ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 8 (2013) 1–10

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Review

The endocytosis and intracellular fate of nanomedicines: Implication for rational design

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ARTICLE INFO

Article history:
Received 5 January 2013
Received in revised form
7 February 2013
Accepted 14 February 2013

Keywords:
Nanomedicines
Endocytosis
Transcytosis
Organelle target
Clathrin
Caveolae

ABSTRACT

Nanomedicines employ multiple endocytic pathways to enter cells. Their following fate is interesting, but it is not sufficient understood currently. This review introduces the endocytic pathways, presents new technologies to confirm the specific endocytic pathways and discusses factors for pathway selection. In addition, some intriguing implication about nanomedicine design based on endocytosis will also be discussed at the end. This review may provide new thoughts for the design of novel multifunctional nanomedicines.

1. Introduction

With the continuous development and progress of human society, people are suffering much more modern diseases than before. Tumors, for example, are characterized by heterogeneity and adaptive resistance. Regarding traditional drugs, they work while trafficking in the blood circulation and the concentration at the lesion site determines the therapeutic efficacy of the drugs. Usually, to achieve high concentration at the lesion site, excess drugs are taken. Simultaneously, however, systemic side effects and disorder of other organic or tissular function would appear. It was an inevitable problem for pharmaceutical scientists to solve until nanomedicines emerge. Compared to traditional small molecule drugs, theoretically speaking, nanomedicines can concentrate at certain organs, tissues and even cells, load more drugs to final targets, deliver macromolecules (like proteins and peptides) and minimize side

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Peer review under responsibility of Shenyang Pharmaceutical University

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http://dx.doi.org/10.1016/j.ajps.2013.07.001

effects or toxicity [1]. Scientists see a future in the nanomedicine.

A series of nano-sized preparations, such as liposomes, nanoparticles, polymeric micelles and polymeric-drug conjugates, have been developed in laboratory, and some of them are undertaking preclinical studies. Some successful nano-sized preparations have already emerged in today's pharmaceutical market and shown better clinical performance than traditional drugs [2]. Traditional drugs with small molecule enter cells mainly through the passive diffusion or active transport while nanomedicines come into cells via endocytosis. Endocytosis helps nanomedicines to enter specific cells and accumulate there. Pharmaceutical scientists showed a great interest in this process and spent much time and energy to study, and they have obtained some achievements. The endocytosis pathway has been classified according to the proteins which play a role in the process. Correspondingly, it has been explained that how nanomedicines interact with cytomembrane, enter cells and travel in the cells in different pathways. Even so, there are still many problems that have not been solved. Some pathways are still insufficiently understood, and the functions of some proteins involved in endocytosis are still uncertain, and the factors that affect the pathway for nanomedicines entering cells are not absolutely proven, etc. It is necessary to study further for a better understanding, and the findings may contribute to the emerging of the novel multifunction nanomedicine.

This review summarizes much important advancement about endocytosis mechanisms and the subsequent intracellular fate of nanomedicines. We will focus on the cellular uptake and intracellular route in different type of endocytosis pathways, the tools used to confirm the specific endocytic pathway, and the effect of physicochemical properties of particles and cell types on the selection of the endocytic routes. In addition, some meaningful implications about rational nanomedicine design depending on endocytosis are also introduced in separate paragraphs. The review may provide new thoughts for the design of novel multifunctional nanomedicine and will be helpful to related workers.

2. Endocytic pathways for nanomedicines to enter cells

Endocytosis is the major route for nanomedicines to transport across the membrane (Fig. 1). It is generally classified into phagocytosis and pinocytosis. Phagocytosis was originally discovered in macrophages. Pinocytosis is present in all types of cells in four forms, such as clathrin-dependent endocytosis, caveolae-dependent endocytosis, macropinocytosis, and clathrin- and caveolae-independent endocytosis [3,4].

2.1. *Phagocytosis*

Phagocytosis is a special endocytic pathway predominantly occurred in phagocytes, such as macrophages, neutrophils and monocytes [5]. Relatively, large particles are more likely to take this way. Nanoparticles which adopt this way of entry into cells need to be recognized by the opsonin firstly, such as immunoglobulin (IgG and IgM), complement component (C3, C4, and C5) and blood serum proteins. Thereafter, the

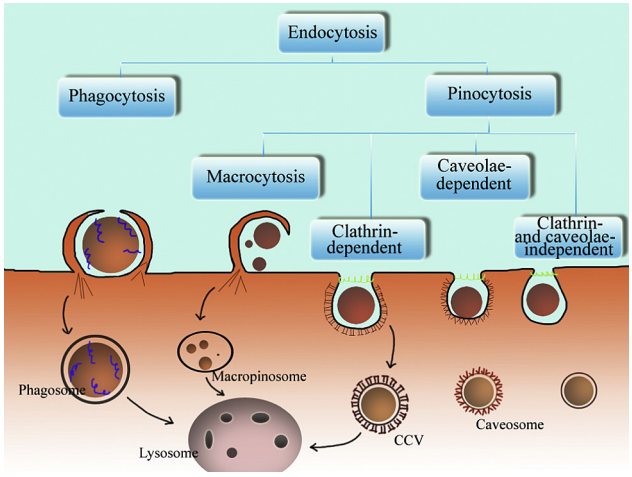

Fig. 1 – Nanoparticles internalization pathways in mammalian cells. The picture briefly shows the classification of endocytic trafficking and different mechanisms of endocytosis. Abbreviation is: CCV, clathrin coated vesicle.

opsonized nanoparticles bind to the cell surface and interact with the receptor, inducing the cup-shaped membrane extension formation. The membrane extensions enclose the nanoparticles and then internalize them, forming the phagosomes which have a diameter of 0.5–10 μm. Finally, the phagosomes move to fuse with lysosomes [5,6]. But the cargo contained in the phagosomes will be destroyed by acidification and enzymolysis in the lysosomes. Therefore, to produce desired effects, nanomedicines must bypass this route to avoid degradation.

2.2. *Pinocytosis*

Pinocytosis is a major route for the cells to drink fluid, solutes and suspensions containing small particles. It is classified to clathrin-dependent endocytosis, caveolae-dependent endocytosis, macropinocytosis and clathrin- and caveolae-independent endocytosis, based on the proteins involved in the pathways [3,4].

2.2.1. *Clathrin-dependent endocytosis*

Clathrin-dependent endocytosis is present in all mammalian cells, occupying an important part in cellar entry. After nanomaterials interact with receptors on the cytomembrane, a kind of cytosolic protein named clathrin-1 polymerizes on the cytosolic side of the plasma where the cargo is internalized [4]. After wrapping the nanoparticles inside, the vesicle is pinched off through the GTPase activity of dynamin, forming a clathrin coated vesicles (CCV) [7]. With energy supplied by actin, CCVs move towards inside the cells, and the route is regulated by the cytoskeleton [8]. The clathrin coat is shed off in the cytosol. Where is the destination of the vesicles? It may be associated with the receptor that nanoparticles' ligands attach to. For example, low-density lipoprotein particles are internalized through LDL receptor and transferred to lysosomes for degradation; while, iron-loaded transferrin is engulfed via transferrin receptor and recycled to the cell

surface [9]. This route can be blocked by its inhibitors or some other factors, such as chlorpromazine, a hypertonic medium or potassium depletion [10,11].

2.2.2. *Caveolae-dependent endocytosis*

Caveolae-dependent endocytosis is also a common cellular entry pathway. It could bypass lysosomes [9], thus many pathogens including viruses and bacteria select this way to avoid lysosomal degradation [12]. For the same reason, this route is believed to be beneficial for enhancement of concentration of targeting position and improvement of therapeutic effect. In this pathway, caveolin, a protein exist in most cells, plays a dominate role. There are three isoforms of caveolin in mammalian cells. Caveolin-3 is muscle specific, while caveolin-1 and -2 are abundant in most nonmuscle cells (such as endothelial cells, fibroblasts and adipocytes) and absent in neurons and leukocytes [9]. By binding to the receptors on the plasma membrane, nanoparticles or pathogens, like Simian virus 40 [13] and cholera toxin [14], can interact with the receptors to induce the formation of the flask-shaped vesicles, which are cut off from the membrane by dynamin. Similar to clathrin-dependent endocytosis, caveolar vesicles require actin to move and intact microtubules to traffic within the cell. The caveolae vesicles traffic to fuse with caveosomes or multivesicular bodies (MTV) which have a neutral pH [15]. The caveosomes containing nanomedicine move along with microtubules to the ER [13,14]. It is thought that nanomaterials in ER penetrate into the cytosol, and then enter nuclear via the nuclear pore complex [16]. Compared to clathrin-dependent endocytosis, this pathway takes longer time and has smaller vesicles in the process [17]. According to those described above, nanomaterials taking this way in some certain avoid a degradative fate and enhance the delivery to a target organelle (such as ER or nucleus), which is critical for improvement of therapeutic delivery [13].

2.2.3. *Macropinocytosis*

Macropinocytosis is commonly defined as a transient, clathrin- and caveolin-independent, growth factor-induced, actin-driven endocytosis that internalizes the surrounding fluid into large vacuoles [9,18]. The cargo absorbed through this way is nonspecific. Actually, macropinocytosis can be found in almost all cells with few exceptions, like brain microvessel endothelial cells. This pathway is generally started with external stimulations which activate the receptor tyrosine kinases. The activation of receptor mediates a signaling cascade that induces the formation of membrane ruffles. However, according to the form of the ruffles, there are different mechanisms of the macropinosomes pinched off from the membrane is different. Circular ruffles are cut off by the multi-functional GTPase of dynamin. In contrast, the lamellipodial macropinosomes separated from the membrane is free of dynamin. The macropinosomes with a diameter of 0.5–10 μm are distinct from other vesicles that formed in other pinocytosis. The surrounding fluid and particles can be internalized into the macropinosomes. In macrophages, after separating from the membrane, macropinosomes move into the cytosol and fuse with lysosomes. In contrast, in human A431 cells, the macropinosomes travel back to the cell surface of the membrane and release the contents to the extracellular space. Therefore, the final fate of macropinosomes depends on the cell type [19].

2.2.4. *Clathrin- and caveolae-independent endocytosis*

This is a distinct pathway, which relies on cholesterol and requires specific lipid compositions. According to GTPases which play a role of regulation in the cellular entry pathway, the clathrin- and caveolae-independent endocytosis is classified to Arf6-dependent, Cdc42-dependent and Rhoa-dependent [19]. Dynamins also play a dominant part in these ways, while it is not deeply understood. This field draws more and more attentions, but unfortunately, it is still far away from deep understanding and need further research. The involved endocytic apparatus may contain clathrin-independent carrier (CLIC) or GPI-anchored protein-enriched early endosomal compartment (GEEC) [9]. Furthermore, their later stages are not yet clearly identified.

3. Study method of the endocytic pathways

The review has introduced the pathways for nanomedicines entering cell. In this section, we will discuss how to study these processes and identify certain pathway that nanomedicines employ. Previous researches usually use endocytic markers to show the location of the nanomedicine, or use endocytic inhibitors to confirm whether the corresponding pathway plays an important role in the uptake of the nanomedicine. Actually, jointly use the two methods, and the results will be more convincing.

3.1. *Markers*

There is a method that uses proper molecular probers or markers to study the intracellular fate of nanomedicines. Mark the specific probers or markers which can show specific fluorescence or color on the nanomedicines, the marked nanomedicines in the intracellular compartments or organelles can be viewed intuitively with the help of the confocal imaging technology. It is important to confirm the destination of the cargo and the pathway employed by nanomedicines. Additionally, combined with a three dimensional confocal technology, we can get more intact information of the whole cell layer by layer [20].

Some classical probers or makers are known to be internalized through specific endocytic pathway. Low density lipoprotein (LDL) [21] and transferrin (Tf) [22] enter cells through clathrin dependent endocytosis (CME), so they are commonly used as markers of CME. Moreover, cholera toxin beta subunit (CTBs) [23], Shiga Toxin [24] and even caveolin-1 are usually used as markers of caveolae dependent endocytosis, and dextran is the marker of macropinocytosis [25]. However, these markers are hard to select, and while entering different cells these markers may use different pathways. For example, when CTBs enter cells that lack caveolae, they cannot use caveolae dependent endocytosis, and a series of electron microscopic assay show that it may be related to a novel clathrin-independent internalization pathway [26].

Some markers can indicate the destination of the nanomedicines within cells. Especially, the proteins contained in specific endocytic vesicles or intracellular organelles, can be

fused with fluorescent proteins, and the formed fusion proteins can show the exact position of the nanomedicines in the cells, like Rab5 in the early endosome [27], Rab7 in the late endosome [28], the lysosome associated membrane protein 1(LAMP-1) around the lysosome [28], caveolin-1 in caveolae, endoplasmic reticulum retention signal KDEL of ER [29]. They can help to solve the problem associated with the intracellular fate of the nanomedicines.

There are also dyes for organelles, such as LysoTracker and LysoSensor for lysosome [30]. They can be used to detect the colocalization of lysosome and the labeled nanomedicine with the confocal microscope. Apart from the confocal imaging technology, electron microscope and atomic force microscope also can be used in this area.

3.2. *Inhibitors*

Inhibitors of endocytosis can be used to block the specific endocytic pathway to confirm whether it is employed by the nanomedicines to enter cells. It can be used along with the markers to confirm the endocytic mechanisms used by the nanomedicines and achieve a more convincing result. Unfortunately, there are some disadvantages for the commonly used inhibitor tools, either. For example, previous researches show that the inhibitor of the specific endocytic pathway always influence on other pathways [31], and the inhibitor can block different endocytic mechanisms in different cell types [32]. Some widely used inhibitors and methods related to inhibition will be introduced in the following paragraph.

As almost all endocytic pathways are energy dependent processes, they can be inhibited by low temperature and an ATPase inhibitor (like sodium azide) at the same time [33]. Therefore, the two factors can be used together to distinguish from the non-endocytic pathways. As respect to the specific endocytosis mechanisms, hypertonic sucrose (0.4–0.5 M), chlorpromazine (50–100 μM) and potassium depletion can be used to inhibit the clathrin dependent endocytosis [34]; methyl-β-cyclodextrin (MβCD), filipin, nystatin and cholesterol oxidase can be used as the inhibitors for caveolae dependent endocytosis [35]; amiloride, cytochalasin D and rottlerin can block macropinocytosis [35]. When an inhibitor is firstly used on the cell, the concentration of sufficient inhibitory efficiency and lowest cytotoxicity need to be detected to make sure a proper concentration to be used in the experiments.

Apart from inhibitors, mutants which lack of the protein involved in a specific endocytic pathway, like knock-out cell lines [36], also can be used to exclude or verify specific endocytic pathways for nanomedicines. This method is becoming increasingly popular.

4. Effect factors of endocytosis pathway selection

Different endocytic pathway varies in the protein involved, the size of the formed vesicles and the cell type where they were found. After engulfed, the intracellular fate of the nanoparticles is dependent upon the selected endocytic pathway. Modern drug delivery systems pay more attention on the nanoparticles' intracellular travel. A growing number of researches show that the selection of nanomaterials transport pathway was affected by the physicochemical characteristics of nanoparticles (size, charge, shape, etc.) and the different endocytic machinery in various cell type [3,4,6]. But, in fact, in the studies of diversified nanoparticles and cell models, there is no common factor. In this paper, we will take an attempt to present these factors and make recommendations for the design of drugs in the future.

4.1. *Size*

In the endocytic process, the size of the vesicles that contain nanoparticles varies with the specific pathway. It has always been believed that the size of nanoparticles may be a considerable factor that affects which pathway will be employed by the nanoparticles [37]. Firstly, keep the particles small enough to enter the vesicles, and the size range from 10 nm to 500 nm and limited up to 5 μm. The large particles are most likely to be engulfed via macropinocytosis. The size of vesicle involved in clathrin mediated endocytosis is about 100 nm, while the size involved in caveolae mediated endocytosis is about 60–80 nm [9]. On the other hand, some researchers suggest that the size may not be that important compared to other factors in the pathway selection of nanoparticles entry into cells [38]. But it is understandable that the small particles may be beneficial to enter the cells rapidly.

4.2. *Surface charge*

It is known that the cytomembrane possess negative charge [39]. Therefore, the cationic nanoparticles may show a strong electrostatic interaction with the cells, which result in a rapid entry (Fig. 2). It's worth mentioning that positively charged nanoparticles can escape from endosomes after internalization and exhibit perinuclear localization because of the 'proton-sponge' effect. The nanoparticles without any charge at physiological pH may interact with the cells with the aid of hydrophobic and hydrogen bond interactions [40]. Additionally, neutral particles coated with hydrophilic polymers can

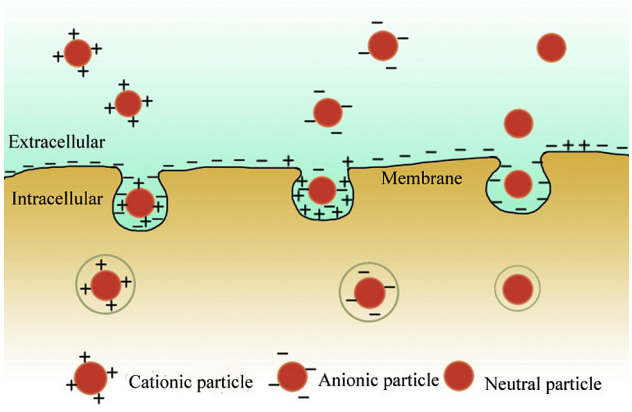

Fig. 2 – Nanoparticles which possess different charge or no internalization into cells. a) Cationic particles strongly interact with the membrane and enter cells rapidly. b) Anionic particles bind the positive site at the membrane and enter cells. c) Neutral nanoparticles also can get into cells. Two different neutral particles will be showed in Fig. 3.

prevent interaction with the cytomembrane leading to less absorption. The anionic nanoparticles may be endocytosed through the interaction with the positive site of the proteins in membrane, and they can be highly captured by cells because of their repulsive interactions with the negatively charged cell surface [41]. Is the charge a parameter to determine which trafficking pathway will be chosen? It seems confused. For cationic nanoparticles, the majority of the reports indicated they mainly enter cells through CME [42,43], while some others show that they utilize macropinocytosis [44] or caveolae- and clathrin-independent endocytosis [45] or even multiple pathways including caveolae mediated endocytosis [46]. The anionic nanoparticles are more likely to use caveolae-dependent endocytosis [45], but there are also some exceptions [47]. In addition, the neutral nanoparticles show no clear preference for specific routes.

4.3. *Surface hydrophobicity*

Hydrophobic nanoparticles have higher affinity for the cell membrane than hydrophilic ones, leading to an improvement of cell uptake in the kinetics and the amount (Fig. 3). Hydrophilic polymers used to modified nanoparticles, such as polyethylene glycol (PEG) [48–50], poly (N-vinyl-2-pyrrolidone) (PVP) [51], poly(amino acids) [52] and dextran [53], form a 'cloud' to suppress the interaction between the nanoparticles and lipid bilayer of cells. On the other hand, it can prolong nanoparticles' life in blood to reach specific site. The chemical composition at the surface nanoparticles determines the surface hydrophobicity which can promote or suppress the interaction with cells, thereby influencing the route of cell uptake. It is worth noting that the kinds of polymer used in the formation of nanoparticles may contribute to the route selection.

4.4. *Shape*

Precious experimental studies have discovered the role of the particle shape in drug delivery, but these mainly focus on phagocytosis (Fig. 4) [54,55] and there is no specific conclusion

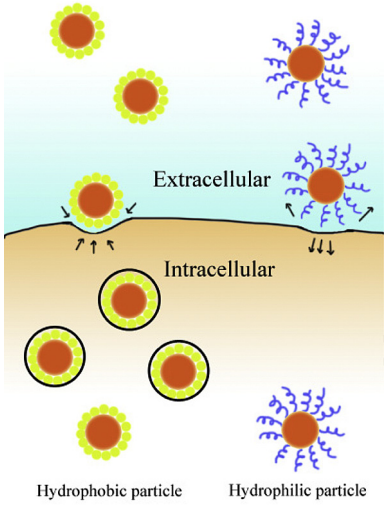

Fig. 3 – Nanoparticles with different hydrophobicity present different affinity with the cell membrane.

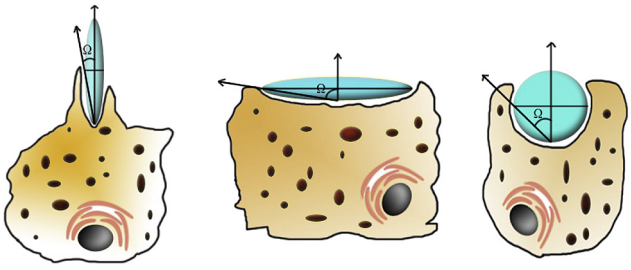

Fig. 4 – The effect of particle shape on phagocytosis. Ω is defined as the angle between the membrane normal at the point of attachment and the line defining the particle curvature at this point. Particles are internalized successfully at Ω ≤ 45°; the internalization of particles can be inhibited at Ω > 45°.

on the pathway selection of nanoparticles. It is notable that particles with a proper aspect ratio enjoy a perceptible advantage as to internalized rate [56–58]. Moreover, a particle fabrication technique called PRINT may be extensively applied in the preparation of nanoparticles with needed size or shape [59].

4.5. *Cell type*

If the cells have no necessary proteins involved in the specific endocytic pathway, it is easy to understand that the endocytic pathway cannot be adopted by this kind of cells. For example, HepG2 cells have no endogenous caveolin, so they are unable to uptake nanoparticles by caveolae mediated endocytosis [60]. In addition, the growing environment of cells, such as cell density and hormones, may affect the phenotype of cell and further affect the endocytic pathway. Notably, there are distinct differences between normal cells and tumor cells, and it is promising to target the tumor based on the different endocytic pathway [61]. However, the current studies fail to focus on the connection between the cell origin and the endocytic pathways. It is necessary to supply a gap in this area.

In fact, all factors work jointly to result in the selected pathway. These factors make their contribution in union to defining nanoparticles' entry into cells and final destination in cells, and it is better to consider these factors as far as possible in design of the desired nanoparticles.

5. Implication for rational design of nanomedicines

There are many interesting phenomena associated with the endocytosis of nanomedicines within our bodies. Some of them enlighten us on rational design of nanoparticles. Next, we will introduce them.

5.1. *Transcytosis*

The medicine is believed to perform well only if it can be delivered to specific organs or cells. For orally preparations, they need to travel across the epithelial cells in gastrointestinal tract and get into blood vessels. But this is not the end. They can work unless they traffic across the vessel endothelial

cells to lesion site. The drugs for lung have the similar path. Transcytosis play a critical role in these processes. From above we know that nanoparticles which take caveolae-dependent endocytosis can bypass the lysosomes and avoid the degradation compared with classic clathrin-dependent endocytosis and other endocytic pathways. After that, the cargo could be released to extracellular matrix. Therefore, the transcytosis of nanoparticles is predominantly mediated by the caveolae, which is determined by the property of caveolae-mediated endocytic pathway [62]. Literature have revealed that clathrin also participate in the transcytosis, but its contribution is less than 1% [63].

Caveolae-mediated transcytosis can be thought in three steps (Fig. 5) [64]. It starts with the formation of the separate caveolae pinched off from the membrane, in short, endocytosis. Following, the caveolae traffic through cytoplasm. Finally, the free caveolar vesicles dock and fuse with specific membranes to release the cargo into the perivascular space, and this process may rely on the presence of members of SNARE complex [65].

The particular character and function of caveolae make itself be an ideal drug target. It has the potential therapeutic value that increasing the absorption of drugs and accumulation in specific site by enhancing transport through the epithelial or endothelial cells. As an obvious obstacle, endothelial cells can forbid many materials from circular blood into underlying tissue cells; likewise they can limit the drug to enter target cells. For example, monoclonal antibody was promising initially, but it is far away from success in target the extravascular sites. This is, in part, because it cannot extravasate across the tight and continuous endothelial tissue [64]. In physiology field, specific ligands, modified ligands and antibodies are usually used to target receptor. An accessible approach to raise uptake of nanomedicines is binding an antibody which can recognize or target the caveolae to the nanoparticles [65]. It provides a method to overcome the barrier of endothelial or epithelial cells for drug and gene delivery. We can achieve theoretical expected value in pharmacokinetics and desired therapeutic effects. Intrinsic antibodies and peptides of tumors only recognize the antigens expressed on the surface of the tumor. As a result, they are restricted by solid tumors partly because of the barrier formed by endothelial cells and the increased pressure in tissue space [66]. The therapeutics which target caveolae at vascular endothelium can overcome the barrier to deliver into tumors successfully. The albumin which can be transported from the luminal to the basal poles of the membrane with the help of caveolae after binding to its receptor gp60, is a best example [67]. Paclitaxel that packed in the nanoparticles with albumin covering can be easily delivered to the tumor tissue after transcytosis. Compared to control paclitaxel, the increased antitumor activity and higher intratumoral paclitaxel level have been conformed according to clinical researches [68].

5.2. *A potential method to minimize first past effect of nanomedicine*

The oral administration of macromolecular drug such as proteins and peptides or nanoparticles is faced with challenges in drug absorption delivery. Recent researches have revealed that M cells in the Follicle Associated Epithelium overlying organized mucosa-associated lymphoid tissues (Fig. 6A), namely the intestinal Peyer's patches, may be necessary in absorption transport of macromolecules and nanoparticles for oral administration [69,70]. The intestinal surface area is physically protected by a layer of tightly joined epithelial cells, which consist of M cells and enterocytes (Fig. 6B). Compared to enterocytes, M cells are characterized with fewer lysosomes, more mitochondria, a lack of mucous glycocalyx covering, poorly organized brush border membrane and a basolateral cytoplasmic invagination that forms a pocket containing lymphocytes and occasional macrophages [71,72]. Otherwise, M cells have reduced levels of membrane hydrolase activity, which can keep the absorbed drug intact [71]. M cells' high transcytotic capacity is most remarkable and interesting. This property indicates that M cells may act as a highly efficient portal for macromolecular drug, nanomedicine and mucosal vaccination. M cells can delivery foreign materials bound to their surface from the intestinal tract to the underlying lymphoid tissues, such as lymphoid follicle or lymphatic vessels, where immune response occurs for vaccine. Studies on polystyrene particles show that the

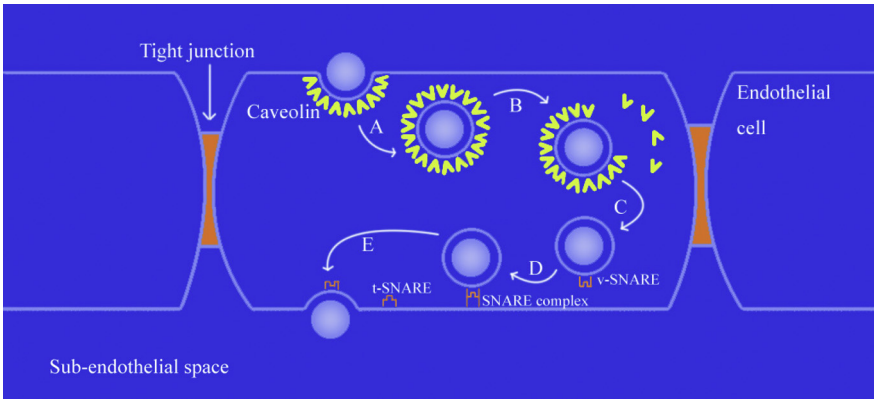

Fig. 5 – Model of caveolae-mediated transcytosis. A) After the fission mediated by dynamin, the free caveolar vesicle forms. This is the first step. B) The formed vesicle is changing. C) v-SNARE forms at the surface of the vesicle. B and C make up the second step. D) v-SNARE contacts with t-SNARE at target membrane, and SNARE complex forms. E) The cargo in the vesicle is released to outside cells. D and E make up the third step.

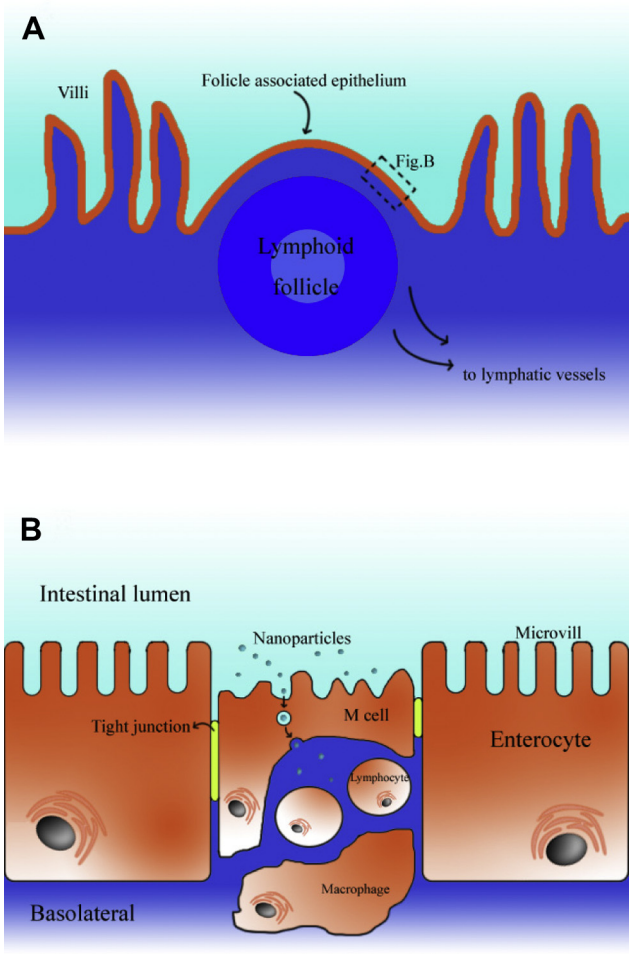

Fig. 6 – A Model of follicle associated epithelium (FAE). B Model of M cells in FAE. (Adapted from the reference [71,72]).

most suitable size range from 50 nm to 100 nm for the particles absorbed into the lymphatic vessels, while particles above 1 μm remain entrapped in the peyer's patches [73].

As for M cells, it is clear that the transport of nanomedicines is predominantly mediated by endocytosis, an energy-dependent mechanism, but the question is which specific mechanism of endocytosis will be employed? Although the process can be considered as transcytosis, the caveolae-mediated endocytosis is unlikely to be solely responsible for M cells [73]. Previous literature in this field remain controversial. Jae Sung Lim, etc, considered that caveolin-1 plays a crucial role in the entry of nanoparticles into M cells, and they found that caveolin-1 has a high level of expression in M-like cells, while not in caco-2 cells (an intestinal epithelial cell simulated model) [74]. Anne des Rieux, etc, suggested that nanoparticle endocytosis of M cells is most likely macropinocytosis [70]. Some other reported that clathrin mediates endocytosis of particles, macromolecules and microorganisms [75]. Sometimes, phagocytosis also is a candidate [76]. Therefore, as for M cells every mechanism may contributes to the endocytosis of nanoparticles to some extent. Physicochemical properties of nanoparticles, such as size, shape, surface potential and hydrophobicity/hydrophilicity balance, etc, may exert their effects on the mechanism selection of nanoparticles uptake of M cells [73].

M cells are not the only gate for the particulates in the gastrointestinal tract. However, their relative high transcytotic capacity and intimate relationship with inductive sites of the mucosal immune system make them to be a perfect target for strategic delivery of nanoparticles and mucosal vaccines. Some literature said that the bulk of particle translocation occurs in the FAE [73], and M cells began to be the subject of intense research. As the specific mechanism employed by M cells is uncertain, it makes little sense to modify the particles with the ligands to target clathrin or caveolae, or other associated component. It is a relative wonderful strategy to decorate nanoparticle surface with a molecule targeting on M cell, which can enhance their absorption transport through specific interactions between nanocarriers and M cells [70,73]. The special interactions might be non-specific interactions or targeting on M cells by a specific ligand or both [77]. Studies have attained significant achievements, such as lectins derived from Sambucus nigra and Viscum album could label the surface of human FAE [74], RGD derivatives could target β1 integrin concentrated at the apical pole of M cells [78], and Claudin 4 highly expressed in peyer's patch M cells also could be a site for oral nanoparticles target delivery [79]. Nanoparticles targeting on M cells could not only enhance the oral bioavailability but also bypass the liver and avoid the first pass effect to some extent owing to their lymphatic delivery. Therefore, the continuous attention given to M cells is not surprising.

5.3. *Organelle target selection*

In contemporary drug therapy, most drugs are designed to target specific sites in the human body. Nevertheless, many therapeutical sites are located in the cells. Like the nucleus, the mitochondria or lysosomes, they all require drugs to be delivered to specific organelles. In ideal circumstances, nanoparticles as carriers could transfer their payload to specific tissues, cells, or even cellular organelles. Therefore, the nanoparticles which can be delivered to specific site may be an answer for organelle target selection. Nanoparticles take various endocytic pathways to come into cells, and the way they employ influences the intracellular fate of the nanoparticles [80]. The intracellular delivery of nanoparticles based on endocytosis will be discussed in detail in the following part.

Except for caveolae-mediated endocytosis, the other pathways all have a relationship with lysosomes, and it is a great idea for drugs to target lysosomes. Enzyme replacement therapy should had perfect therapeutic effect on lysosomal storage disease, but the enzyme can be eliminated easily in the blood circulation, which result in the dramatic decrease of drug in desired site [81–83]. Encapsulated in the nanocarriers, the enzyme could be protected from clearance and keep stable. After endocytosis, the drug will deliver to lysosomes and produce improved therapeutic effects. While, as regard to drugs used for other diseases, the lysosomes will be their hell. These drugs will be entrapped in endosomes and degraded in lysosomes and lost their activity in the end.

In the process of caveolae-mediated endocytosis, the nanoparticles do not fuse with lysosomes after their entry into

Table 1 – Overview of strategies based on nanotechnology for organelle targeting mentioned in this article.

Target organelle	Strategy	Ref.
Lysosomes	Increase drug accumulation in lysosomes by load the drug in liposomes	[82,83]
Endoplasmic reticulum	pH-sensitive liposomes improve therapeutic efficiency	[85]
	Nanoparticles decorated with ER-targeting peptides	[86]
Cytosol	Nanoparticles modified with CPPs	[89–91]
	pH-sensitive nanoparticles enhance endosomal escape	[87,88]
	Transferrin modified nanoparticles interact with the receptor at the surface of cell	[92]
Nucleus	Nuclear localization signal modified nanoparticles	[93]
	Nanoparticles modified with CCPs	[96]
Mitochondria	Mitochondria targeting sequence modified quantum dots	[95]
	Polymer micelles show mitochondria targeting property	[97]

cells, which not only prevent the drugs from degrading but also give them a chance to arrive other organelles. After engulfed in the cells, the vesicles containing nanoparticles fuse with caveosomes or multivesicular body (MTV). Thereafter, the payload can be delivered to endoplasmic reticulum or Golgi complex, or even released to outside the cells [84]. So the drugs that work by targeting ER or Golgi complex can be designed to employ caveolae mediated endocytosis. This strategy may be helpful to increase the accumulation of the drugs in ER or Golgi apparatus [85,86].

Also, there are still some targets at other organelles need to be treat, like cytosol, nucleus and mitochondria. How can we deliver the drugs to that desired sites? As mentioned, nanoparticles which transferred to ER via caveolae-mediated endocytosis can penetrate into cytosol and have a chance to pass through nuclear pore complex and get into nucleus. In addition, if released from the endosomes, particles could target the sites at cytosol and other organelles. Literature show that the application of pH-sensitive polymeric blocks in nanoparticles design can help the particles to be released from the endosomes [87,88]. Additionally, nanoparticles that modified with cell penetrating peptides [89–91] or ligands which interact with the receptor at the surface of membrane [92] also could enter cells. To reach the desired organelle sites, Drugs are still confronted with some barriers, such as cytoskeletal proteins in cytosol and membrane structure of target organelle. With the help of signal sequence, like nuclear localization sequences [93] and mitochondrial localization sequences [94,95], drugs can target the appointed organelle. Additionally, modified nanoparticle [96] or polymer micelles [97] also present organelle target property.

As a new promising approach for optimizing the drug pharmacological activities, organelle targeted drug delivery will draw more attention (Table 1). The different endocytic pathway and their intracellular fate may enlighten the researchers who are interested in organelle targeting preparation.

6. Conclusion

Nowadays, nanomedicines are increasingly showing its outstanding advantages in diagnosis and treatment of the diseases. As for the design of nanomedicines, it is critical to understand their uptake pathways. We have summarized the characteristics of the endocytic pathways, the tools to dissect the specific mechanism and the factors affecting the selection of pathway employed by nanomedicines. In addition, some rational designs associated with endocytosis in human body have been introduced in this review.

Although considerable achievements have been acquired, this field is still in the infancy. The existing results are mostly based on the *in vitro* experiments, so the crux of the research is to study the complex endocytic process of nanomedicine entering into cells *in vivo*. Besides, there are still many controversial points and unstated field. A deep and elaborate study on this field is still necessary.

In the near future, organelle target will be a hotspot. Drugs will be delivered to lesion site in the specific organelles. If so, it is not difficult to imagine the dose of medication will dramatically decrease. Correspondingly, unwanted adverse effects will be minimized. In the design of nanocarriers targeting on specific organelle, researchers will spend much more time on the study of their intracellular fate. What's more, everybody looks forward to applying the designed nanomedicine to treat diseases and achieve desirable therapeutic effect.

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