, $l_b$ equals 150 nm and $l$ equals 0.34 nm. Assuming the random coil behaves as a sphere with a radius equal to the radius of gyration, $R_c$, we find the temperature dependent diffusion coefficient as [30]

$$D = \frac{0.196k_bT}{\sqrt{6}\mu R_c}$$

(10)

For open ended coils

$$R_c = \frac{<d_{2,2}^2>^{1/2}}{\sqrt{6}}$$

(11)

For finding the diffusion coefficient of drug molecule, we use the temperature dependent Wilkie–Chang correlation [31] given by

$$D = 7.4 \times 10^{-10} \left( \frac{\Gamma(\phi M)^{0.5}}{\mu V^{0.6}} \right)$$

(12)

where $V$ is the molar volume of the solute (cm$^3$/mole) at its normal boiling point, $\mu$ is the viscosity of solution, $M$ is the molecular weight of the solvent and $\phi$ is an association parameter for the solvent. The diffusion coefficients of these species are used to find the modified diffusion coefficient in bound condition. A size averaged harmonic mean was used to find the effective diffusion coefficient of the assembly as diffusion coefficient inversely varies with size of species.

$$V_{1+2} = V_1 + V_2$$

$$D_{1+2} = D_1 + D_2$$

(13)

where $V_{1+2}$, $V_1$ and $V_2$ are the volume of species $1$ linked with $2$, volume of species $1$ and volume of species $2$, respectively. $D_1$, $D_2$ and $D_3$ are the diffusion coefficient of species $1$ linked with $2$, diffusion coefficient of species $1$ and diffusion coefficient of species $2$, respectively. From this equation it is clear that if the size of one species is very small resulting in high diffusion coefficient, the term corresponding to that species equals zero and the resultant diffusion of the combined species is determined by the other species, which is expected from physical consideration.

We used Doxorubicin as the test drug molecule as the data for this drug is abundant in medical literature. Doxorubicin is a common anticancer agent used in the treatment of a number of neoplasia.

3. Computation and validation

We approximated the 3D tumor model for this simulation by considering tumor as a sphere resting over the cylindrical muscle covered by skin as shown in the Figure S-5(a) (see supplementary material). The metabolic heat generation has been taken to be 400 W/m$^3$. The other thermophysical properties are given in Table S-2 (see supplementary material). COMSOL multiphysics software based on the finite element method has been used to solve the model by a direct matrix inversion technique with temperature tolerance of 0.0001. After the creation of the 3D model, it has been meshed, as shown in Figure S-5(b) (see supplementary material). The mesh structure contains 157 elements, 330 mesh points. To confirm the robustness of our solution and its non-dependence on mesh elements, we have performed several simulations with identical system parameters but with variable number of mesh elements and points. Consequently maximum 0.07% change has been noted. Thus the meshed model has been solved transiently by conjugate gradients linear system solver with algebraic multigrid preconditioner.

Next, the simulation results have been compared with the experimental trends obtained by the Hilger group [32]. In a representative set of experiments on tumor-bearing mice, the animals have been subjected to alternating magnetic field (frequency 400 kHz, amplitude 8.8 kA/m) for 242 s after homogeneous intratumoral injection of 21 mg $\pm$ 9 of magnetite sample of diameter 10 nm per 299 mm$^3$ (70 mg/cc) of tissue. Due to the heat by magnetic nanoparticles, within the treatment time, the intratumoral temperature has been found to increase from 26 $\pm$ 1 to 71 $\pm$ 8 $^\circ$C. Including the heating history (Figure S-5c) (See supplementary material). Once our model has been validated, we further endeavor to propose an optimized therapeutic protocol, which should augment the
efficacy of nanoparticle mediated drug delivery system. In the actual model for the drug delivery simulation, we took a spherical tumor of radius 4 mm implanted deep inside the healthy tissue. As the magnetic therapy is applied to tumors well embedded in tissue, for further computations, the tumor has been modeled as situated inside tissue.

4. Results and discussions:

4.1. Effect of nanoparticle diameter

The values for the relevant parameters used in this work have been tabulated in Tables 1, S-1, S-2, S-3 and S-4. Of several process parameters on which the current therapeutic protocol relies, the pattern of rise in temperature field has been noted to be the most influencing. It is evident that with the decrease of nanoparticle diameter, the heat generation per unit volume increases drastically due to the strong size dependence of $\sigma$ and the relaxation times incorporated in blocking temperature. For example, with small decrease in nanoparticle size from 10 to 9 nm, the power generation significantly increases causing extremely heated tissue ($T > 500 ^\circ C$). On reverse side, with 12 nm diameter nanoparticles, the power generation is seemingly not adequate for effective temperature ($T < 40 ^\circ C$) rise. Interestingly, most of the experiments conducted till date, presumably by trial and error, deals with 10 nm nanoparticles which, according to the present simulation results, is nearly the optimum for hyperthermia and drug delivery applications.

4.2. Effect of temperature on the quantum of drug delivered

Here, it is relevant to narrate that temperature itself imparts both increasing and decreasing effects on heat generation as shown from denominator of Eq. (6). However, in mutually opposing influence of terms involving $T$ and $\exp[25TB/T]$, the power generation increases considerably with temperature, which leads to further increase of the system temperature to attain a favorable condition for drug delivery or ablation. Again, attributing to the specific localization of nanoparticles on tumor site, the temperature increase has been found to be extremely confined and on the healthy tissue away from the tumor site, the effective rise in temperature field has been observed to be negligible (Figure S-5c; see supplementary material). The only means by which temperature rise is possible in this regime is through heat diffusion, which is anticipated to lack adequate transmission efficiency to implore a deleterious side-damage. As we can see from the sensitivity study (Table 2) the magnetizing field has a significant effect on temperature generation and this in turn changes the amount of drug released considerably.

4.3. Space specificity of drug delivery

Another factor that perceptibly dictates the success of therapeautic treatment is the localized concentration of oligonucleotide conjugated drug molecules, which is, in turn, governed by the accumulation of nanoparticle-oligo-drug assembly and oligonucleotide dissociation kinetics in the rising system temperature. In the representative theoretical scheme where the magnetic field was set on for $t = 1300$ s, temperature rise up to $42 ^\circ C$ has been recorded (Fig. 3). Subsequently, this has been probed to be adequate for melting of significant fraction of double stranded oligonucleotides and release of drug molecules (Figure S-3; see supplementary material). In this scenario, there exists a difference between concentration profiles of liberated chemical species that essentially possess identical rate of generation. This is attributed to the difference in diffusivities, which arises from the intrinsic disparity in molecular sizes. One must note that, within the purview of coupled thermofluidic events, the nature and the forces involved in the transport of each type of molecular assembly can also be of immense importance. As it becomes evident, on application of magnetic field, oligo-drug compound diffuses faster into the tumor-site than any other molecular assembly. Here, within the interstitial spaces of tumor, transport process is strongly diffusion dependent (Fig. 2c and d), which is in congruence of the experiment [25].

<table>
<thead>
<tr>
<th>Table 2</th>
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<td><strong>Summary of sensitivity analysis.</strong></td>
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<table>
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<tr>
<th>Input parameters</th>
<th>Maximum drug concentration (mol/m³)</th>
<th>Total drug delivered (mol)</th>
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<tbody>
<tr>
<td>Magnetizing field ($H$)</td>
<td>$\pm 3.48 \pm 5% + 26.82 + 2.77 + 20.55$</td>
<td>$\pm 3.71 \pm 24.98 \pm 2.93 \pm 19.27$</td>
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<tr>
<td>Number of DNA base pairs ($n$)</td>
<td>$-0.46 - 1.62 - 0.35 - 1.37$</td>
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<td>Void fraction ($c$)</td>
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<td>$-0.23 - 0.64 - 0.26 - 0.44$</td>
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<td>Diffusion coefficient of drug</td>
<td>$-0.464 - 0.696 - 0.41 - 0.464$</td>
<td>$-0.464 - 0.465 - 0.39 - 0.334$</td>
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<table>
<thead>
<tr>
<th>Table 1</th>
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<td><strong>List of input parameters.</strong></td>
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<table>
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<td>Void fraction ($c$)</td>
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<td>m$^{-1}$</td>
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<tr>
<td>Diffusion coefficient of Doxorubicin</td>
<td>$5.01 \times 10^{-11}$</td>
<td>m$^2$ s$^{-1}$</td>
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back flow induced by interstitial fluid pressure gradient, the convective transport is effectively restricted only within the peripheral domains of tumor-site. This divergence in mode of transport across the depth of interstitial space, reserves the intra-tumor penetration only to free drug molecules while nanoparticle-oligo-drug or nanoparticle-oligo compounds can accumulate on encircling region with the aid of both convective and diffusive flux. Appreciating the specificity of delivery process, the biocompatibility and the intracellular permeability of drug molecules, the afore-illustrated phenomenon can be of utter significance and should be subjected to further experimental investigations towards the desired betterment of the entire process.

4.4. Choice of proper DNA sequence

Finally, the protocol is anticipated to rely upon the oligo sequence that forms chemical connection between the nanoparticle and the drug molecules. On the process of drug delivery, it imparts two way influences. While the size of oligonucleotide decides the net diffusivity of the released oligo-drug complex through tumor site, both of its size and sequence should determine the temperature induced dissociation kinetics. As delineated in Fig. 4(b), the drug diffusivity increases with decreasing length of oligo-chain, implying short oligo-sequence to be suitable for this kind of therapeutic applications. However, the accessible degree of reduction in oligo size is very much limited by the required dissociation kinetics. Oligos of very short length \((n < 20)\) have very low melting temperature and high probability of dissociation even at ambient temperature, which would imply an undesirable non-specificity and ineffectuality of the delivery system. Hence, we propose oligonucleotides of 30–40 bases to be optimum for this purpose. For sequence specification, it is conjectured that oligonucleotides with bubble-in-the-middle (i.e. AT rich middle region flanked by GC rich sequences on either side) configuration should be the most suitable for the present therapeutic purpose. From the very nature of oligo-melting kinetics, as demonstrated by Das and Chakraborty [26], flanking GC regions prevent strand dissociation at ambient temperature, instead of formation of transient “melting bubble” in the AT rich middle region. However, with increasing temperature beyond 40°C, the bubble becomes stabilized and generates sufficient conformational strain through inter-nucleotide phosphate linking to decouple two complementary strands within a very short temperature window. This event, therefore, concurrently inhibits non-specific release in ambient temperature and yet, accelerates the drug release kinetics within physiologically tolerable range of temperature increase, justifying our proposition. Interestingly, for same oligo sequence, the concentration of drug is approximately same for different nanoparticle diameters within relevant range as the process is essentially dissociation kinetics limited and temperature increase occurs at reasonable short time.

4.5. Sensitivity to various parameters

As the complete model consists of numerous process and parameters we conducted a sensitivity analysis (Table 2) to specify the effect of the important parameters and to test the robustness of this predictive model. As we can see the error in porosity has minor effect on the total drug delivered as well on the maximum drug concentration at a point. As the pore scale is not well defined as pointed out earlier, it was worth studying to measure the robustness of this work to the parameter porosity.

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![Fig. 3. At t=1300 s after the application of magnetic field: (a) top left: temperature profile (unit K), (b) top right: concentration profile of species 1(unit mole/m³), (c) bottom left: concentration profile of species 2 (unit mole/m³), (d) bottom right: concentration profile of species 3(unit mole/m³). Species 2 and 3 have similar profiles. But the scale shows their different values of concentration. This occurs due to different diffusion coefficient.](image)
The diffusion coefficients and the parameters affecting them were also used as input parameters for sensitivity study. The DNA length and in turn the number of base pairs is a deciding factor for the evaluation of diffusion coefficient and this was one of the input parameters. The diffusion coefficient of drug, DNA and nanoparticles all entangle to give the effective diffusion coefficients and it is clear from the Table 2 that the model is almost stable to the variation of these parameters. It is noteworthy that magnetizing field has quite high sensitivity on the temperature generation and subsequent drug delivered. But as it is a parameter that can be precisely controlled from outside during the therapy this sensitivity should not be an impediment for the application of the modeling strategy.

4.6. Limitations

To discuss the limitations it is worth mentioning that this work is an attempt to computationally predict a truly multi-physics phenomena and as the drug delivery process encompasses many more intricate phenomena, several effects should be studied coupled with this generalized framework like double-layer effects, osmosis, hydration forces and steric effects on the diffusion parameters to name a few. Moreover we wish to point out regarding hydraulic permeability that small structural change in tissue can lead to large variation in hydraulic parameters [36]. So, proper characterization of tissue and quantification of this parameter is required to make the model better. An application of poroelastic model can be a good approach along with this characterization [23].

5. Summary

We have developed a fundamental mathematical model for superparamagnetic nanoparticle assisted drug delivery under external low frequency magnetizing field applicable for DNA-mediated therapy. The model has been rigorously tested to match experimental results. Most of the magnetic nanoparticle heating and drug delivery studies till date are experiment based and not suitable for development of quantitative therapeutic protocol. However, we have attempted here to understand the superparamagnetic nanoparticle assisted heating mechanism from a fundamental viewpoint. We have successfully modeled the denaturation phenomena and extremely complicated transport process in biological system though leaving several intricate details. Our present studies reveal that with the advents in high-speed computing, enormous opportunities and potentials do exist for developing a truly fundamental-based approach towards analyzing superparamagnetic nanoparticle mediated treatment instead of employing tunable parameters like specific absorption rate (SAR), diffusion coefficients determined mostly from experiments. This effort is expected to be contributory towards the medical treatment under critical biophysical conditions,

Fig. 4. (a) Drug profile with varying magnetizing fields for nanoparticle diameter 10 nm and DNA sequence L60B36. (b) Drug profiles with different DNA sequences for magnetizing field 350 kA and nanoparticle diameter 10 nm.
in which there is likely to be a natural emphasis on the optimization and control of various therapeutic parameters, along with the minimization of drug quantity purely experience based treatment protocols. Towards that, the present study is expected to unveil a deeper understanding of physico-thermal processes involved with magnetic nanoparticle-electromagnetic field interaction, by quantifying the role of type, radius and concentration of nanoparticle, magnetizing field, oligonucleotide type, drug type and exposure time on the treated volume, thereby enabling one to control the treatment by suitably regulating these parameters as well as pre-observing the medical treatment through simulations in real time with an unprecedented accuracy, as a complementary to purely experience driven clinical trials.

Conflict of interest statement

The authors do not have any conflict of interest with anyone or any institution.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.compbiomed.2011.06.013.

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