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Rational Design of Nanoparticles for Cancer Drug Delivery

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Abstract. A plethora of nanoparticles for biomedical applications have been presented in the literature and are being developed by several laboratories with differences in composition, surface physico-chemical properties, size and shape. Mathematical modeling and in-vitro/in-vivo testing can help in identifying general rules for optimizing the performance of intravascularly injected nanoparticles. This paper presents a review of the research activity developed over the last years on the rational design and vascular dynamics of nanoparticles for biomedical applications and provides perspectives on future directions for the research activity within the field.

1. INTRODUCTION

Organic, inorganic and biological particulate systems are being developed by several laboratories for different biomedical applications ranging from early disease detection to imaging, therapy and follow-up. Particulate systems sufficiently small to be administered at the systemic level and transported by the blood flow along the circulatory system can reach potentially any tissue and organ (biological target) within the host. In sharp contrast to freely administered drug molecules and monoclonal antibodies, particles can be engineered to carry a large number of drug molecules and contrast agents providing simultaneously a therapeutic and imaging function (particle multi-functionality). Moreover, the particle surface can be conjugated with several antibodies and/or ligand molecules to enhance the specific recognition of the biological target through the formation of ligand-receptor bonds, as well as polymer chains of different length and type to effectively "camouflage" the particle from the reticular-endothelial system (RES) recognition and sequestration.

In oncology, the main strategy currently followed for the delivery of particulate systems has relied on

exploiting the well known enhanced retention and permeability (EPR) effect, in which sufficiently small particles can passively cross the tumor endothelial barrier through fenestrations with a characteristic size of the order of a few hundreds of nanometers. This strategy poses a limit to the maximum size of the particles to about 100 nm – 300 nm, because larger particles would be less likely to pass through the fenestrations passively. Moreover, such an approach relies exclusively on the presence of vascular fenestrations whose size is known to change over time, being negligibly small at an early stage of the disease; to be affected by the type of tumor and site of tumor growth; and to be potentially reduced by anti-angiogenic therapies. Moreover, for cardiovascular, hemorrhagic and other diseases, particle delivery can not rely on EPR which therefore is just limited to cancer application.

Recent studies are confirming that biological and biophysical differences exist between normal and abnormal endothelium, for cancer as well as for other diseases. Differences have been observed in the organization of the vascular network and mean blood velocity; and in the expression of specific markers whose surface density can be much higher on the abnormal compared to the regular endothelial cells. Such experimental evidence has encouraged the definition of an alternative delivery strategy that aims at exploiting vascular biodiversity to optimally design particulate systems able to (i) navigate within the vascular network transported by the blood flow; (ii) recognize the abnormal endothelium and (iii) adhere firmly to it under flow. From this privileged position, the vascular target particle can release towards the extravascular space smaller nanoparticles, loaded with therapeutic and imaging agents, or drug molecules. This approach, known as vascular targeting, has two main advantages compared to the EPR based strategies: (i) it can be employed for any pathology involving the endothelium, without requiring any alteration in the blood vessel permeability; (ii) large particles with much higher payloads can be used.

2. MODELING THE VASCULAR DYNAMICS OF NANOPARTICLES

The intravascular 'journey' of a systemically injected nanoparticle can be broken down into three fundamental events: (i) transport and margination dynamics, (ii) firm adhesion and (iii) control of internalization/translocation (Fig.1). Mathematical models, supported by in vitro and in vivo analysis, have been developed over the last years for predicting the margination propensity, probability of adhesion and rate of internalization for nanoparticles

as a function of their size, shape and surface physico-chemical properties (the 3-S problem).

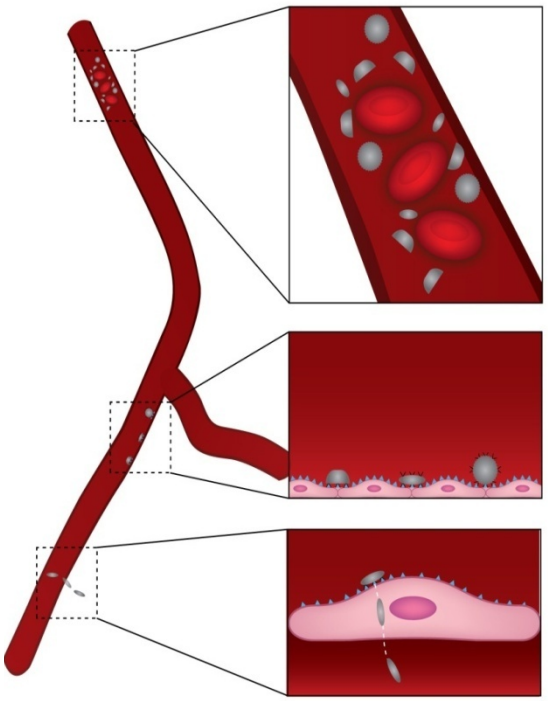


Figure 1. Transport and margination, adhesion and internalization of nPS within an authentically complex vasculature.

Margination is a well-known term in physiology conventionally used to describe the lateral drift of leukocytes and platelets from the blood vessel core towards the wall. Similarly, marginating nanoparticles are designed to move preferentially in close proximity to the blood vessel walls which can therefore be more efficiently 'inspected' for biological and biophysical diversities. Marginating nanoparticles would tend to accumulate in the cell free layer (a few micron thick) where an almost linear shear laminar flow is observed. However, whilst for leukocytes and platelets margination is an active process requiring an interaction with fast moving red blood cells and the dilatation of inflamed vessels with blood flow reduction [1]; nanoparticle margination can only be achieved by proper 'rational design'. Our computational module for margination can predict the trajectories of nanoparticle with an arbitrary size and shape within a laminar flow in proximity of the vessel walls.

The motion of spherical beads in a linear laminar flow has been described by Goldmann et al. [2], who showed that no lateral drift can be generated in the absence of external forces, such as gravitational, magnetic or short-ranged van der Waals and electrostatic interactions [3]. In other words, neutrally buoyant spherical beads cannot drift laterally. Non-spherical particles exhibit more complex motions with tumbling and rolling which can be exploited to control margination. The Navier-Stokes equation for a Newtonian fluid and mass conservation govern the flow field evolution over time t

$$\rho\left(\frac{\partial \boldsymbol{V}}{\partial t}+\boldsymbol{V}\cdot\nabla\boldsymbol{V}\right)=-\nabla p+\mu\nabla^2\boldsymbol{V}$$

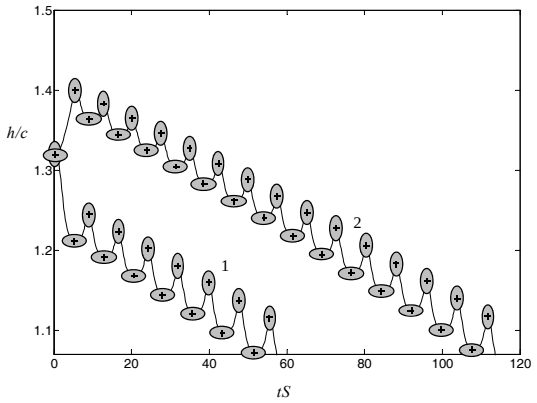
$$\text{and } \nabla\cdot\boldsymbol{V}=0 \qquad (1)$$

where ρ and μ are the density and viscosity of the fluid, respectively; $\boldsymbol{V}$ is the fluid velocity vector and p the fluid pressure. The motion of the particle, assumed to be confined within the 2D plane x-y, is governed by Newton's law as

$$m\frac{d\boldsymbol{u}}{dt}=\boldsymbol{F} \quad \text{and} \quad I\frac{d\boldsymbol{\omega}}{dt}-I\boldsymbol{\omega}\times\boldsymbol{\omega}=\boldsymbol{T} \qquad (2)$$

where m and I are the mass and rotational inertia of the particle, respectively; $\boldsymbol{u}$ is the particle velocity; $\boldsymbol{\omega}$ is the particle angular velocity; and $\boldsymbol{F}$ and $\boldsymbol{T}$ are the force and torque exerted on the particle including both surface forces, as the hydrodynamic forces, and external volume forces, as gravitation and electromagnetic.

By considering a rigid ellipsoidal particle (Fig.2), it can be shown that, regardless of the initial conditions, the propensity for margination increases with moderate Stokes number [4]. Similar observations have been derived for other geometries, as hemispherical and discoidal beads [5]. The theoretical predictions are also supported by in vitro experiments that showed how moving from discoidal, to hemispherical and spherical the margination propensity reduces [6] (Fig.3). The computational model for margination can also be used to predict the behavior of few nanoparticles, even with different shapes and sizes, moving together in proximity of the vessel wall, taking into account the interactions among sufficiently close nanoparticles.



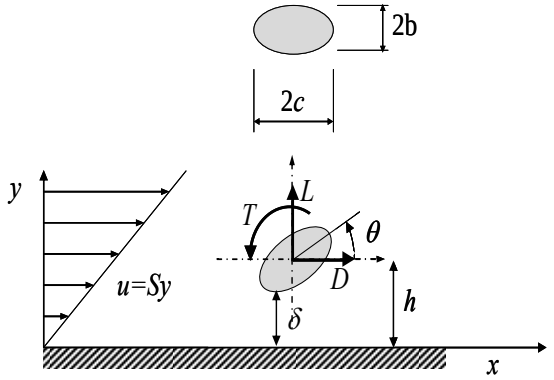


Figure 2. (Top) An ellipsoidal bead in close proximity to a vessel wall in a linear laminar flow. (Bottom) Trajectories of an ellipsoidal bead with two different initial orientations (parallel and orthogonal to the flow direction). [4]

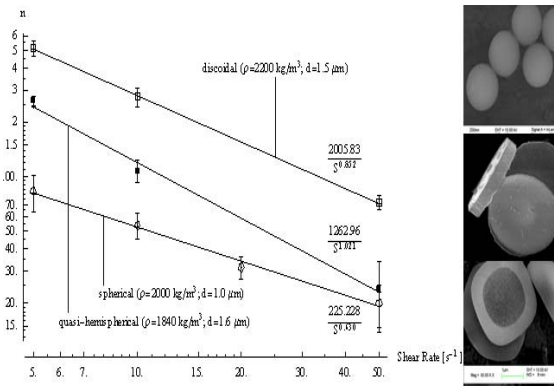


Figure 3. Experimental results for the number of particles sedimenting under flow in a microfluidic chamber as a function of the shape. [6]

The nanoparticles moving in close proximity to the blood vessels can interact both specifically and non-specifically with the endothelial cells and adhere firmly to it, once suitable conditions are met in terms of hydrodynamic shear stress at the wall and type and level of expression of receptor molecules. Firm wall adhesion is ensured as long as the dislodging forces (hydrodynamic, magnetic and other forces) acting to detach the particle from the target cell are balanced by specific ligand-receptor interactions and non-specific adhesion forces arising at the cell/particle interface. The strength of adhesion can be expressed in terms of an adhesion probability P_a, defined as the probability of having at least one ligand-receptor bond formed under the action of the dislodging forces. The adhesive model can predict the strength of adhesion P_a as a function of biophysical features as the wall shear stress μS, biological features as the surface density and family of endothelial receptor molecules m_r, and nanoparticles design parameters as the size, the shape, the surface density and family of ligand molecules distributed over the surface, the surface charge and presence of polymer linkers. The receptor-ligand binding is treated as a chemical reaction with forward (kon) and reverse (koff) rates, which are affected by the mechanical forces exerted on the bond through appropriate constitutive relations. The probability of having n bonds close at time t can be described invoking the kinetics of small systems, and is governed by the general equation.

$$\frac{dp_n(t)}{dt} = z_1(f_b)p_{n+1}(t) - z_2(f_b)p_n(t) + z_3(f_b)p_{n-1}(t); \qquad f_b = F/n$$

$$z_1(f_b) = (n+1)k_r(f_b)$$

$$z_2(f_b) = (A_c m_r - n)(A_c m_l - n)\frac{k_f(f_b)}{A_c} + nk_r(f_b)$$

$$z_3(f_b) = [A_c m_r - (n-1)][A_c m_l - (n-1)]\frac{k_f(f_b)}{A_c}$$

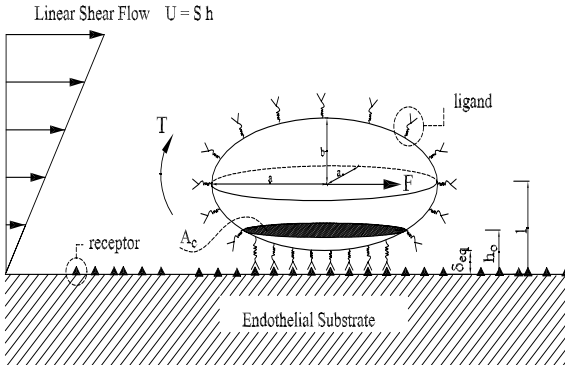


Figure 4. (Left) A spheroidal bead in specific adhesion to a rigid wall under a linear laminar flow. (Right) The probability of adhesion P_a as a function of t

Interestingly, it has been shown [7] that for each particle shape, a characteristic size can be identified for which the P_a has a maximum (Fig.4): for small particles, the area of adhesion at the particle/cell interface is so small that an insufficient number of ligand-receptor bonds are formed to prevent particle detachment (area-limited regime); whereas for large particles, the hydrodynamic dislodging forces are so large than even a large number of ligand-receptor bonds cannot prevent the particle to be dislodged away (force limited regime). The optimal size for adhesion, that is the size for which P_a has a maximum, falls between these two limiting conditions. As an example, when considering a capillary with a shear stress at the wall of μS = 1 Pa and a surface density of receptors m_r=100 μm^2, the optimal radius for a spherical particle would be of about 500 nm with a total volume of 0.05 μm^3, whereas the optimal volume for an oblate spheroidal particle with an aspect ratio γ = 2 would be more than 50 times larger (3.5 μm^3), thus emphasizing the importance of the shape in nanoparticles adhe-

sion under flow. A comparison between spherical and hemi-spherical particles adhering specifically to the cell membrane on the endothelial cells under the hydrodynamic forces has also been performed (9). In the simple case of spherical beads, the mathematical predictions on the strength of adhesion have been shown to be in good agreement with experiments both in vitro and in vivo.

3. CONCLUSIONS AND FUTURE PERSPECTIVES

The performance of nanoparticles administered at the systemic level is influenced by physiological and biophysical properties, as the hydrodynamic conditions and surface density of receptor molecules at the site of potential adhesion, and by design properties that can be controlled during manufacturing as the geometry (size and shape) and the surface features (density of ligand molecules, density and length of polymer chains, electrostatic charge). Clearly, the number of possible combinations is extraordinarily large and a 'rational design' approach is therefore required to select the best particle just as in an engineering optimization problem where the best solution has to be found within constraints and objective functions. Combining the models for vascular transport, margination and adhesion design maps can be derived which allow to predict the full performance of a nanoparticle as a function its design parameters. Mathematical modeling should combined with *ad-hoc* in-vitro assays and in-vivo experiments to generate an integrated approach aimed at validating and refining the accuracy of the theoretical predictions.

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