Mechanical characteristics of human red blood cell membrane change due to C\textsubscript{60} nanoparticle infiltration

Xiaoyue Zhang,\textsuperscript{ac} Yong Zhang,\textsuperscript{ab} Yue Zheng\textsuperscript{*abc} and Biao Wang\textsuperscript{*abc}

The mechanical characteristics of human red blood cell (RBC) membrane change due to C\textsubscript{60} nanoparticle (NP) infiltration have been investigated in the present work. Using experimental approaches, including optical tweezer (OT) stretching and atomic force microscopy (AFM) indentation, we found that RBCs in the presence of C\textsubscript{60} NPs are softer than normal RBCs. The strain–stress relations of both normal and C\textsubscript{60} infiltrated RBC membranes are extracted from the data of AFM indentation, from which we proved that C\textsubscript{60} NP infiltration can affect the mechanical properties of RBC membrane and tend to weaken the tensile resistance of lipid bilayers. In order to explain this experimental phenomenon, a mechanical model has been developed. Based on this model, the strain–stress relations of both normal and C\textsubscript{60} infiltrated lipid bilayers are calculated with consideration of intermolecular interactions. The theoretical results are in great agreement with the experimental results. The influence of C\textsubscript{60} NP concentration on the mechanical properties of RBC membrane is successfully predicted. Higher concentrations of C\textsubscript{60} NPs in the lipid bilayers will lead to increased damage to the cell membrane, implying that the dosage of C\textsubscript{60} NPs should be controlled in medical applications.

1 Introduction

It is well known that the mechanical characteristics of cell membranes are significant to the biological functions of cells.\textsuperscript{1–3} Changes in the mechanical characteristics of cell membranes can result in the progression of certain diseases. Typically, sickle cell disease is caused by a defect in the hemoglobin of human red blood cells (RBCs). The abnormality of hemoglobin alters the mechanical properties of the RBC membrane, which adversely affects its deformability and biorheology.\textsuperscript{4} The inelastic sickle cells can easily jam-up the capillary vessels, cause the tissues surrounding the blood vessels to receive insufficient oxygen and cause severe pain. As another example, malaria is caused by the protozoa of the genus Plasmodium falciparum.\textsuperscript{5} During the maturation of the parasite in the cell, the molecular constitution of its membrane is altered, then the structure of the cell cytoplasm and the shape of the cell are changed, and the cell progressively loses its function.\textsuperscript{6,7} Generally speaking, many recent works have indicated that the mechanical information of cell membranes is an important indicator in the study of bio-processes, pathology and toxicology.

In recent years, carbon nanomaterials, including carbon nanotubes (CNTs) and carbon nanoparticles (NPs) e.g. C\textsubscript{60} (fullerene), have been proven to possess important applications in biomedical engineering.\textsuperscript{8,9} For example, due to their particular electrical and mechanical properties, CNTs have been considered to be a promising material for nano-biosensors and molecular carriers.\textsuperscript{10} C\textsubscript{60} (fullerene), on the other hand, has potency for induction of reactive oxygen species after photoexcitation. This property makes it possible to explore C\textsubscript{60} as a new antimicrobial agent.\textsuperscript{11} However, adverse effects and certain diseases may be caused due to the complexity of interaction between biological cells and nanomaterials.\textsuperscript{12} For example, studies have suggested that CNTs can induce lipid peroxidation, oxidative stress and mitochondrial dysfunction in living cells. The cytotoxicity of water-soluble fullerene is the biggest obstacle to exploring it as a carrier for the delivery of drugs. Consequently, for the safety of applying nanomaterials as biomedicine, inspecting the effects of nanomaterials on living cells has become a new topic of considerable scientific interest and medical significance. Typical work such as that by Shi \textit{et al.}\textsuperscript{13} has investigated the mechanism of cell entry for CNTs. The article also suggests that modifying the tips of CNTs may help avoid uptake by cells and achieve material safety by design. In this work, we were especially concerned about the fact demonstrated by recent
works that C₆₀ can easily infiltrate living cells, disturb functions of cells and potentially cause nanotoxicity by changing the molecular structure of the cell membrane and organelles. Based on theoretical molecular dynamics (MD) simulations, researchers have reported the ability of C₆₀ to infiltrate into the lipid bilayers.¹⁴⁻¹⁷ Results of the MD simulations indicated that C₆₀ particles have the ability to easily infiltrate into the lipid bilayers, stay between the lipids, and finally alter the molecular structure and interactions between lipids.¹⁴ In fact, other recent works have also shown that C₆₀ NPs might exhibit cytotoxicity in people and animals.¹⁸⁻²⁴ However, the pathogenesis of the toxicity of C₆₀ NPs has not yet been made clear. Other than chemical transformation processes, i.e. oxidation, the cytotoxicity of C₆₀ NPs results from changes in the cell membrane mechanical characteristics. Therefore, it is necessary and important to clarify any differences of mechanical properties between the cell membrane with and without C₆₀ infiltration, respectively.

In this paper, the mechanical characteristics of RBCs with and without C₆₀ NP infiltration have been investigated. An optical tweezers (OT) approach has been firstly used to perform stretching on integrated RBCs with and without C₆₀ presence to directly investigate the effects of C₆₀ NP infiltration on the mechanical properties of RBCs. In order to apply a larger load on RBCs, atomic force microscopy (AFM) was used to perform indentation on both normal and C₆₀ infiltrated RBCs, and gain further data of mechanical characteristics of RBC membrane changing due to C₆₀ NP infiltration. At the same time, a molecular theory has been established to explain the experimental results, and demonstrate how the C₆₀ NPs affect the lipid bilayer mechanical characteristics.

2 Experimental

Preparation of RBC simples

RBCs have a relatively simple structure that readily facilitates single cell mechanical deformation experiments.²²⁻²⁴ Therefore, we used human RBCs and their membranes as samples. Human RBCs were separated from the blood of a healthy donor using centrifugation. Two groups of samples were prepared, which were normal RBCs and RBCs with C₆₀ infiltration. The normal RBC sample can be easily obtained by adding 20 μl RBCs into 10 ml Alsever’s solution. In preparing the sample of RBCs with C₆₀ presence, 5 mg C₆₀ NPs were first added to 10 ml Alsever’s solution. The C₆₀ NPs were then uniformly dispersed in the mixture using supersonic-vibrating. Hereafter, 20 μl RBCs were added into the mixture. After quiescence under room temperature for 20 min, RBCs with C₆₀ NP infiltration can be obtained. Although the quantity of C₆₀ NPs in the mixture is high for the sample with C₆₀ NP presence, not all the C₆₀ NPs actually entered the cell membrane. Therefore, the high concentration of C₆₀ NPs in the mixture is necessary.

Optical tweezer setup

For measuring the mechanical properties of an integrated cell, an approach based on OT is very useful.⁶⁻⁷ The schematic diagrams of the approach are shown in Fig. 1a. A 75 mW He–Ne laser source (Coherent, Inc.) is used for laser generation. The collimated laser beam is connected to an inverted microscope (Olympus Corporation, IX71). To create a laser trap with high quality, we use a 100×/0.75 oil-immersed objective with NA = 1.1 (Olympus Corporation). Two polystyrene micro-beads with a diameter of 5 μm are attached to the surface of the RBC sample. One of the beads was attached to the slip, the other bead and the cell are free standing. With the photons from the laser beam passing through the high-refractive-index dielectric bead, the change of the momentum can induce the external force on the bead, which is then “trapped” in the focal point of the laser. By trapping the free standing bead and pulling it away from the bead that stuck on the slip, the RBC sample can be stretched. The trapping force applied on the bead is determined by the laser power. Calibrated by counter flow method,⁷ the maximum trapping force in our system is 70 pN. A laser filter is used to adjust the laser power, which made trapping force of the OT tunable. To acquire the mechanical properties of integrated RBC, stretching ratios S₁/S₀ under different trapping force F have been recorded, where S₀ is the original diameter of RBC and S₁ is the length of RBC after stretching. Both normal and C₆₀ infiltrated RBCs have been stretched using the OT system to investigate the effect of C₆₀ infiltration on the mechanical properties of integrated RBCs.

AFM indentation

AFM indentation has been widely used in biomechanics research.²⁵⁻²⁹ In our work, this technique has been used to investigate the mechanical characteristics of RBC membranes with and without C₆₀ presence. The sketch map of the AFM indentation is shown in Fig. 1b. A single layer of RBCs with or without C₆₀ infiltration was placed on the piezoelectric sample stage, respectively. By moving the spherical tip to approach and press the RBCs samples at 20 nm s⁻¹, local deformation is generated on the cell surface. The resulting deflection of the cantilever tip can be calibrated to estimate the value of the applied force and indentation depth, from which the force-indentation depth (F–H) curves of the samples can be obtained for studying the mechanical properties of cell membrane.³⁰⁻³¹ In this work, a CSPM5500 Scanning probe microscope system (Being Nano-Instrument, Ltd.) is used to perform AFM indentation. The piezoelectric sample stage of our AFM system has a piezoelectric coefficient of 17 nm V⁻¹. The force constant of the probe is 0.02 N m⁻¹ and the curvature radius of its tip is R = 20 nm. The maximum force that can be applied on a RBC in AFM indentation is 5 nN. In total, 100 F–H curves of both normal and C₆₀ infiltrated RBCs have been obtained.

3 Results and discussion

3.1 Experimental results

As shown in Fig. 2a, the data of the OT stretching experiment reveal the effects of C₆₀ NP infiltration on the mechanical properties of integrated RBCs. The slope of the F–S₁/S₀ curve of RBCs with C₆₀ NP presence is smaller than that of the normal one. Under the same value of force, the deformation of RBCs
with C_{60} presence is considerably larger than that of the normal one, which proves that C_{60} NP infiltration can affect the mechanical properties of RBCs and tends to weaken the tensile resistance of the cells.

The results of AFM indentation show similar phenomena. In total, 100 $F-H$ curves of both normal and C_{60} infiltrated RBCs were integrated to form the results. As shown in Fig. 2b, it can be seen that the slope of the $F-H$ curve of normal RBCs is greater than that of C_{60} infiltrated RBCs. The $F-H$ curves reflect the samples’ resistance to the tip. The greater slope in the $F-H$ curves indicates that the resistance of the sample to the indentation is relatively small. Consequently, it is easy to deduce that the RBCs with C_{60} infiltration are softer than normal RBCs. It can also be seen in Fig. 2b that the $F-H$ curves of C_{60} infiltrated samples have a broader distribution range. We believe that this difference is relevant to the concentration of C_{60} NPs that enter the RBC. The RBC is softer if more C_{60} NPs enter the cell.

3.2 The strain–stress relation of normal and C_{60} infiltrated RBC membrane obtained by AFM indentation

Because AFM indentation only affects the surface of RBC, and considering the simplicity of the structure of RBCs, combined with the conclusions recently reported by MD simulations,$^{14-17}$ there are reasons to conjecture that the effects of C_{60} NP infiltration on the mechanical properties of RBCs are mainly focused on the cell membrane. In order to study the mechanical characteristics of RBC membrane change due to C_{60} NP infiltration, comparing the strain–stress relations of normal membrane with that of C_{60} infiltrated membrane is essential. In this work, we manage to extract the strain–stress relations of both normal and C_{60} infiltrated membrane from the data of AFM indentation. To achieve this, the cell membrane structure is considered to be a two-dimensional, continuum material,$^{33,34}$ as shown in Fig. 3a. The strain–stress relation of the two-dimensional elastic membrane can be expressed as:$^{35}$

$$T_{ij} = 2 \left( \frac{\rho}{\rho} \right) \left[ B_2 \left( \frac{\partial E}{\partial B_1} \right) \delta_{ij} + \left( \frac{\partial E}{\partial B_1} \right) \beta_{ij} \right],$$  \hspace{1cm} (1)

where $\rho$ and $\rho$ are the material densities in the final and initial states, respectively, $E$ is the potential energy of the unit area, $B_1 = \lambda_1^2 + \lambda_2^2$ and $B_2 = -\lambda_1^2 \lambda_2^2$ are the invariants of the two-dimensional tensor, where $\lambda_1$, $\lambda_2$ are the principal extension ratios of the membrane in $x$ and $y$ direction (Fig. 3b), respectively, and $\beta_{ij}$ is the Finger strain tensor, which represents area change. The bending stresses are several orders of magnitude less than the membrane stresses,$^{36}$ therefore the equations of
the relation between $T$ and $\lambda$ of the contact area is considered to be the representative strain–stress relation of the cell membrane.

To calculate the stress $T$ of the contact area, one can establish the z-direction equilibrium equation of the contact area to be $T^2 \pi \sin^2 \phi_0 = F - P_0 R^2 \sin^2 \phi_0$, where $P_0$ is the excess intracellular pressure and $\phi_0$ is the tangent at the contact area boundary. Therefore, expression of the stress can be given by:

$$T = \frac{F}{2 \pi R \sin^2 \phi_0} - \frac{P_0 R}{2}.$$  \hfill (3)

The only unknown is $\phi_0$, which can be determined by analyzing the x-direction and moment equilibrium equations of isolated body ABCD (Fig. 3c) as:

$$T_b x_b d \theta = P_0 (R \sin \phi_0 + x_m) H_1 d \theta + T (R \sin \phi_0 \cos \phi_0) d \theta,$$

$$T_b x_b H_1 d \theta = \int_0^{H_1} P_0 [R \sin \phi_0 H + R \sin \phi_0] H d H d \theta$$

$$+ \int_{x_m}^{x_b} P_0 x (x - R \sin \phi_0) d x d \theta,$$

where $H_1$ can be calculated from $H_1 = H - R (1 - \cos \theta)$; $x_b$ and $T_b$ are defined to satisfy that at boundary $x = x_b$, the boundary stress $T_b$ of the membrane has only the x-direction component. Therefore, $x_b$ can be calculated from $P_0 x_b^2 = F$. It is hard to write an expression of $\sin \phi_0$ as a function of $H$ and $F$; however, from eqns (3)–(5), it is easy to calculate the value of $\sin \phi_0$ corresponding to each point in the $F$–$H$ curves (Fig. 1b). Thus, the stress $T$ of the contact area can be obtained as a function of $H$. Establishing the relation between $H$ and the principal extension ratios $\lambda$ of the contact area is more complex; however, the relation can be approximated by power relation, $H = A (\lambda - 1)^B$. For the reason that the indentation depth $H$ on the left of the power relation should be the same magnitude as the value of $H$ determined from the experimental data, we approximately chose $A = 3500$ and $B = 1$ in our calculation. Although the values of parameters $A$ and $B$ are not absolutely precise, this hypothesis do not seriously affect the conclusion.

The strain–stress relations of both normal (blue dots) and C$_{60}$ infiltrated RBC membrane (red and green dots) calculated from the experiment data are shown in Fig. 3d. It can be seen that the strain–stress relation of RBC membrane is significantly changed by C$_{60}$ infiltration. The tensile resistance of RBC membrane with C$_{60}$ infiltration is smaller than the normal one. Decrease of the tensile resistance explains why the indentation depth $H$ of C$_{60}$ infiltrated RBCs is larger than that of normal RBCs under the same value of force, implying that the RBCs with C$_{60}$ presence are softer than normal RBCs.

### 3.3 A molecular theory to explain how the C$_{60}$ NPs affect the lipid bilayer mechanical characteristics

In order to explain how C$_{60}$ NPs change the strain–stress relation of the RBC membrane, investigating the changes in the molecular structure of lipids bilayers due to C$_{60}$ infiltration
is of first importance. According to a recent MD simulation study, the infiltration of C_{60} NP into the lipid bilayer was observed and the equilibrium position of C_{60} NP in the lipids bilayers was also determined. After the C_{60} molecule entered the phospholipid bilayers, instead of the central plane of the membrane, its most probable z-position is $z = 1$ nm, very close to the boundaries $z = \pm 1.5$ nm, right below the head group (Fig. 4a). Due to the presence of C_{60} NPs, the relative positions of lipids are affected, which means the molecular structure of lipids bilayers is altered.

Inspired by the fact that molecular structure is crucial to the mechanical properties of cell membrane, we developed a mechanical model based on intermolecular interactions to explain how the C_{60} NPs affect the mechanical characteristics of lipids bilayers. In our model, the lipid is simplified to consist of a head group and tail group, which is shown in Fig. 4a. Without the presence of C_{60}, the interaction between the two lipids can be described by the potential $P_1$, which has a Lennard-Jones type potential:

$$P_1(r_1) = 4\alpha_{hh} \left[ \left( \frac{\beta_{hh}}{r_1} \right)^{12} - \left( \frac{\beta_{hh}}{r_1} \right)^{6} \right] + 4\alpha_{tt} \left[ \left( \frac{\beta_{tt}}{r_1} \right)^{12} - \left( \frac{\beta_{tt}}{r_1} \right)^{6} \right].$$

(6)

where the parameters with subscript $hh$ belong to the interaction between two head groups, and the parameters with subscript $tt$ belong to the interaction between two tail groups; $z$ is the depth of the potential well, $\beta$ is the finite distance at which the inter-particle potential is zero, and $r$ is the distance between the lipids. At $r_1 = D_1$, the potential is the minimum, and the force is zero.
The strain–stress relation described in eqn (1) can be given by calculating the elastic potential \( E \) from \( P_\nu \). The micro-unit of the cell membrane is shown in Fig. 4b. The two principal stresses satisfy \( T_{\theta \theta} = T_{\varphi \varphi} = T \). The material is isotropic as discussed, which implies that the principal extension ratios must satisfy \( \lambda_1 = \lambda_2 = \lambda_3 \) which gives \( B_1 = \lambda_1^2 + \lambda_2^2 = 2\lambda^2, B_2 = \lambda_1^2 \lambda_2^2 = -4\lambda^2 \). The molecular arrangement in the membrane element is also shown in Fig. 4b. The actual position of each lipid may not be as accurate as in the diagram; however, due to the uniformity of the membrane, it is reasonable to believe the distance between each two lipids must be almost the same. For a uniform area expansion, the principal extension ratios can always be written as \( \lambda = r/D \), where \( D \) is the mean equilibrium distance between two head groups in the initial state, and \( r \) represents the mean distance between two head groups in the final state. The elastic potential can be obtained by summing all the potentials between each lipid as \( E = \frac{1}{2} \sum_{i<j}^n P_\nu \), where the factor \( \frac{1}{2} \) prevents double counting of the interaction, and where \( n \) is the total number lipids. For simplicity, in the analysis of the intermolecular interactions, we assume that long range interactions can be ignored and only short range interactions are considered. Short range interaction is defined to be the interaction between two adjacent molecules and long range interaction refers to the interaction between two nonadjacent molecules. This simplification is acceptable because the short range interactions contribute the main part of the total elastic potential of the lipid bilayers. Therefore, the total elastic potential can be rewritten as \( E = \frac{4}{3}nnp(r) = 3np(r) \), where \( n \) of the initial state can be easily obtained by geometry as \( n = \frac{2}{\sqrt{3}D^2} \). Hence the equation should be:

\[
E = \frac{6}{\sqrt{3}D^2}P(r). \tag{9}
\]

The density changes of lipid bilayers during the deformation can be given by \( \frac{P}{\rho} = M/\left( \frac{M}{\lambda^2} \right) = \lambda^2 \). By substituting all the equations above into eqn (1), the strain–stress relations of cell membrane with and without C_{60} molecules as a function of principal extension ratios can be theoretically given as:

\[
T_\nu = \frac{16\sqrt{3}}{D^4\lambda^3} \left[ -2\varphi_{hh}^6 + \varphi_{hh}^2 + \varphi_{hh}^2 \right] + D^2 \left( \varphi_{hh}^2 \right)^2 \tag{10}
\]

and

\[
T_\varphi = \frac{16\sqrt{3}}{D^4\lambda^3} \left[ -2\varphi_{hh}^6 + \varphi_{hh}^2 + \varphi_{hh}^2 \right] + D^2 \left( \varphi_{hh}^2 \right)^2 \tag{11}
\]

The interaction between C_{60} NP and the lipid as a function of their distance \( r_2 \) can also be described in Lennard-Jones potentials form:

\[
P_2(r_2) = 4\alpha_{hh} \left[ \frac{\varphi_{hh}}{r_2^6} - \left( \frac{\varphi_{hh}}{r_2^6} \right)^{2} \right] + 4\alpha_{tt} \left[ \frac{\varphi_{tt}}{r_2^6} - \left( \frac{\varphi_{tt}}{r_2^6} \right)^{2} \right] \tag{7}
\]

where the parameters with subscript \( hh \) belong to the interaction between C_{60} NP and head group, the parameters with subscript \( tt \) belong to the interaction between C_{60} NP and tail group.

Combining the C_{60}–lipid and lipid–lipid potentials, and noticing \( r_2 \) can be obtained by the geometrical relation and written as \( r_2 = 0.5r_1 \), an equivalent potential \( P_\nu \) of interaction between lipids with C_{60} presence can be obtained to be function of \( r_1 \) as:

\[
P_\nu(r_1) = P_1 + P_2 = 4\alpha_{hh} \left[ \frac{\varphi_{hh}}{r_1^6} - \left( \frac{\varphi_{hh}}{r_1^6} \right)^{2} \right] + 4\alpha_{tt} \left[ \frac{\varphi_{tt}}{r_1^6} - \left( \frac{\varphi_{tt}}{r_1^6} \right)^{2} \right] + 4\alpha_{hh} \left[ \frac{\varphi_{hh}}{0.5r_1^6} - \left( \frac{\varphi_{hh}}{0.5r_1^6} \right)^{2} \right] + 4\alpha_{tt} \left[ \frac{\varphi_{tt}}{0.5r_1^6} - \left( \frac{\varphi_{tt}}{0.5r_1^6} \right)^{2} \right] \tag{8}
\]

The density changes of lipid bilayers during the deformation can be given by \( \frac{P}{\rho} = M/\left( \frac{M}{\lambda^2} \right) = \lambda^2 \). By substituting all the equations above into eqn (1), the strain–stress relations of cell membrane with and without C_{60} molecules as a function of principal extension ratios can be theoretically given as:

\[
T_\nu = \frac{16\sqrt{3}}{D^4\lambda^3} \left[ -2\varphi_{hh}^6 + \varphi_{hh}^2 + \varphi_{hh}^2 \right] + D^2 \left( \varphi_{hh}^2 \right)^2 \tag{10}
\]

and

\[
T_\varphi = \frac{16\sqrt{3}}{D^4\lambda^3} \left[ -2\varphi_{hh}^6 + \varphi_{hh}^2 + \varphi_{hh}^2 \right] + D^2 \left( \varphi_{hh}^2 \right)^2 \tag{11}
\]

eqn (10) and (11) are the strain–stress relations of normal cell membrane and C_{60} infiltrated cell membrane, respectively. However, it should be pointed out that in the derivation of eqn (11), it is assumed that all “bonds” between lipids in the
membrane are affected by C_{60} NPs. Therefore, eqn (11) describes the weakest tensile resistance of lipid bilayers due to C_{60} infiltration with highest concentration. According to our experimental results, the concentration of C_{60} NPs is one of the factors that influence the strain–stress relations. Therefore, we have also derived the strain–stress relations to correlate with the concentration of C_{60} NP presence in the lipid bilayers. Supposing that the proportion of the number of “bonds” affected by C_{60} NPs is \( \omega \), then because the total number of “bonds” per unit area is \( \frac{6}{\sqrt{3}D^2} \), the number of normal “bonds” affected by C_{60} NPs is \( \frac{6\omega}{\sqrt{3}D^2} \), and the number of normal “bonds” that are not affected by C_{60} NPs is \( \frac{6(1-\omega)}{\sqrt{3}D^2} \). The potential energy \( E \) in eqn (1) then can be considered to contain two parts and can be rewritten as \( E = E_a + E_c \), where \( E_a \) represents the total energy of normal “bonds” and \( E_c \) represents the total energy of C_{60} NP affected “bonds”. Thus, eqn (1) can be rewritten as:

\[
T_{\text{rel}} = 2 \left( \frac{E}{\rho} \right) \left\{ B_2 \left[ \frac{\partial (E_a + E_c)}{\partial B_2} \right] \delta y + \left[ \frac{\partial (E_a + E_c)}{\partial B_1} \right] \delta y \right\},
\]

where \( E_a \) and \( E_c \) can be approximately calculated as:

\[
E_a = \frac{6(1-\omega)}{\sqrt{3}D^2} P_a(r),
\]

\[
E_c = \frac{6\omega}{\sqrt{3}D^2} P_c(r).
\]

Therefore, eqn (11) can be rewritten to contain the information of concentration of C_{60} NP presence as:

\[
T_{\text{rel}} = \frac{16\sqrt{3}(1-\omega)}{D_1^4} \left\{ -2(z_{hh} \beta_{h}^2 + z_{ct} \beta_{c}^2) + D_1^2 (z_{hh} \beta_{h}^2 + z_{ct} \beta_{c}^2) \rho r \right\}
\]

\[
+ \frac{16\sqrt{3}\omega}{D_2^4} \left( -2(z_{hh} \beta_{h}^2 + z_{ct} \beta_{c}^2) + z_{ch} \left( \frac{\beta_{c}}{2} \right)^{12} \right)
\]

\[
+ D_2^2 \left( z_{hh} \beta_{h}^2 + z_{ct} \beta_{c}^2 + z_{ch} \left( \frac{\beta_{c}}{2} \right)^{6} \right) \right\}.
\]

By comparing the theoretical strain–stress relation and experimental data of AFM indents, the values of parameters \( \alpha \) and \( \beta \) can be determined. Based on the strain–stress relation deduced by AFM indentation, choosing \( z_{ct} = 0.0003 \) nN nm, \( \beta_{ct} = 0.4 \) nm and \( z_{hh} = z_{hbh} = 1.1 \beta_{hh} \) enables eqn (10) to describe the strain–stress relation of normal lipid bilayers correctly. In determining the values of \( z_{ct}, z_{hh}, z_{hbh} \) and \( \beta_{ct} \), the experimental strain–stress relations of the softest membrane with C_{60} infiltration, as shown with red dots in Fig. 3d, were used as the benchmark. However, even in the softest RBC with C_{60} presence, not all “bonds” between lipids in the membrane are affected by C_{60} NPs. Therefore, the influence of C_{60} NPs on the mechanical properties of RBC membrane will be underestimated. To remedy this deviation, the parameters in eqn (7) should be chosen to make the slope of theoretical strain–stress relation smaller than the benchmark, which then is chosen to be \( \beta_{ct} = 0.5z_{ct}, z_{hh} = 0.5z_{hbh}, \beta_{ct} = 0.5\beta_{ct} \) and \( \beta_{ct} = 0.75\beta_{ct} \). Because \( r = 0.5r_1 \), the parameters satisfy the condition that the forces between C_{60} NP and the head group tend to be repulsive, which is reflected in the formulation that \( \beta_{ct} r_2 \) is larger than \( \beta_{ct} r_1 \). The chosen parameters also reflect that tail groups tend to attract C_{60} NPs. Comparisons of the potentials and forces between normal lipids and between C_{60} infiltrated lipids are shown in Fig. 5. Three differences should be noticed. First, with C_{60} presence, the equilibrium distance between lipids increases to \( r = D_2 \), which will surely change the density of the cell membrane; Second, the slope of the potential decrease, which implies that the interactions between lipids are weakened. The maximal constraint force between lipids is also reduced, which influences the ultimate strength of the cell membrane. It can be considered that lipids in the cell membrane are connected with “bonds”, and the presence of C_{60} NPs weakens the “bonds”.

After determining the values of these parameters, the strain–stress relations of both normal and C_{60} infiltrated RBC membranes can be calculated, which are shown in Fig. 6 with lines. According to the theoretical results, the tensile resistance of lipid bilayers decreases with the increase of infiltrated C_{60} NP concentration, and the slope of the strain–stress curve is reduced to approximately 2/3 of that in normal cell membrane. The strain–stress relations induced by AFM indentation data shown in Fig. 3e are in great agreement with the theoretical strain–stress curve of the normal RBC membrane, in which 25 and 75% of “bonds” are affected by C_{60} NPs, respectively. This proves that our theory can accurately predict the mechanical characteristic changes due to C_{60} NP infiltration.
The decrease in tensile stress resistance implies that it may involve risk to use NPs in medical treatment with mass dosage. The effect of mass dosage of C$_{60}$ NPs on RBC membrane might be similar to malaria, a disease resulting from merozoites change and the destruction of the molecular structure of RBC membranes$^{40}$ that causes fever and disseminated intravascular coagulation (DIC). The results also imply that the ultimate strength of the cell membrane is reduced by C$_{60}$ NPs. The ultimate strength corresponds to the largest force the element can provide to resist the applied load. It can be seen in Fig. 6 that the ultimate stress of the cell is reduced from 0.08 nN to 0.04 nN mm$^{-1}$ due to the infiltration of C$_{60}$ NPs. RBCs experience different obstacles in blood circulation. If their ultimate strength decreases, many of them might dehisce in circulation. In that case, hemolytic anemia may happen, of which the consequences are serious and may be fatal. Not limited to RBCs, most human cells have a similar membrane structure; therefore, C$_{60}$ also has the ability to change their mechanical characteristics and have different affects on their functions. For example, our results provide the explanation for the experimental results reported by ref. 22 that human skin (HDF) and liver carcinoma (HepG2) cells begin to exhibit signs of leaky membranes after 30 h of exposure to C$_{60}$ NPs. Also, considering that the infiltration of C$_{60}$ NPs does not depend on any receptor on a cell membrane, it is reasonable to speculate that other NPs have the same ability to infiltrate into lipid bilayers as long as they are small enough$^{41}$ and have a hydrophobic surface. However, the question of whether the infiltration of these NPs can affect the cell membrane in the same way as C$_{60}$ still remains to be answered by further studies. So far, our parallel work suggests that the effect of NP infiltration on mechanical properties might be related to the size of the NPs.

Although there are potential risks in the medical applications of C$_{60}$ NPs, the ability of C$_{60}$ NPs to change the mechanical characteristics of cell membranes might also be valuable for therapies of certain diseases. In fact, it is because of its photocytotoxicity that C$_{60}$ is able to be explored as an antimicrobial agent. Analogously, the mechanical properties of cell membranes are essential to the functions of bacteria or cancer cells. C$_{60}$ NPs might provide an alternative method to kill bacteria or cancer cells without resulting in increased drug resistance by changing the mechanical properties of the cell membranes. However, the crucial issue of how to target C$_{60}$ NPs on the right cells without disturbing healthy ones remains to be solved. So far, little work has been devoted to utilizing the ability of C$_{60}$ NPs to change the mechanical characteristics of cell membranes, and further research is needed on these issues.

4 Conclusions

In this paper, we investigated the mechanical characteristics of human RBC membrane change due to C$_{60}$ NP infiltration based on an experimental method combined with theoretical analysis. Results performed by OT and AFM indicate that the mechanical properties of human RBC membrane can change due to the presence of C$_{60}$ NPs. The tensile resistance of C$_{60}$ infiltrated RBC membrane decreases significantly. To explain this phenomena, a mechanical model based on the intermolecular interactions was developed. Based on this model, structural change of the lipid bilayers caused by the infiltration of C$_{60}$ was discussed, the interactions among molecules were analyzed, and the strain–stress relation of the RBC membrane was derived theoretically. The theoretical results are in good agreement with the experimental data, which approve the conclusion that the mechanical properties of RBC membrane are changed by C$_{60}$ NPs infiltration. We believe that infiltration of C$_{60}$ NPs will make the cell membrane ‘softer’ and easier to break. Our results might provide an important reference to the safe application of C$_{60}$ NPs and to the development of new therapies using NPs in bio-medical engineering.

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