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Effect of the surface charge density of nanoparticles on their translocation across pulmonary surfactant monolayer: a molecular dynamics simulation

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ABSTRACT

Interaction between nanoparticles (NPs) and pulmonary surfactant monolayer plays a very significant role in nanoparticle-based pulmonary drug delivery system. Previous researches have indicated that different properties of nanoparticles can affect their translocation across pulmonary surfactant monolayer. Here we performed coarse-grained molecular dynamics simulation aimed at nanoparticles’ surface charge density effect on their penetration behaviours. Several hydrophilic nanoparticles with different surface charge densities were modelled in the simulations. The results show that NPs’ surface charge density affects their translocation capability: the higher the surface charge densities of NPs are, the worse their translocation capability is. It will cause the structural changes of pulmonary surfactant monolayer, and inhibit the normal phase transition of the monolayer during the compression process. Besides, charged NPs can be adsorbed on the surface of the monolayer after translocation as a stable state, and the adsorption capability of NPs increases generally with the increase of surface charge densities. Our simulation results suggest that the study of nanoparticle-based pulmonary drug delivery system should consider the nanoparticles’ surface charge density effect in order to avoid biological toxicity and improve efficacy.

1. Introduction

It is well known that inhaled nanoparticles (NPs) have obtained intense interests for their extensive biomedical applications, such as specific targeting, bio-imaging, drug delivery, etc. For example, using inhaled NPs as pulmonary drug delivery carrier has showed comprehensive promises for treating lung diseases due to NPs’ good administration, higher bioavailability and low side effects [1,2]. Besides, NPs are able to enter the respiratory system because of the large surface area, abundant vascular tissue and low acidity in the human lungs. However, accumulating researches show that some inhaled NPs may cause serious adverse effect to the human beings, such as blood disease, pneumonia and nephrotic syndrome, presumably similar to the toxicological effect of ultrafine particulate matters [3,4]. Therefore, understanding the interaction between NPs and pulmonary surfactant monolayer has attracted great attention for improving the nanoparticle-based pulmonary drug delivery system and avoiding NPs’ potential toxicological effect.

As mentioned above, inhaled NPs are an excellent drug carrier to the pulmonary drug delivery system. Hence, much attention should be paid to the translocation mechanism of NPs across the lung barrier and the relative side effects of NPs. In the present work, the most studied lung barrier is the pulmonary surfactant. The pulmonary surfactant bathes the inner surface of the lung, which lines the entire alveolar surface and is considered to be the first line of lung defense. It can reduce alveolar surface tension at the air—liquid interface in order to maintain the normal respiratory mechanics characteristics, hence it can effectively prevent the collapse of the alveolar structure during the respiration process [5]. Moreover, as a complicated network of extracellular membranes that overlies the alveolar epithelium and alveolar macrophages, pulmonary surfactant can also defend against inhaled pathogens and protect against injury to be an anti-inflammatory and antioxidant. Therefore, lack of pulmonary surfactant, whether caused by premature birth, lung injury, or mutations in genes critical to surfactant production or function, causes respiratory failure [6]. The pulmonary surfactant is a lipoprotein complex consisting of approximately 90% lipids and 10% proteins [7]. Among them, dipalmitoyl phosphatidylcholine (DPPC) is the main component of lipids [5,8], therefore, DPPC in the gas—liquid interface is often used to simulate the pulmonary surfactant. Both experiments and computer simulations [6,8], the normal phase transition of the lipid monolayer has been proved to play a quite important role in regulating the biophysical function of the pulmonary surfactant. During the expansion process, the lipid monolayer becomes a liquid-expanded (LE) phase from a liquid-condensed (LC) phase. During the compression process, the lipid monolayer changes the direction of its phase transition and becomes an LC phase from an LE phase. The normal phase transition continues throughout the entire respiratory system cycles.
Previous researches [9–11] have indicated that different properties of NPs can affect their translocation across pulmonary surfactant monolayer, including size, hydrophobicity, shape, surface charge density. Surface charge density has an important impact on the interaction between NPs and pulmonary surfactant monolayer [12,13]. It can determine the behaviour of NPs in the cellular or extracellular environment [14,15]. For example, Lin et al. [16] have indicated that NPs with different surface charge densities can be adsorbed on the surface of cell monolayer after they have an interaction. Hong et al. [17] have showed that charged NPs can penetrate the lipid bilayer and cause an irreparable hole. Though many researchers have studied how surface charge density affects the interaction between NPs and a lipid bilayer [18,19], few works focus on the effects of charged NPs on pulmonary surfactant monolayer, regardless of simulations or experiments.

Currently, the studies of the interactions between NPs and pulmonary surfactant monolayer are mainly in vitro experiments. However, the results in different laboratories sometimes cannot achieve absolute consensus due to the difficulty in controlling experimental parameters that affect the behaviour of NPs and pulmonary surfactant monolayer. Hence, there is a need to use theory or simulations to compensate for the disadvantages of experiments [20]. Moreover, it is impossible for traditional experiments to explore various related properties of the complex system in the molecular level due to the limitation of time or space scale [21]. As an important research tool in many fields, molecular dynamics simulation can build the similar models of the real experiments on the computer and generally improve the size of space according to research needs. Besides, it has been successfully used to study the interactions between NPs and cell membranes [22,23]. Therefore, it is necessary and also effective to research NPs’ surface charge density effect on their translocation across pulmonary surfactant monolayer with molecular dynamics simulation and these research studies can promote the design, the optimisation and the applications of the nanoparticle-based pulmonary drug delivery system.

In this paper, we study the effect of the surface charge density of NPs on their translocation across pulmonary surfactant monolayer with molecular dynamics simulation. Simulation details are given in the methods, and simulation results and analysis are given in the results and discussion.

2. Methods

2.1. Coarse-grained model

In this paper, the coarse grained (CG) force field was performed in all the molecular dynamics simulation. Compared to atomistic models, CG models can allow simulations undergo a larger length scale and a longer time period because they map several atoms into one interaction site. The CG force field, MARTINI, is one of the most popular CG models, which can be used to study for lipids, peptides and proteins at the level of time and space [24,25].

MARTINI can build the model according to four main types of interaction sites: Polar (P), nonpolar (N), apolar (C) and charged (Q). Particles of type N and Q contain four subtypes (0, d, a, da), which are used to distinguish the hydrogen-bonding capabilities. Particles of type C and P contain five subtypes (1–5), which are used to indicate the degree of polarity. Besides, a coulomb interaction potential energy function and a Lennard-Jones (LJ) potential energy function are applied for the non-bonded interactions. A weak harmonic potential function is used to describe the bonded interactions. The CG DPPC and water models can be downloaded from http://md.chem.rug.nl/cgmartini/.

2.2. Simulation details

The simulated symmetric system consisted of 784 DPPC molecules and 47,040 water molecules with the box dimensions of 14.72 × 14.72 × 70.00 nm$^3$, including a gas slab sandwiched by two non-interacting surfactant monolayers with individual waterside, and the nanoparticle was introduced from the air layer (Figure 1(a)). Moreover, we added Na$^+$ or Cl$^–$ into the simulated system to neutralise the surface charge of the nanoparticles in order to keep the system in a neutral state. Many recent researches have experimentally proved that similar simulated symmetric systems can be used to simulate certain biological properties of the pulmonary surfactant monolayer, such as the surface activities [26], outflow of phospholipids during the compression [27]. In the CG force field, DPPC molecule model is comprised of 12 beads including the headgroup (NC3, PO4), glycerol ester linkage (GL1, GL2) and two tails (C1A-C2A-C3A-C4A, C1B-C2B-C3B-C4B) (Figure 1(b)). Besides, water molecule model is comprised of four polar beads (P4). More details can be found in the paper of Marrink et al. [28].

We established several hydrophilic NPs with different surface charge densities, and all the NPs were the face-centred cubic structure. The surface charge densities of NPs were, respectively, 0 e/nm$^2$, ±4.0 e/nm$^2$, ±10.0 e/nm$^2$ and ±18.6 e/nm$^2$ and all the NPs (diameter: 5 nm) were consisted of 856 CG beads (Figure 1(c)). The CG bead type P2 was used for constructing the neutral hydrophilic NPs and Qda was used to modify the surface charge of the particle [29]. Cationic, anionic and neutral beads were shown in yellow, blue and black, respectively. Besides, to restrict bonds and bond angles among beads of NPs, the force constants applied to NPs equalled to what was used for DPPC molecules [30].

To facilitate the following discussion, we defined mid-plane of DPPC layer as x−y plane, with z axis perpendicular to the layer. The system was kept same and had only single NP in each simulation. All simulations were performed with GROMACS 4.5.4 simulation package [31]. A cutoff of 1.2 nm was used for van der Waals interactions. The default value of relative dielectric constant was 15 and the system temperature was set at 310 K with a coupling constant 1 ps. Berendsen coupling schemes for both pressure (semi-isotropic, coupling constant of 4.0 ps, compressibility in the x−y plane of 3e-5 bar$^{−1}$ and in the z axis of zero bar$^{−1}$) were used to establish a NPT ensemble.

After energy minimisation, an equilibration of 50 ns was performed, and the final equilibrium configuration was used as the starting state for the next simulation. Then 120 ns molecular dynamics (MD) simulations have been performed to obtain a stable configuration.
3. Results and discussion

After 120 ns molecular dynamics simulations, we analysed the simulation trajectory for getting the density profile of DPPC layer along z axis and area per lipid of the simulation system under the compression process over time. As shown in Figure 2(a) and (b), the thickness of lipid layer is about 2.05 nm, and the area per lipid decreases gradually until stability under the compression process over time, which can be found that the area per lipid is about 0.552 nm² in the expansion state and about 0.471 nm² in the compression state, corresponding to the results with the given methods in Ref. [32].

3.1. Surface charge densities of NPs affect their translocation across pulmonary surfactant monolayer

We first experimentally compared the effects of neutral NPs and charged NPs on their translocation across pulmonary surfactant monolayer. As shown in Figure 3(a) and (b), the NP without surface charges can penetrate the monolayer easily, but the NP completely coated by surface charges was only wrapped by lipids partially instead of penetrating the monolayer in both of expanded and compressed states. Besides, all the NPs did not leave the surface of the pulmonary surfactant monolayer or cause a lipid pore on the pulmonary surfactant monolayer in the stable state. It is that pulmonary surfactant monolayer has the ability to heal [33]. Therefore, surface charges of NPs might determine their fate of translocation. Lee et al. [34,35] have reported that PAMAM dendrimer can induce pore formation in DPPC bilayer, which is similar to the results in this paper that NPs completely coated by surface charges have the poor translocation ability and will cause the monolayer to appear a disturbance more easily.

To understand the interaction mechanism between pulmonary surfactant monolayer and NPs on the different surface charge densities, we then established some partial charged MD...
models to simulate NPs’ translocation with different surface charge densities. As shown in Figure 3, all the NPs with different surface charge densities (except for NPs completely coated by surface charges) can penetrate the monolayers in spite of the expanded state or the compressed state. And all the NPs are adsorbed on the surface of pulmonary surfactant monolayer instead of entering the water phase. These results are in line with the results of the experiment [36,37]. Besides, it is worth noting that these above results will not change, although NPs switched the surface charge. In other words, positive or negative charge will not make any difference to the translocation results, which can be considered that DPPC is a zwitterionic molecule and it will not cause any apparent differences for cationic or anionic NPs to cross through the pulmonary surfactant monolayer. Hu et al. [29] have reported that cationic NPs can adsorb more palmitoyl-oleoyl phosphatidylglycerol (POPG) than DPPC compared anionic NPs or neutral NPs on their translocation through pulmonary surfactant monolayer, which is similar with our simulation results.

Moreover, NPs with different surface charge densities can cause a disturbance to the monolayer during their translocation across pulmonary surfactant monolayer, but it is not permanent and will tend to heal slowly. Besides, it is more or less bucking or folding due to curvature of the NPs in contact with the soft monolayer only in the compressed state even if the system has been stable. Figure 4 shows local membrane thickness distribution
3.2. Surface charge densities of NPs cause NPs’ adsorption on the pulmonary surfactant monolayer

Lipids usually have different interactions with NPs, such as adsorption [39,40], insertion [41], translocation [42,43] and aggregation [44]. Among them, surface charge properties play an important role on the NPs’ adsorption on the lipid layer [45]. We have known that all the NPs are adsorbed on the surface of pulmonary surfactant monolayer instead of entering the water under the different stable systems, and a red line has divided them into two parts. It can be found that there is a striking contrast on the photos of the above part, which represents a disturbance on the monolayer, and photos in the below part represent that the monolayers are relatively smooth. The results exactly agree with the results in Figure 3, which is said that NPs with different surface charge densities can cause the structural changes of pulmonary surfactant monolayer when they cross through the monolayer. Besides, the huge disturbance to the monolayer might cause DPPC out of the gas–liquid interface and further affect the dynamic equilibrium of pulmonary surfactant monolayer [38]. Therefore, NPs without surface charges might be more suitable to nanoparticle-based pulmonary drug delivery system.

Figure 5. (Colour online) Trajectories of NPs with different surface charge densities along z axis through a pulmonary surfactant monolayer under the different compression states.

Notes: The left and right columns are corresponding to the expansion state and compression state, respectively. The four rows from top to bottom represent the translocation process of NP with 0, 4.0, 10.0 and 18.6 e/nm² surface charge densities. Inset (A) shows the side view of the simulation system when NPs just touched the monolayers, and the upper side of the monolayer is water and the lower side of the monolayer is air. Inset (B) shows the histogram of the distance distribution between NPs and the monolayer after NPs enter the monolayer.
In addition, the slightly shorter distances for anionic NPs than cationic NPs denote that the adsorption ability of anionic NPs is stronger. It might be resulted from the positive charged head group of the DPPC molecule exposed to the outside can attract the anionic NPs more easily [24,32]. It is also remarkable that the distances in the expansion state are longer than those in the compression state. The main reason is that charged NPs cause more or less structural changes of lipids in the compression, which leads to the increase of the average thickness of the monolayer (Figure 3(b)) and the decrease of the average distances between NPs and the monolayer. All the results are very consistent with previous researches [16]. Besides, our results suggest that surface charge densities of NPs not only affect their absorption ability but also play an important role in regulating subsequent biomolecular exchange and designing the nanoparticle-based pulmonary drug delivery system.

3.3. Surface charge densities of NPs inhibit the normal phase transition of pulmonary surfactant monolayer during the compression process

NPs’ nonspecific adsorptions may induce the change of pulmonary surfactant monolayer in thickness, therefore there is a large possibility to affect the monolayer’s related properties, such as order parameter [46]. To better understand the NP–PS interaction mechanism, we analysed the order parameter of the monolayer in the different NP’s translocation process over time. As shown in Figure 7, in the expansion process, the order parameter values of NPs with different surface charge density have a sharp depression, but the order parameter values of NPs with 0, 4.0 and 10.0 e/nm² surface charge density keep stable generally, and only the order parameter value of the NP with 18.6 e/nm² surface charge density decreases and finally becomes stable over time. It is that only the NP with 18.6 e/nm² surface charge density cannot penetrate through the monolayer. Respectively, in the compression process, the order parameter values of NPs with different surface charge density also have a sharp depression, and then only the order parameter value of the NP with 0 e/nm² surface charge density increases generally and finally keeps stable over time, others decrease firstly and...
4. Conclusion

With coarse-grained molecular dynamics simulations, we investigated NP’s surface charge density effect on translocation across the DPPC layer. Several NPs with different surface charge densities were considered in our simulations. The results show that NPs’ surface charge density affects their translocation capability: higher surface charge density NPs inhibit the translocation capability and facilitate more structural changes of pulmonary surfactant monolayer, especially disrupt the normal phase transition of pulmonary surfactant monolayer during the compression process. Besides, charged NPs can be adsorbed on the surface of the monolayer after translocation as a stable state, and the adsorption ability becomes stronger generally as NPs’ surface charge densities increase. Our data therefore suggest that any in vitro study of inhalation nanotoxicology or NP-based pulmonary
drug delivery system should consider the nanoparticle's surface charge density effect.

**Disclosure statement**

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