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# Design Rules for Cancer Nanomedicines

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***Abstract*— The use of nanotechnology has offered new hope for cancer detection, prevention and treatment. Nanoparticle formulations are advantageous over conventional chemotherapy because they can incorporate multiple diagnostic and therapeutic agents and are associated with significantly less adverse effects due to selective accumulation to tumor tissue. Despite their great promise, however, only a few nanoparticle formulations have been approved for clinical use in oncology. The failure of nano-scale drugs to enhance cancer therapy is in large part due to inefficient delivery. Indeed, physiological barriers posed by the tumor micro-environment inhibit homogeneous distribution of drugs to the interstitial space of tumors and compromise the efficacy of the treatment. To overcome this outstanding problem, a better understanding of how the physical properties (i.e., size, and surface charge) of nanoparticles affect their transport in tumors is required. Here we use a mathematical model to provide basic design guidelines for the optimal delivery of nanoparticle formulations.**

***Keywords — Drug delivery; solid tumors; EPR effect; vascular permeability; interstitial transport***

## I. INTRODUCTION

Systemic administration of drugs to solid tumors is a three-step process [1, 2]. Any blood-borne diagnostic or therapeutic agent has to first travel through the blood vessels to reach the tumor tissue. Subsequently, it will cross the tumor vessel wall to enter the interstitial space, and finally, it will travel through the interstitial space to attack cancer cells. The enhanced permeability and retention (EPR) effect has served as a key rationale for the use of nanoparticles for solid tumors. It is based on the fact that the vascular wall of neoplastic tumors exhibit much larger pore sizes than that of normal vessels. We have previously shown that the pore size ranges from 100 to 2,000 *nm* in various tumors growing in animal models, while the pore size of normal vessels is less than 10 nm [2,3]. As a result, any nanoparticle formulation with a diameter larger than the pore size of normal vessels will not be able to enter normal tissues and it will selectively accumulate to tumor tissue, enhancing the therapeutic outcome and diminishing potential adverse effects.

Recent advances in nanotechnology have permitted the incorporation of multiple diagnostic and therapeutic agents, and offered a great promise for detection, prevention, and treatment in oncology [4,5]. Nanomedicines, with a size range of 1-1000 nm, for cancer therapy are advantageous over conventional medicine because they have the potential to enable i) preferential delivery of drugs in tumors due to the EPR effect, ii) delivery of more than one therapeutic agents for combination therapy, iii) specific binding of drugs to targets in cancer cells or tumor micro-environment, and iv) simultaneous visualization of tumors using innovative imaging techniques. Furthermore, many widely used conventional chemotherapeutics are associated to severe toxicities and adverse effects that compromise the quality of life of cancer patients. Nanomedicine has the potential to reduce adverse effects and improve quality of life.

Given the advantages of nanomedicines, nanoparticle formulations that have been approved for clinical use in oncology include liposomes (pegylated liposomal doxorubicin, liposomal daunorubicin), albumin-bound paclitaxel, and polymeric particles (Methoxy-PEG-poly($_{D,L}$-lactide) taxol) (Table 1), while many more formulations are in preclinical or clinical trials [1]. These agents have a diameter in the range of 100-130 nm and exhibit significantly less adverse effects than conventional chemotherapy, presumably due to the EPR effect. However, they might still exhibit a profile of other adverse effects (e.g. stomatitis and palmar-plantar erythrodysesthesia for pegylated liposomal doxorubicin and sensory neuropathy and nausea for albumin-bound paclitaxel). Moreover, the increase in overall survival in many cases is modest (Table 1), which is in large part due to inefficient and heterogeneous delivery [6-10]. Therefore, while the EPR effect allows the transvascular transport of large nanoparticles, which are excluded by the pores of normal vessels, it cannot ensure that sufficient amounts of these drugs will be homogeneously delivered to the tumor and cause complete treatment. Another interesting observation of From Table 1 is that in many cases nanomedicines have been clinically approved mainly because they improve the quality of life of the patient reducing the severity of adverse effects and not because they increase the overall survival.

A better understanding of the barriers that prevent the uniform delivery of nanoparticles into tumors is required to develop strategies to improve treatment. Most importantly, an understanding of how the physical properties of nanoparticles (size, and surface charge density) affect their delivery is required for the development of design guidelines for optimal distribution in tumors. In this paper, we seek to provide guidelines for the optimal design of nanoparticles with the use of mathematical modeling.

TABLE I. CLINICALLY APPROVED NANOPARTICLES FOR SOLID TUMORS[A]

| *Generic name* | *Trade name(s)* | *Indication* | *Benefit vs. conventional therapy* |
|---|---|---|---|
| PEGylated liposomal doxorubicin | Doxil® and Caelyx® | HIV-related Kaposi's sarcoma | Not statistically significant [5] |
| | | Metastatic ovarian cancer | Statistically significant Overall Survival (108 vs. 71.1 weeks) [6] |
| | | Metastatic breast cancer | Not statistically significant [7] |
| Liposomal daunorubicin | DaunoXome® | HIV-related Kaposi's sarcoma | Not statistically significant [8] |
| Albumin-bound paclitaxel | Abraxane® | Metastatic breast cancer | Statistically significant Overall Survival (56.4 vs. 46.7 weeks) [9] |

a. The polymeric platform Methoxy-PEG-poly($_{D,L}$-lactide) taxol with the trade name Genexol-PM (Samyang Co., Seoul, Korea) has been approved in Korea for the treatment of metastatic breast cancer.

The table was adapted from [1]

## II. MATHEMATICAL MODELING OF NANOPARTICLE TRANSPORT IN SOLID TUMORS

Mathematical modeling of the transport of nanoparticles in solid tumors requires the solution of a fluid mechanics problem in order to determine the fluid pressures in the vascular and interstitial spaces and a transport problem, which describes the delivery of nanoparticles.

### *A. Coupling of Fluid flow in the vascular and interstitial space*

The equations that govern the fluid flow in the vascular, transvascular and interstitial spaces are the following.

Fluid flow through blood vessels can be assumed to follow Poisseuille's law and to be given by the equation:

$$Q_{vascular} = -\frac{\pi d^4}{128\mu}\nabla p_v \,, \qquad (1)$$

where $Q_{vascular}$ is the volumetric flow rate of the blood in the vessels, $d$ is the vessel diameter, $p_v$ is the vascular pressure and $\mu$ is the blood viscosity.

Volumetric fluid flow rate across the vessel wall ($Q_{transvascular}$) follows Starling's law [11]:

$$Q_{transvascular} = L_p S\left(p_v - p_i\right), \qquad (2)$$

where $L_p$ is the hydraulic conductivity of the vessel wall, $S$ is the surface area of the vessel and $p_i$ is the interstitial fluid pressure. Notice that in Eq. 2 we neglect osmotic pressures since in solid tumors they have a negligible effect to fluid flow across the vessel wall.

Interstitial volumetric fluid flow rate ($Q_{tissue}$) follows Darcy's law [12],

$$Q_{tissue} = -K_t A_C \nabla p_i \,, \qquad (3)$$

where $K_t$ is the hydraulic conductivity of the interstitial space, $p_i$ is the interstitial pressure and $A_c$ is the tissue cross-sectional area. The tissue cross-sectional area is related to the vascular density, $S_v$, and the diameter of the vessel, $d$, by $A_C = \pi d / S_v$ .

### *B. Coupling of nanoparticle transport in the vascular and interstitial space.*

Coupling of nanoparticle transport between the vascular and interstitial spaces is based on the following assumptions.

Inside the blood vessels diffusion is negligible and the mass balance is governed by convection only:

$$\frac{dc_v}{dt} = -v\nabla c_v \,, \qquad (4)$$

where $v$ is the fluid velocity which is determined by dividing $Q_{vascular}$ in Eq. 1 by the cross sectional area of the vessel and $c_v$ is the intravascular concentration of the nanoparticle.

In the interstitial space transport of nanoparticles is governed by the convection-diffusion equation,

$$\frac{dc_i}{dt} + v_i\nabla c_i = D\nabla^2 c_i \,, \qquad (5)$$

where $c_i$ is the concentration of the nanoparticle in the interstitial space, $D$ is the diffusion coefficient, and $v_i$ is the interstitial fluid velocity which is calculating by dividing $Q_{tissue}$ in Eq. 3 by $A_c$.

Transport across the tumor vessel wall, $\varphi$, is given by Starling's approximation as [11]

$$\varphi = L_p S(p_v - p_i)(1-\sigma)\frac{c_v e^{Pe} - c_i}{e^{Pe} - 1} \,, \qquad (6)$$

where $P_e = L_p(1-\sigma)\dfrac{\left(p_v - p_i\right)}{P}$ is the Péclet number across the vessel wall, and $P$ is the vascular permeability of the nanoparticle through the pores of the wall.

Using theory for transport of particles through cylindrical pores we calculate the hydraulic conductivity, $L_p$, vascular permeability, $P$, and reflection coefficient, $\sigma$, by the equations [13]:

$$L_p = \frac{\gamma r_o^2}{8\mu L}, P = \frac{\gamma H D_o}{L}, \sigma = 1 - W, \quad (7)$$

where $\gamma$ is the fraction of vessel wall surface area occupied by pores, $r_o$ is the pore radius, $L$ is the thickness of the vessel wall, and $D_o$ is the diffusion coefficient of the particle in free solution at 37 $^{o}$C.

Finally, The parameters $H$ and $W$ account for hydrodynamic and electrostatic interactions and for dilute solutions are given by the equations [13]:

$$H = 2\int_0^{1-\lambda} K^{-1} e^{-E/kT} \beta d\beta, \quad (8)$$

$$W = 4\int_0^{1-\lambda} G\left(1-\beta^2\right) e^{-E/kT} \beta d\beta, \quad (9)$$

where $\lambda$ is the ratio of the particle size over the pore size, $E$ is the electrostatic energy of interaction between the nanoparticle and the pore, $k$ is the Boltzmann's constant, $T$ is the absolute temperature, $K(\lambda,\beta)$ and $G(\lambda,\beta)$ are hydrodynamic functions, and $\beta$ is the radial distance in the pore divided by the pore radius.

The set of equations 1-9 provides a complete mathematical framework that describes vascular, transvascular and interstitial transport of nanoparticles to solid tumors taking into account the size and surface charge of the particles as well as physiological parameters of the tumor micro-environment that inhibit transport (e.g. vessel wall pore size and charge, hydraulic conductivity of vessel wall and interstitial space). This framework is general and can be applied to any vascular geometry. We employed a finite difference method for the solution of this system of equations as described in [2].

## III. Effect of Nanoparticle Size on Delivery

For optimal efficacy, a therapeutic agent must reach tumors in amounts sufficient to kill cancer cells but at the same time should not have adverse effects in normal tissues. Obviously, the smaller the particles the better the transport; however, small molecules, such as chemotherapeutics, generally extravasate in most normal tissues potentially causing adverse effects [1]. The combination of the two constraints suggests that increasing the size of the nanoparticle will provide selectivity, but at the cost of limiting transport from some pores of tumor vessels. Therefore, the size of the particle needs to be optimized for each tumor and its metastases. The challenge is that the tumor micro-environment is not spatially homogeneous and it changes with time and in response to treatment.

Given the temporal and spatial heterogeneity of the tumor micro-environment we used the mathematical model to predict the amount of particles that cross the tumor vessel wall from the vascular into the interstitial space, i.e., the transvascular flux. We varied the diameter of the particles from 1 to 250 nm, while and the diameter of each pore was derived by a unimodal distribution with a mean diameter ranging from 40 to 1000 nm and a standard deviation of 60 nm. The model parameters are presented in Table 2 and the predictions in Fig. 1.

TABLE II. Physiological Parameters of Tumor Micro-Environment

| ***Physiological parameters*** | ***Value*** |
|---|---|
| Vessel wall pore size | 40 - 1000 nm [2,3] |
| Blood viscosity | 0.04 Pa-sec [13] |
| Vascular density | 100 $cm^{-1}$ [2, 13] |
| Vessel wall thickness | 5 μm [2] |
| Vessel diameter | 15 μm [2, 13] |
| Interstitial space conductivity | $6x10^{-13}$ $m^2$/Pa-sec [2,13] |

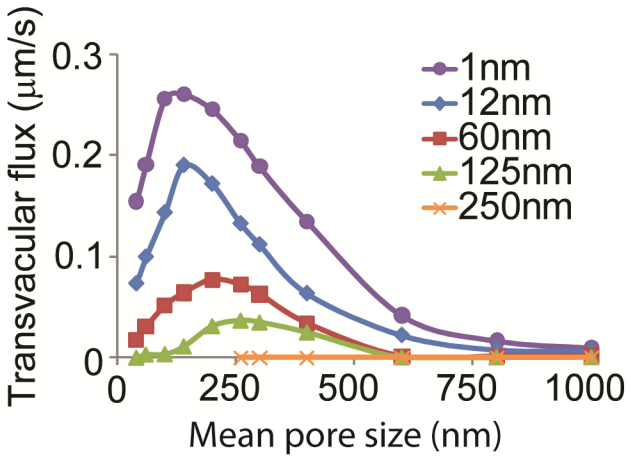


Figure 1. Transvascular flux of nanoparticles as a function of mean pore size. Pore size standard deviation is 60 nm. Particles with sizes 1, 12, 60, 120, and 250 nm are considered. Figure adapted from [2].

Generally, smaller particles (1-12nm) demonstrate the most rapid transvascular flux into tumors, while the largest particles (125-250nm) did not appreciably leave the vasculature. It is also important to notice that there is a value of mean pore size where the transport of nanoparticles is optimized. At very small pores steric and hydrodynamic interactions hinder the transport of nanoparticles. As the pore size increases these interactions become less important and transvascular flux increases. However, further increasing mean pore size past a point will

eventually hinder drug delivery due to rising interstitial fluid pressure leading to limited fluid flux.

## IV. Effect of Nanoparticle Charge on Delivery

Not only the size but also the surface charge of therapeutic nanoparticles play a crucial role in extravasation and interstitial transport. On the one hand, it has been shown that cationic nanoparticles interact with the negatively-charged pores of the tumor vessel walls and exhibit a higher vascular permeability compared with their neutral or anionic counterparts [14, 15]. On the other hand, neutral nanoparticles diffuse faster and distribute more homogeneously inside the tumor interstitial space than cationic and anionic particles, because the latter form aggregates with negatively-charged (for example, hyaluronan) or positively-charged (for example, collagen) matrix molecules [16].

Figure 2 depicts model predictions for the transvascular flux of nanoparticles as a function of the mean pore size and for negatively-charged, neutral, and positively-charged particles. The surface charge density of the pores of the vessel walls is set to -0.05 C/m$^2$. In the figure, q denotes the surface charge density of the particles.

Again, we observe that there is a value of the mean pore size for which transvascular transport of nanoparticles becomes optimal. Electrostatic attraction between cationic nanoparticles with the negatively-charged vascular pores result in an increase in the flux, while electrostatic repulsion between anionic particles and the pores of the vessel walls results in inhibition of the transport.

At smaller pores the benefit of cationic nanoparticles is less dramatic than at larger pores. For cationic particles, there is a competition between steric and hydrodynamic forces that hinder transport and electrostatic forces that enhance transport. At small pores steric and hydrodynamic forces must dominate and for that reason we do not see dramatic effects, while for larger pores the effect of electrostatic interactions might become dominant. Finally when pores are getting very large compared to the size of the particles all these types of interactions diminish and for that reason we do not see any effect of the charge.

In general, Many components of the tumor micro-environment have an electric charge. The vascular glycocalyx renders the blood vessels negatively-charged, while in the interstitial space the hyaluronic acid consists of highly anionic molecules and the collagen fibers have a slightly positive charge. Therefore, electrostatic interactions between nanoparticles and components of the tumor micro-environment could play an important role on drug delivery. Transvascular transport of negatively-charged particles is hindered only when the pore size is comparable to the Debye length. Of note, for pores of tumor vessel walls, whose size is on the order of hundreds of nanometers, the effect of electrostatic repulsion must be negligible. On the contrary, electrostatic attraction, caused by positively-charged particles, can significantly increase transvascular flux (Fig. 2). It seems that for every nanoparticle size, there is a value of surface charge density above which a steep increase in transvascular transport is predicted. But again, as the size of the pores increases, the benefit of electrostatic attraction disappears.

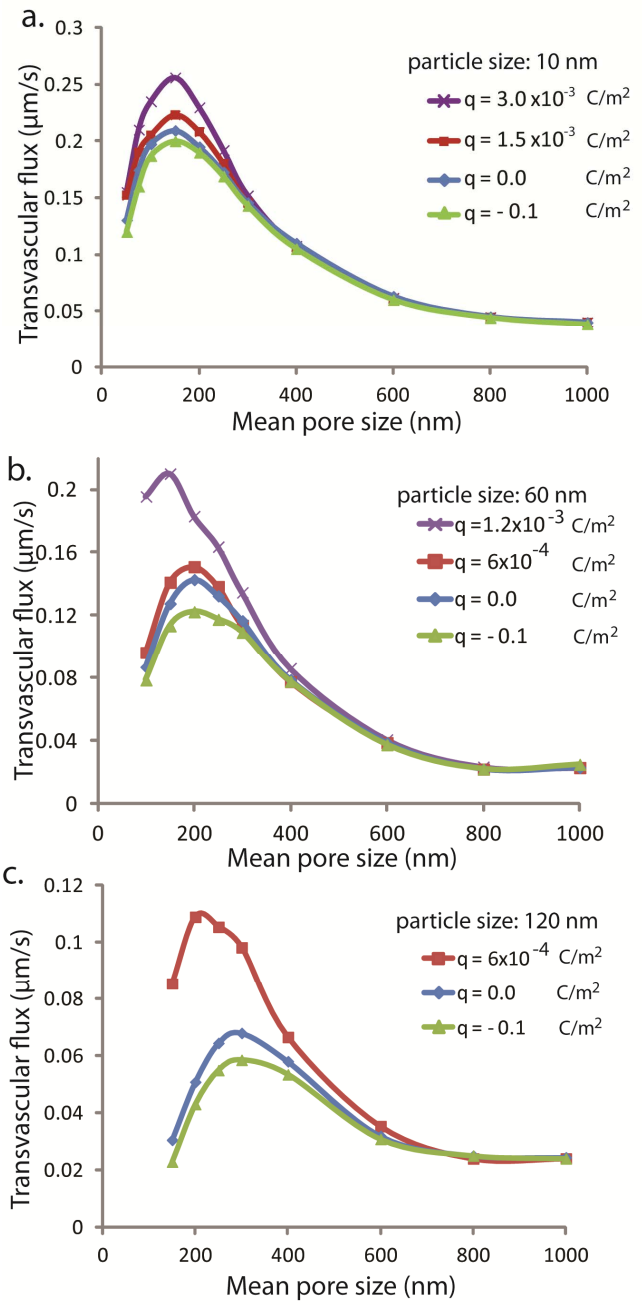


Figure 2. Transvascular flux of charged nanoparticles as a function of mean pore size. Pore size standard deviation is 60 nm. Particles with sizes (a) 10, (b) 60 and (c) 120 nm are considered. Figure adapted from [25].

## V. OTHER DESIGN CONSIDERATIONS

Apart from transvascular transport, the size and charge of nanoparticles affects other steps of drug delivery such as the circulation time in the blood. In general, the longer time the particle remains in the blood stream, the more efficient it will interact with the pores of the vessel walls. Therefore, we want to prolong the circulation time, provided the nanoparticle formulation is not toxic to normal tissues. Nanoparticles, like any other blood-borne agent are cleared by the kidney. Renal clearance is inversely related to the hydrodynamic diameter of the particle. Particles with a hydrodynamic diameter smaller than 5–6 nm are rapidly cleared, while larger particles can significantly increase the half-life in the blood [17]. In addition to the kidneys, interactions between nanoparticles and the reticuloendothelial system in the liver and the spleen plays an important role in nanoparticle clearance. Clearance from the reticuloendothelial system depends not only on particle size but also on surface modification and can vary significantly among the different types of nanoparticles [18]. As the surface charge becomes larger (either positive or negative), interactions with the reticuloendothelial system increase and lead to greater clearance of the particle. To prolong circulation times, the surface of the nanoparticles is coated with polyethylene glycols (PEGylation). Nanoparticles are stabilized by attaching PEGs to the surface which converts their surface charge density to near neutral charge. PEGylated particles have minimal electrostatic interactions with cells and proteins in the blood or the interstitial space. As a result opsonization by serum proteins and phagocytosis by Kuppfer cells or hepatocytes is prevented [19, 20].

Furthermore, in many cases the nanoparticle has to be internalized by cancer cells in order to cause treatment, as it is the case for oncolytic viruses. Cellular internalization would depend on size, shape and charge. For spherical particles, internalization is faster for smaller particles, while for larger particles it is slower and might follow a different mechanism [21]. In addition, there is evidence that cellular internalization is maximized for particle sizes ranged from 40 to 50 nm, while internalization was less effective for particles outside of this range [22]. For non-spherical particles, internalization is affected by the angle of contact between the particle and the cell [23]. Rod-like particles that are aligned perpendicular to the cell surface forming a contact angle of 90 degrees can be internalized more effectively than particles that align parallel to the cell surface. Finally, internalization is favored for cationic particles with higher aspect ratios [24].

## VI. CONCLUSIONS

The last two decades more than 30,000 articles have been published in the area of cancer nanomedicine but to-date only three nanoparticle formulations have been approved for clinical use (Table 1). Anti-cancer drugs, including both chemotherapeutic agents and nanomedicines, are potent enough to eradicate cancer. The scientific effort has to focus on enhancing the accumulation and homogeneous distribution of these drugs to solid tumors, so that the promise of nanomedicines to cancer patients be realized. With increasing numbers of nano-scale drugs in preclinical and clinical studies for cancer detection and therapy, it is critical to consider the physiological barriers that hinder their delivery and develop strategies that can overcome these barriers. Given the highly heterogeneous and continuously evolving nature of the tumor micro-environment, the optimal design of nanoparticles is likely to be disease-specific. This is a formidable task, especially considering the difference from one tumor to the next, from primary tumor to its metastasis, from one day to the next in the same tumor and the changes induced by treatment.

Mathematical modeling can play a crucial role in indentifying the optimal properties of nanoparticle formulations at each stage of tumor growth. Further improvements of our modeling approach are required though so that our model can deal with the highly heterogeneous tumor microenvironment but also with the internalization of nanoparticles by cancer cells. The mathematical framework presented here is general and can be applied to any vascular network. Therefore, to deal with the heterogeneity of solid tumors we propose to use high resolution, three-dimensional images of the tumor vascular network taken with intravital microscopy in order to construct the computational domain. In addition, the model could be augmented by accounting for multiple therapeutic/diagnostic agents that can be loaded on the nano-carrier, their release from the nanoparticle and their internalization by cancer cells. Such an advanced model that takes pharmacokinetics and pharmacodynamics into account would significantly increase the predictive capability of our approach.

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