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Nano-Enabled Delivery of Intracellular Therapeutics

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Abstract Many diseases that plague the modern medical world have their origins at the cellular or molecular level and, as such, require greater specificity to be effectively combated and cured. A number of recent advances in understanding the biology and biochemistry have enabled researchers to develop the specialized tools and techniques needed to detect and provide therapy for these debilitating conditions. Many of these treatments take advantage of the way that cells behave and interact with their environment or various properties of the cell's structure and form. Researchers are able to surpass a number of cellular hurdles, such as the cell membrane, endosomal escape, and intracellular targeting to begin the arduous task of understanding, diagnosing, and treating diseases like cancer.

Keywords Cell-penetrating peptides, Intracellular delivery, Nanoparticles, Therapeutics

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Abbreviations

ATP	Adenosine triphosphate
CIE	Clathrin-independent endocytosis
CLIC	Clathrin- and dynamin-independent carriers
CME	Clathrin-mediated endocytosis
CPP	Cell penetrating peptides
CPT	Camptothecin
DNP	Dinitrophenol
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptors
ER	Endoplasmic reticulum
ETC	Electron transport chain
FR	Fc receptor
GUV	Giant unilamellar vesicle
HP	Hematoporphyrin
NLS	Nuclear localization sequence
NP	Nanoparticle
NPC	Nuclear pore complex
PC	Phophatidylcholine
PCI	Photo-chemical internalization
PE	Phosphatidylethanolamine
PEG	Poly-ethylene glycol
PS	Phosphatidylserine
PSMA	Prostate-specific membrane antigens
RES	Reticulo-endothelial system
ROS	Reactive oxygen species
TCHD	*Trans*-cyclohexane-1,2-diol
TIM	Transporter inner membrane
TOM	Transporter outer membrane
TPP	Triphenylphosphonium
TR	Transferrin receptors

1 Introduction

For predominantly fatal and life-shortening diseases in modern society such as Alzheimer's, diabetes, and cancer, the paradigm shift in treatment has been toward the cellular and molecular levels and away from systems and tissue levels. This shift is largely due to the many discoveries of the molecular origins for these specific disease pathways [1–3] which include hereditary risk factors, mutations, and result changes in molecular pathways, which cannot be identified or treated until their pathological effects are on the systemic or tissue levels. Additionally, since current medical capabilities are unable to target, locate, or treat these cellular events effectively, these diseases remain largely undetected until they have progressed to tissue level. This shortcoming can potentially lead to disease spread among other tissue systems and thus shortens the patient's life.

Advances in nanotechnology have allowed for potentially earlier identification and treatment of pathologies that are of cellular and molecular origin ex vivo. Nanoparticles come in various forms such as soft particles (e.g., liposomes [4, 5] dendrimers [6], polymers [7]), hard particles (e.g., quantum dots [8], gold [9, 10], magnetite [11]), or naturally occurring species (e.g. proteins [12], micelles [13, 14], viral envelopes [15]). Compared to molecular or capsule drug emission therapeutic delivery, nanoparticles can deliver higher local concentrations of cytotoxic drug with minimal systemic concentrations [7, 16, 17]. Current nanoparticle-based treatments are capable of combining modality-specific imaging contrast with high drug payload and large surface area targeting ligands for an advanced multipurpose therapy agent. The combinatory relationship between treatment and localization of disease models is exclusively exploited in the nanomedicine field with the new approach for combating disease models known as "theranostics" (a portmanteau of therapy and diagnostics). Theranostic nanoparticles allow for a more appropriate application of personalized medicines as the imaging contrast provided allows the researcher or clinician to track the efficacy of the therapy throughout the application. This personalization with concurrent monitoring of medical treatment becomes especially critical when considering diseases that are largely heterogeneous in nature such as cancer, whose current treatments are associated with emaciation and suffering, almost as highly as the disease.

Although nanoparticles have huge potential in molecular medicine, drug delivery optimization and cellular targeting are bottlenecks in their efficient exploitation. These barriers include human efficiency, such as cost; external barriers, such as skin or mucosa; en route efficiency, such as blood; and cellular barriers that must be overcome in order for a treatment to be successful. Nanomedicine offers solutions to the problems presented by cellular barriers, which offer some of the most varied and difficult challenges in drug delivery, as well as many of the most promising methods for future drug delivery approaches.

2 Crossing the Cell Membrane and Internalization

A primary barrier preventing successful cellular delivery is the cellular membrane. This membrane is composed of a phospholipid bilayer with embedded proteins selectively permeable for ions and organic molecules and is crucial for cell communication and adhesion. Successful translocation across this membrane is critical for further intracellular drug targeting. Endocytosis, the formation of new cytosolic membrane-bound vesicles from the cell plasma membrane, is the primary method of internalization of extracellular components (Fig. 1). The two principal endocytotic pathways utilized by cells are phagocytosis and pinocytosis. Phagocytosis is used by a multitude of cell types to engulf foreign particles as part of the immune response. Interaction of cell-surface receptors with factors that recognize the foreign body or with the foreign body itself triggers phagocytosis. Receptors that have been identified as facilitating phagocytosis include the Fc receptor (FR) family and complement receptors [19].

In the case of nanoparticles, attractive forces such as van der Waals, electrostatic, ionic, and hydrophobic/hydrophilic between nanoparticles and cells facilitate internalization via phagocytosis [20, 21]. These forces are affected by the contact angle between the nanoparticle and host cell membrane [21]. Differences in nanoparticles' geometry have been shown to affect the success of internalization

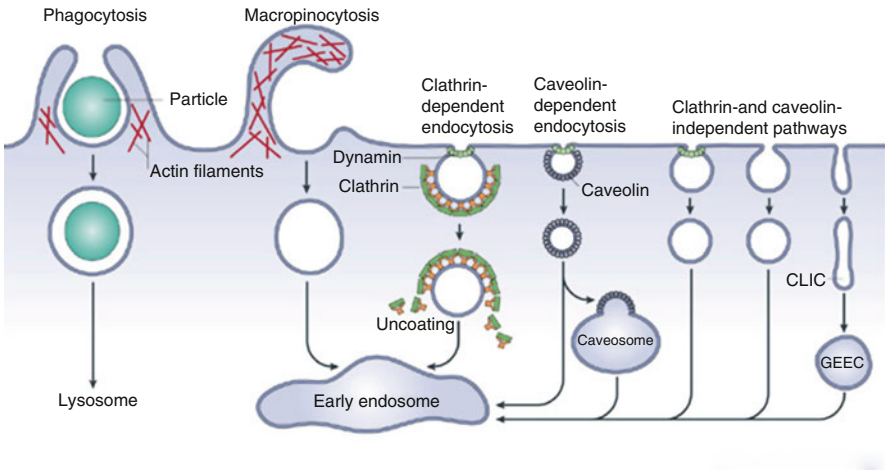

Fig. 1 Pathways of entry into cells. Large particles can be taken up by phagocytosis, whereas fluid uptake occurs by macropinocytosis. Numerous cargoes can be endocytosed by mechanisms that are independent of the coat protein clathrin and the fission GTPase dynamin. Most internalized cargos are delivered to the early endosome via vesicular (clathrin- or caveolin-coated vesicles) or tubular intermediates known as clathrin- and dynamin-independent carriers (CLICs) that are derived from the plasma membrane. Some pathways may first traffic to intermediate compartments, such as the caveosome or glycosylphosphatidylinositol-anchored protein-enriched early endosomal compartments (GEEC), en route to the early endosome. Reproduced with permission from [18]

via phagocytosis, due to the varying contact angles at the cell membrane surface caused by different particle shapes [22]. In a comparison of nanoparticles of various shapes and aspect ratios, it was found that particles that were elongated with higher aspect ratios were less likely to be internalized via phagocytosis [23]. Concurrently, a similar study found that particles with higher aspect ratios were more prone to endosomal and lysosomal localization [24]. Nanoparticle size and shape tunability is thus an important tool in developing targeted nanomedicines, but must be carefully controlled in order to achieve the desired outcome, whether it is phagocytosis or specific intracellular targeting [25]. Modulation of particle properties also has been shown to affect internalization via pinocytosis. Pinocytosis is clathrin mediated (CME), clathrin independent (CIE), or caveolae mediated [26]. Nanoparticles can be made more susceptible to these internalization pathways by modulating size, shape, and surface charge. Positively charged nanoparticles have been shown to be preferentially taken up through CME, while particles with negative surface charges are associated with internalization via caveolae [27, 28].

Nanomedicine presents an attractive option because it has no cargo size limitations and can specifically be targeted to certain cellular receptors [29]. Carbon nanoparticles have been identified as possible carriers of DNA molecules and have shown a high transfection efficacy in breast cancer cells, as shown in Fig. 2 [30]. Nanoparticles have the potential to be effective carriers for a large variety of different materials which help to increase cargo uptake by the cells. Additionally, when compared to delivering small molecule drugs alone, nanoparticles can increase delivery efficiency, leading to lower effective dosages and fewer side effects [31].

More recently, dendrimers have been shown to function as effective intracellular carriers for therapeutic and imaging agents. The new generation of dendrimer-based delivery systems has shown to be capable of bypassing efflux transporters to enable the efficient transport of drugs across cellular barriers.

Receptor-mediated endocytosis is an internalization method that is used to deliver nanoparticles to disease sites by exploiting the overexpression of cell-surface receptors on target disease cells. This method of active targeting has been utilized for the delivery of both small molecule drugs and nucleic acids and is achieved via the functionalization of nanoparticle surfaces with targeting ligands including small molecules, peptides, antibodies, and aptamers. In the context of tumor targeting, folate receptors (FR), epidermal growth factor receptors (EGFR),

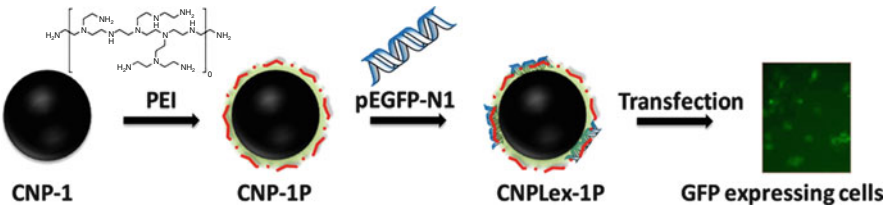

Fig. 2 Carbon nanoparticles used for gene delivery. Image of cells transfected with the pEGFP-N1 reporter gene plasmid in breast cancer cell line MDA-MB231. Reproduced with permission from [30]

transferrin receptors (TR), prostate-specific membrane antigens (PSMA), and integrins have been implicated in different types of cancer and thus used as targeting ligands in order to specifically deliver therapeutic nanoparticles to tumor sites with minimal off-target toxicity [32].

2.1 Endosomal Escape and Cytosolic Delivery

After the payload has been successfully internalized, it must still pass the subcellular obstacles such as the early endosome, late endosome, and lysosome. This critical moment in the subcellular delivery of nanoparticles can either result in lysosomal degradation, exocytotic release, or trafficking of particles to the desired organelle [33]. Specifically, it is of paramount importance that nanoparticles escape the endosome because vesicular sequestration impedes delivery of the cargo and leads to degradation of the nanoparticle [23]. Vesicular entrapment is widely regarded as an undesirable phenomenon, unless targeting lysosomal storage disorder. Several strategies have been developed to circumvent vesicular entrapment such as fusogenic peptides [34, 35], pH-sensitive polymers, pH-sensitive core shell nanoparticles [36], and pH-sensitive liposomes. Cationic liposomes, polypeptides, amine-containing polymers, and cationic lipids have been shown to be efficient in non-viral gene therapy. These materials interact electrostatically with membrane glycoproteins, proteoglycans, or other anionic membrane components efficaciously as non-viral vectors [34, 37, 38].

2.2 Cationic Escape

There are two methods that enable cationic materials to undergo endosomal escape. One strategy involves the material's interaction with endosomal membrane and subsequent pore formation facilitating the transport to the cytosol. A second cationic endosomal escape strategy utilizes the "proton sponge effect" during which the endosomal membrane ruptures and the cargo is released directly into the cytosol [10]. Through endosomal maturation, the pH significantly decreases from 6 to 4 and an excess of protons can be sequestered by the contribution of the protons from amine groups in cationic polymers, maintaining the action of the proton pumps. A parallel influx of Cl^- and water takes place so as to keep a neutral pH of the environment, resulting in swelling and subsequent rupture of the endosome [33]. Although a viable platform for direct release of cargo into cytosol is protonated, they are claimed to be cytotoxic and unstable in biological buffers or culture media and are cleared rapidly upon exposure to the extracellular environment by the reticuloendothelial system (RES) [33]. These concerns have partially been resolved via surface passivation by materials such as polyethylene glycol (PEG), dextran, Pluronics, and human serum albumin [39]. In addition, there exists a complementary method called photochemical internalization (PCI) during which

a photosensitizing molecule conjugated with drug is photochemically illuminated, subsequently triggering the formation of reactive oxygen species (ROS) and ultimately causing endosomal rupture [40]. However, this method has some limitations such as potential damage to the drug due to singlet oxygen exposure. Coupling of PCI with pH-responsive systems in which photosensitizing agents can become active only in low pH has been utilized to enhance the overall efficacy [41]. For instance, very recently, Pasparakis et al. [42] developed a novel self-assembling polymer of the polyacetal family, which is degradable by light and pH. They used this polymer to indicate the potential of photochemical internalization in a multimodal therapy approach combining chemo- and photothermal therapy. The phototoxic drug hematoporphyrin (HP) and the chemotherapeutic anticancer agent camptothecin (CPT) were incorporated within the polymeric nanoparticles which can subsequently be activated using visible wavelength leading to cancer cell death due to light and pH-mediated intracellular delivery of drug payload. The polymer was synthesized via acid-catalyzed polycondensation reaction of 2-nitroresorcinol and cyclohexyl divinyl ether which was further capped by poly(ethylene glycol). The spherical particles had hydrodynamic size of 190 nm and were found to be stable in slightly alkaline solutions for weeks. The CPT release profile of the polymer under both acidic (pH 5.2) and light irradiation condition was significantly enhanced (>90%) compared to non-irradiative condition (~52%), indicating the role of HP in generating the reactive oxygen species. Furthermore, preliminary cytotoxicity studies on HeLa cells revealed that the drugs acted more effectively in the samples under both irradiative and low pH conditions compared to the non-irradiative case (death rate of 52% vs 27%). In addition, fluorescence microscopy investigation of the developed nanoparticles confirmed the uptake of nanoparticles by strong absorption at characteristic absorption of HP at 400 nm. The authors contended that the mechanism for cellular uptake consists of NP endocytosis pathway and translocation to the late endosome where the cargo gets hydrolyzed and the effect is further boosted due to laser photolysis leading to endosomal degradation and release of CPT. Overall, this study utilizes clever chemistry alongside with nanoparticle approach to cross the endosome compartment through pH and ROS generation at visible range as two dominant factors. Figure 3 summarizes the role of CPI as a viable method to cross the endosome barrier.

Fusogenic Peptide Escape: Some synthetic peptides containing fusogenic peptides (such as GALA99 or KALA sequences) are also capable of enhancing endosomal escape. At physiological pH, these peptides coil as they are rich in anionic carboxyls, while they form α-helix secondary structure upon protonation at a lower pH, such as inside the endosome. This α-helix secondary structure can interact with and destabilize the lipid bilayer, leading to endosomal escape [43–45].

Cell-Penetrating Peptides: Another strategy for endosomal escape is the use of cell-penetrating peptides (CPPs) which facilitate the translocation of cargo along the membrane and make the direct release of the drug into the cytosol feasible [46, 47]. Despite being studied extensively, their mechanism of traversing the membrane remains highly elusive. Studies suggest that the interaction of CPPs'

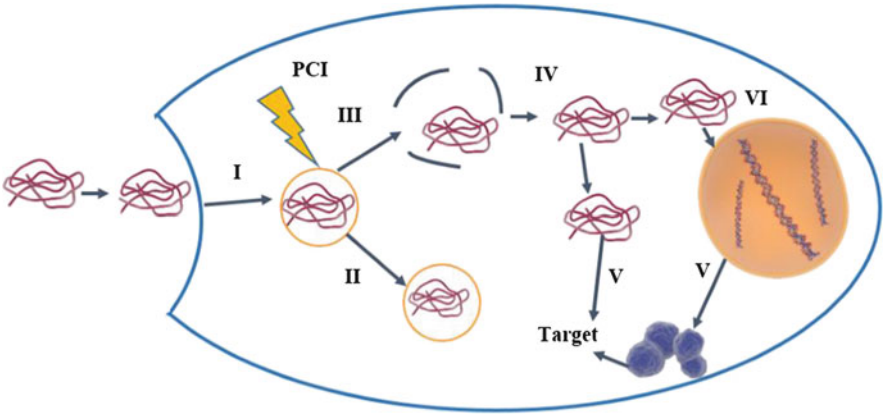

Fig. 3 Photochemical internalization pathway. (I) Endocytosis, (II) light exposure, and singlet oxygen generation (III) rupture of vesicular membrane due to oxidative damage (IV) release of the payload into the cytosol which can be either targeted to (V) cytoplasm or (VI) nucleus leading to (V) transgene activation. Alternative route is (II) hydrolytic degradation by endosome and lysosome

cationic lipid region with phospholipid membrane and conformational changes can facilitate the lipid head insertion, while other studies refer to endocytosis as the dominant mode of internalization [48–50]. The current scientific understanding is that CPPs induce various types of endocytosis using some of their physicochemical properties, such as molecular length, charge delocalization, and hydrophobicity. CPPs have gained much attention recently and are currently being investigated in preclinical studies, where they have shown to be successful for helping to address a wide variety of conditions [51]. It should be acknowledged that the low specificity of CPP is the main limiting factor in their application. This low specificity has been remedied through conjugation with other more specific ligands. Furthermore, to boost their efficacy and ameliorate the cytotoxic effects, modification with fatty acids (such as cholesterol, cysteamine, CPP-like ligands, and various guanidine-rich transporters) has been investigated [45]. For example, the TAT peptides derived from HIV1 have the ability to penetrate the cell membrane and deliver cargoes into the cytoplasm without endosomal or lysosomal degradation. TAT proteins have been effective at delivering a variety of molecular cargoes, including proteins with a mass greater than 100 kDa, 40 nm nanoparticles, and 200 nm liposomes [52, 53]. TAT proteins also have the potential to generate pores in model membranes. Giant unilamellar vesicles (GUVs) were constructed as model membranes that were made of only phophatidylcholine (PC), PC and phosphatidylserine (anionic) (PS), or PC and phosphatidylethanolamine (cationic) (PE). Each membrane also contained cholesterol to better mimic physiological membranes. TAT was effectively able to translocate across both the PC/PS and PC/PE membranes, but not the PC alone. Each membrane had a different interaction with TAT based on the charges present in the membrane. In PC/PS GUVs, these interactions

would cause the GUVs to deform after 20–30 min, and they would eventually rupture, releasing their contents. In PC/PE GUVs, these interactions were only seen at 20% and 30% PE after 30 min, but not at 10%. The GUVs never burst when the membrane composition was PC/PE. This study showed that TAT peptides accumulated on anionic membranes and were very rapidly internalized by the GUVs. It was also observed that these peptides were able to translocate across membranes containing lipids that induce negative curvature to the membrane such as PE [54].

2.3 pH-Sensitive Liposomes

pH-sensitive liposomes are designed to be endocytosed, but facilitate lysosomal escape of their drug cargo upon acidification during endosomal maturation. The exact mechanism for drug lysosomal escape to the cytoplasm via pH-sensitive liposomes is unknown, but theories include liposome-facilitated destabilization of the lysosomal membrane, passive diffusion across the lysosomal membrane, and pH-triggered fusion of liposomal and lysosomal membranes [55]. As a recent example, Turk et al. [56] developed folate-targeted liposomes incorporating pH-sensitive peptides. The peptide was designed with specific arrangement of hydrophobic and hydrophilic amino acid residues to disrupt the liposomal membrane at lower peptide concentrations than previously used peptides. At neutral pH, the peptides are in a mostly random coil conformation; upon acidification to pH values of around 5, the peptides adopt an amphipathic alpha helical structure. This structural change allows the peptides to insert themselves into membranes in a cooperative, self-aggregating manner, inducing permeabilization of the liposomal and subsequently lysosomal membranes. When loaded within these pH-sensitive liposomes, cytosine arabinoside showed a 30-fold increase in potency compared to the free drug [56].

2.4 Intracellular Targeting

In delivering drug within the cell, the cytoplasm acts as an additional barrier which the drug must overcome. This barrier presents itself in two ways. The first is the degradation that may occur as a particle passes through the cytoplasm, and the second is the route that has to be taken to get from one place to another in the cytoplasm.

Cells use the ubiquitin proteasome pathway to degrade proteins in the cytoplasm. This can pose an issue in drug delivery if proteins in the drug delivery particle are marked for degradation. This can degrade all or part of the drug, rendering it ineffective, or it can destroy the nanoparticle, leading to the premature release of the drug before it has reached its final target.

The transport of a drug can also be inhibited by the drug carrier particle's size. Moving through the cytoplasm is only possible passively with smaller particles. This is due to the high density of organelles and macromolecular crowding in the cytoplasm. Larger particles must interact with molecules in order to form a cytoplasmic sieve. This allows the larger molecules to pass through the cytoplasm [57].

Recent research has shown there are ways to avoid hindrance in the transport of drug through the cytoplasm. PEGylation of nanoparticles has been found to decrease the number of particles that are hindered in their transport through the cytoplasm. It has been shown that PEGylation doubles the diffusion rate across the cytoplasm and decreases the amount of hindered particles from 79.2% to 48.8%. It is believed that PEGylation reduces nonspecific adhesion to the cytoskeleton, allowing the nanoparticle to move freely within the cytosol [58].

As mentioned previously, intracellular targeting poses multiple challenges, which can open access to the vast number of highly significant targeting moieties once overcome. Nanomedicine targeting inside human cells has focused on inhibiting or causing a change to natural biochemical reactions contained in organelles or directly within the cytoplasm. The nucleus, mitochondria, lysosome, endoplasmic reticulum, and the Golgi apparatus are popular organelles to study because of the high traffic of cellularly dependent reactions.

The mitochondria serve as the cell's power plant, providing the necessary Adenosine triphosphate (ATP) for many enzymatic reactions and active transport. This double-membrane enveloped organelle is believed to have originated as an extracellular organism which forms a symbiotic relationship with prokaryotes and eukaryotes, thus explaining the existence of its own internal genome.

In addition to ATP synthesis, the mitochondria also play a role in calcium homeostasis regulation and initiation of programmed cell death [59]. Intramitochondrial issues are considered markers for cancer, Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis, thus highlighting the importance of accessing mitochondrial processes for therapeutic nanomedicine [60–63].

The transport proteins transporter inner membrane (TIM) and transporter outer membrane (TOM) provide access to the inner and outer mitochondrial membranes, respectively, and have become attractive ports for drug delivery. Size limitations of these beta-barrel porin-like transport proteins have been reported to restrict passage to molecules smaller than 6 kDa. Once inside the intermembrane space, multiple targeting moieties are open for interaction, for instance, the ATP synthesis factory electron transport chain (ETC). This highly negative system of proteins built into the inner membrane attracts positively charged molecules such as triphenylphosphonium (TPP), dequalinium, or the fluorescent dye rhodamine [52, 64]. Additionally, the protein cytochrome C becomes accessible. As cytochrome C is a crucial component in delivering electrons to the final hydrogen pump, it is directly involved in the apoptosis pathway.

Current commercially available mitochondrial targeting drugs include lonidamine, alpha tocopheryl succinate for cancer, curcumin for Alzheimer's, and Dinitrophenol (DNP) for obesity [4, 7]. Potential future treatments can involve the

mitochondrial delivery of antioxidants, proapoptotic factors, drugs, proteins, and nucleic acids [64].

Nuclear Delivery: The nucleus holds the cell's genetic information necessary for protein building which in turn determines the cell function and fate. Targeting this organelle with gene delivery, drugs, or various activators and inhibitors can lead to a multitude of induced therapeutic processes which can be utilized to combat gene-related illnesses. The nucleus is also considered to be one of the most challenging yet significant subcellular organelle targets in nanomedicine. Once a drug or other nanomedicine substance is inside the cell, the next barrier to overcome is the double-membrane nuclear envelope which separates DNA from the cytosol. A well-known strategy for targeting a cell's DNA involves precise timing of cell stage development and delivery. Specifically, the mitotic phase of the cell cycle is where the nuclear envelope breaks down and leaves DNA accessible to cytosolic payloads [65]. Another common approach to cross the nuclear envelope is via the nuclear pore complex (NPC), a receptor-mediated transport protein for RNA and ribosomal proteins as well as a passive diffusion port for the small molecules. The passive diffusion properties have been investigated, and it has been reported that using the amphipathic alcohol trans-cyclohexane-1,2-diol (TCHD) results in pore dilation, effectively increasing the nucleus's passive diffusion ability [66–69].

The specific ligand studied for NPC active transport is a chain of consecutive lysines (or PKKKRKV), also known as the nuclear localization sequence (NLS) which has been taken advantage of and labeled across plasmid DNA and nanoparticles [70]. Karyopherin-beta-mediated transport is an additional method that works as an NLS for different proteins [71]. However, the limitations for transport across the NPC have been reported as 60 kDa (10 nm) [68, 72]. A final approach for crossing the nuclear envelope is through passive diffusion across the lipid membrane, which is governed by the same laws as the main cellular membrane, diffusible only to small molecules and ions [73, 74].

Golgi Apparatus: The Golgi body and the endoplasmic reticulum (ER) are also of great interest for researchers pursuing subcellular-targeted nanoparticles. The Golgi body is associated with Alzheimer's, Parkinson's, and several other lethal congenital diseases. Malfunctions in Golgi body have been linked to prostate cancer. ER mutations have also played a role in diabetes insipidus, chronic pancreatitis, and cancer. Work has begun to target the mTOR pathway, which plays a crucial role in cancer cell growth and which exists mainly in the ER and Golgi body [75]. Viruses have been used to target the nucleus, as well as the ER and the Golgi apparatus. Specifically, the Simian vacuolating virus 40 is particularly adept at targeting these organelles. However, as with all viral-mediated delivery, there is a high risk of toxicity and immune reaction. Nanoparticles outfitted with some of the same sequences and peptides that allow for viral targeting could be very useful in avoiding this immune response but retaining organelle specificity [25].

3 Conclusion

In this review, we have highlighted hurdles in crossing the cellular membrane, endosomal escape, and intracellular targeting. Although nanomedicine and extra-/intracellular targeting have been studied for over a decade, these hurdles have historically been the limiting factor on nanomedicine achieving clinical implementation. One of the primary hurdles to overcome is improving and optimizing internalization and endosomal escape which is a crucial step before any payload delivery or organelle targeting takes place. Once such a structure is designed, the modalities involved in nanomedicine delivery should be perfected. This includes studying the travel mechanics of the payload within microfluidic-like environments and product interactions with endothelial lining on a three-dimensional plane. Extracellular matrix (ECM) gels and microfluidic platforms are excellent tools for this type of investigation. Once localization within the body is well established, the next significant challenge is the simultaneous optimization of both extracellular and intracellular targeting techniques. Biomimicry of viruses is a great modality to improve the design of synthetic subcellular targeting systems, essentially using a virus as a guide in the design of a nanoparticle. Similarly, surface treatments such as PEGylation may address some of these concerns mentioned, which may otherwise inhibit favorable cellular interactions. Depending on molecular weight, polarity, and surface charge of the nanoparticles, some membrane penetrating routes may be preferred over others.

Once inside the cell, the next challenge is decreasing cytotoxicity while improving therapeutic efficacy, or overcoming additional membranes for organelle-specific targeting. Image capable ligands can also be incorporated in nanomedicine to allow for the visualization of drug transport and action. Future research on the horizon includes the physicochemical characterizations and bioproducts of nanoparticles and clinical determinants in the human body. Despite major advances, there is still significant work ahead to be done, but the progress does not seem to be slowing down and instead is increasing its speed of innovation within the realm of therapeutic nanomedicine.

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