Mathematical Biosciences 295 (2018) 55–61

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

An investigation of endocytosis of targeted nanoparticles in a shear flow by a statistical approach

Fan Bai, Jie Wu, Ren Sun*

Department of Engineering Mechanics, Shanghai Jiao Tong University, Shanghai 200240, China

ARTICLE INFO

Keywords:
Endocytosis
Shear flow
Nanoparticle
Receptor-ligand binding

ABSTRACT

The receptor-ligand mediated endocytosis of nanoparticles by endothelium cells in a shear flow is investigated theoretically. A set of population balance equations is used to calculate the number of endocytosed nanoparticles of diameters about 100 nm for a given period of time. Hydrodynamic analysis reveals that whether a wash-out procedure is effective to remove incompletely endocytosed nanoparticles depends on the bond formation and rupture rates rather than the shear rate since the rupture rate of bonds linking nanoparticles and endothelium cells does not change with the shear rate appreciably. Furthermore, it is shown that critical bond formation and rupture rates, above which the elevation in ligand expression on cell surfaces stimulated by the shear flow will increase the number of endocytosed nanoparticles significantly, exist for typical average endocytosis durations.

1. Introduction

Study of the receptor-ligand mediated endocytosis of nanoparticles (NPs) by endothelium cells (ECs) is important to understand drug delivery in human bodies [1,2]. A NP with receptors on its surface is usually called a targeted NP. When such NPs filled with drug travel with blood flow, the ones near blood vessel walls may adhere to and penetrate through the membranes of vascular ECs with the help of the formation of receptor-ligand bonds. In vitro, a parallel-plate flow chamber with ECs covering its bottom wall is usually used to simulate this process [3-6], shown in Fig. 1. Two factors may significantly influence the bond mediated endocytosis: one involves the properties of receptors and ligands, and the other the shear rate near the wall. In this paper, we focus mainly on the shear rate.

Although previous researchers have conducted several experimental studies in order to understand how the shear flow affects the endocytosis of targeted NPs [3-7], two aspects still require further investigations. Firstly, the bond mediated endocytosis in a shear flow was thought to be difference from that in a static environment because a high shear rate may lead to sufficient hydrodynamic loads on NPs to wash them out from the surfaces of cells, inferred from the fact that the number of endocytosed non-targeted NPs decreases significantly with the shear rate [6]. However, the experimental results in [4,6,7] show that the decrease in number of endocytosed targeted NPs with the shear rate appear to be inappreciable. The formation of bonds can indeed enhance the attachment of targeted NPs to ECs, but whether the NPs can resist the flushing of the blood flow for all possible shear rates under physiological conditions is not known. Also, clarifying this issue will tell us whether washing the ECs with a flow of an extremely high shear rate can remove the incompletely wrapped NPs, a common procedure often performed in experiments before counting the number of fully internalized NPs (see, e.g., [3]). Secondly, the number of ligands per unit surface area of a cell, referred to as the level of ligand expression, can affect the likelihood of bond formation when a NP touches the cell and the time needed for a complete wrapping. Several former measurements reveal that a high shear rate can increase the expression of certain ligands on cell surfaces [8-12]. However, whether this factor will cause a significant rise in the number of endocytosed targeted NPs has not yet been theoretically analyzed.

Up to now, there are several theoretical and numerical investigations on the endocytosis of targeted NPs. Chou [13] adopted the master equations of the birth and death process to model the competition between fusion and endocytosis during the entry of a virion. Zhdanov [14] improved this method by establishing connections between the coefficients in the equations and the energies of bond, membrane bending and cytoskeleton. Gao et al. [15] took advantage of the balance between the gain and dissipation in the free energy of a NP-membrane system to explore how the size of the NP affects its endocytosis. Yue and Zhang [16] used the dissipative particle dynamics to simulate the simultaneous endocytosis of two neighboring NPs, showing that they would be internalized independently or cooperatively, depending on their sizes. However, all these researches do not take account of the shear flow. To investigate the flow effects on endocytosis of targeted NPs, Tseng and Huang [17] simulated numerically the endocytosis of a

* Corresponding author.
E-mail address: drrsun@sjtu.edu.cn (R. Sun).

https://doi.org/10.1016/j.mbs.2017.11.004
Received 3 March 2017; Received in revised form 1 November 2017; Accepted 6 November 2017
Available online 07 November 2017

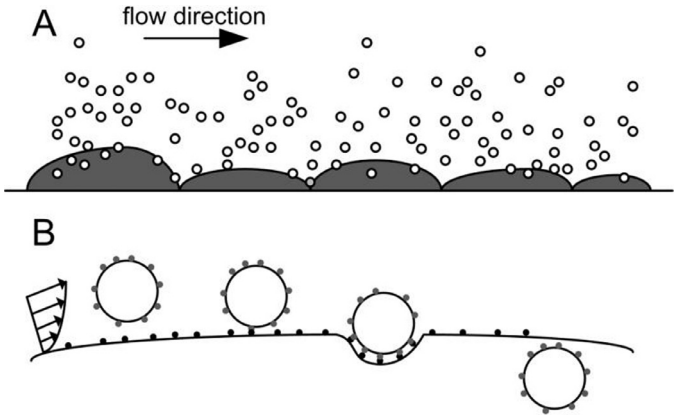

Fig. 1. Diagrams of endocytosis of NPs: (A) A solution of NPs (small white circles) flows over the bottom wall of a parallel-plate flow chamber covered by ECs (depicted as large irregular gray particles). (B) A NP adheres to and enters an EC by the formation of receptor-ligand bonds between their surfaces. The leftmost velocity profile shows the nearby shear flow over the membrane of the EC. The receptors (small gray filled circles) on the NP can link to the ligands (small black filled circles) on the membrane.

single targeted NP in a shear flow using the immersed boundary method, demonstrating that the shear flow affected the process significantly, and the dissipation of energy caused by the flow could not be neglected. But this cannot explain the inappreciable decrease of the endocytosed NPs with the shear rate mentioned above [4,6,7].

In the present study, a statistical approach dealing with endocytosis of targeted NPs in a shear flow is proposed. The approach is based on a set of population balance equations describing the numbers of adhered and endocytosed NPs. The relationship between the coefficients in the equations and the shear rate is analyzed to understand the effect of hydrodynamic forces on NPs and the efficiency of wash-out procedures. Besides, the flow-induced increase of expression of ligands is considered and its influence on the number of endocytosed NPs with different bond formation and rupture rates is investigated.

2. Method

2.1. Population balance equations

Considering a NP suspension flowing over an EC monolayer, we assume that the formation of the first bond stops the NP, making it attached to a cell. If the bond breaks, the NP will detach from the cell, otherwise more bonds will form subsequently and the NP will be wrapped gradually by the cell membrane. For convenience, we call a NP to be "in state i" if i bonds has formed. We also neglect the lateral interaction among ligands, receptors and the bonds, and assume that only one bond can form at a time. Thus, a NP in state i will only go to state $i+1$ or $i-1$ ($i = 1, ...,n_e - 1$), where n_e is the number of bonds when a NP is fully wrapped. n_e does not necessarily equal to the number of receptors on a NP. When a NP is in state 0, it can only go to state 1; while arriving at state n_e, it will remain in this state afterwards, indicating that a completely wrapped NP cannot come out of a cell.

When multiple NPs are conveyed to the EC monolayer, some of them will attach to the ECs and be endocytosed. To describe this process, we denote the number of NPs in state i ($i = 1, 2, ...,n_e$) on an area S of a cell membrane to be N_i and use the conventional population balance equations which have been widely used in the modeling of chemical reaction [18] and nucleation [19], as well as for virion attachment [20]. The equations in the present case take the form of

$$\frac{dN_1}{dt} = (2R + l_b)c_{NP}(t)\left(S - S_{NP}\sum_{k=1}^{n_e-1} N_k\right)\lambda_0 - (\lambda_1 + \mu_1)N_1 + \mu_2 N_2, \tag{1}$$

$$\frac{dN_i}{dt} = \lambda_{i-1}N_{i-1} - (\lambda_i + \mu_i)N_i + \mu_{i+1}N_{i+1} \text{for} i = 2, 3, ...,n_e - 2, \tag{2}$$

$$\frac{dN_{n_e-1}}{dt} = \lambda_{n_e-2}N_{n_e-2} - (\lambda_{n_e-1} + \mu_{n_e-1})N_{n_e-1}, \tag{3}$$

$$\frac{dN_{n_e}}{dt} = \lambda_{n_e-1}N_{n_e-1}, \tag{4}$$

where λ_i ($i = 0, 1, ...,n_e - 1$) and μ_i ($i = 1, ...,n_e - 1$) are the forward and backward rate constants related to bond formation and rupture respectively, l_b the bond length, and $c_{NP}(t)$ the number of NPs per unit volume (named as the bulk concentration of NPs in the following) within a thin flow layer over the cell membrane with a thickness of $2R + l_b$. t denotes time and S_{NP} the area occupied by a bound NP. The first term on the right-hand side of Eq. (1) means that the number of NPs which are able to attach to the EC monolayer decreases with the occupied area by bound NPs. The maximum number of NPs on S, denoted by N_0, equals to S/S_{NP}. We define $\alpha_\lambda(t)$ by

$$\alpha_\lambda(t) = (2R + l_b)c_{NP}(t)S_{NP}. \tag{5}$$

Then Eq. (1) can be rewritten as

$$\frac{dN_1}{dt} = \alpha_\lambda(t)\left(N_0 - \sum_{k=1}^{n_e-1} N_k\right)\lambda_0 - (\lambda_1 + \mu_1)N_1 + \mu_2 N_2. \tag{6}$$

Here $\alpha_\lambda(t)$ is clearly smaller than or equal to 1 by its definition, and means that the area S is saturated with NPs.

A typical experiment of endocytos usually lasts from about 10 minutes to 3 hours. This time scale is much larger than the time scale for N_i (1, 2, ...,$n_e - 1$) to reach its steady value N_i^* which is obtained by solving the following algebraic equations

$$\alpha_\lambda\lambda_0\left(N_0 - \sum_{k=1}^{n_e-1} N_k^*\right) - (\lambda_1 + \mu_1)N_1^* + \mu_2 N_2^* = 0, \tag{7}$$

$$\lambda_{i-1}N_{i-1}^* - (\lambda_i + \mu_i)N_i^* + \mu_{i+1}N_{i+1}^* = 0, \tag{8}$$

$$\lambda_{n_e-2}N_{n_e-2}^* - (\lambda_{n_e-1} + \mu_{n_e-1})N_{n_e-1}^* = 0, \tag{9}$$

where α_λ is the steady value of $\alpha_\lambda(t)$ determined by a balance among the attachment, detachment and bulk diffusion of NPs and is proportional to the bulk concentration of NPs far from the monolayer. The detailed balance leads to a linear increase of N_{n_e} with time because $dN_{n_e}/dt = \lambda_{n_e-1}N_{n_e-1}^*$. With the solution of $N_{n_e-1}^*$ obtained from Eqs. (7) to (9), we have

$$\frac{dN_{n_e}}{dt} = N_0 \Bigg/ \left[\frac{1}{\lambda_{n_e-1}} + \frac{1}{\alpha_\lambda\lambda_0} + \frac{1}{\alpha_\lambda\lambda_0}\sum_{j=1}^{n_e-1}\prod_{k=1}^{j}\frac{\mu_k}{\lambda_k} + \sum_{i=1}^{n_e-2}\left(\frac{1}{\lambda_i} + \frac{1}{\lambda_i}\sum_{j=i+1}^{n_e-1}\prod_{k=i+1}^{j}\frac{\mu_k}{\lambda_k}\right)\right]. \tag{10}$$

Similar to that given by [18,19], the denominator of the right-hand-side of Eq. (10) is the average time for the complete endocytosis of a NP, denoted by t_e. To see this, let w_i ($0 \le i \le n_e$) be the average time for a NP to reach state n_e from state i. Then, $w_{n_e} = 0$ and $w_0 = t_e$. According to [21], the equations governing all w_i's are

$$w_0 = \frac{1}{\lambda_0 + \mu_0} + \frac{\lambda_0}{\lambda_0 + \mu_0}w_1, \tag{11}$$

$$w_i = \frac{1}{\lambda_i + \mu_i} + \frac{\lambda_i}{\lambda_i + \mu_i}w_{i+1} + \frac{\mu_i}{\lambda_i + \mu_i}w_{i-1} \text{ for } i = 1, 2, ...,n_e - 2, \tag{12}$$

$$w_{n_e-1} = \frac{1}{\lambda_{n_e-1} + \mu_{n_e-1}} + \frac{\mu_{n_e-1}}{\lambda_{n_e-1} + \mu_{n_e-1}}w_{n_e-2}. \tag{13}$$

Solving Eqs. (11)-(13) yields