Review

Interplay of Nanoparticle Properties during Endocytosis

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Abstract: Nanoparticles (NPs) have been widely applied as drug carriers in drug delivery, due to their unique physical and structural properties. To achieve the drug delivery purpose, receptor-mediated endocytosis is a primary explored mechanism to internalize NPs into tumor cells. During the endocytosis process, properties of NPs, including size, shape, and surface functionality, play an important role in determining the final drug delivery efficacy. Many of these NP properties have been extensively explored individually. However, the multiple NP properties naturally interplay with each other in the endocytosis process to determine the internalization efficiency together. Therefore, it is significantly important to understand the interplay of different NP properties to improve the NP's final delivery efficacy. In this review, we focus on the interplay of NPs properties on the endocytosis process to summarize the relevant experimental observations and physical mechanisms. Particularly, three different aspects are discussed in detail, including the interplay between size and shape; size and elasticity; shape and elasticity. We have summarized the most recent works and highlighted that building up systematic understandings for the complex interplay between NP properties can greatly help a better design of NP platforms for drug delivery.

Keywords: nanoparticle; endocytosis; nanoparticle properties

1. Introduction

Nanoparticles (NPs) have been widely used to deliver drugs by virtue of their unique physical and structural properties [1–5]. NPs as drug delivery vehicles allow an efficient drug accumulation and precise payload dispensing at the targeted sites, and thus, hold great promise to improve therapeutic index [6–8]. For example, utilization of NPs decorated with tLyp1 peptide is shown to enhance tumor inhibition and regression in vivo, elevating the survival ratio to 40% after 60 days in a melanoma mouse model [8,9]. Generally, drug-loaded NPs can translocate from their site of deposition to the disease sites by the blood circulation system after intravenous injection [10,11]. When NPs reach the site of action, tumor cells can take up these engineered NPs and unload drug molecules at the desired intracellular compartment. The cellular uptake of NPs can significantly elevate the drug concentration and act as an intracellular reservoir for long-term drug release [12,13]. However, there are many biological barriers during the drug delivery process to achieve such a purpose. One significant challenge for the further development of NP-based therapies is the low level of cellular uptake and drug delivery [14,15]. To this end, a detailed understanding of the NP cellular uptake mechanisms is crucial for efficient therapeutic applications.

Receptor-mediated endocytosis is a primary route exploited in drug delivery to deliver the NPs and drug molecules into tumor cells [16,17]. In general, receptor-ligand complexes form when mobile receptors on the cell membrane diffuse to bind ligands on the NP's surface. Binding between receptors and ligands drives engulfing to overcome resistance to membrane deformation and changes in configuration entropy of receptors,

thus contributing to membrane wrapping and cellular uptake [18–23]. Depending on the type of involved proteins, the receptor-mediated endocytosis can be classified as clathrin-mediated endocytosis, caveolae-mediated endocytosis, and calthrin/caveolae-independent endocytosis [16,24–26]. In addition to the receptor-mediated endocytosis, NPs can also be internalized by cells via pinocytosis and phagocytosis, depending on the cargo type, internalization route, and scission mechanism [26]. Pinocytosis is commonly involved in the internalization of fluids and molecules from the external environment of a cell by small vesicles called pinosomes, and is ubiquitous to almost any eukaryotic cell [27]. Phagocytosis is an actin-dependent process by which the specialized cells, such as macrophages, monocytes, dendritic cells, and neutrophils, engulf large particulate matter with the formation of vesicles called phagosomes [28]. In contrast to receptor-mediated endocytosis, both pinocytosis and phagocytosis are non-selective modes of taking up foreign materials [26].

Accumulating evidence indicates that NP uptake through receptor-mediated endocytosis depends strongly on their physical and chemical characteristics, including the size, shape, stiffness, and surface functionality [17,29–32]. For instance, it has been well recognized that there is an optimal size of NP during the endocytosis process, at which the cellular uptake reaches a maximum in a cell type-dependent manner [33–35]. Chithrani et al. investigated the size-dependent uptake of gold NPs by Hela cells, showing that the NPs with a diameter of 50 nm were taken up into cells more effectively than those with diameters of 14, 30, 74, and 100 nm [36]. Win and Feng found that the 100 nm polystyrene NPs showed the most efficient cellular uptake compared with other sizes (50, 200, 500, and 1000 nm) [37]. Gao et al. developed a mathematical framework to uncover the mechanism underlying the size effect. They found that the optimal particle diameter is determined by the competition between receptor diffusion kinetics and thermodynamic driving force. For NPs larger than the optimal size, receptors need to diffuse longer distances, leading to increased wrapping time. For NPs smaller than the optimal size, it needs to overcome more elastic bending energy of membrane, resulting in the decreased driving force for wrapping, hence decreased efficiency of cellular uptake [38]. Apart from size, the effects of the other properties of NP (such as shape, stiffness, and surface functionality) on the cellular uptake have also been extensively investigated, and discussed in detail in many excellent reviews [29,39–41].

It has been indicated that NPs uptake by cells through endocytosis is actually determined by an intricate interplay between physicochemical particle properties. For instance, the cellular uptake of NPs depends strongly on the collaborative effects of shape and size [42,43]. Studying the contribution made by the interplay of NPs properties to the cellular uptake can not only deepen our understandings of the endocytosis and underlying mechanisms, but also provide guidelines and strategies for the NP design to improve the drug delivery efficacy. Here, we review representative works regarding the interplay of NP properties on the endocytosis process mainly from three aspects: (1) Size and shape, (2) size and elasticity, (3) shape and elasticity.

2. Size and Shape

As mentioned above, size-dependent cellular uptake of NPs has been extensively investigated in various cell lines because particle size is known as a key determinant of the cellular uptake pathways; see reviews by the authors of [16,44,45] for more detail. Additionally, NPs can be of various shapes, such as spheres, triangles, stars, rods, prolate and oblate spheroids, which have been demonstrated to have a significant effect on cellular uptake of NPs [39,46–48]. Li et al. showed that the spherical NPs were internalized to a much greater extent than the cylindrical NPs using RAW 264.7 (Figure 1a). This difference can be attributed to their different endocytosis pathways: Uptake of spherical NPs was based on clathrin- and caveolin-mediated endocytosis, while cylindrical NPs mainly depended on clathrin-mediated endocytosis [49]. Xie et al. studied the efficiency of cellular uptake of the gold NPs with different shapes by RAW 264.7, which was found to rank as stars < rods < triangles. Further studies revealed that the various endocytosis pathways adopted by NPs

are responsible for their shape-dependent uptakes [50]. Using dynamic molecular modeling combined with free energy calculations, Dasgupta et al. showed that NPs' entry modes of membrane wrapping strongly depend on the NP shape. Their work suggested that an increased NP aspect ratio is unfavorable for complete membrane wrapping (Figure 1b). Especially, the NP enters the cell via a submarine mode for high aspect ratios and round tips and via a rocket mode for small aspect ratios and flat tips. They further showed that for cube-like and rod-like particles, the energy barriers for transitions during the wrapping process increase with the sharpness of the edges [51]. A question naturally arises, that is—how the NPs are internalized by cells if simultaneously considering the size and shape.

Yue et al. studied the effect of the structural features of NP size and shape on the ability of siRNA-conjugated gold NPs to enter U87 cells. Experimental results indicated that 50-nm spheres, and 40-nm stars have much higher cellular uptake efficiency compared to 13-nm spheres (Figure 1c). They also showed that the siRNA delivery level after 24 h of 50-nm spheres is 50-fold and 1.6-fold higher than that of 13-nm spheres and 40-nm stars, respectively, suggesting that larger NPs have higher potential as nanocarriers for the siRNA delivery. They suggested that the more efficient uptake of spheres over that of stars is caused by the differences in the interactions between oligonucleotides and scavenger receptors [43]. A more direct study to explore the interplay of size and shape of NPs was performed by Nambara et al. They investigated the uptake of triangular NPs with a side length of 46, 55, 72, and 94 nm and spherical NPs with a diameter of 22, 39, and 66 nm by RAW264.7 and HeLa cells. They found a reverse size-dependence of the cellular uptake of triangular and spherical gold NPs: Cellular uptake increases with the side length of triangular NPs, but decreases with the diameter of spherical NPs (Figure 1d). Particularly, the triangular NPs with 72 nm in side length show 20-fold more efficient uptake compared to spherical NPs with a similar surface area. They proposed that the edges and vertices of triangular NPs with high local curvature are responsible for this reverse size-dependence [42]. Furthermore, Ding et al. found that the endocytosis mechanism of gold NPs with different sizes and shapes is totally different (Figure 1e). The uptake of spherical particles with diameters of 15 and 45 nm, and rod-shaped particles is a clathrin-mediated endocytosis pathway. The internalization of star-shaped particles depends on both the clathrin-mediated and caveolin-mediated endocytosis pathways. By contrast, the uptake of spherical particles with a diameter of 80 nm is mainly through macropinocytosis, due to the bigger size [52]. There are also many computational studies exploring the interplay of NP size and shape on the endocytosis process. Using coarse-grained molecular dynamics, Huang et al. studied the receptor-mediated endocytosis of NPs, which is demonstrated to be size-dependent and shape sensitive. For spherical NPs, there is an optimal size at which cellular uptake maximizes. For the uptake of an upright spherocylindrical NP endocytosis proceeds through a laying-down-then-standing up sequence (Figure 1f). According to free energy analyses, they showed that NP size and shape affect the endocytosis in different ways: NP size primarily determines whether endocytosis can complete, while NP shape dictates the endocytic pathway and the angle of entry [53]. These results highlight the importance of the interplay between NP size and shape on their interactions with cells, and contribute to a complete understanding of cellular uptake and the design of NP platform.
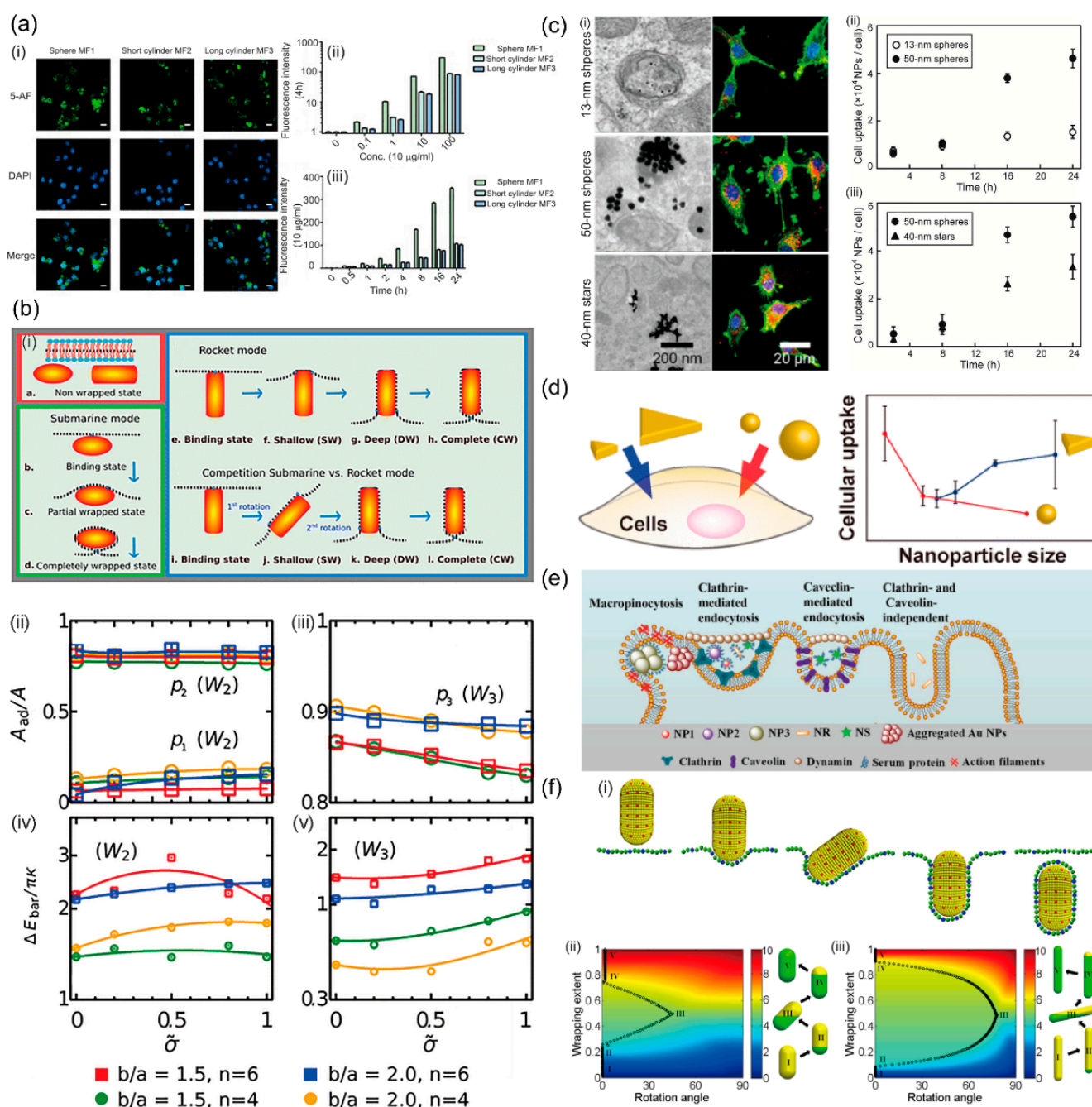
Figure 1. The interplay of NP size and shape on endocytosis. (**a**) (i) Confocal fluorescence microscopy images showing the uptake of various gold NPs by RAW 264.7 macrophages after 4 h of incubation. (ii) Dose dependence and (iii) time dependence of the binding of gold NPs with macrophages [49]. (**b**) (i) Submarine and rocket modes of entry for uptake of NPs with different shapes. (ii,iii) Wrapping fractions for stable states for the discontinuous wrapping transitions W2 (transition from shallow- to the deep-wrapped state) and W3 (transition from the deep-wrapped to the complete-wrapped state), respectively. (iv) and (v) correspond to the energy barriers for both transitions, respectively [51]. (**c**) (i) TEM images and confocal fluorescence images of U87 cells treated with NPs with different sizes and shapes. (ii) Size- and (iii) shape-dependent cellular uptake of siRNA-conjugated gold NPs [43]. (**d**) Reverse size-dependent effect of triangular and spherical NPs on cellular uptake [42]. (**e**) Schematic illustration of the endocytosis mechanism of gold NPs with different sizes and shapes [52]. (**f**) (i) Schematics of the laying-down-to-standing-up process. (ii,iii) The endocytic pathways for spherocylindrical NPs with an aspect ratio $\rho = 2$ and $\rho = 5.5$, respectively [53].
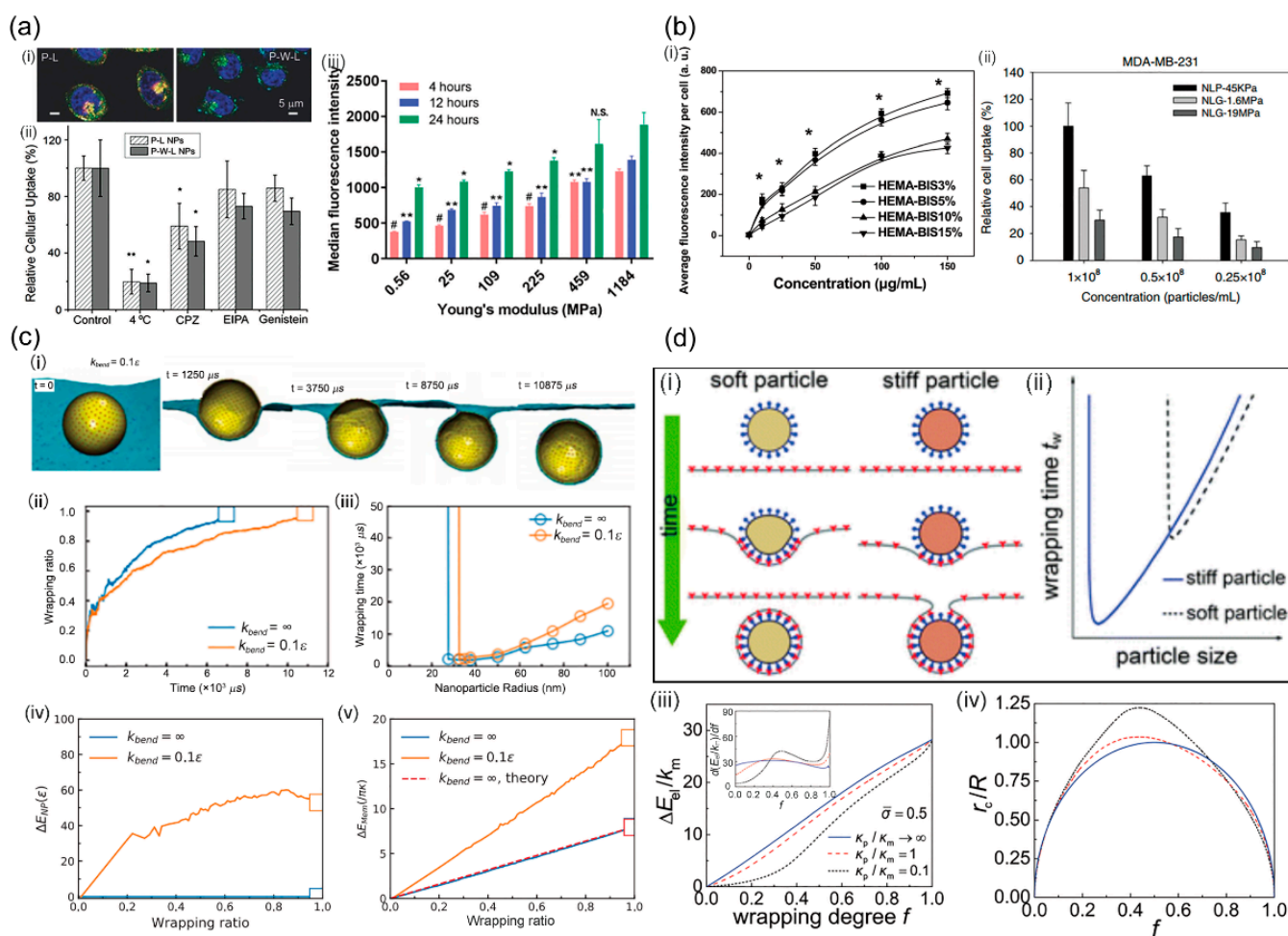
3. Size and Elasticity

The mechanical property of NPs is another important factor in determining the endocytosis process, as proved by extensive experimental research [54–56]. However, there are often seemly controversial results regarding the effect of NP elasticity. On the one hand, Sun et al. synthesized core-shell poly (lactic-co-glycolic acid) (PLGA)-lipid NPs of varying rigidities with the same chemical composition, size, and surface properties and showed that cellular uptake of rigid NPs is more efficient than soft ones in HeLa cells (Figure 2a(i,ii)) [57]. Hui et al. studied the effect of elasticity on cancer cell uptake by synthesizing silica nanocapsules (SNCs) with a wide range of elasticity (Young's modulus ranging from 560 kPa to 1.18 GPa) [58]. They reported a four-time higher uptake of the stiff folate-poly (ethylene glycol) (FA-PEG)-modified SNCs than their soft counterparts. Using transmission electron microscopy, they revealed that soft SNCs are deformed during endocytosis, which reduces the endocytosis rate (Figure 2a(iii)) [58].

On the other hand, Liu et al. synthesized four types of poly (2-hydroxyethyl methacrylate) (HEMA) hydrogel particles with the different compressive modulus (15–156 kPa), but similar size and surface property by an emulsion-precipitation. Their results revealed that softer NPs are internalized by HepG2 cells at a faster rate and larger amount than the stiffer NPs (Figure 2b(i)). They proposed that this difference results from the different cellular uptake mechanisms: The softer particles are internalized by cells mainly through micropinocytosis, while the stiffer particles enter cells via caveolae- and clathrin-mediated endocytosis, as well as macropinocytosis pathways [59]. Similarly, Guo et al. investigated in vitro and in vivo cellular uptake of nanolipogels (NLGs) [60]. The elasticity of NLGs is regulated by an alginate core to obtain different Young's moduli ranging from 45 ± 9 to $19,000 \pm 5$ kPa. They reported that uptake of softer NLGs is significantly greater than their rigid counterparts by Neoplastic and non-neoplastic cells (Figure 2b(ii)). They also suggested that different endocytic pathways are the main reason for the different efficiencies observed in soft and rigid NPs [60].

These seemingly controversial results might be caused by the different mechanical properties of the NP itself. Particularly, soft NPs can be characterized into two different categories based on their materials: (1) Soft NPs that preserve their volume during their cellular uptake process. It is represented by lipid-based NPs [15]. The amphiphilic lipid membrane surfaces are impermeable for the water molecules inside. Therefore, the volume of lipid-based NPs is usually considered as constant during the membrane wrapping process; (2) Soft NPs that can easily change their volume. Hydrogel-based capsule NPs are representative of the second type NPs [61,62]. The mechanical properties of these NPs are maintained by the outside layer of hydrogel materials, which is permeable for the water molecules inside. Therefore, the volume of these soft capsule NPs can be easily changed during the membrane wrapping process. The difference in a volume change of NPs can significantly influence the contact area between NPs and cell membrane during the endocytosis, which further affects their endocytosis efficiency and their size effect during the cellular uptakes.

In the simulation work, Shen et al. applied the coarse-grained molecular dynamics simulations to investigate the membrane wrapping of soft NPs. In simulations, soft NPs have different bending constants and constant volume and area to regulate mechanical properties of lipid-based NPs. They first compared the membrane wrapping of soft NPs with rigid NPs. It is found that the membrane wrapping process of soft NPs is slower than rigid NPs. Further energy and kinetic analysis revealed that wrapping soft NPs needs to overcome a higher energy barrier, which requires recruiting and bind more receptors. Additionally, soft NPs and rigid NPs have a similar contact edge length with the lipid membrane, due to the controlled volume and area. The similar contact edge length induces similar receptor recruiting speeds for soft and rigid NPs. Therefore, rigid NPs are more efficient than soft NPs. They further investigated the receptor-mediated endocytosis of elastic spherical nanoparticles (NPs) with different radii (R = 25 to 100 nm) [63]. Their results showed that the minimum fully wrapped size was shifted from 27.5 nm for rigid

spherical NPs to 30 nm for soft spherical, due to the increased energy barrier in soft NPs and similar contact length (Figure 2c). They further demonstrated that all soft spherical NPs are less efficient to be fully wrapped. Moreover, the wrapping efficiency difference between soft and rigid spherical NPs increased with NP sizes. Their analysis further revealed that the energy barrier of soft NPs increases as the NP size increases. However, the energy barrier of rigid NPs keeps as constant. This computational work explained the aforementioned experimental work for lipid-based NPs [64,65].

Figure 2. The interplay of NP size and elasticity on endocytosis. (**a**) (i) Confocal fluorescent images of HeLa cells incubated with poly(lactic-co-glycolic acid)-lipid (P-L) NPs and poly(lactic-co-glycolic acid)-water-lipid (P-W-L) NPs. (ii) The effect of hypothermia (4 °C) treatment and endocytic inhibitors (CPZ, EIPA, and Genistein) on the cellular uptake of P–L and P–W–L NPs [57]. (iii) Four-, 12-, and 24-h cellular uptake of FA-PEG–modified silica NPs in RAW264.7. All values are means ± SD ($n = 3$, with * $p < 0.05$, ** $p < 0.01$, and # $p < 0.001$; N.S., not significant) [58]. (**b**) (i) 24-h cellular uptake of hydrogel NPs by HepG2 cells as a function of particle concentration. Asterisk indicates significant difference at $p < 0.05$ level [59]. (ii) Elasticity dependence of cellular uptake of nanoliposomes by MDA-MB-231 cells [60]. (**c**) (i) Snapshots showing the membrane wrapping of soft spherical NPs. (ii) Wrapping ratio evolution for rigid and soft spherical NPs with the same radii. (iii) Wrapping time for rigid and soft spherical NPs of different radii. (iv,v) The NP elastic energy change and membrane energy change during the wrapping process, respectively [63]. (**d**) (i) Schematics of the receptor-mediated endocytosis of spherical soft and stiff NPs. (ii) The wrapping time as a function of the particle size for soft and stiff NPs. (iii,iv) The variation of elastic energy change and contact radius during endocytosis for different particle-membrane stiffness ratios κ_p/κ_m, respectively [66].

The trend of the wrapping efficiency between soft and stiff NPs in the simulations by Shen et al. seems to conflict with the theory study carried by Yi and Gao [66]. In their theoretical model, soft NPs can easily change their volume to mimic the soft elastic capsule. Due to the easy volume change, they showed that a soft NP has a larger contact area between cell membrane compared to the rigid counterparts. This large contact area can induce higher receptor recruiting speeds, which offsets the required receptors caused by the increased energy barrier. Therefore, their study indicated that the wrapping of soft NPs is kinetically faster than that of the stiff counterparts (Figure 2d). They also showed that as the particles get softer, the minimum particle size required for full internalization increases [67]. This simulation work is consistent with experimental observations on hydrogel nanoparticles [59], polymer microcapsules [68,69], in which soft NPs can be more easily deformed into a flattened configuration to enhance cell-particle interaction during the membrane wrapping process.

4. Shape and Elasticity

It is quite common that elastic NPs have different shapes in experiments. Thus, NP shape and elasticity may be closely intertwined in affecting the endocytosis of NPs. Based on the self-assembly of amphiphilic α-lactalbumin (α-lac) peptides from partial enzymolysis and cross-linking, Bao et al. prepared peptosomes (PSs) with various shapes and rigidities [70]. The combined experimental results and the molecular simulations demonstrated that short nanotube exhibits the highest cellular uptake and transmembrane permeability. Particularly compared with the other counterparts, including long nanotubes, big nanospheres, small nanos pheres, and cross-linked short nanotubes (Figure 3a). More importantly, their results indicated that tubular and flexible nanocarriers penetrate more efficiently through mucus than the spherical and stiffer ones, which may be attributed to their more flexible structure and the easier conformational change [70]. Palomba et al. investigated discoidal polymeric nanoconstructs (DPNs) with varying geometrical and mechanical properties to resist cellular uptake by professional phagocytic cells, including RAW 264.7 and primary cells [71]. Using confocal fluorescent microscopy (FM) and flow cytometry (FC) analysis to quantify cellular uptake of DPN, they revealed that soft circular nanoconstructs (sCPN) with a size of 1000 nm (185 ± 35 kPa) and 2000 nm (56 ± 6 kPa) are both poorly internalized compared with spherical polymeric nanoconstructs (SPNs) and ellipsoidal nanoconstructs (EPNs). While the uptake level increases by two to four times as the increase of the rigidity of rigid circular nanoconstructs (rCPNs) and rigid-rigid circular nanoconstructs (rrCPNs) (Figure 3b). Upon a critical analysis, Palomba et al. identified three different internalization regimens based on the bending stiffness ratios between particles and cells. When the particle bending stiffness is lower than cells, it facilitates internalization; while a slightly higher bending stiffness than cells is detrimental to internalization; however, when the bending stiffness is much higher than cells, it favors internalization again [71].

Utilizing a two-dimensional triangulation method, Chen et al. developed a coarse-grained model for elastically deformable NP with tunable shape and mechanical properties, wherein the interactions between NPs and membrane are described by the surface area-dependent adhesion energy with no explicit consideration of the specific types of ligands and receptors [72]. Their simulation results indicated that the effects of elasticity on the cellular uptake of deformable NPs strongly depend on their shape: The increase in softness slows down the uptakes rate of spherical and prolate NPs, but speeds up that of oblate NPs (Figure 3c). Further analyses of endocytosis mechanism and free energy calculations revealed that, due to the flexibility and the morphology deformation of soft NPs during the reorientation step, the greatly decreased curvature of oblate edge, i.e., the less membrane bending energy required for wrapping NPs, leads to a higher uptake rate compared with the stiff NPs [72]. Shen et al. investigated the receptor-mediated membrane wrapping process of elastic NPs with different shapes based on coarse-grained molecular dynamics simulations to explore the interplay between geometry and elasticity in cellular uptake of

NPs [63]. In contrast to the model developed by Chen et al., the receptor diffusion kinetics has been incorporated. With comparable ligand and receptor densities as experimental values in the simulations, their results revealed three major points regarding the membrane wrappings of elastic NPs with different shapes: (1) The membrane wrapping efficiency of NPs with the same bending constant is ranked as oblates > spheres > prolates. This shape-dependent uptake is because oblate NPs have the largest contact edge lengths, followed by spherical and prolate NPs; (2) For each geometry, the wrapping time of NPs decreases with the increase in their bending constant, due to the smaller energy barrier of more rigid NPs; (3) the oblate ellipsoid is found to be the least sensitive geometry to the bending constant change, since these soft oblate NPs with significantly larger contact edge length accelerate the formation speed of receptor-ligand complexes to overcome the increased energy barrier (Figure 3d). Their results clearly showed that the membrane wrapping efficiency of NPs during receptor-mediated endocytosis depends both on receptor diffusion kinetics and thermodynamic driving force [63].
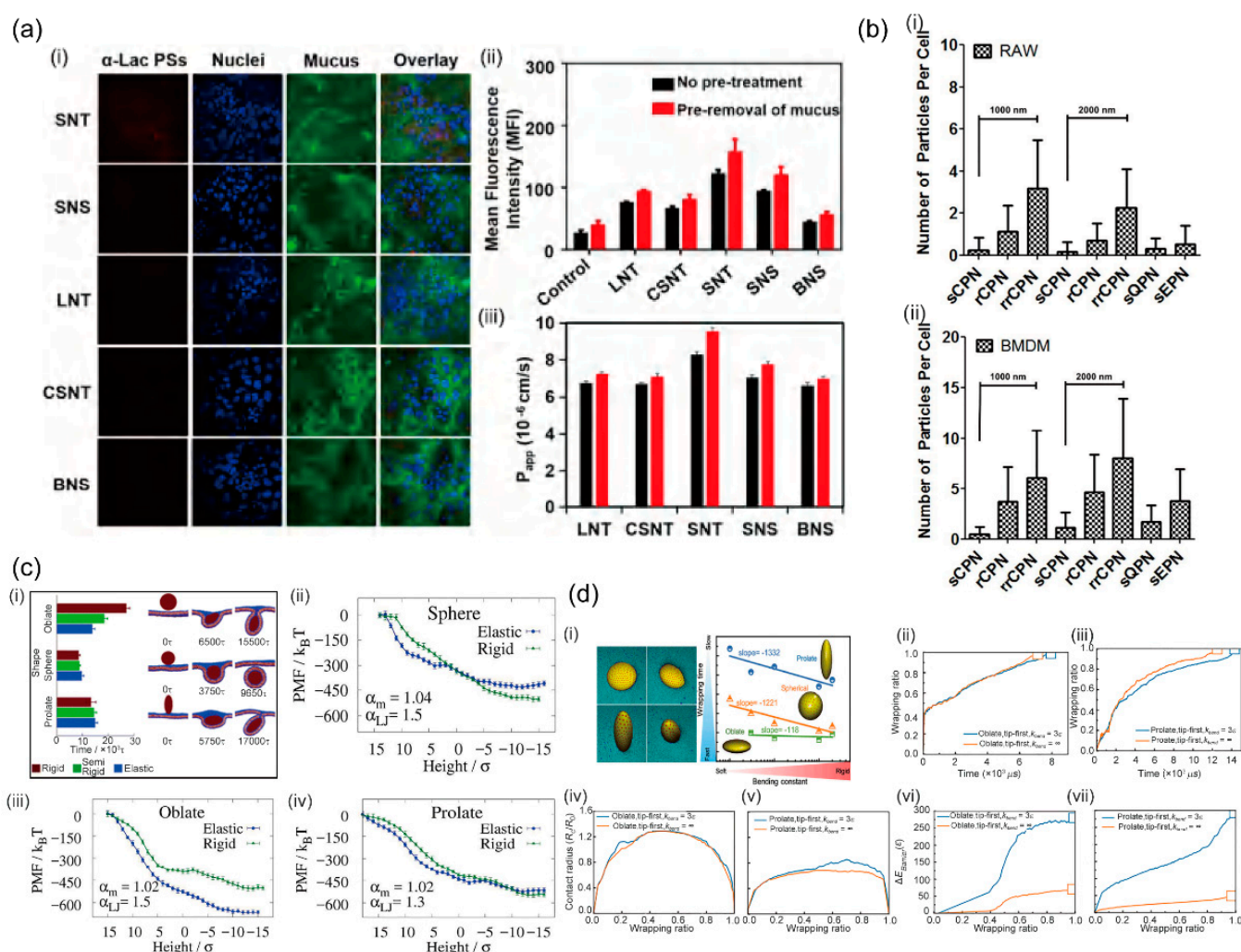
Figure 3. The interplay of NP shape and elasticity on endocytosis. (**a**) Different cellular uptake behavior of peptosomes with different shapes and rigidity as indicated by (i) confocal laser scanning microscope images, (ii) mean fluorescence intensity (MFI), and (iii) apparent permeability coefficients (P_{app}) [70]. (**b**) Number of particles internalized by (i) RAW cells and (ii) BMDM cells for discoidal polymeric NPs with different geometries and mechanical properties [71]. (**c**) (i) Endocytic time for oblate, spheric, and prolate NPs with varied stiffness; (ii–iv) Profile of potential of mean force (PMF) for elastic and rigid NPs with different shapes [72]. (**d**) (i) Wrapping time as a function of bending constant for oblate, prolate, and spherical NPs. (ii–vii) Comparisons of the (ii,iii) wrapping ratio, (iv,v) contact radius, and (vi,vii) energy barrier for rigid and soft oblate and prolate NPs [63].

Contributions made by the interplay between NP properties to the biological performance of NPs has attracted increasing interest. In fact, besides the three combinations discussed here, there also exists interplay among other various properties of NP, which has been demonstrated to be of crucial significance for the NP uptake during the endocytosis process. For example, using confocal laser scanning microscopy, Bhattacharjee et al. investigated how the size and surface charge of NPs affect their cellular uptake and found that smaller NPs (45 nm) with a positive charge are more easily internalized by NR8383 and Caco-2 cells than the bigger NPs (90 nm) with a positive or negative charge. Furthermore, their results indicated that the NPs with different sizes and charges are taken up through different endocytic pathways, determined by the specific interactions with cell membrane-bound receptors, including clathrin, caveolin, and mannose [73]. Jiang et al. quantified the cellular internalization of different sized-NPs featuring neutral, anionic, and cationic headgroups. They showed that the cellular internalization of neutral, anionic NPs decreased with increasing their size, whereas the opposite is true for cationic NPs. They proposed that this contrasting behavior is ascribed to the surface-dictated shift in uptake pathways [74]. In addition to the interplay between NP size and surface charge, other properties of NPs, such as size and surface chemistry, shell thickness, and size, have also been demonstrated to interact in an interrelated fashion to modulate cellular uptake [75,76]. Recent studies focus on the more complicated multiparameter system where distinct properties ranging from size, shape, stiffness, surface chemistry, and composition are simultaneously investigated to establish the optimal feature of NP for efficient cellular uptake [77,78].

5. Summary and Perspective

Engineering NPs to deliver drug molecules has attracted extensive attention in the past decades, due to their great potential to improve cancer therapy, vaccination, and treatment of genetic disorders. One significant obstacle for developing effective NP-based drug therapies is their limited ability to cross the plasma membranes of cells. Endocytosis is a primary exploited route in NP cellular uptake for drug delivery purposes. There is a growing appreciation that a comprehensive understanding of the endocytosis mechanisms responsible for the cellular uptake will play a central role in nanomedicine. It has been well established that the cellular uptake of the NPs via endocytosis depends strongly on the physicochemical characteristics of NPs such as size, shape, stiffness, which have been investigated individually. In practice, it is the properties of NP that interact in an interrelated fashion to regulate their cellular uptake together. This review summarizes the advances regarding the interplay of NP properties on the cellular uptake during endocytosis mainly from three aspects: (1) Size and shape, (2) size and elasticity, (3) shape and elasticity. For case (1), the efficiency of cellular uptake is determined together by both size and shape. The difference in uptake efficiency is proposed to lie in the receptor-ligand binding and endocytic pathways. For case (2), it indicates that softer NPs can be internalized at a faster or slower rate than the stiffer NPs, depending on the volume constraint, and this wrapping efficiency difference increased with NP size. For case (3), the collaborative effects of shape and elasticity on the uptake efficiency are complicated, some of which are proposed to be a result of competition between the receptor diffusion kinetics and driving force provided by the receptor-ligand complexes. These results highlight the importance of interplay between properties of NPs and shed light on the better NP design in drug delivery.

To develop more efficient intracellular NP-dependent drug-delivery nanosystems, further studies on the properties of NPs based on more complicated multiparameter systems are needed. Accumulating evidence indicates that the cellular uptake of NPs is both property- and cell type-dependent, and there is a difference in the uptake of NPs between cell types, e.g., cancer vs. normal cells, phagocytic vs. nonphagocytic cells, and monocytes vs. macrophages [79–83]. The underlying mechanisms accounting for this cell type-dependent manner are manifold. For example, cancer cells can express different numbers and types of receptors on their surfaces compared with normal cells, thus affecting

cellular uptake of NPs by regulating the available binding sites of cargos. In addition, the membrane composition, metabolic activity, cell size, and endocytic pathway can also affect the cellular uptake of NPs [79,80]. There is still much work to be done to understand the cell type-dependent uptake of NPs with various properties. Coupled with innovations in materials science, the results of further studies will continue to contribute to the rational design of clinically useful NPs and the realization of transition from a promising field to the medical application for the NP-based drug-delivery.

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