A Model-Based Analysis of Tissue Targeting Efficacy of Nanoparticles

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This work was supported by National Science Foundation CBET-CDS&E grant No. 1609642.

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Abbreviations used in this paper: CFT, Colloidal Filtration Theory.

Abstract

Tissue targeting is a critical challenge for systemic delivery of drug nanocarriers. To overcome this challenge, major research efforts have been undertaken to design ligand-conjugated nanoparticles. However, limited work has been done to quantitatively assess the effectiveness of such approach. In this work, using a mechanistic spatiotemporal model, we investigate the effectiveness of ligand-directed tissue targeting. By applying an approach from the colloidal filtration theory (CFT), we develop a Brownian Dynamics model of nanoparticle-cell interaction. The model incorporates a single cell and its surrounding flow field. It considers both specific (receptor-mediated) and nonspecific (bare cell surface-mediated) recognition of nanoparticles subject to convective and diffusive motion. Using the model, we investigate how the specific and nonspecific interactions compare in determining the overall targeting efficacy. Our analysis provides some interesting findings that contradict with the general notion that effective targeting is possible based upon the differential receptor expression in cancer and non-cancer cells. We show that such strategy may yield only a marginal gain in the targeting efficacy. Moreover, nonspecific interaction may have an important influence on particle recognition by cells even at high receptor expression levels.

Key words: Colloidal Filtration, Brownian Dynamics, Drug Delivery, Creeping Flow

Introduction

Tissue targeting remains to be a critical challenge for nanotechnology-based drug delivery. A very small fraction of intravenously-injected nanoparticles can reach the target tissues (**?** **?**), while most of them are lost in a variety of mechanisms (**?** **?** **?**). To overcome this challenge, significant efforts have been made to develop ligand-conjugated nanoparticles (**?**). The key idea of ligand conjugation relies on the fact that cancer cells overexpress certain receptor proteins that are found minimally expressed in the normal (non-malignant) cells (**?**). It is believed that attachment of receptor-specific ligand on the nanoparticle surface can direct the particle to the target tissue while avoiding nonspecific recognition by the normal cells.

However, despite the expansive research on developing ligand-conjugated nanoparticles, the delivery and therapeutic effectiveness of such strategy remain poorly investigated (**?** **?**). There might be several caveats. Nanoparticles in a systemic delivery could be lost even before encountering the target tissue. Therefore, it is quite misleading to think that ligand attachment can direct a particle to a tissue; rather, a circulating particle first arrives at the target site and retained through the ligand-receptor binding. Another caveat is the formation of protein biocorona on the surface of a circulating nanoparticle (**?**). Biocorona modifies the surface of a particle and may compromise the ligand-receptor specificity (**?**). In addition, it is possible that particles get captured by a cell through nonspecific interactions. In a tumor microenvironment, a variety of non-malignant cells, which are devoid of the receptor proteins, can capture a particle through nonspecific binding. Moreover, the bare regions of a cancer cell surface should be able to mediate nonspecific interaction like the non-cancer cells. The effects of such nonspecific particle capture on targeting remain poorly understood.

In this modeling work, we investigated the effectiveness of receptor-directed nanoparticle targeting. We focused on the following questions: to what extent differential receptor expression may affect tissue targeting of particles? What roles does the nonspecific particle-cell interaction play in determining the overall targeting efficacy? More specifically, we investigated parameter k in Eq. **??** at different levels of cellular expression of receptors and nonspecific particle-cell interactions:

$$\frac{\partial C}{\partial t} = \nabla \cdot [D_e \nabla C] - \nabla \cdot (\boldsymbol{u} C) - kC \tag{1}$$

Eq. **??** describes particle transport in a heterogeneous advection-diffusion-reaction system, such as a porous medium or biological tissue. In a biological tissue, C represents particle concentration, D_e represents effective diffusion constant, and k represents the rate constant for particle capture by cells. The value of k is directly related to the targeting efficacy. Our goal was to dissect this constant based on the contributions from the specific (receptor-mediated) and nonspecific (bare cell surface mediated) particle-cell interactions. For effective targeting, k should be sensitive to the level of cell-surface receptor expression, which is the key rationale behind targeted drug delivery. On the other hand, for cells devoid of receptors (or particle lacking the targeting ligand), k should account for a weak nonspecific recognition.

In order for the ligand-directed targeting to be effective, the value of k in a cancer tissue overexpressing the receptors should be large compared to a normal tissue.

To investigate above questions, we developed a detailed spatiotemporal model of particle-cell interaction. The model is an extension of a theoretical framework called Happel Sphere-in-Cell model (**?**). In colloidal filtration theory (CFT), the framework has been extensively used for studying particle deposition in porous media (**? ? ? ? ?**). It has also been used to model particle transport in biological tissues (**?**). In a Happel model, a porous medium or biological tissue is represented by a unit spherical body (cell) and its surrounding flow field. Particles entrained into the flow field can get captured upon making a contact with the surface of the spherical cell. The surface of the spherical cell is considered to be smooth (homogeneous) that capture a particle in a single contact. However, in our model, we explicitly incorporate cell-surface features that represent receptor molecules and non-receptor (bare) regions. We accounted for both receptor-mediated (specific) and bare cell-surface-mediated (nonspecific) particle-cell interaction. Applying a time-adaptive Brownian Dynamics approach, we simulated particle motion in the flow field and particle uptake by the cell via the specific and nonspecific interactions.

Using the model, we analyzed the effects of receptor expression on the tissue targeting efficacy of finite-sized nanoparticles. Our analysis led to several interesting findings, which contradict with common perceptions about receptor-directed nanoparticle targeting. We show that the differential expression of receptor proteins in cancer and healthy cells may lead to a marginal difference in the targeting efficacy. The model indicates that nonspecific interaction may serve as a major determinant of the overall targeting efficacy even when cells overexpress the receptor proteins. Our analysis sheds light on the mechanisms whereby specific and nonspecific interactions determine cellular recognition of nanoparticles at various receptor expression levels.

1 Materials and Methods

We developed a particle-cell interaction model based on the Happel model (**?**). A time-adaptive Brownian Dynamics algorithm was used to simulate particle transport and particle-cell interaction. The model and simulation approach are discussed below.

1.1 Computational Domain

The computational domain of our model is illustrated in Fig. **??**. The domain consists of a unit spherical cell of radius $a = 10$ μm and its surrounding fluid. Based on the Happel model, for a tissue with porosity ϵ, the thickness of the fluid surrounding the unit cell is $l = a[(1-\epsilon)^{-1/3} - 1]$. We consider tissue porosity $\epsilon = 0.4$ (**?**). Therefore, $l = 1.86$ μm, and the radial distance between the center of the cell to the periphery of the domain, $b = a + l = 10.186$ μm (Fig. **??**).

Based on the Happel model, at any point (r, θ) (Fig. **??**A), where $b \leq r \leq a$, we calculate the radial and azimuthal velocity components by using the equations below.

$$v_r = -|\boldsymbol{U}_\infty| \cos\theta \Big(\frac{K_1}{r^{*3}} + \frac{K_2}{r^*} + K_3 + K_4 r^{*2} \Big), \text{ and} \tag{2}$$

$$v_\theta = \frac{1}{2} |\boldsymbol{U}_\infty| \sin\theta \Big(-\frac{K_1}{r^{*3}} + \frac{K_2}{r^*} + 2K_3 + 4K_4 r^{*2} \Big), \tag{3}$$

where $\boldsymbol{U}_\infty$ is the local mean fluid velocity in the tissue, $K_1 = \frac{1}{K_5}$, $K_2 = -(3 + 2\omega^5)/K_5$, $K_3 = (2 + 3\omega^5)/K_5$, $K_4 = -\omega^5/K_5$, $K_5 = 2 - 3\omega + 3\omega^5 - 2\omega^6$, $\omega = \frac{b}{a}$, and $r^* = r/a$.

1.2 Cell and Nanoparticle Boundary

We explicitly mark the cell boundary with receptors and bare regions, as illustrated in Fig. **??**B. We model each receptor molecule as a small circular spot of 7.5 nm radius. The center of the spot lies on the cell boundary. Therefore, ignoring the curvature of the cell boundary, only half of each spot is exposed to the interstitial region and therefore accessible to a particle.

We scale receptor number for the two-dimensional cell boundary. Considering N receptors on the surface of a cell in three dimensions, the equivalent number is $n = (\pi N)^{1/2}$. We vary n in the range 12-396, which corresponds to 45-50,000 receptors per cell in three dimensions. A larger number of receptors ($n > 396$) is not used because the model remains insensitive at $n > 396$, as will be discussed in the Results section.

We consider nanoparticles as finite-sized circles of 10 nm radius. We do not explicitly incorporate ligand molecules on the particle boundary. To purposefully favor receptor-mediated (specific) particle recognition, we consider the entire particle boundary to be reactive to a receptor. In other words, we implicitly consider the particle surface to be densely popu-

lated with ligand molecules and therefore its orientation with respect to a receptor is not important.

1.3 Adaptive Brownian Dynamics

Similar to (**?**), we use a time-adaptive Brownian Dynamics algorithm to simulate particle transport in the fluid around the cell. The sole purpose of the time-adaptive feature is to accelerate computation while capturing high-resolution details of the particle-cell interaction. Our analysis involved many realizations of particle trajectories, which would be computationally too expensive without the adaptive feature. Below we briefly discuss the adaptive algorithm.

Just like in a typical Happel model, in our simulations, a particle is initiated at $r = b$ (domain boundary) and $-\pi/2 \leq \theta_s \leq \pi/2$. Simulation is continued until either the particle leaves the domain boundary or gets captured by the cell. In each Brownian Dynamics time step Δt, a particle is advanced by a distance $\boldsymbol{S} = \boldsymbol{v}\Delta t + \sqrt{4D\Delta t}\boldsymbol{e}$, where $\boldsymbol{S}$ is displacement, $\boldsymbol{v}$ is local fluid velocity, D is particle diffusivity, and $\boldsymbol{e}$ is a unit vector in a random direction. We consider $D = 10^{-9}$ cm^2/s. The local fluid velocities are determined using Eqs. **??** and **??**. Fig. **??**A shows the velocity field around the unit cell. Fig. **??**B illustrates particle advancement based on the diffusion and local velocity in the flow field.

In the adaptive algorithm, a particle takes relatively larger steps away from the cell boundary. Near the cell boundary, it takes smaller steps to capture particle-cell interaction at the molecular scale. We impose an upper limit on the particle jump size, $\Delta S_{max} = 10$ nm, while we set a lower bound on the time step $\Delta t_{min} = 10^{-5}$ seconds. This smallest time step is applied when a particle is in the vicinity of the cell boundary, where $|\boldsymbol{v}| \approx 0$. Thus, the smallest jump size permitted is $\Delta S_{min} \approx \sqrt{4D\Delta t_{min}} = 2$ nm. Time step Δt is adapted to maintain particle jump sizes in the range 10 nm $\geq |\Delta \boldsymbol{S}| \geq 2$ nm. This conservative limit on the particle jump size is applied and a larger ΔS_{max} is not chosen due to the possibility of violating the local velocity field.

In each Brownian Dynamics step, the adaptive Δt is calculated as follows. We first evaluate l_c, the shortest distance (collision distance) between the particle boundary and the cell boundary. If $l_c \geq \Delta S_{max}$, we set $\Delta t = (\Delta S_{max} v + 2D)/v^2 - \sqrt{(\Delta S_{max} v + 2D)^2 - \Delta S_{max}^2 v^2}/v^2$, where $v = |\boldsymbol{v}|$ is the magnitude of the local fluid velocity. However, if $l_c < \Delta S_{max}$, we calculate a tentative time step $\Delta t_t = (l_c v + 2D)/v^2 - \sqrt{(l_c v + 2D)^2 - l_c^2 v^2}/v^2$. If $\Delta t_t < \Delta t_{min}$, which can happen only when the particle is very near to the bare cell boundary or a receptor, we set $\Delta t = \Delta t_{min}$. Otherwise, we set $\Delta t = \Delta t_t$.

1.4 Particle-Cell Interaction

Near the vicinity of a receptor feature or cell boundary, a particle in our simulation is advanced with subnanometer-scale steps, as discussed above. While taking such small jumps,

a particle can make a contact with a receptor or the bare cell boundary. If the particle makes a contact with a receptor (specific interaction), we consider the particle is captured immediately. However, if the particle makes a contact with the bare boundary (nonspecific interaction), we consider the particle is captured with probability $\rho < 1$. In each contact between the particle and the cell boundary, we sample a uniform random number $0 < U(0,1) < 1$. If $U(0,1) < \rho$, we assume the particle is captured. Otherwise, we simply reject the particle move.

The single-collision model for particle capture by receptors may overestimate particle recognition via the specific interaction. However, as mentioned in Section **??**, we purposefully favor the specific interaction (receptor targeting) to maximize the contribution from the receptor-directed targeting. On the other hand, we can change the parameter ρ between 0 and 1 to tune particle recognition via the nonspecific interaction.

1.5 Targeting Efficacy

Despite being a single-cell system, the Happel model can be used to evaluate parameter k in Eq. **??** using the following equation (**? ?**):

$$k = \frac{3(1-\epsilon)}{2\epsilon a}\eta_c |\boldsymbol{U}_\infty| \tag{4}$$

In colloidal filtration theory, η_c is called unit cell collector efficiency. It measures the efficiency of the unit spherical cell to capture particles entrained into its flow field. Below we first discuss how η_c is calculated in a typical Happel model where the cell surface is featureless and homogeneous. We then explain corresponding calculation in our model to account for the specific and nonspecific interaction of particles with the heterogeneous cell boundary.

In a Happel model, if a particle makes a single contact with the cell, the particle is assumed captured. However, the probability of making a contact depends on the starting angle of the particle $0 \le \theta_s \le \pi/2$ (due to symmetry, $-\pi/2 \le \theta_s \le 0$ is simply a mirror image). A particle starting from a smaller θ_s has a higher chance to make a contact with the cell. However, even all particles starting from the same θ_s do not make a contact because of the random component (diffusion) in their motion. Therefore, for a given θ_s, the probability of a particle getting captured is less than 1. This probability, denoted as $p(\theta_s)$, can be computed from large number of Monte Carlo realization of particle trajectories. Based on (**? ?**), the collector efficiency η_c is defined as:

$$\eta_c = \int_0^{\pi/2} 2p(\theta_s)\sin(\theta_s)\cos(\theta_s)d\theta_s. \tag{5}$$

Here, $2\sin(\theta_s)\cos(\theta_s)$ is the weight associated with particles starting from the corresponding angle. The integration can be numerically evaluated by simulating large number of particles with starting angles $0 \le \theta_s \le \pi/2$.

However, unlike a typical Happel model, the cell boundary in our model is heterogeneous and a particle is captured through specific (receptor-mediated) and nonspecific interactions.

As mentioned before, if the particle makes a contact with a receptor, the single contact leads to particle capture with probability 1. However, if the particle makes a contact with the bare cell boundary, it leads to a success with probability $\rho < 1$. Therefore, in our model, $p(\theta_s)$ depends on ρ and n, where n is the number of receptors on the cell boundary:

$$\eta_c(\rho, n) = \int_0^{\pi/2} 2p(\theta_s, \rho, n) \sin(\theta_s) \cos(\theta_s) d\theta_s. \tag{6}$$

For a given ρ and n, we can evaluate η_c (Eq. **??**), and then derive the rate constant $k(\rho, n) = \frac{3(1-\epsilon)}{2\epsilon a} \eta_c(\rho, n) |\boldsymbol{U}_\infty|$ (Eq. **??**). The upper limit on $k(\rho, n)$ or $\eta_c(\rho, n)$ corresponds to the condition where a single particle-cell contact results in a particle capture. This situation may arise in a hypothetical case where $n = \infty$ (cell surface contains infinite number of receptors) or $\rho = 1$. We denote corresponding k and η_c as k_{max} and $\eta_{c,max}$ respectively. Finally we define particle targeting efficacy by the following normalized quantity:

$$\psi(\rho, n) = \frac{k(\rho, n)}{k_{max}} = \frac{\eta_c(\rho, n)}{\eta_{c,max}} = \frac{\int_0^{\pi/2} 2p(\theta_s, \rho, n) \sin(\theta_s) \cos(\theta_s) d\theta_s}{\int_0^{\pi/2} 2p(\theta_s, \rho, n)|_{\rho=1} \sin(\theta_s) \cos(\theta_s) d\theta_s} \tag{7}$$

Given the above definition, we can consider few special cases. If the cell is devoid of receptors ($n = 0$), and particle uptake is mediated by nonspecific interaction only, $\psi(\rho, n)|_{n=0} = \alpha < 1$. If particle capture is mediated through specific (receptor) interaction only and nonspecific interaction plays no role at all ($\rho = 0$), then $0 < \psi(\rho, n)|_{\rho=0} \leq 1$. If particle capture is mediated by both nonspecific and specific interactions, $\alpha < \psi(\rho, n) \leq 1$. Finally, in order for the receptor-directed delivery to be effective, $\psi(\rho, n)/\alpha >> 1$.

1.6 Model Parameter Values

Table **??** summarizes the model parameter values mentioned in the above sections.

1.7 Code Implementation

The model and simulation algorithm was written in C++. The C++ code and necessary instructions are provided in the Supporting Material.

Results

Our analyses primarily focus on how variable receptor expressions may impact the tissue targeting efficacy of a nanoparticle. We consider two distinct cases. First, we consider a case where there is no nonspecific particle recognition by cells; particles can be recognized only by the cell-surface receptors. Second, we consider the case T. Below we describe our key findings.

Targeting in the Absence of Nonspecific Recognition

We first investigated how receptor expression might impact the targeting efficacy in the absence of nonspecific particle recognition by cells ($\rho = 0$). This is an extreme case, where the nonspecific interaction does not play any role in particle uptake, and the targeting efficacy is solely determined by the level of receptor expression. In Fig. **??**A, we show $p(\theta_s, \rho, n)|_{\rho=0}$ at various levels of receptor expression (n/n_{max}). As defined in Section **??**, $p(\theta_s, \rho, n)$ represents capture probability for particles with starting angle θ_s (Fig. **??**). Each black curve in the figure represents a finite level of receptor expression, as indicated in the figure legend. The black curves are compared against the red curve, which represents a hypothetical case of infinite receptor expression ($n/n_{max} = \infty$). The red curve represents the maximum probability where a particle is captured in a single contact with the cell boundary.

The interesting thing to note in Fig. **??**A is that $n/n_{max} = 1$ and $n/n_{max} = \infty$ (the red curve) give rise to equivalent level of particle recognition. Therefore, a further increase in n/n_{max} beyond 1 would not change the efficiency of particle capture by the cell. As explained in Section **??**, in our model, $n/n_{max} = 1$ represents 396 receptors on the two-dimensional cell boundary. This is equivalent to having 50,000 cell-surface receptors in three dimension. Because the model becomes insensitive at $n > 396$, we used this as the maximum number of receptors in our subsequent analysis.

In Fig. **??**B, we show targeting efficacy ($\psi(\rho, n)|_{\rho=0}$) corresponding to each level of receptor expression in Fig. **??**A. The calculation associated with each point involves numerical evaluation of $\eta_c(\rho, n)|_{\rho=0}$ (Eq. **??**), and then calculation of $\psi(\rho, n)$ (Eq. **??**). From the figure, in the range $n/n_{max} = 0.03 - \infty$, the gain in the targeting efficacy could be approximately 5 fold. In our two dimensional model, $n/n_{max} = 0.03$ corresponds to 12 receptors on the cell boundary. This is equivalent to having 45 receptors per cell in three dimension (based on the inter-receptor distance). Importantly, in the range $n/n_{max} = 0.3 - \infty$ (4,500 to infinite number of receptors per cell), there was barely any difference in the targeting efficacy.

Effective Receptor Recognition of Particles

According to our results in the previous section, there was no significant change in the targeting efficacy in the range $0.3 \leq n/n_{max} < \infty$ (Fig. **??**A). This was quite surprising

because at $n/n_{max} = 0.3$ ($n = 119$), receptors are still sparse on the cell boundary. In our model, each receptor has a radius of 7.5 nm. Thus, at this level of receptor expression, the aggregate (two dimensional) area occupied by the receptors is only $\sim 3\%$ of the cell boundary. In contrast, at $n/n_{max} = \infty$, the entire cell boundary is reactive in the sense that it captures a particle in every single contact. It was not apparent how these two cases could be almost equally efficient in capturing a particle. To investigate the reason, we analyzed the trajectories of individual particles in our simulation.

In Fig. **??**A, we show trajectories of 50 individual particles. The entry point (angle) of each particle was randomly selected at $r = b$. From the figure, particles hitting the cell boundary made many repeated contacts and explored large patches of areas. In general, a particle initiated at a smaller θ_s was able to explore larger areas compared to a particle initiated at a larger θ_s.

The result underscores the multi-collision interaction between a particle and a cell, which are very different in size. To a particle, the cell boundary is a very large and almost a flat obstacle. When a particle is in the vicinity of this boundary, it can either collide or diffuse away. However, due to the directional bias from the fluid velocity, a particle is persistently driven towards the cell boundary. This may force it to make many collisions and travel along the cell boundary to search for the sparsely populated receptors (Fig. **??**B). In the absence of the fluid velocity and under pure diffusion, there could be a higher chance for a particle to move away from the cell boundary.

Effects of Nonspecific Interactions on Targeting Efficacy

In our analysis so far, we ignored nonospecific particle recognition by setting $\rho = 0$. In Fig. **??**, we investigate how nonspecific particle recognition ($\rho > 0$) may impact the receptor targeting efficacy of a particle. In Fig. **??**A, we performed the same analysis as in Fig. **??**A setting $\rho = 0.001$. The figure shows the probability distribution $p(\theta_s, \rho, n)|_{\rho=0.001}$ at various levels of receptor expression, n/n_{max}, as indicated in the figure legend. Comparing Fig. **??**A with Fig. **??**A, the effect arising from the difference in receptor expression is significantly reduced despite this apparently weak nonspecific interaction, where a particle on average requires 1,000 contacts (collisions) with the cell boundary to be captured.

In Fig. **??**B, we compare the targeting efficacies $\psi(\rho, n)|_{\rho=0.001}$ at different levels of receptor expression. As seen in the figure, the targeting efficacy in the absence of any receptor ($n/n_{max} = 0$) was almost half of the maximum possible efficacy ($n/n_{max} = \infty$). To further investigate the effects of the nonspecific interaction, we considered four different values of ρ in our simulations: 0, 0.001, 0.003, and 0.01. Fig. **??** compares $\psi(\rho, n)$ for these four cases. The result clearly indicates that the nonspecific interaction becomes the dominant mechanism of particle recognition beyond $\rho = 0.003$. It also indicates that a very low probability of nonspecific capture (ρ) can make a significant impact on the overall targeting efficacy.

Targeting in the Presence of Nonspecific Recognition

We next investigated what might cause the nonspecific particle-cell interaction to be so effective even at a small value of ρ. The particle trajectory analysis in Fig. **??** revealed that the particles may make a large number of contacts with the cell boundary. This gave us a clue to further investigate whether such multicollision interactions could lead to a large effective probability of particle capture (ρ_{eff}) despite a small value of ρ. For example, if a particle on average makes 1,000 contacts with the cell boundary at $\rho = 0.001$, the effective probability of capture (ρ_{eff}) should be equal to 1. A further increase in ρ should make no difference in targeting efficacy.

We initiated 100,000 particles from $\theta_s = 0$ and analyzed the number of contacts of these particles with the cell boundary. We set $n/n_{max} = 0$ and $\rho = 0$ so that particle can only collide with the cell boundary but never get captured by the cell. Only 32% of the particles made at least one contact with the cell boundary, while the rest of the particles were lost without making a contact. We distributed the particles making contacts into 1,000 uniform bins based on the number of contacts they made. We then normalized the population size of each bin by the bin width. The resulting distribution (Fig. **??**) is the probability distribution of number of particle-cell contacts for particles with starting angle $\theta_s = 0$. The distribution shows that the particles make thousands of contacts with the cell boundary before escaping from the system boundary.

The area of the distribution in Fig. **??** is equal to 0.32 (the fraction of particles making contacts with the cell boundary). This area also represents the upper limit of effective capture probability, i.e., $\rho_{eff,u} = 0.32$. This upper limit can be achieved in a hypothetical case where every single nonspecific contact results in a particle capture ($\rho = 1$). However, because of the multicollision interactions, it might be possible to reach this upper limit even at $\rho << 1$. The smallest value of ρ yielding $\rho_{eff,u} = 0.32$ is: $\rho_u = \rho_{eff,u} / \int p_c m dm$, where p_c and m are the Y- and X-axis of Fig. **??**A, respectively. Our estimation led to $\rho_u = 5.2 \times 10^{-4}$. For any ρ greater than this value, the capture rate of particles should remain unaltered because the effective maximum capture probability cannot exceed 0.32. In Fig. **??**B, we show the distribution for particle capture probability at $\rho_u = 5.2 \times 10^{-4}$ and two other values smaller than ρ. The figure shows how the multi-collision particle-cell interactions contribute to the overall effective capture probability for particles with starting angle $\theta_s = 0$.

Overall, the analysis underscores an important feature of particle-cell interaction. The multi-collision interaction between a particle and cell may lead to a large effective particle capture rate despite a poor specificity. This might be a general phenomenon where the reactive bodies are too different in size.

Discussion

In this work, we presented a mechanistic spatiotemporal model of nanoparticle-cell interaction in a biological tissue. Using the model, we analyzed the effectiveness of receptor targeting of nanoparticles. Our overall analysis indicates that such strategy may not have a remarkable impact on the particle targeting efficacy. In the presence of nonspecific particle recognition, the gain in the targeting efficacy from ligand attachment could be marginal. We have provided a detailed analysis on the mechanisms whereby a normal cell and a cancer cell having significantly different levels of receptor expression could recognize particles with comparable efficiencies. Our analysis indicates that the size difference between a particle and a cell may enable a mobile particle to make numerous contacts with the cell surface. Such multi-collision interaction could lead to a high effective rate of particle uptake despite a poor nonspecific recognition.

In our model does not incorporate cellular heterogeneity of a tumor microenvironment (**?**). The model is based on a single-cell system assuming the tissue is homogeneous. In reality, a significant fraction of a tumor may be composed of various non-malignant cells (**?**) that may not express the target receptor proteins. Therefore, the aggregate cell-surface area available for nonspecific interaction could be larger than what has been considered in our model. As a result, our analysis may actually underestimate nonspecific recognition. Consideration of cellular heterogeneity in the model would further diminish the significance of receptor-directed targeting.

It should be noted that our model does not incorporate explicit ligand molecule to mediate receptor interaction. Rather, the entire particle surface was assumed capable of recognizing a receptor. This consideration favored the particle uptake via the specific (receptor-directed) interaction because every single contact between the particle and a receptor lead to a capture of the particle. The implicit assumption was that the particle surface could be densely populated (to achieve efficient targeting) so that a ligand-receptor contact was possible in every contact between the particle and a receptor. In reality, ligand-receptor binding may require proper orientation of the ligand and receptor binding sites. For a finite-sized particle with sparsely populated surface ligands, steric constraints and geometrical restrictions may play important roles.

We considered particle capture an irreversible process in both specific (receptor-mediated) and nonspecific particle-cell interaction. Again, the irreversible specific capture was to make the receptor-directed targeting more efficient. On the other hand, the irreversible nonspecific capture accounted for the possibility of intracellular particle uptake via endocytic trafficking or other mechanisms (**? ?**). The small value of ρ accounted for the numerous transient (reversible) nonspecific contacts that may eventually culminate in an irreversible intracellular uptake. In the first part of our analysis (Fig. **??** and **??**), we considered zero possibility of particle uptake via the nonspecific interaction ($\rho = 0$). This may represent an unrealistic extreme case because numerous experimental evidence indicate that nonspecific interaction accounts for an appreciable amount of particle uptake by cells (**? ? ? ? ?**). Therefore, our

subsequent analyses with a non-zero ρ in the range $10^{-4} - 10^{-2}$ may portray a more realistic scenario. In these latter case, we found the nonspecific interaction had a strong influence on particle recognition that marginalized the efficacy of receptor-directed targeting.

In our model, we consider a two-dimensional geometry. A three-dimensional form of the model would be more realistic but computationally expensive given a large number of simulations needed to generate the statistics. By proper scaling, the inter-receptor distance in our model was kept consistent with a three-dimensional system. In three dimensions, a particle would also display similar multicollision interaction, explore the two-dimensional cell surface, and avail sparsely-distributed receptors. Overall, key findings and insights should not change appreciably if the dimension of the system is changed.

Our study underscores the effects arising from the multi-collision interactions between two mutually reactive bodies of dissimilar sizes. Given the size difference between a nanoparticle and a cell, the former gets numerous opportunities to make contacts with the latter. Therefore, a very low probability of particle capture per contact (ρ) may translate into a high effective probability of capture (ρ_{eff}) (Fig. **??**). Our analysis shows that a value of ρ in the-the range $10^{-2} - 10^{-3}$ can translate into $\rho_{eff} \approx 1$. It should be noted that, nonspecific particle-cell interactions might be governed by several factors, such as particle and cell-surface charges (**?**), van der Waals attraction (**?**), and hydrodynamic lift (**?**). Explicit incorporation of these components in the model was possible. However, the exact contributions of these components should vary depending on the particle and cell. Therefore, we adopted the simpler but more general probabilistic construct where the value of ρ defines the lumped contributions from these factors in addition to the possibility of irreversible particle uptake by cells.

The approach developed in our model provides a unique capability to bridge the link between molecular-scale interaction to macroscale particle dispersion (Eq. **??**). In an earlier Happel model by Su et al. (**?**), the cell surface was considered featureless and homogeneous. Our extended model explicitly incorporates receptor features on the cell boundary and takes into account the molecular-scale details of the particle-cell interaction. It is possible to further extend the model by incorporating particle surface attributes, including targeting ligands. Such extension will enable simulation of the transport behavior of multivalent particles and biomolecules (ligands, antigens, and growth factors) in biological tissues or porous media. Currently, only the rule-based approach provides the capability to consider site-specific details of multivalent reactive species (**? ?**). However, most of the existing rule-based approach use nonspatial graphs to define protein molecules and complexes. Therefore, the rule-based models provide limited capability to explicitly investigate the spatiotemporal effects arising from diffusion and geometry of multivalent species. Extension of our approach may enable development of realistic tissue models bridging spatiotemporal complexities at distinct time and spatial scales.

2 Conclusion

In this study, we have developed a spatiotemporal model of nanoparticle-cell interaction. Using the model, we analyzed the effectiveness of receptor-directed tissue targeting of nanoparticles. Our analysis indicates that a targeted nanoparticle can be barely more efficient compared to a non-targeted particle. Our model indicates that nonspecific interactions play a significant role in particle recognition due to the large surface area of cells available for nonspecific recognition of particles. A particle having a much smaller size makes numerous contacts with the interstitial cell boundaries devoid of receptor proteins. Such multicollision interactions may allow the particle to be captured with high probability despite having a very low specificity for nonspecific recognition per contact with the cell boundary.

3 Funding

Research presented in this work was supported by the National Science Foundation CBET-CDS&E grant No. 1609642. No additional external funding was received to support this work. The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. The author declares no existing conflict of interest.

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Figure Legends

Figure 1: Illustration of the particle-cell interaction model. (A) A porous medium (left) and corresponding Happel-Sphere-in-Cell model (right) are shown. (B) The particle-cell interaction model based on the Happel model.

Figure 2: Brownian Dynamics simulation of particle motion. (A) Interstitial velocity field around the spherical calculated using Eqs. **??** and **??**. The length and direction of each arrow represent the magnitude and direction of velocity at the indicated points. (B) Particle motion in the interstitial space. The net particle displacement $\boldsymbol{S}$ in a single time step is the sum of the two vectors, $\boldsymbol{S}_v$ and $\boldsymbol{S}_d$. These vectors represent the convective and diffusive jump of the particle, respectively. The particle takes smaller jump sizes are adapted based on the distance from the cell boundary, as discussed in Section **??**.

Figure 3: Effect of receptor expression on the targeting efficacy in the absence of nonspecific particle uptake ($\rho = 0$). Only receptor-mediated particle uptake is allowed. (A) $p(\theta_s)$ represents the probability of capture of a particle with starting angle θ_s (Fig. **??**). The red curve represents a hypothetical case of infinite receptor expression ($n/n_{max} = \infty$). The black curves represent finite levels of receptor expression, as indicated in the legend. $n/n_{max} = 1$ correspond to 396 receptors (maximum number of receptors in the model). Each discrete point (value of $p(\theta_s)$) is calculated from 1,000 particle trajectories starting from the corresponding θ_s. The solid lines represent polynomial fits. (B) Targeting efficacy (ψ) (Eq. **??**) at different levels of receptor expression (n/n_{max}).

Figure 4: Particle trajectories in the flow field. (A) The trajectories of 50 individual particles are shown. Each color corresponds to a distinct particle trajectory. The curved boundary represents part of the cell boundary. The red spots on this boundary represent receptor locations. (B) A zoomed-in view of a small region of Panel A. Simulations are done setting $\rho = 0$ and $n/n_{max} = 0.03(12 receptors along the cell boundary)$.

Figure 5: Effect of receptor expression on the targeting efficacy in the presence of nonspecific particle uptake ($\rho = 0.001$). Both specific- and nonspecific particle uptake are allowed. (A) As in Fig. **??**, $p(\theta_s)$ represents the probability of capture of a particle starting from an angle θ_s (Fig. **??**). The red curve represents a hypothetical extreme case, where $n/n_{max} = \infty$. The black curves represent finite receptor expression levels, as indicated in the legend. (B) Targeting efficacy (ψ) (Eq. **??**) at different levels of receptor expression (n/n_{max}).

Figure 6: Comparison of targeting efficacy at different levels of nonspecific particle uptake (ρ). Each of the four curves corresponds to a distinct value of ρ, as indicated in the figure legend.

Figure 7: Analysis of multi-collision particle-cell interaction. (A) Distribution of number of particle-cell contacts for particles with starting angle $\theta_s = 0$. A total of 10^5 particles, all with starting angle $\theta_s = 0$, were simulated considering no particle capture by the cell ($\rho = 0$ and $n/n_{max} = 0$). Based on the number of contacts with the cell boundary, the particles were distributed in 1,000 uniform bins, and the population in each bin was normalized, as discussed in Section **??**. (B) The effective probability of particle capture: $p_a = \rho m(p\Delta m)$, where p is the corresponding Y-axis value in Panel A. The probability distribution is shown for three distinct values of ρ, as indicated in the legend.

Table 1: Model Parameter Values

Parameter	Comment
$R = 10^4$ nm	Cell radius (a typical cell size assumed).
$r_p = 10$ nm	Nanoparticle radius.
$n = 12 - 396$	Number of receptors.
$r_{rec} = 7.5$ nm	Radius of a receptor.
$\epsilon = 0.4$	Tissue porosity (**?**).
$D = 10^5$ nm^2/s	Particle diffusion constant (**?**).
$U_\infty = 10^3$ nm/s	Mean (bulk) fluid velocity in the tissue. (**?**)
$\rho = 0 - 10^{-4}$	Probability of particle capture per nonspecific collision.
$\Delta S_{max} = 10$ nm	Largest particle jump size.
$\Delta t_{min} = 10^{-5}$ s	Smallest time step.