Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Nano Today 40 (2021) 101279

Contents lists available at ScienceDirect

Nano Today

journal homepage: www.elsevier.com/locate/nanotoday

Review

Endocytosis of abiotic nanomaterials and nanobiovectors: Inhibition of membrane trafficking

Pooyan Makvandi[a,*,1], Meiling Chen[b,1], Rossella Sartorius[c,1], Ali Zarrabi[d], Milad Ashrafizadeh[d,e], Farnaz Dabbagh Moghaddam[f], Jingzhi Ma[b], Virgilio Mattoli[a], Franklin R. Tay[g,**]

[a] Istituto Italiano di Tecnologia, Centre for Materials Interfaces, Viale Rinaldo Piaggio 34, 56025 Pontedera, Pisa, Italy
[b] Department of Stomatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[c] Institute of Biochemistry and Cell Biology (IBBC), National Research Council (CNR), Naples 80131, Italy
[d] Sabanci University Nanotechnology Research and Application Center (SUNUM), Tuzla, Istanbul 34956, Turkey
[e] Faculty of Engineering and Natural Sciences, Sabanci University, Orta Mahalle, Üniversite Caddesi No. 27, Orhanlı, Tuzla, 34956 Istanbul, Turkey
[f] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran 1477893855, Iran
[g] The Graduate School, Augusta University, Augusta, GA 30912, United States

ARTICLE INFO

Article history:
Received 10 December 2020
Received in revised form 5 August 2021
Accepted 19 August 2021
Available online 8 September 2021

Keywords:
Cell internalization mechanisms
COVID-19
Nanoparticles
Metal-based nanomaterials
SARS-CoV-2
Viruses

ABSTRACT

Humans are exposed to nanoscopical nanobiovectors (e.g. coronavirus SARS-CoV-2) as well as abiotic metal/carbon-based nanomaterials that enter cells serendipitously or intentionally. Understanding the interactions of cell membranes with these abiotic and biotic nanostructures will facilitate scientists to design better functional nanomaterials for biomedical applications. Such knowledge will also provide important clues for the control of viral infections and the treatment of virus-induced infectious diseases. In the present review, the mechanisms of endocytosis are reviewed in the context of how nanomaterials are uptaken into cells. This is followed by a detailed discussion of the attributes of man-made nanomaterials (e.g. size, shape, surface functional groups and elasticity) that affect endocytosis, as well as the different human cell types that participate in the endocytosis of nanomaterials. Readers are then introduced to the concept of viruses as nature-derived nanoparticles. The mechanisms in which different classes of viruses interact with various cell types to gain entry into the human body are reviewed with examples published over the last five years. These basic tenets will enable the avid reader to design advanced drug delivery and gene transfer nanoplatforms that harness the knowledge acquired from endocytosis to improve their biomedical efficacy. The review winds up with a discussion on the hurdles to be addressed in mimicking the natural mechanisms of endocytosis in nanomaterials design.

Contents

* Correspondence to: Istituto Italiano di Tecnologia, Centre for Micro-BioRobotics, Viale Rinaldo Piaggio 34, 56025 Pontedera, Pisa, Italy.
** Corresponding author.
E-mail addresses: Pooyan.makvandi@iit.it (P. Makvandi), ftay@augusta.edu (F.R. Tay).
[1] Equal contributors to this work.

https://doi.org/10.1016/j.nantod.2021.101279
1748-0132/

Introduction

Nanomaterials have been used in nanomedicine as diagnostics and drug carriers [1]. They are recognized by cells via specific receptors present on the cell membrane and are internalized through endocytosis [2]. The dimensions of nanomaterials are roughly equivalent to the intracellular organelles. The ability of nanomaterials to interact directly with cells makes them unique tools for influencing biological pathways and processes. Interactions between living organisms and nanomaterials affect cell physiology and trigger both positive and negative reactions [3]. As the interaction between nanomaterials and cells becomes better understood, new materials are designed for specific cell-material interactions. The plethora of natural and synthetic nanomaterials and their intricate interactions with different cell types justify preparing a compendium on the information accumulated over the past five years to create a backdrop for interested parties to continue the legacy in this exciting research arena.

The intracellular milieu contains compounds for cell growth, proliferation, differentiation and death that are distributed throughout the cytoplasm, nucleus, mitochondria, endoplasmic reticulum and Golgi complex [4]. Endocytosis opens a window of opportunity for communication of materials, energy and information between the inside and outside of cells; such a process provides the essential link for life and physiological activities [5,6]. The size, charge and surface composition of nanomaterials determine their internalization pathways. The low pH and enzyme-rich intracellular environment as well as the presence of lysosomes may result in degradation or non-specific distribution of nanomaterials [7,8]. The ability of nanomaterials to overcome these obstacles has led to the development of platforms to improve their bioavailability [9]. Nanomaterials may be optimized by adjusting their physicochemical properties [10]. Understanding the mechanisms involved in cellular uptake is also critical for evaluating the fate and toxicity of nanomaterials.

Many infectious diseases in humans are caused by biovectors such as viruses and bacteria that enter cells for replication. Pathogens have evolved efficient strategies to be internalized into host cells, as exemplified by the entry of the coronavirus SARS-CoV-2 into pneumocytes that accounts for the COVID-19 pandemic [11,12]. As natural nanoscale biovectors, viruses typically range from 10 to 300 nm in dimensions and are the blueprints for the synthesis of many bioengineered nanomaterials [13]. Viruses are highly variable in shape and are coated with ligands. Most non-enveloped and enveloped viruses enter host cells by protein-mediated endocytosis [14,15]. Virus entry is a highly active process involving a series of biochemical signaling pathways [16]. The efficacy and specificity of viral interaction with host cells have stimulated enormous research to uncover the physical and biochemical mechanisms exploited by viruses to enter cells. These strategies may be adopted for designing nanomaterials for disease targeting. Answering fundamental questions on cellular intake of viruses will pave the way for a more efficacious design of biomimetic nanomaterials [17]. Similar to viruses, bacteria have surface ligands that induce specific intracellular signaling cascades [18]. The recent discovery that bacteria can access cells via clathrin-mediated pathways suggests that endocytosis plays an important role in the entry of these biovectors into cells [19]. Participation of bacteria in endocytosis will not be covered in the present review because they are too large to be classified as nanoscale objects.

Intracellular uptake and transport pathways of nanomaterials will be summarized in the present review. The properties of nanomaterials such as size, shape, charge and surface chemistry, as well as the effects of the intracellular microenvironment on nanomaterial internalization, will be discussed. Understanding the physicochemical properties of nanomaterials and their cellular absorption mechanisms will facilitate scientists to design better functional nanomaterials for biomedical applications. Such knowledge will also provide important clues for the control of viral infections and the treatment of virus-induced infectious diseases.

Serendipitous and intentional human encounter with nanomaterials

Humans are exposed to an extensive array of nanoscopical biovectors as well as metal-based and carbon-based nanomaterials that enter the internal milieu of cells unintentionally. Nanomaterials that are designed for targeting specific tissues such as tumors may accumulate in non-cancerous cells, resulting in undesirable cytotoxicity. Because nanomaterials are much smaller in size compared to cells, they can penetrate human cells by inhalation, ingestion or skin absorption during human activity. Such untoward uptakes often

result in unanticipated interaction of the biovectors and nanomaterials with cellular proteins and intracellular macromolecules [20].

Apart from serendipitous entries, humans also uptake nanomaterials intentionally, often in the form of bioengineered entitles that are developed for medical diagnosis and therapy. Some of these nanomaterials are used as carriers for drugs and biomolecules to improve their release kinetics [21]. These carriers protect and stabilize their payloads and enhance therapeutic bioavailability for two purposes: tuning the rate of delivery and precision delivery of the therapeutic agent to a destined site within the human body. For example, polymeric nanomaterials such as poly(lactic-co-glycolic acid) and ceramic-based nanomaterials such as mesoporous silica have been tuned to load and release biomolecules at a designer-optimized rate to a targeted site. Apart from solely functioning as carriers, some nanomaterials are also utilized as diagnostic/therapeutic agents themselves. The most well-known examples of these functional nanomaterials are silver nanoplatforms, which are used as antimicrobial materials [22], and gold nanoplatforms, which are used as biomarkers and anti-cancer photothermal agents [23]. Nanomaterials may also be conjugated with pharmaceutical compounds to enhance their therapeutic efficacy via synergistic interactions. For example, polyethylene glycol-stabilized gold nanorods containing doxorubicin are used as photothermal agents and as carriers for the delivery of the anti-cancer drug. The aforementioned nanosystems are intentionally introduced into human body via the bloodstream along the trajectory that they are exposed to and interact with different types of cell proteins [24].

An in-depth understanding of the mechanisms of endocytosis enables the development of strategies for inhibiting the undesirable effects of serendipitous cell entry, as well as for minimizing the cytotoxicity of intentionally administered nanosystems to healthy cells and optimizing efficacious delivery of the loaded biomolecules to the cytosol of targeted cells.

Endocytosis mechanisms for nanomaterials

Functional and nonfunctional nanomaterials find their way into cells via endocytosis. This method of intracellular ingress involves the formation of phagocytic membrane invaginations and intracellular vesicles [25,26]. Endocytosis may be divided into pinocytosis and phagocytosis. Pinocytosis is the major route of uptake for smaller particles and phagocytosis is the preferred route for the ingress of larger particles (> 500 nm). Pinocytosis is subdivided into clathrin-mediated endocytosis, caveolae-mediated endocytosis, as well as clathrin-and-caveolae-independent endocytosis [27]. The endocytosis pathways for the intake of nanomaterials are summarized in Fig. 1a.

Clathrin-mediated endocytosis

Clathrin-mediated endocytosis (CME) is a vesicular transport event that facilitates internalization and recycling of receptors engaged in processes such as signal transduction, nutrient uptake and synaptic vesicle formation. This is the canonical internalization process for most nanomaterials in non-macrophages. The pathway enables intracellular absorption of plasma membrane components and nutrients such as cholesterol through lipoprotein receptors, and iron through transferrin receptors [28]. According to the interaction during internalization, CME may be divided into non-specific adsorption and receptor-mediated absorption. In non-specific receptor-independent CME, absorption occurs via hydrophobic or electrostatic interactions, eventually resulting in internalization [29]. In contrast, receptor-mediated CME is initiated only after a specific macromolecule (ligand) or ligand-encased nanomaterial binds to a receptor on the surface of the cell membrane.

Non-specific CME adsorption is triggered by binding of cationic particles or proteins to the negatively-charged cell surface. Unlike adsorption, receptor-mediated CME is highly-selective and specific [25]. The process involves the polymerization of clathrin, a triskelion-shaped scaffold protein, around the cytoplasmic side of an invaginated pit [30]. The polymerized clathrin coat functions as a reinforced mold in which the membrane vesicle eventually forms and enlarges (Fig. 1b). Initiation of a clathrin complex requires the accumulation of phosphatidylinositol-4,5–bisphosphate (PIP2) and adapter proteins such as AP-2 at the pinching site [31,32]. After the coat is assembled, the actin filament network polymerizes at the endocytosis site to form an actin module [33].

Contraction and scission of the invaginated necks of the clathrin-coated vesicles are mediated by BAR domain (Bin, amphiphysin and Rvs) proteins, dynamins and dephosphorylation of PIP2 [34]. The vesicles are then transported and sorted, based on the receptor type or membrane composition, to various intracellular destinations such as the trans-Golgi network, endosomes or vacuoles. Dynamins, a family of membrane fission guanosine-5′-triphosphatases (GTPases), bind to and assemble into a helical polymer around the neck of a clathrin-coated vesicle. Constriction of the dynamin helix around the vesicle neck via GTP hydrolysis results in the formation of a hemi-fission membrane state that ultimately results in membrane scission [35].

Clathrin-mediated endocytosis, which transports a large number of different cargoes from the plasma membrane into the cell, plays an important role in maintaining cell membrane homeostasis and regulating intercellular signaling. The protein components of clathrin coatings bind to specific binding sites in the cytosolic parts of different transmembrane cargo molecules and recruit them to the region of the plasma membrane to form vesicles. This results in specific cargo enrichment within the forming vesicles. Within the vesicles, cargo-adapter interactions occur via ubiquitination or phosphorylation of the cargo proteins [36]. Receptor ligands that are internalized through receptor-mediated CME include low-density lipoprotein, transferrin, growth factor, G-protein, tyrosine kinase receptors and insulin [36]. Nanomaterials such as Ag and TiO_2 nanoparticles are also internalized by the endocytic pathways mediated by clathrin [37,38].

Caveolae-mediated endocytosis

This is a clathrin-independent, receptor-mediated endocytosis pathway that involves the participation of 50–60 nm diameter, bulb-shaped plasma membrane invaginations known as caveolae (little caves) [39]. Caveolae are abundant in endothelial cells, adipocytes, fibroblasts and muscle cells. They are a subset of caveolin-containing lipid rafts, the latter being dynamic cholesterol- and sphingolipid-rich plasma membrane microdomains in which cellular processes such as endocytosis and signal transduction are compartmentalized [40].

Formation of caveolae is driven by integral membrane proteins known as caveolins as well as peripheral membrane proteins known as cavins (Fig. 2a) [41]. Caveolins such as caveolin-1 are proteins essential for the biogenesis of caveolae [42]. Cavins form homo- or hetero-oligomers with each other and account for membrane curvature [43]. The caveolae endocytic machinery also include proteins such as dynamins that are necessary for vesicle scission [44].

Caveolae-mediated endocytosis is involved in cellular signaling and regulation of membrane proteins, lipids and fatty acids [45]. Caveolae are dynamic endocytic carriers; they bud off from the plasma membrane during caveolae-mediated endocytosis [41]. Caveolae budding is mediated by dynamin while Epsl5-homology domain-containing protein 2 (EHD2), a dynamin-related ATPase, negatively regulates caveolar endocytosis [46]. Endocytosis commences with tyrosine phosphorylation of the associated protein,

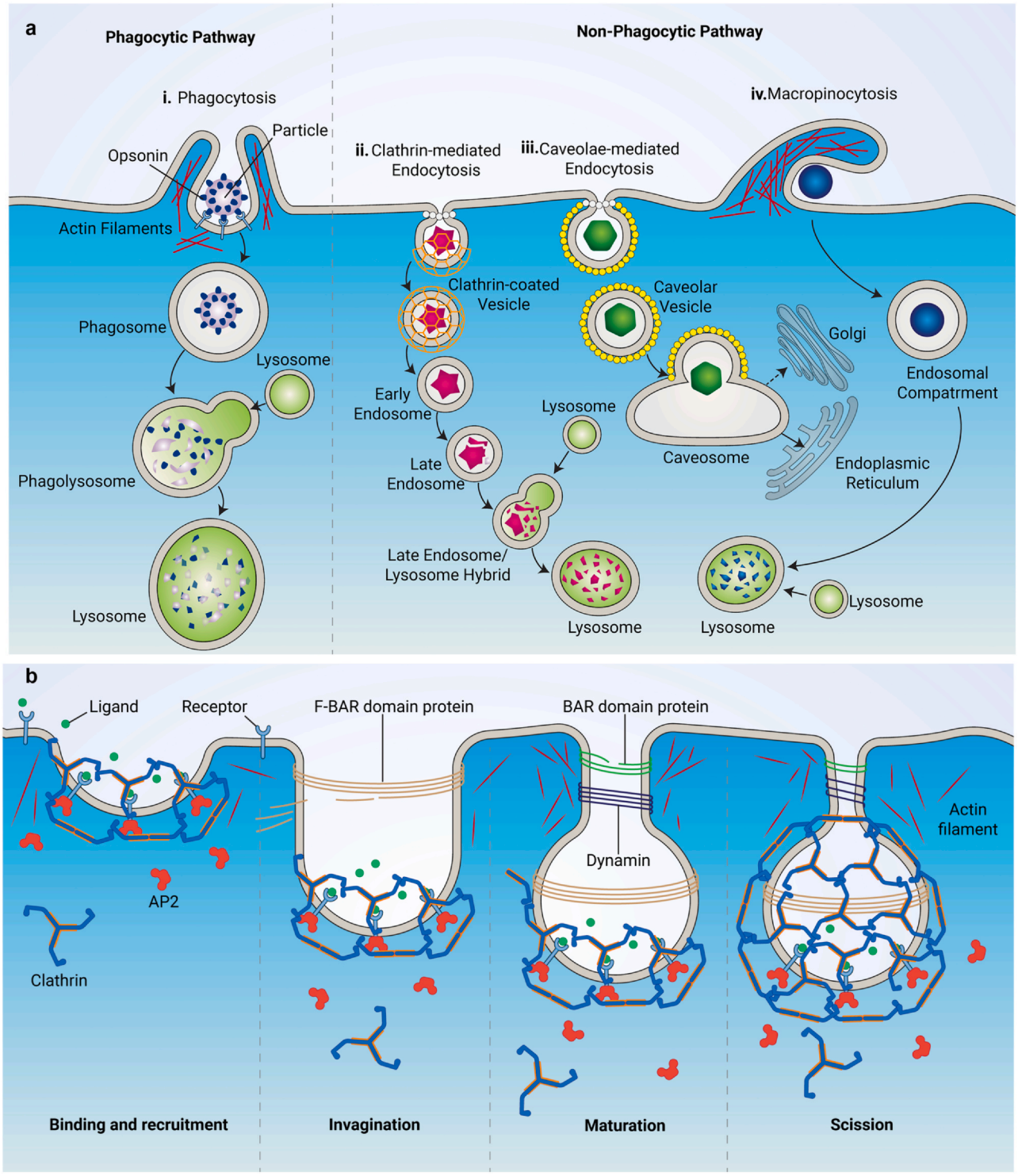

Fig. 1. a. Overview of phagocytic and non-phagocytic pathways. i) *Phagocytosis* occurs through an actin-based mechanism that involves interaction with specific cell surface receptors. Opsonin-coated nanoparticles are internalized into phagosomes. Fusion of phagosomes with lysosomes produces phagolysosomes, within which foreign particles such as bacteria are degraded by lysozymes (glycoside hydrolases). ii) *Clathrin-mediated endocytosis* involves the formation of vesicles from the clathrin-coated regions of the plasma membrane. The ingested material moves from the early endosome to the late endosome, and finally fuses with a lysosome to form a lysosome-endosome hybrid. These substances are subsequently degraded within the intravesicular low pH and enzyme-rich environment. iii) *Caveolae-mediated endocytosis* involves internalization by caveolin-enriched invaginations. The caveolar vesicle transfers its contents to an endosome, forming a caveosome that evades lysosomal fusion and digestion. The caveosome is transported along the cytoskeleton to the endoplasmic reticulum/Golgi complex. iv) *Macropinocytosis* is a pathway independent of clathrin and caveolin. A pseudopodium protrudes through the plasma membrane driven by actin filaments, forming a macropinosome. The contents are degraded after fusion with lysosomes (modified from [53] with permission from Springer), b. Different stages of clathrin-mediated endocytosis: binding and recruitment, invagination, maturation and scission (modified from Mechanobiology Institute, National University of Singapore under a Creative Commons Attribution-NonCommercial 4.0 International License; https://www.mechanobio.info/what-is-the-plasma-membrane/what-is-membrane-trafficking/what-is-clathrin-mediated-endocytosis/invagination-and-maturation-of-the-clathrin-coated-vesicle/).

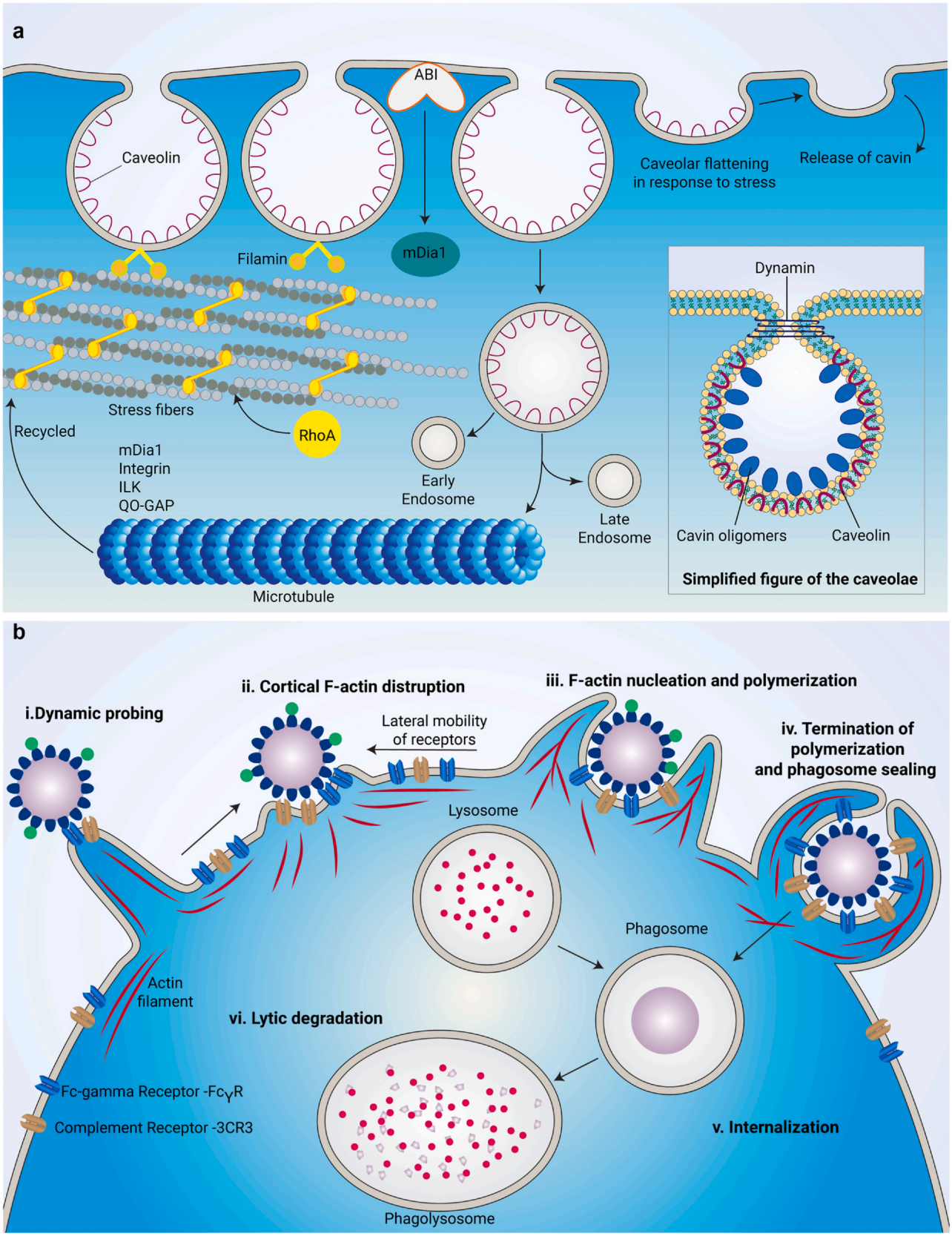

Fig. 2. a. Details of caveolae-mediated endocytosis. Caveolae bud off the plasma membrane. Some of the caveolae attempt to fuse with the plasma membrane, but most reach the early endosome before recycling back to the plasma membrane. Caveosomes are late endosomes modified by overexpression of caveolin. The cell cytoskeleton plays an important role in caveolar organization and trafficking. Actin stress fibers affect the linear distribution of many types of caveolae in the plasma membrane. The actin binding protein filamin A also plays a key role in trafficking of caveolae connected to the actin. Microtubules stabilize the vesicles locally (modified from Mechanobiology Institute, National University of Singapore under a Creative Commons Attribution-NonCommercial 4.0 International License; https://www.mechanobio.info/what-is-the-plasma-membrane/what-is-membrane-trafficking/what-is-caveolar-endocytosis/#what-is-caveolar-endocytosis). b. Different stages of phagocytosis from nanoparticle detection to the formation of a phagolysosome (modified from Mechanobiology Institute, National University of Singapore under a Creative Commons Attribution-NonCommercial 4.0 International License; https://www.mechanobio.info/what-is-the-plasma-membrane/what-is-membrane-trafficking/what-is-phagocytosis/).

which causes depolymerization and mobilization of actin. The cytoskeleton plays an important role in the organization and trafficking of caveolae. Actin stress fibers affect the linear distribution of caveolae along the plasma membrane [47]. Regulation of actin and microtubular components of the cytoskeleton is crucial for the internalization and circulation of caveolae [48].

Caveolins play a vital role in triggering the invagination of plasma membrane. Substance internalized via caveolae-mediated endocytosis is susceptible to opportunistic escape via lysosomal degradation. Such a property has been harnessed for gene or protein delivery [49]. Caveolin vesicles fuse to form multi-caveolar structures called caveosomes. Caveosomes are capable of evading lysosomes, thereby protecting the contents from lysosomal degradation. Such a mechanism is utilized by viruses to prevent degradation by lysosomal enzymes [50,51].

Clathrin-and-caveolae-independent endocytosis

Clathrin-and-caveolae-independent endocytosis (CIE) is mediated by flotillins, ADP-ribosylation factor 6 (Arf6), endophilins or tubular structures known as clathrin-independent vectors. Depending on the effectors, CIE pathways are presently classified as flotillin-dependent, Arf6-dependent, RhoA-dependent, Cdc42-dependent, clathrin-independent carrier/glycosylphosphatidylinositol-anchored protein (GPI-AP)-enriched early endosomal compartments (CLIC/GEEC) endocytic pathway and micropinocytosis [52]. These pathways appear to require specific lipid compositions and are dependent on cholesterol. The CIE pathways occur nonspecifically without the need for receptors. They may be further divided into dynamin-independent and dynamin-dependent pathways, with most of the pathways being dynamin-independent.

The CIE pathway is utilized by extracellular fluid, growth hormones, interleukin-2 and GPI-APs to enter cells. The mechanisms involved in CIE pathways are still under intense debate. Except for the CLIC/GEEG pathway and macropinocytosis, other CIE pathways do not contribute significantly to the intracellular uptake of nanoparticles and biovectors and will not be elaborated further.

The CLIC/GEEC pathway is mediated by uncoated tubulovesicular primary carriers known as clathrin-independent carriers (CLICs). These carriers are derived from the plasma membrane and subsequently mature into tubular early endocytic compartments known as GPI-AP enriched early endosomal compartments (GEECs). The GEECs are routed to the endosomes for fusion with lysosomes. This pathway is responsible for the uptake of fluid, transmembrane proteins, toxin subunits as well as some types of viruses [41,53].

Macropinocytosis is a type of clathrin-and caveolae-independent pinocytosis in which cells take in a large volume of extracellular fluid by forming a 0.5–10 µm diameter vesicle known as the macropinosome [53]. The macropinocytosis pathway can internalize micron-sized nanomaterials which cannot be uptaken by other pathways. Nanomaterials in the extracellular fluids are encapsulated inside macropinosomes via nonspecific extracellular fluid uptake [53].

Phagocytosis

Phagocytosis is a complex process responsible for the elimination of pathogens and apoptotic cells. This process is the basis of tissue homeostasis [54]. Phagocytosis may be divided into four stages: identification of target particles, signaling that activates internalization mechanisms, phagosome formation and phagolysosome maturation [55]. Phagocytosis involves the recognition and uptake of particles larger than 0.5 µm into plasma membrane-derived vesicles known as phagosomes. The process requires the participation of the cytoskeleton in membrane rearrangement [56]. Specialized phagocytes of the immune system, including neutrophils, macrophages, monocytes and dendritic cells, utilize this process to internalize invading organisms, pathogens, dead cells and debris [57]. Other cells such as endothelial cells, epithelial cells and fibroblasts also undergo phagocytosis.

Phagocytes have to recognize a large number of different foreign particles. Receptors present on the plasma membrane of these phagocytes may be divided into non-opsonic receptors and opsonic receptors [58]. Non-opsonic receptors directly recognize molecular groups on the surface of phagocytic targets. Opsonic receptors recognize host-derived opsonins, which bind to foreign particles such as bacteria, enabling them to be recognized. After identifying the target particles, the receptors activate a signaling cascade to reshape the membrane lipids and regulate the actin cytoskeleton to expand the peripheral membrane to surround the particles. Membrane remodeling and rearrangement of the actin cytoskeleton result in the formation of pseudopodia. After surrounding the target particle, the pseudopodia close at the end to produce a phagosome. In the final stage of phagocytosis, the phagosome fuses with a lysosome and becomes a phagolysosome. The ingested particle is digested by hydrolases derived from the lysosome. Phagosome maturation involves continuous fusion and fission interactions between the early endosomes (*aka* sorting endosomes), late endosomes and finally, the lysosomes (Fig. 2b). The phagolysosome contains a highly acidic degradation environment with complex mechanisms for the direct elimination and degradation of microorganisms [59].

Both abiotic nanomaterials and nanobiovectors penetrate cells through endocytic pathways. Despite the similarity of these materials and biovectors in utilizing endocytosis for cell entry, there are subtle differences among them. The majority of nanoparticles, owing to their positive charge, can interact with negatively-charged cell membranes to penetrate into cells through invaginations and engulfments. Penetration of nanostructures into cells through receptor-mediated endocytosis occurs after their surfaces are modified by specific ligands. However, viruses penetrate cells predominantly through receptor-mediated endocytosis, by binding to specific proteins on the cell membrane. Furthermore, viruses can use uncommon forms of endocytosis that are dependent on actin cytoskeleton reorganization.

Cells and the surrounding microenvironment

Diverse cell types

Cellular uptake of nanomaterials involves highly-regulated mechanisms via the interaction of complex biomolecules with ligands present on the nanomaterial surface. It is important to understand how nanomaterials enter cells because the uptake pathways involved determine their intracellular fate as well as the biological responses of the cells to their ingress [60]. Nanomaterials circulating in the bloodstream are internalized into many types of cells. The plasma membrane is a selectively-permeable membrane used for transporting substances that are necessary to sustain cell life. Substances obligatory for cell survival such as ions and proteins pass through the lipid bilayer through specific membrane transporter channels. The plasma membrane of a cell chooses its endocytosis pathway according to the size, shape and surface chemistry of the nanomaterials. Examples of nanomaterial ingress into specific cell types will be elaborated below. Table 1 represents examples of cell lines that are affected by endocytosis pathways.

Fibroblasts

A quantitative model has been developed to correlate the rate of endocytosis with the geometry of nanomaterials. The model suggests that nanomaterials aggregate on the cell membrane to reach a size that generates large enough enthalpy through receptor-ligand interaction to overcome the elastic energy and entropy barriers associated with vesicle formation. The uptake mechanism of gold

Table 1
Endocytosis pathways for different cell lines.

Cell line	Nanomaterials	Size (nm)	Geometry	Functional groups	Endocytosis pathway	Ref.
Human cervical carcinoma cells HeLa	Lipid nanoparticles	60	Spherical	Alexa647 (including sulfate and phosphate functional groups) and Gold	Macropinocytosis	[82]
Mouse embryonic fibroblast cells NIH3T3					Clathrin-mediated endocytosis	
Murine macrophage cell line J774A.1	Polystyrene nanoparticles	40	Spherical	Carboxylic acid	Clathrin-mediated endocytosis; phagocytosis	[83]
Human type II cell alveolar epithelial cell line A549					Clathrin-mediated and caveolae-mediated endocytosis	
Bovine oviductal epithelial cells/Human colon fibroblasts BOEC/HCF	Polystyrene nanoparticles	45	Spherical	carboxylic acid	ATP-independent endocytosis	[84]
Human renal cortical epithelial HRCE					Multiple mechanisms of ATP-dependent and ATP-independent internalization pathways	[85]
Human hepatocellular carcinoma cells HepG2/C3A/Primary IRHCs	Carboxylated polystyrene nanoparticles	20	Spherical	carboxylic acid	Not through early endosomes	[86]
Human astrocytoma cells/Human type II cell alveolar epithelial cell line 1321N1/A549		40			Clathrin-mediated endocytosis	[87]
Human astrocytoma cells/Human type II cell alveolar epithelial cell line 1321N1/A549	Carboxylated polystyrene nanoparticles	200	Spherical	carboxylic acid	Clathrin-mediated endocytosis	[87]
Human cervical carcinoma cells HeLa		40 and 200			Clathrin-mediated endocytosis and macropinocytosis	
Human breast adenocarcinoma cell line/Normal human epithelial mammary cell line/Human cervical carcinoma cells MCF-7/MCF-10A/HeLa	Carboxylated quantum dots	15–20	Dot	Carboxylic acid and amine	Clathrin-mediated endocytosis	[88,89]
293T cells/Monkey kidney fibroblast-like cell line 293T/COS-7	Alginate–chitosan nanoparticles	160	Spherical	Amine functional groups	Clathrin-mediated endocytosis	[90]
Chinese hamster ovary cell line CHO					Caveolae-mediated endocytosis	
Human mesenchymal stem cells Porcine chondrocytes	Hyaluronic acid-coated gold nanorods	51 nm in diameter 12 nm in length	Rod	Thiol functional group	BMP-receptor mediated endocytosis	[91]
Mesenchymal stem cells	Citrate zinc hydroxyapatite nanorods	0.345 and 0.693 nm	Rod	Citrate functional group	Endocytosis	[92]
BxPC-3 and HPSCs cells	Cancer cell membrane-coated nanorods	120–130 nm	Rod	Cancer cell membrane based liposomes	Caveolin-mediated endocytosis	[93]
HUVECs, HMDMs, HeLa and RAW cells	Gold nanorods	70 nm in length 10 nm in width	Rod	PEG, poly(styrene sulfonic acid) (PSS), poly(allylamine hydrochloride) (PAH), silica, aminated silica	Macropinocytosis Clathrin-mediated and caveolae-mediated endocytosis	[94]
Brain vascular endothelial cells	PLA-PEG nanoparticles	100 nm	Diblock	PLA-PEG, 100 nm nanoparticles tagged with fluorescent pyrene butanol and coated with PEG chains	Macropinocytosis; clathrin-mediated endocytosis	[94]

nanoparticles by “Medical Research Council cell strain-5” fibroblasts was studied using endocytosis inhibitors. Fibroblasts treated with concanavalin A and chlorpromazine, inhibitors of clathrin-mediated endocytosis, demonstrated significant decrease in Au nanoparticle uptake. The results pointed to the involvement of this endocytic pathway in the uptake of Au nanoparticles [61].

Self-assembled glycol chitosan nanoparticles are a useful platform for drug delivery to targeted cell types. The mechanism of intracellular delivery of chitosan nanoparticles to human lung fibroblasts was examined in the presence of type I collagen matrix. The lung fibroblasts were pretreated with chlorpromazine to inhibit clathrin-mediated endocytosis, genistein to inhibit caveolae-mediated endocytosis and amiloride to inhibit macropinocytosis. Amiloride pretreatment significantly reduced the uptake of chitosan nanoparticles by the fibroblasts. The results indicated that intracellular uptake of chitosan nanoparticles by lung proceeded predominantly via macropinocytosis in response to a collagen-rich extracellular matrix. The chitosan nanoparticles were found to be useful as a drug delivery system for targeting fibrotic lung fibroblasts in the treatment of potentially fatal lung diseases such as idiopathic pulmonary fibrosis [62].

Dexamethasone, a glucocorticoid that inhibits inflammation, has been used extensively for the treatment of rheumatoid arthritis, osteoarthritis and patients with severe COVID-19 symptoms. Nevertheless, prolonged use of high-doses of dexamethasone produces severe adverse effects such as adrenal insufficiency, hyperglycemia and osteoporosis. To circumvent this problem, dexamethasone has been conjugated to polyethylene glycol-coated carbon nanotubes for the suppression of human arthritis synovial fibroblast inflammation. This was achieved by increasing caveolae-mediated endocytosis of the synovial fibroblasts and preventing mitochondrial disruption by recovery of their mitochondrial membrane potential. With the use of this experimental low-dose glucocorticoid-releasing system, there was greater uptake by the fibroblasts and more efficient intracellular release of dexamethasone from intracellular endosomes [63].

Epithelial cells/endothelial cells

Human body organs are composed of four basic tissue types: epithelial, connective, muscular and nervous tissues. Epithelium may be divided into two major groups: covering (or lining) epithelium and secretory (glandular) epithelium. Covering epithelia are organized into one or more layers that cover the surface or line the cavities of an organ. Common types of covering epithelia are simple epithelium, stratified epithelium and pseudo-stratified epithelium. Examples of simple epithelium are lining of blood vessels (endothelium) and serous lining of cavities (pericardium, pleura and peritoneum). Epithelial cells cover both the inner and outer surfaces of internal organs while endothelial cells cover the inner surfaces of blood vessels and lymphatic vessels [64]. The clinical applications of nanomaterials invariably require their crossing over the epithelial cell barrier. The transport mechanism of nanoparticles through epithelial cells is dynamic and is significantly influenced by the adhesion pattern of nanoparticles on the cell membrane [65]. Systemically-administered nanoparticles enter endothelial cells via caveolae formation and cross the endothelium by transcytosis, a type of transcellular transport in which macromolecules are transported across the interior of a cell. Macromolecules are captured in vesicles on one side of the cell, drawn across the cell, and ejected on the other side via exocytosis. This caveolae-based shuttle mechanism transports nanoparticles and their payloads actively across the endothelial barrier. The mechanism also improves the efficacy of delivery of therapeutic nanoparticles and their cargoes to diseased tissues in the body [65].

Apart from drug delivery, viral infections are clinically relevant examples of the passage of nanoscopical biovectors through the epithelial barrier. This is exemplified by the diversity of endocytic pathways that SARS-CoV-2, the coronavirus responsible for the COVID-19 pandemic, uses to enter the human lung epithelium. Current evidence suggests that the entry mode of coronavirus varies with the types of virus and host cells. These pathways include CME, caveolae-mediated endocytosis, as well as clathrin-and-caveolae-independent endocytosis that involves lipid rafts [66,67]. In cultured cell lines, entry of both SARS-CoV and SARS-CoV-2 into the cultured cells appear to involve endocytosis. Endocytosis of SARS-CoV proceeds through a variety of pathways depending on the cell line [68]. A large number of endocytic markers are expressed in nasal epithelial cells, indicating the existence of multiple active pathways [69]. In contrast, pneumocytes, the surface epithelial cells of the alveoli, show more limited expression patterns, with low or null expression of some of the key proteins associated with traditional endocytosis. More importantly, large GTPase dynamin, which is required for the scission of clathrin-coated vesicles and caveolae, is present in large numbers in the nasal epithelium but is not detectable in pneumocytes. Proteins associated with macropinocytosis, such as C-terminal binding protein (CtBP)-1, CtBP-2, and p21 activated kinase 1, are expressed at medium to high levels. Accordingly, macropinocytosis may be an important pathway for endocytosis by pneumocytes [12]. The observation that macropinocytosis in alveolar epithelial cell lines is upregulated by water-pipe smoke condensate suggests potential association of COVID-19 morbidity with smoking [70]. Identification of the endocytic route of SARS-COV-2 in primary pulmonary epithelial cells has significant translational implications. Specifically, establishment of a cell-based SARS-CoV-2 infection model will contribute to the establishment of drug development and screening platforms aiming at identifying compounds that can block or hinder the SARS-CoV-2 endocytic pathway (Fig. 3) [71,72].

Mesenchymal stem cells

Stem cells provide the ammunition for the flourish of contemporary regenerative medicine. The most well-studied subpopulation of pluripotent stem cells is the mesenchymal stem cell (MSC). The MSCs can differentiate into mesoderm-derived bone, cartilage, adipose tissue and muscle tissue [73]. A nanoscopical core-shell contrasting agent was synthesized for magnetic resonance imaging using perfluorooctyl bromide as core, poly(lactic-co-glycolic acid) as shell and a polystyrene sulfonate surface coating. These hybrid nanoparticles were internalized by MSCs via caveolae-mediated endocytosis without adversely affecting cell proliferation and their subsequent differentiation into osteoblasts [74].

Mesenchymal stem cells have an inherent migratory capacity toward tumorous tissues in vivo. Capitalizing on this inherent tumor-homing characteristic, engineered MSCs may be used as vectors for delivering diagnostic and therapeutic nanoparticles into a tumor. For example, 4 nm-diameter Au nanoparticles have been fed to MSCs and uptaken via endocytosis. These engineered MSCs act as contrasting agents in magnetic resonance imaging for tracking tumors via homing of the Au nanoparticle-containing MSCs in vivo [75]. Similarly, carboxyl-coated fluorescent quantum dots have been fed into human skin MSCs via clathrin-mediated endocytosis for long-term fluorescence imaging of tumors [76].

With respect to therapeutics, DNA or RNA gene vectors may be encapsulated and protected from degradation by nanoscopical liposomes and delivered to MSCs via different endocytic pathways [77]. Polymeric nanoparticles such as polyethyleneimine may also be used for gene delivery into stem cells. Genetically-engineered MSCs that contain pro-angiogenic and anti-apoptotic genes provide the basis for stem cell-based cardiac repair in ischemic heart diseases by improving angiogenesis, relieving ventricular remodeling and enhancing overall heart function [78]. The processes involved in

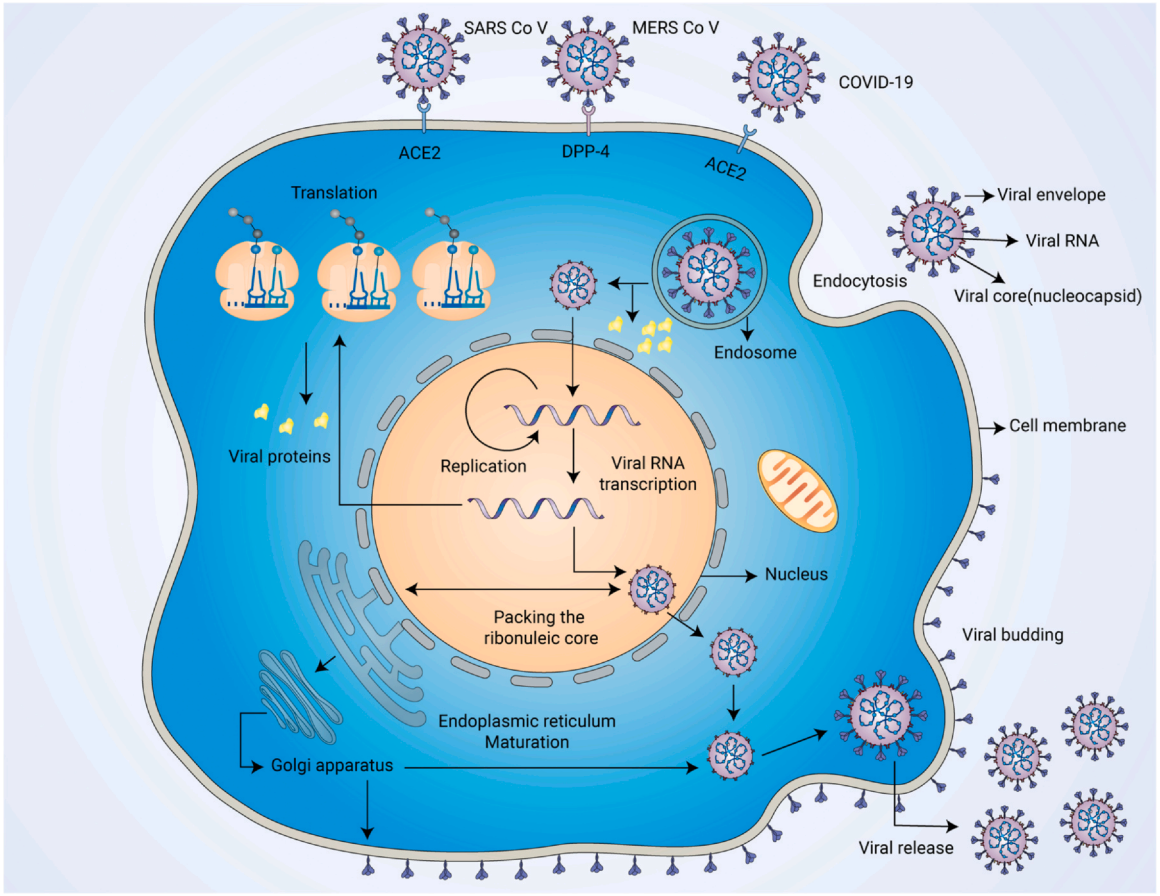

Fig. 3. Pathogenesis of coronavirus infections. *Abbreviations* – ACE2: Angiotensin-converting enzyme 2 cell-surface receptor; CoV: coronavirus; DPP-4: dipeptidyl peptidase-4 cell-surface receptor; MERS: middle east respiratory syndrome; SARS: severe acute respiratory syndrome [72].

nanoparticle-based gene transfer into stem cells are summarized in Fig. 4.

Macrophages

Surgical implantation of nanomaterials initiates a series of cellular and biochemical events that ultimately determine the quality of their integration within the human body. Macrophages play a key role in these events by regulating the body's immune response during inflammation and healing. Macrophages are derived from monocytes and are distributed throughout the body. They function as janitor cells (for engulfing dead cells, microbes and foreign materials) as well as antigen-presenting cells [78].

Hyperfunction or dysfunction of macrophages has been linked to the pathogenesis of diseases such as osteoporosis, rheumatism, arteriosclerosis and tumors. Specific macrophages known as tumor-associated macrophages are found in tumors. For treating these diseases, macrophage-targeting drug delivery systems have been developed by utilizing receptors on the surface of macrophages as cellular targets. Because all conventional drug delivery nanocarriers reply on endocytosis for entering target cells, it is necessary to develop phagocytosis-inducing nanocarriers for faster and more efficient drug internalization in macrophages. A macrophage-targeting, phagocytosis-inducing bio-nanocapsule-based nanocarrier has been developed. These nanocarriers consisted of a hepatitis B virus-enveloped particle outwardly displaying protein G-derived IgG Fc-binding domains and protein L-derived IgG Fab-binding domains in tandem. This enables the nanocarriers to aggregate and trigger phagocytosis in macrophages. When the nanocarriers are fused with liposomes, they may be used to deliver drugs to the targeted macrophages for treating diseases associated with these defense cells [79].

A major obstacle to gene transfer in vivo is its uptake process. Lysosomal degradation of the genetic material ultimately results in their extracellular excretion. Nanosystems that have the capacity to bypass endocytosis are highly-esteemed for gene transfer. For example, porous silicon nanoparticles that contain an outer sheath of homing peptides and fusion liposomes (fusogenic pSi nanoparticles) selectively target macrophages and directly introduce the oligonucleotide payload into the cytosol of macrophages via direct membrane fusion with the liposomes, thereby evading endocytosis and vesicular fusion with lysosomes (Fig. 5) [80]. Introduction of the small interfering RNA oligonucleotide into the macrophages enhances their clearance capability and improves their survival in a murine pneumonia model caused by *Staphylococcus aureus*.

The interface between cell surface and nanomaterials profoundly affects the interaction between the two entities, and even determines cell fate. When graphene is introduced into the human body, they are recognized as "foreign" and are uptaken by macrophages for removal. This adversely affects the biomedical applicability of graphene and graphene oxide (GO). To address this issue, the surface of carboxylated nanographene oxide complexes (nGO) were decorated with polyethylene glycol (PEG), bovine serum albumin (BSA) or polyetherimide (PEI) (Fig. 6a), to examine the effects of surface chemistry on their uptake by macrophages. Compared with highly negative charged pristine nGO, reduced negative zeta-potentials were recorded for PEG-decorated nGO (nGO-PEG) and BSA-decorated nGO (nGO-BSA). These two surface modifications inhibited endocytosis of the surface-decorated nGOs by macrophages. The inhibitory effect was more effective for nGO-PEG, in

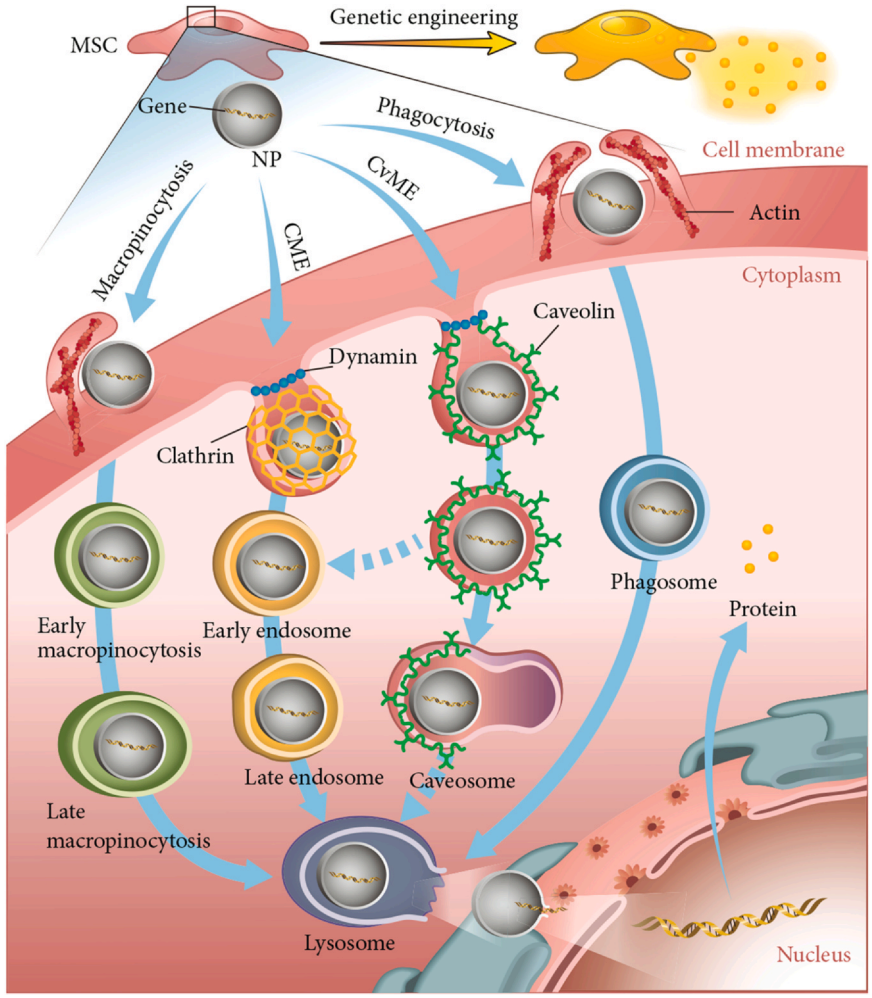

Fig. 4. The processes involved in nanoparticle-based gene transfer through the cell membrane and cytoplasm into the nucleus of stem cells. *Abbreviations* – CME: clathrin-mediated endocytosis; CvME: caveolae-mediated endocytosis.
Reprinted from [77] with permission from Hindawi under a Creative Commons Attribution-NonCommercial 4.0 International License.

which endocytosis was almost completely halted. In contrast, modification of nGO by PEI created a two-dimensional surface with positive zeta potential, which facilitated endocytosis. Macrophage viability was reduced after uptake of nGO-PEI because of their electrostatic interaction with mitochondria within the macrophages. Release of reactive oxygen species and cytochrome C by the mitochondria further activated the caspase cascade, ultimately resulting in apoptosis of the macrophages (Fig. 6b) [81]. The results indicate that the interaction of graphene oxide with macrophages may be tuned by surface functionalization to dampen their removal by these patrolling janitor cells.

Local environmental effects

Endocytosis may be driven by nonspecific interactions when the nanoparticles do not bind to specific cell surface receptors. Substrate stiffness plays an important role in changing the cellular uptake pattern of nanoparticles. The regulatory effect of substrate stiffness and morphology on cell uptake may be attributed to the change in cell membrane mechanical properties and cell diffusion area [95]. Studies in mechanical biology have demonstrated that various local physical cues regulate cellular responses, resulting in changes in cellular morphology and surface mechanics that, in turn, influence cellular uptake of nanoparticles [96]. Cell uptake increases linearly with cell membrane surface area because it represents the area assessable to the nanoparticles. Cell surface area is the predominant factor that governs membrane tension. Hence, absorption of each cell escalates with increase in matrix stiffness [97]. Mechanosensing induction refers to the ability of a cell to sense mechanical signals in its microenvironment. These signals include not only all components of force, stress and strain, but also rigidity, topological structure and adhesion. This ability is crucial for cells to respond to mechanical cues around them and to adapt to changing environments. Examples of response and adaptation include cell (de)activation, proliferation/apoptosis and (de)differentiation. Receptor-mediated cellular mechanical induction is a multi-step process that commences with binding of cell surface receptors to the extracellular matrix or to ligands on adjacent cell surfaces. Mechanical cues are provided by ligands and received by receptors at the binding surface [98].

Another issue to be considered is the sedimentation effect. Aggregation and deposition of nanomaterials involve diffusion and Brownian movement, which causes variations in the concentration of nanomaterials along the cell surface [99]. Although static cell culture is extensively used in in vitro studies, the study of nanoparticle-cell interactions under dynamic flow conditions is more appropriate in simulating in vivo conditions. Anisotropic nanomaterials such as polymer worms can be stretched and undergo morphological rearrangement under dynamic flow. Strong hydrodynamic shear may disrupt the interaction between nanoscopical cylinders and the cell surface and reduce the chance of cell uptake. In contrast, cell uptake of spherical nanomaterials is less affected by dynamic fluids [100].

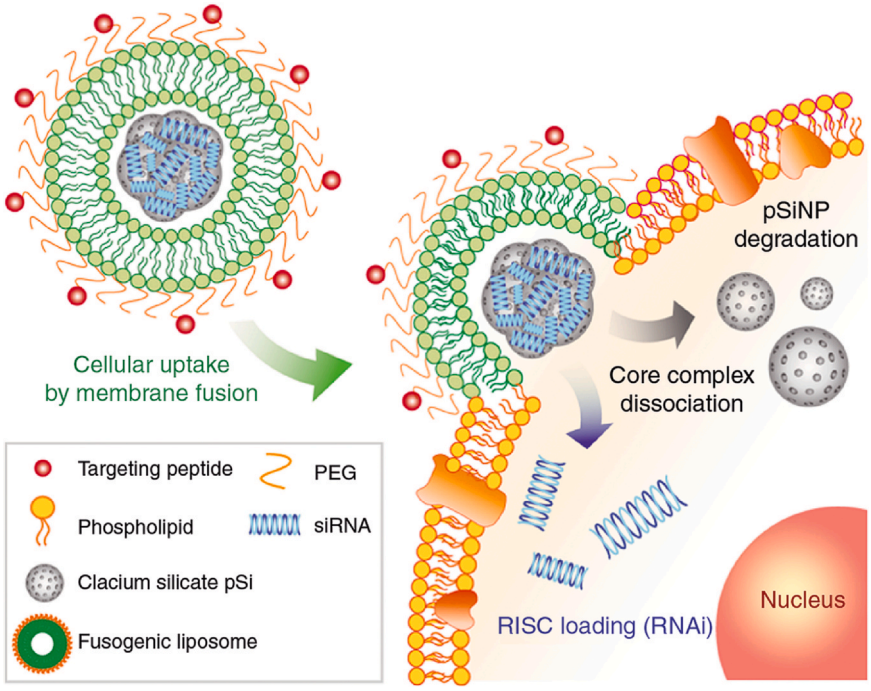

Fig. 5. Fusogenic porous silica nanoparticle system with small interfering RNA (siRNA) encapsulated by a liposome with a lipid bilayer and coated with macrophage-specific surface targeting peptides. Because of the similarity in composition between the liposome and the cell membrane of the macrophage, the nanocarriers system can be uptaken by the macrophage via membrane fusion without undergoing endocytosis and vesicular formation. After cell entry, dissociation of the core complex of the fusogenic nanoparticle releases the gene transfer material directly into the cytoplasm and evading vesicular fusion and degradation by the intracellular lysosomes. The remnant calcium silicate porous nanoparticle undergoes degradation within the cell. *Abbreviations* – PEG: polyethylene glycol; pSi: porous silica.
Reprinted from [80] with permission from Nature Publishing Group.

Attributes of man-made nanomaterial that affect endocytosis

An important facet in the medical application of nanomaterials is the correlation between the physicochemical properties of these materials and endocytosis. Size, shape and the type of surface functional groups are the three most important attributes that influence endocytosis, which will be highlighted first prior to discuss other attributes. Table 2 represents the impact of nanomaterials properties on the internalization mechanism.

Size and agglomeration

Internalization of nanostructures into cells significantly depends on their size. Many of the properties of nanoparticles in the human body such as lifetime, targeting potential, cell internalization and clearance from the body are dependent on their size.

There has been considerable efforts in determining the optimal size of nanostructures for internalization. The latter has important implications in the field of targeted drug delivery. In receptor-mediated endocytosis, complete encapsulation of a nanoparticle depends on sufficient adhesion strength and ligand density to cross the energy barrier. The forward tracking diffusion model shows that the time required for nanoparticle internalization is closely related to its size [124].

The effect of size varies for nanomaterials of different shapes, in terms of the quantity of uptaken nanoparticles and the endocytosis mechanism. For example, small graphene oxide nanosheets (~ 200 nm) enter cells via CME while bigger sheets (~ 1 μm) are uptaken by phagocytosis [125]. The endocytosis mechanism is also affected by size. For instance, nanoparticles with size around 125 nm are uptaken by cells via CME [126]. Another study investigated the relationship between arginine-terminated Au nanoparticles of different sizes and the method of uptake (Fig. 7a). Gold nanoparticles with size less than 10 nm underwent energy-independent direct membrane penetration. The cellular uptake strategy was altered as the size of the nanoparticles increased from 10 nm; the larger arginine-terminated Au nanoparticles penetrated cells through energy-dependent endocytosis (Fig. 7b) [127]. Likewise, small carboxylated polystyrene nanoparticles were uptaken into cells via CME, whereas larger nanoparticles were uptaken via caveolae-mediated endocytosis (Fig. 7c) [128]. Increase in size reduces the level of internalization of nanoparticles by endocytosis. Loading cargoes on nanoparticles increases their particle size, which may result in alteration of their endocytosis mechanism. This effect should be taken into consideration during gene or drug delivery [129]. In addition, nanoparticles with larger sizes demonstrate agglomeration, which adversely interferes with endocytosis [130].

The size of nanomaterials affects their toxicity [131]. The size and polydispersity of nanoarchitectures increase as they aggregate in biological solutions. Aggregation of nanoparticles before entering a cell affects their internalization. For accurate assessment of the association between nanostructure size and endocytosis, it is imperative that the colloidal stability of the nanoparticles is tested to ascertain that they remain solitary in the biological medium prior to cell entry [130]. Usually, the culture medium employed for analyzing endocytosis of nanoparticles is different from the original medium used for the synthesis of nanoparticles. Metal nanoparticles synthesized by chemical or green methods may contain salts, surfactants, reducing agents, stabilizing agents or organic solvents used for their synthesis. The culture media used for analyzing cell internalization contain proteins instead of the aforementioned chemicals [132]. This difference may result in aggregation of the nanoparticles, which alter their size range and distribution. The same attributes should be considered for in vivo applications because cells and their surrounding media affect nanoparticle aggregation.

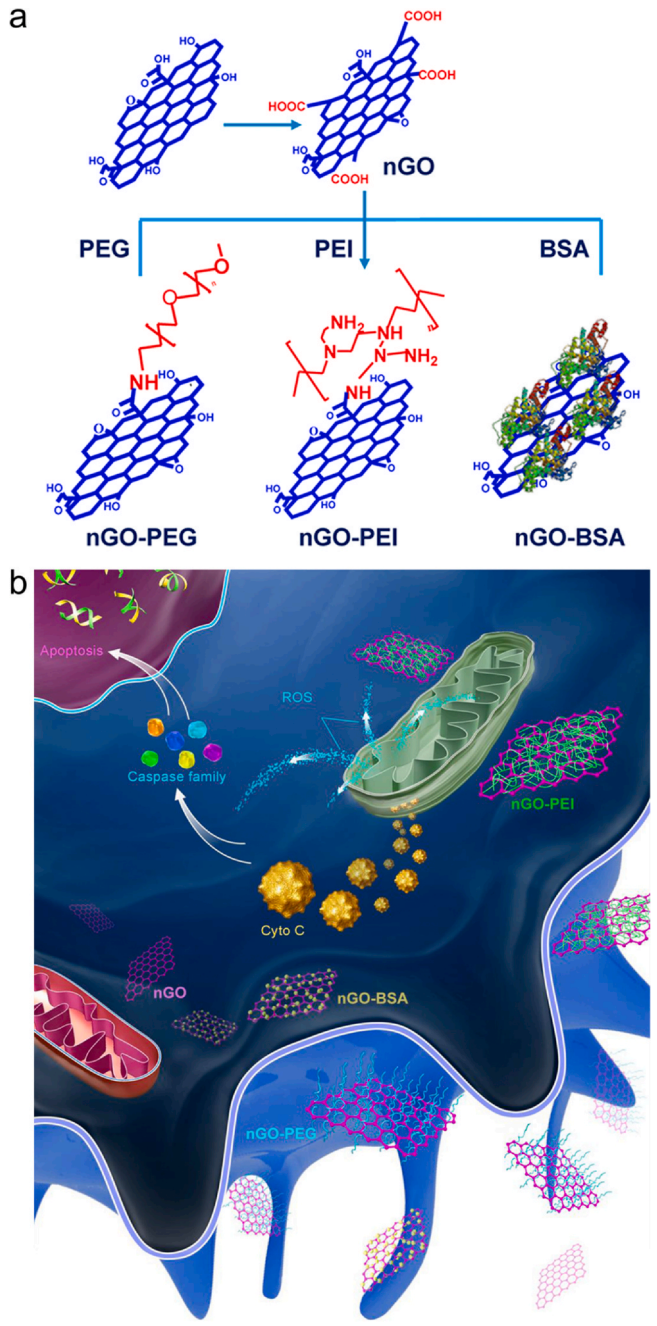

Fig. 6. a. Preparation of nanographene oxide (nGO) complexes and their surface decoration with polyethylene glycol (PEG), polyetherimide (PEI) or bovine serum albumin (BSA). b. Schematic of the different intracellular pathways in a macrophage after exposure to different nGO complexes. Pristine 2-D nGO were uptaken by the macrophage via endocytosis. Decoration of nGO with PEG almost completely prevented endocytosis. Endocytosis was compromised with nGO-BSA but not as severely as nGO-PEG. Unlike nGO-PEG and nGO-BSA, nGO-PEI were readily uptaken by endocytosis. However, the uptaken nGO-PEI interacted with mitochondria, causing the latter to release reactive oxygen species (ROS) and cytochrome C (Cyto C), which, in turn, activated the downstream caspase signaling cascade to induce apoptosis. Reprinted from [81] with permission from American Chemical Society.

Shape

Studying the effect of nanoparticle shape on the mechanism of internalization is challenging because of different confounding factors. First, changes in shape can also affect the size of nanomaterials. Second, non-spherical objects can interact with cell membranes in different directions. The contact area between the nanoparticle and the cell surface differs, depending on the direction of interaction with the cell membrane [133]. The shape of nanoparticles as well as their angle of entry determine the mechanism of endocytosis [134].

There is increasing evidence showing that spherical nanoparticles have higher cellular uptake compared with rod-shaped nanoparticles [26,135,136]. For example, spherical Au nanoparticles were more readily uptaken by HeLa cells than rod-shaped Au nanoparticles [135]. This is probably attributed to the longer time required for membrane wrapping of rod-shaped nanoparticles, as well as absorption of surfactant molecules to the longitudinal axis of the rod-shaped nanoparticles [136]. For rod-shaped nanoparticles, cellular uptake via endocytosis is more profuse for shorter nanoparticles than longer nanoparticles [114]. The uptake of Au nanospheres, nanostars and nanorods of different sizes was investigated in an in vitro study (Fig. 8) [137]. The quantity of all three sizes of nanospheres uptaken by different cell lines exceeded those of nanostars and nanorods. In addition, Au nanospheres and nanorods were predominantly uptaken via CME, while Au nanostars were uptaken by both CME and caveolae-mediated endocytosis. Another study reported that Au nanotriangles had higher cellular uptake than Au nanorods and Au nanostars. Gold nanotriangles were uptaken via micropinocytosis whereas Au nanorods were internalized through caveolae/lipid raft-mediated endocytosis [138]. These results indicate that even for the same material (Au), different endocytosis mechanisms are involved in the uptake of nanomaterials with different shapes.

Similar to metal nanoparticles, carbon nanomaterials also demonstrate shape-dependent cellular uptake [139]. Although CME is the major pathway for uptake of carbon nanomaterials, caveolae-mediated endocytosis and micropinocytosis are also involved in their cellular uptake [140]. For single-walled carbon nanotubes, the endocytosis mechanisms responsible for their uptake are in the ascending order: micropinocytosis, caveolae-mediated endocytosis and CME [141].

Surface functional groups and charge

Apart from the size and shape, functional groups and charge also determine cellular uptake of nanostructures. Positively-charged nanomaterials demonstrate higher cellular intake than their neutral or negatively-charged counterparts due to interaction of the positively-charged entities with negatively-charged plasma membranes. Indeed, changing the surface charge from zwitterionic to positive charge in a tumor microenvironment improves internalization of Au nanoparticles into cancerous cells [142,143]. An experiment was conducted to examine the cellular intake of Au nanoparticles with different surface functionalities. The findings indicate that changing the surface charge from zwitterionic to positive charge in a weakly acidic pH such as that present in the tumor microenvironment improves internalization of Au nanoparticles by HeLa and HMEC-1 cells (Fig. 9) [143].

Functionalization of nanomaterials with biomolecules such as genes or drugs causes increase in their particle size. Although large particle size adversely affects nanoparticle internalization and endocytosis, it has been observed that drug- or gene-decorated nanodevices have better cellular uptake. This disparity indicates that size is not the only factor that influences nanostructure internalization; other attributes also play significant roles. Surface charge is one of those attributes. Although increase in size reduces internalization, charge has the opposite effect. Increased surface charge, either positive or negative, improves nanoparticle internalization. This is due to increase in the interaction between the nanoparticles and the plasma membrane. Recently, Au nanoparticles have been examined for experimental delivery of oligonucleotides across brain endothelial cells. Addition of oligonucleotides increases the size of the Au nanoparticles, which is inimical for internalization. The

Table 2
Effect of abiotic nanomaterials on the mechanism of cellular uptake.

Architecture	Nanomaterial	Size/dimension (nm)	Cells examined	Experimental concentration	Cell uptake	Ref.
Nanorod	Ag	58	Murine macrophage cell line (J774A.1)	1.2 μg/mL, 4.5 μg/mL, 8.7 μg/mL; 24 h	Scavenger receptor-mediated endocytosis	[101]
Nanocages	Au	45	PC3 Cells	2.5 μg/mL, 5 μg/mL, 10 μg/mL, 25 μg/mL, and 50 μg/mL; 24 h	Caveolae-mediated endocytosis	[102]
Nanorod	Au	113 × 16; 84 × 18, 62 × 24; 55 × 29	A549 and HeLa line (J774A.1)	40 μg/mL; 4 h or 24 h	Endocytosis	[103]
Nanowire	CuO	50–70	MDAMB-231 cells	0–6 μg/mL; 24 h	Not reported	[104]
Nanowire	NiO	diameter of 31 with a length of 153	Cells derived from *Drosophila melanogaster*	0–10 mM; 24 ± 2 h	Perforation of the chitinous barrier	[105]
Nanocage	Au	25–70	BEL-7402 cell line; orthotopic hepatocellular carcinoma (HCC) mouse models.	(0.01 nM and 0.02 nM Au nanocages); incubated for 48 h.	Endocytosis	[106]
Nanowire	Ni	diameter 33, the length 5.4 μm	human cell line HCT 116	1000:1 nanowire to cell ratio; 24–72 h	Phagocytosis	[107]
Nanorod	Ag	40	human skin fibroblasts	4.3 μg/mL, 1–4 day	Membrane-bound vesicles	[108]
Nanrod	Au	61	human skin fibroblasts	23.4 μg/mL, 1–4 day	Membrane-bound vesicles	[108]
Nanospike	Au	54	Human oral epidermoid carcinoma (KB) cell lines		Not reported	[109]
Nanorod	Au	50 and 12 (with an aspect ratio of around 4:1)	Human oral epidermoid carcinoma (KB) cell lines	3–200 μg/mL, 24 h,	Not reported	[109]
Nanocage	Au	length: 50; wall thickness: 5	U87MGwtEGFR cells	3 h with 0.02 nM	Uptake was mediated by nonspecific adsorption of serum proteins on the surface of Au nanoparticles	[110]
Nanocage	Au	length: 46; wall thickness: 7	Breast Cancer stem cells; MDAMB-435 cells	0.2 nM, 3 h	AuNCs bound to sigma-2 receptors on the cell membrane and enter the cell via SV119-mediated endocytosis	[111]
Nanowire	Ag	72 nm in diameter 1.5 μm long	human alveolar type 1 epithelial cells	25 μg/mL, 1 h–7 day	Macrophage phagocytosis	[112]
Nanowire	Ag	70 nm diameter 1.5 μm length	Human alveolar type-I and type-II epithelial cells	0.1–25 μg/mL, 4 and 24 h	Not reported	[113]
Nanorod	Au	33:30, 40: 21, 50:17 and 55:14 (length:diameter)	Human breast adenocarcinoma cell line (MCF-7)	~ 70 pM Au nanorods with 100 mL of medium, with or without 10% serum, in 96-multiwell plates; 72 h	Not reported	[114]
Nanowire	Fe	50 nm in diameter; 2 and 5 mm long	HeLa cells	10:1–10 000:1; 72 h	Vesicle	[107]
Nanocage	Au	61	RAW264.7	10 μg/mL, 4 h	Not reported	[115]
Quantum dot	Graphene	4.1 nm diameter; 0.720 nm thickness	NR8383 cells	50, 100 and 200 μg/mL	Phagocytosis; caveolae-mediated endocytosis	[116]
Quantum dot	Graphene	1–8 (small nanoparticles) 50–60 (large nanoparticles)	L929 cells	Not reported	Caveolae-mediated and clathrin-mediated endocytosis	[117]
Nanosheet	PEG- and cetuximab-modified magnetic	136.4–215.7	CT-26 cells	10–100 μg/mL	Receptor-mediated endocytosis	[118]
Nanosheet	Mannosylated graphene oxide nanoparticles	Diameter: 50–300 Height: 5–10	THP-1 cells	10 and 20 μg/mL	Mannose receptor-mediated endocytosis	[119]
Nanosheet	Graphene oxide	363–384.6	Neutrophils	Not reported	Macropinocytosis and actin-dependent phagocytosis	[120]
Quantum dot	Graphene	Particle size: 4–6 Thickness: 0.7	NR8383 cells	100 μg/mL	Endocytosis and phagocytosis	[121]
Nanopherical	PEG-PDPA nanoparticles	125	Ishikawa cells	Up to 80 μg/mL	Endocytosis	[122]
Nanopherical	PEG-b-PCL nanoparticles	80–110 nm	HeLa cells	Not reported	Clathrin-mediated and caveolae-mediated endocytosis	[123]

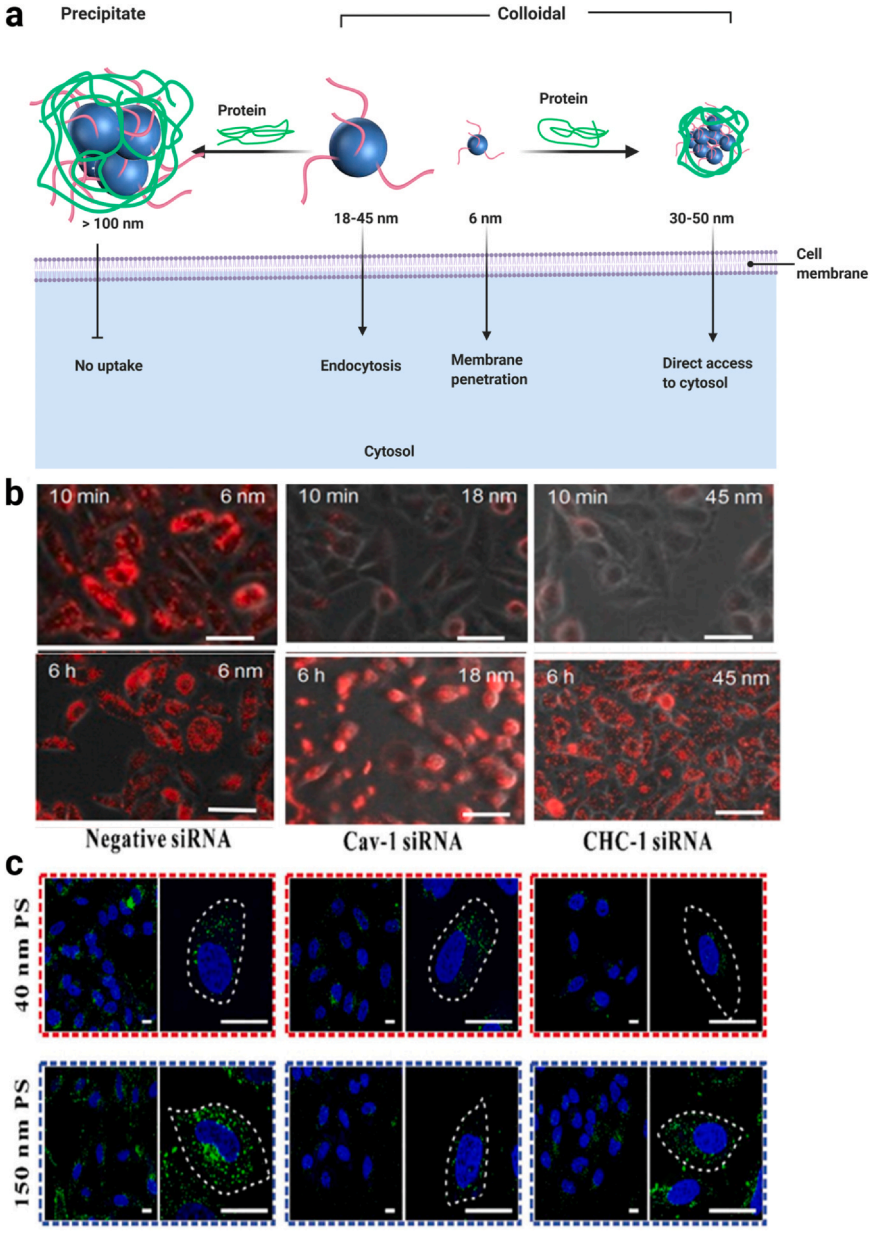

Fig. 7. The effects of nanoparticle size on the method of internalization and crossing the cell membrane in protein delivery. a. Arginine-terminated colloidal nanostructures of various sizes were employed for the experiment. b. Size-dependent uptake kinetics of arginine-terminated Au nanostructures. Gold nanoparticles with 6 nm hydrodynamic diameter were uptaken into cells within 10 min by direct membrane penetration. However, Au nanoparticles that are 18 nm and 45 nm in diameter took 1 h to be uptaken by endocytosis and to direct access the cytosol, respectively; reprinted from [127] with permission from American Chemical Society. c. 40 nm carboxylated polystyrene nanoparticles were internalized via clathrin-mediated endocytosis (clathrin labeling) while 150 nm carboxylated polystyrene nanoparticles were uptaken via caveolae-mediated endocytosis (caveolin-1 labeling). Reprinted from [128] with permission from American Chemical Society.

oligonucleotides augment the negative charge of nanoparticles and ameliorate their endocytosis via interacting with positively-charged proteins on the cell surface [129]. It has to be stressed that this represents the ideal conditions; the result may be different when nanoparticles are exposed to body conditions. This is because negatively-charged Au nanoparticles bind to positively-charged serum proteins that completely alter the overall surface charge of the Au nanoparticles [144]. Likewise, positively-charged Au nanoparticles can also interact with negatively-charged endothelial glycocalyx or endothelial surface proteins [145,146].

Surface chemical modification is an important step in biomedical applications for reducing cytotoxicity, increasing stability and expediting nanoparticle internalization into cells. Functionalized nanoparticles have better targeting efficacy than non-functionalized nanoparticles. Functionalized nanoparticles have a higher rate of cell uptake, thereby enhancing therapeutic efficacy. Surface coating is a crucial factor that determines cell absorption rate. Surface functionalization also determines the endocytosis pathway through which nanomaterials are internalized [147]. Generally speaking, positively-charged nanomaterials demonstrated higher cellular intake than their neutral or negatively-charged counterparts [26,143]. This phenomenon is possibly due to the better attraction of positively-charged nanomaterials to the negatively-charged cell membrane [26].

Architectures such as nanosheets show more complex behavior than nanospheres or nanorods. To investigate the effect of surface charge, graphene nanosheets were functionalized with positive ($-NH_3^+$) and negative ($-OSO_3^-$) charged functionalities. Neutral nanosheets did not have significant uptake while both positively-charged and negatively-charged graphene nanosheets demonstrated

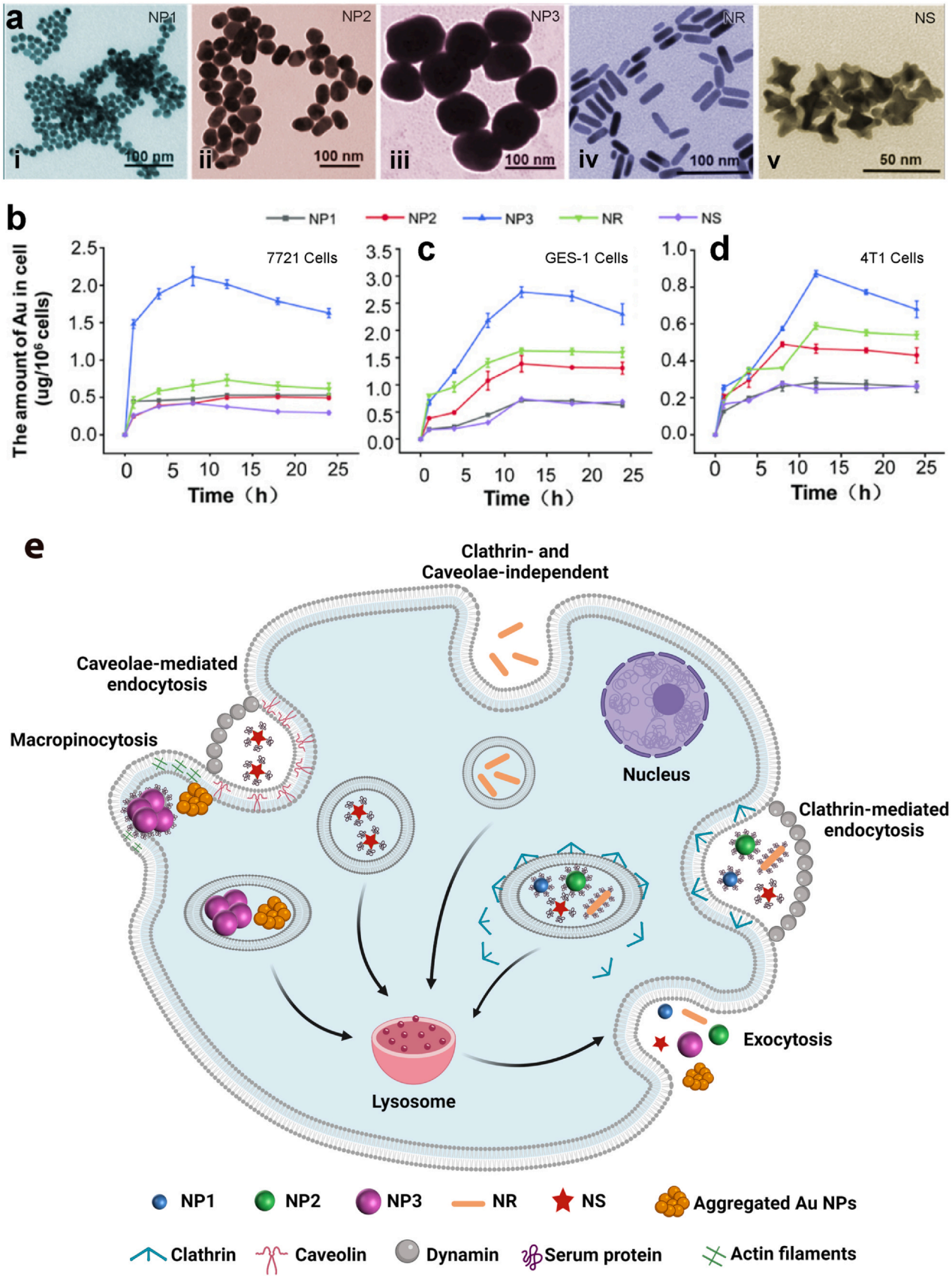

Fig. 8. a. Transmission electron microcopy images of gold nanoparticles: (i) spherical nanoparticles with an average diameter 15 nm (NP1); (ii) spherical nanoparticles with an average diameter 45 nm (NP2); (iii) spherical nanoparticles with an average diameter of 80 nm (NP3); (iv) 33 × 10 nm nanorods (NR); (v) nanostars with an average diameter 15 nm (NS). b–d. Cellular uptake curves of gold NP1, NP2, NP3, NR and NS in 7721 cells (b), GES-1 cells (c) and 4T1cells (d). e. Endocytosis mechanisms involved in the uptake of the Au nanoparticles.
Part a-d reprinted from [137] with permission from Wiley.

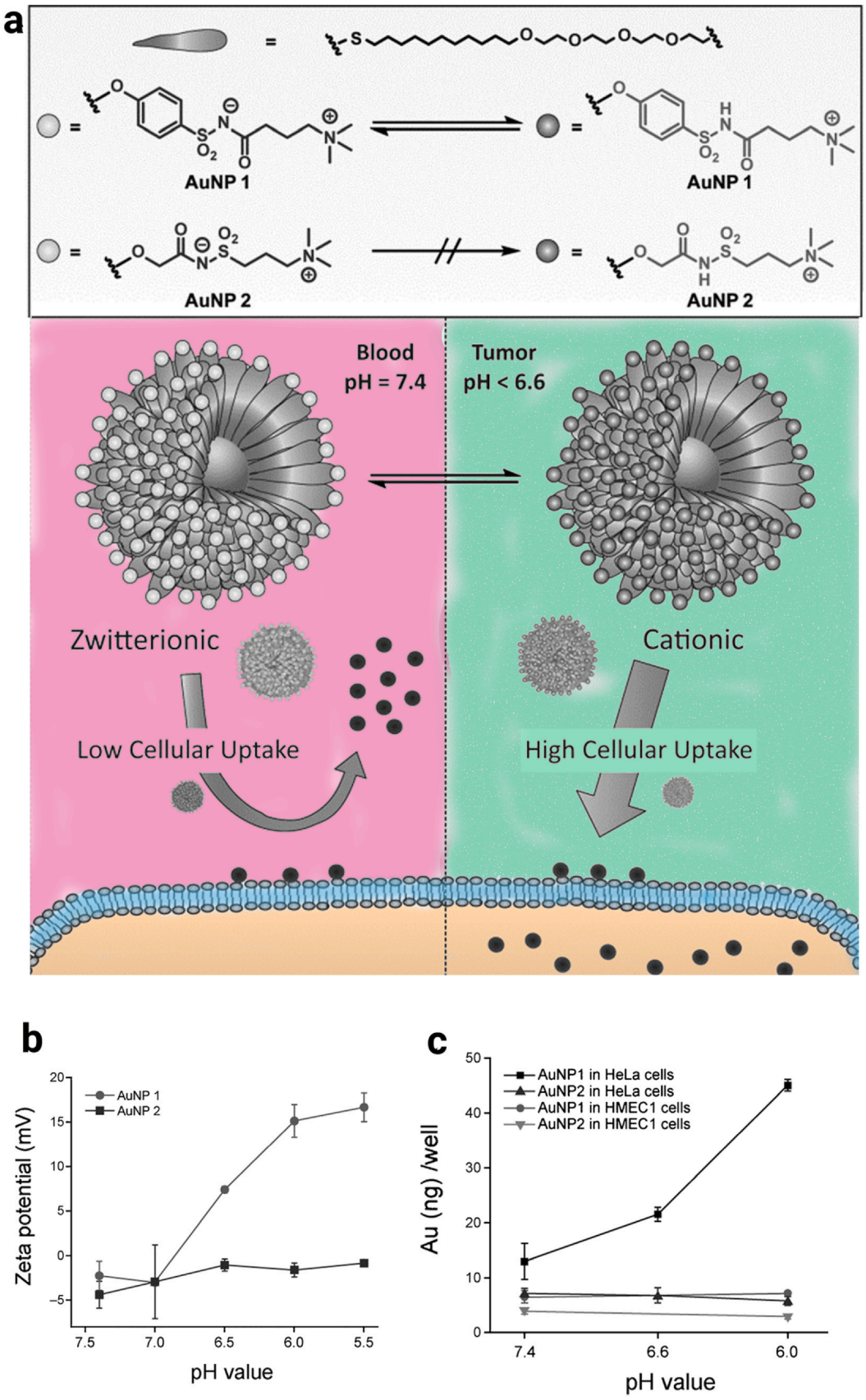

Fig. 9. a. Chemically-modified pH-sensitive Au nanoparticles for selective targeting of cancer. b. Zeta potential plotted against pH values for Au nanoparticles (AuNP) 1 and AuNp 2 (both 1 micromolar). c. Cellular uptake of AuNP 1 and 2 (both 1 micromolar) after incubation with HeLa (30,000 cells/well) or HMEC-1 cells (100,000 cells/well) in the presence of 10% serum for 3 h.
Modification from [143] with permission from Wiley.

energy-dependent uptake (i.e. cellular uptake of ~ 90% of the graphene nanosheets was through energy-dependent endocytic pathways) [125]. Because endocytosis is an energy-dependent process and the major pathway of entrance for extracellular components [148–150], it is also the predominant strategy for internalization of graphene nanosheets. Large functionalized graphene sheets (~ 1 μm) penetrate cells via phagocytosis regardless of the surface functional groups. In contrast, surface charge is the predominant parameter that influences the internalization of graphene nanosheets that are smaller than 200 nm [125].

Surface functional groups may also be employed for nanocarriers to deliver therapeutic agents into a designated part of the cell. To reduce endolysosomal accumulation and to increase cytosolic localization, nanocapsules were functionalized with glycoprotein H derived from the *Herpes simplex virus* 1 (gH625). Emulsions containing curcumin-embedded nanocapsules were directly internalized into the cytosol instead of the lysosomes [151]. These findings indicate that surface functionalization affects the amount of cellular uptake as well as the fate of nanomaterials inside cells.

Incubation of nanoparticles in biological fluids results in development of a new layer on the nanostructure surface that is known as the protein corona. Accordingly, it is necessary to understand the process of protein corona formation and how it is associated with the uptake of nanoparticles using biological fluids to simulate body conditions. Sixty nanometer pristine Au nanoparticles required minimum time for endocytosis. Formation of a protein corona increased the time required for endocytosis. In addition, the presence of a protein corona significantly reduced ligand density on the Au nanostructure surface, resulting in defective engulfment [4]. Surface alteration is not always a negative factor for cellular uptake of nanoparticles. For example, surface modification of nanoparticles by folic acid ligands promoted their cellular uptake by receptor-mediated endocytosis [152].

Elasticity and stiffness

The elasticity of nanoparticles affects their internalization as well as their circulation and biodistribution [153]. Elasticity refers to a material's resistance to change under pressure and the capability to return to its native state after pressure is removed [97]. Stiffness refers to a material's capacity to resist deformation during application of a load. E elasticity and stiffness are the intrinsic and extrinsic features of nanostructures. In addition, the geometry of a nanomaterial, including size and shape, relies on stiffness [154]. Elasticity and stiffness is the most important mechanical characteristics of nanostructures that can be determined using atomic force microscopy. These devices evaluate surface topography by exerting delicate forces. The atomic force microscope has minute cantilever beams that touch the surface of nanoparticles via their sharp tip. Displacement of surface relative to cantilever tip is measured by piezoelectric actuators [155]. Atomic force microscopes are used extensively for determining the stiffness and viscoelasticity of nanoparticles using dynamic mechanical testing principles, generating important information such as the storage modulus, loss modulus and tan delta [156]. The storage and loss modulus in viscoelastic materials measure the stored energy, representing the elastic portion, and the energy dissipated as heat, representing the viscous portion. The ratio of the loss modulus to storage modulus in a viscoelastic material is defined as the tan delta, which provides a measure of damping in the nanomaterial.

Tweaking the elasticity of nanoparticles to enhance their internalization by cells is a valuable tool for nanocargo delivery [157]. Different strategies for tuning the elasticity of nanoparticles are available, including the use of colloidal microgel/nanohydrogels and layer-by-layer systems [153]. Altering the cross-linking density is a popular method to control the elasticity of microgels/hydrogels, which affects cellular uptake and uptake kinetics [158]. For example, microgels with cross-linking densities between 1.7 and 15 mol% were internalized into cells through several mechanisms, which result in high uptake rates [159]. In another study, poly(*N*-isopropylacrylamide)-based microgels were used to investigate the effects of nanoparticles with different cross-linking densities on interaction with the plasma membrane [160] Microgels with low cross-linking densities were softer than those with high cross-linking densities. Charged microgels had fast cellular uptake, in the range of seconds to minutes. Microgels that were larger than 800 nm in diameter and had cross-linking densities of 10–15 mol% could not be translocated into cells [160].

Nanoparticle elasticity may also be tuned by controlling the amount of polymer added during synthesis. For example, hydrogels with tunable elastic moduli (0.255 kPa to 3 MPa) but uniform size (~ 200 nm) and surface charge (~ −35 mV) were prepared by using different amounts of poly(ethylene glycol) diacrylate via a nanoemulsion templating method [161]. Both soft (10 kPa) and hard (3 MPa) nanostructures demonstrated long circulation times with acceptable pharmacokinetics. The hard nanoparticles were phagocytosed more profusely (> 3.5 times) by macrophages compared with the soft nanoparticles. The hard nanoparticles remained less in circulation because of their rapid clearance by phagocytic immune cells [161].

In addition to elasticity, stiffness directly influences the interactions between nanomaterials and cells. Several experimental and theoretical studies have recently been conducted to examine the role of stiffness on nanomaterial internalization. To eliminate the effect of nanomaterial physicochemical characteristics such as size and surface features on cell internalization, a two-stage microfluidic chip was developed that is capable of producing a series of core-shell nanoparticles with the same features but with different stiffness. These nanoparticles were exposed to both cancerous and non-cancerous cells to evaluate the impact of material stiffness on cell internalization. The results showed that rigidity has a significant effect on the uptake of nanoparticles; the more rigid nanoparticles are more easily uptaken by the cells (Fig. 10) [162].

Layer-by-layer particles and capsules are carriers with high elasticity that are promising for drug delivery applications. Typically, layer-by-layer particles consist of sequential layers of proteins and polymers, or are separately layered on a sacrificial polymeric or inorganic core surface [163]. Different approaches may be used to modulate the elasticity of such particles, including controlling the materials used in the layers, the number of layers, the density of layer crosslinking, and removing the core of the capsules [164]. For example, core-shell particles that consist of a solid polymeric core inside a lipid shell with cylindrical polymer brushes exhibited excellent elasticity [165–167]. Layer-by-layer capsules synthesized using a silica template exhibited increased stiffness values after replacement of the core with Au nanoparticles [168]. The stiffness of the capsules was 0.183 N/m without replacement of the core by Au nanoparticles. The stiffness of the capsules increased with increases in the concentration of the Au nanoparticles; stiffness increased to 0.259 N/m, 0.293 N/m and 1.447 N/m, respectively, for low, medium and high loading of Au nanoparticles. In addition, stiffness may be improved by incorporating multiple layers. For example, the stiffness of hyaluronic acid capsules prepared from silica matrices increased from 7.5 N/m to ~ 27.2 N/m by increasing the layers of hyaluronic acid from one layer to 4 layers [169]. Likewise, the stiffness of poly (styrenesulfonate)/poly(allylamine hydrochloride) and dextran sulfate/poly-L arginine capsules prepared using $CaCO_3$ cores increased from ~ 0.25 to 9 N/m by increasing the number of layers [170].

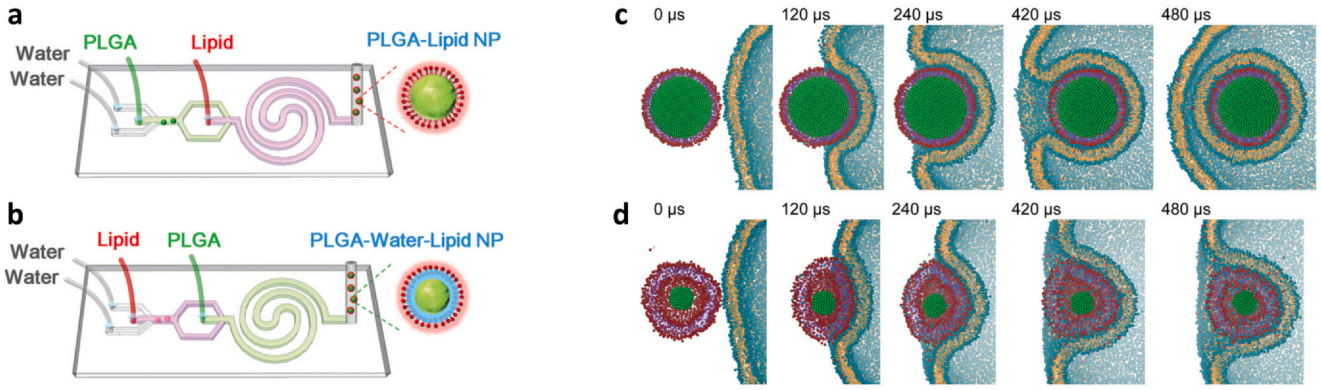

Fig. 10. The two-stage microfluidic chip for fabrication of a. polymer-lipid core shell nanoparticles as a "rigid" assembly, and b. polymer-water-lipid core shell nanoparticles as a "soft" assembly; c, d. Molecular dynamics simulation showing the effect of material stiffness on the uptake of the nanoparticles. The "rigid" assemblies were internalized into cells smoothly via cellular membrane wrapping, whereas the "soft" assemblies were internalized with deformation and were trapped on the cell surface.
Reprinted from [162] with permission from Wiley.

Other parameters

Apart from global physiochemical attributes including size, shape, chemical modification, surface charge and elasticity, other variables such as nanoparticle orientation and energetic interactions also affect cellular uptake [171]. Coating of a nanostructure by the plasma membrane is modulated by the adhesion energy between the nanocarrier and the plasma membrane, and subsequently by the deformation energy consumed for crossing the lipid bilayer [172]. Binding occurs when the adhesion strength is high enough to compensate for the deformation energy consumed at the membrane curvature. This attachment transition is continuous without a potential energy barrier [173,174]. If the adhesion strength between the nanostructure and the plasma membrane increases, discontinuous binding with a potential energy barrier occurs between two partially-wrapped states, or between a completely-wrapped state and a partially-wrapped state. For spherical nanoparticles, the wrapping process is continuous [134]. The stability of partially-wrapped states, however, increases for nonspherical nanoparticles such as ellipsoidal and spherocylindrical nanoparticles [175–177]. The effect of nanorods and nanocubes with varying aspect ratios and edge curvatures on membrane wrapping and cell internalization had been investigated [178]. Nanorods had stable endocytotic states with high wrapping fractions. Increasing their aspect ratio is not conducive to complete wrapping. Nanoparticles with high aspect ratios and round tips enter side-wise via a "submarine" mode, with their long edge parallel to the plasma membrane. Conversely, nanoparticles with small aspect ratios and flat tips enter tip-wise via a "rocket" mode (Fig. 11a).

A membrane model was used to theoretically examine the cellular interactions of nanoparticles through Brownian dynamics simulation [179]. Uptake of nanoparticles with tilted orientation was non-monotonic, whereas uptake of nanoparticles orientated normal to the membrane was monotonic (Fig. 11b). This phenomenon was attributed to the transition energy between membrane adhesion and nanoparticle kinetics. Table 3 represents some examples of the effect of nanoparticle geometry, size and functional groups on endocytosis.

Virus as a biovector nanoparticle

Viruses are specialized intracellular parasites that require a host cell to process and propagate their genetic information. Viral nucleic acids are protected by a proteinaceous coat, the capsid, which is composed of repeated units of one or more structural proteins. Enveloped viruses possess an additional lipid membrane comprising viral membrane glycoproteins. The lipid membrane is capable of binding to specific host cell receptors and induces fusion of the virus with the host cell membrane. Some viruses such as paramyxoviruses [184] and Human Immunodeficiency Virus-1 (HIV-1) [185] are capable of entering the host cell cytosol by direct penetration of the plasma membrane. Other viruses capitalize on intracellular endosomal vesicles to move through the cytoplasm of the host cell and for routing to other intracellular organelles, gradually sensing the changes in the pH of the environment that are necessary for viral uncoating [14].

The initial step of virus penetration into the host cell usually begins with the engagement of the structural proteins of the virus to specific host cell receptors, carbohydrates or lipids. Common viral receptors are sialylated glycans, cell adhesion molecules such as selectins, cadherins and integrins, the immunoglobulin superfamily receptors and the phosphatidylserine receptors that include T-cell immunoglobulin and mucin domain (TIM) and TYRO3, AXL, as well as the MER family of receptor tyrosine kinases (TAMs) [186]. Binding of viruses to the host cell surface receptors triggers a series of cellular signals that cause the host cell to internalize the viruses through one of the endocytic mechanisms: a) CME, which is utilized extensively by many viruses; b) caveolae/lipid raft-mediated endocytosis, which may be used as a substitute to or supplement the clathrin route; c) macropinocytosis, and d) a variety of other still poorly-characterized mechanisms (Fig. 12) [14,187]. Dynamin participates in membrane fission to generate endocytic vesicles and is required for many endocytic pathways. These endocytic pathways may be divided into sub-pathways that are dependent or independent of dynamin. Dynamin-dependent mechanisms include CME, caveolae-mediated endocytosis and clathrin-independent dynamin-mediated pathways. Pathways independent of dynamin encompass macropinocytosis, lipid raft-mediated endocytosis and clathrin-and-caveolae-independent endocytosis [188]. Usually, viruses do not utilize a single entry mechanism into the host cell. They utilize multiple uptake routes, depending on the host cell and virus size and shape (Table 4).

Clathrin-mediated pathway

The CME route is the most common endocytic pathway taken by small and medium-sized viruses. Clathrin-mediated endocytosis is also largely exploited in biomedical applications such as targeted-drug delivery and diagnostic imaging, to deliver ligand-coated nanoparticles to diseased cells and tissues [16,212,213]. Clathrin-mediated virus internalization commences with the binding of virions to host receptors. The subsequent signals mediated by the receptors induce the binding of adapter proteins to the cytoplasmic tail

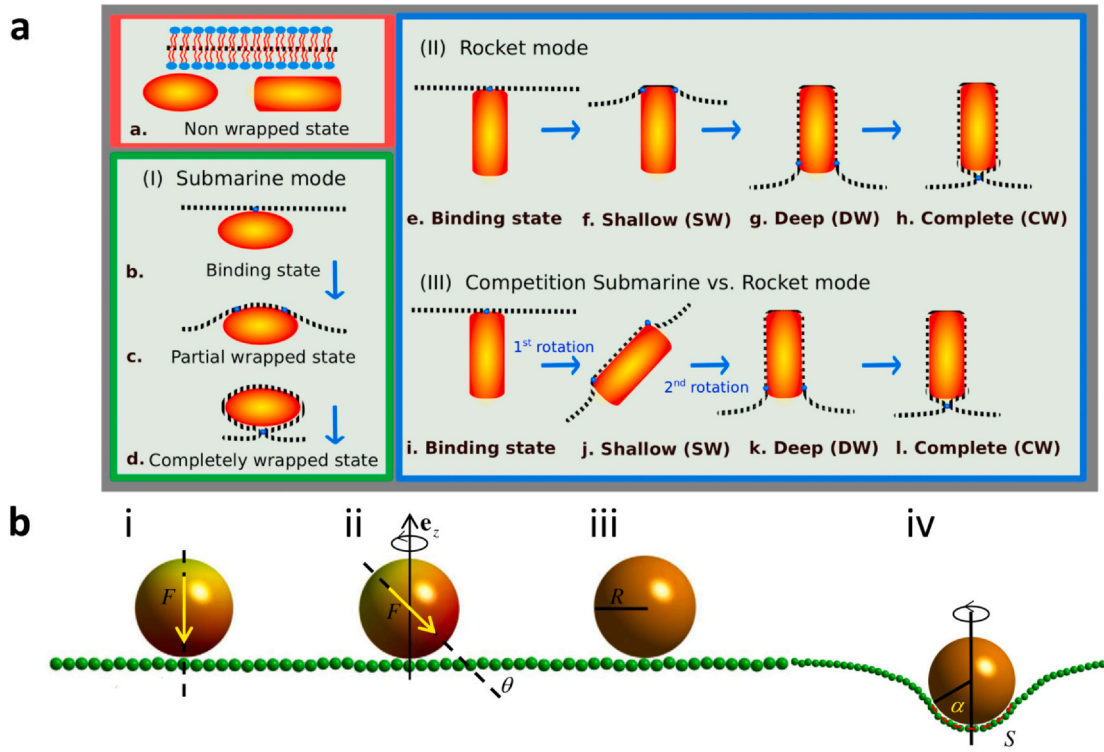

Fig. 11. a. Possible modes of nanostructure uptake by membrane wrapping: (i) submarine mode with the longitudinal axis of the nanoparticles oriented parallel to the membrane, (ii) rocket mode with the longitudinal axis oriented perpendicular to the membrane, and (iii) competition between the submarine mode and the rocket mode as observed for rod-like particles with high aspect ratios. The completely-wrapped nanoparticle is connected by an infinitely small catenoidal neck to the membrane; in this state, particle orientation is irrelevant; reprinted from [178] with permission from American Chemical Society. b. Initial configurations of (i) an active nanoparticle oriented normal to the membrane (ii) an active nanoparticle with tilted orientation, (iii) a passive nanoparticle, and (iv) partial wrapping for a passive nanoparticle. Reprinted from [179] with permission from American Physical Society.

of the receptors. Clathrin proteins are recruited and their binding to adapter proteins induces clathrin multimerization and invagination of a clathrin-coated pit. The GTPase dynamin-1 mediates scission of the vesicles and releases the clathrin-coated vesicles to the cytosol [28]. Viruses are delivered to early endosomes and other intracellular organelles by vesicular transport through the cytoplasm for fusion with endosomes.

The vesicular stomatitis rhabdovirus (VSV) uses CME to infect human cells. The endocytic receptor for VSV belongs to the low-density lipoprotein (LDL) receptor family. Mutations of LDL receptors abolish VSV infectivity [189]. Because VSV is used extensively as a vector for gene therapy and oncolytic viral therapy, this finding is useful for the design of recombinant viruses with modified tropism for biomedical virus retargeting [214,215].

Although many components of CME have been well characterized, other factors remain unidentified. Proteins involved in the entry of large cargoes such as viruses are still largely unknown. Flaviviruses are a large group of viruses responsible for the emergence and re-emergence of mosquito-borne tropical diseases such as Dengue, Zika or West Nile Virus diseases. Flaviviruses utilize CME as an internalization route into the host cell. A conserved domain in the glycoprotein E of the flavivirus envelope is responsible for virus receptor-mediated binding and viral entry into the host cell [216]. Despite the similarity with E protein, the binding receptor exhibits differences among different cell types. Dengue virus employs several cell membrane receptors for cell entry. Carbohydrate molecules such as glycosaminoglycans, glycosphingolipids, heat shock proteins and C-type lectin receptors such as mannose receptor and DC-SIGN have been reported as Dengue receptors [190,191]. Unlike small endogenous cargoes such as transferrin, flaviviruses entry is promoted by the small interferon-inducible glycosylphosphatidylinositol-anchored lymphocyte antigen 6E (LY6E). Large-cargo internalization requires substantial changes in the microtubule compartment of the host cell cytosol. Viral infection promotes the formation of LY6E tubule-like structures that are dependent on ribonuclease K and microtubule end-binding proteins [217,218]. The involvement of LY6E in enhancing virus entry has also been shown for other viruses such as HIV-1 [219], influenza A virus, measles virus, VSV [220] and hepatitis B virus [221].

Influenza A viruses utilize different endocytosis pathways for cell entry, depending on cell type: CME and dynamin-independent micropinocytosis [192]. The influenza A virus uses its major surface glycoprotein, hemagglutinin, to bind to different sialylated receptors on the host cell surface, including the sialic acid-containing, voltage-dependent Ca^{2+} Cav1.2 channels [222]. This multivalent attachment by multiple copies of trimeric hemagglutinin triggers endocytosis of the influenza virus, which is eventually transported to the endosome. Acidification of the lumen during endosome maturation induces conformational changes in the hemagglutinin receptors. This, in turn, causes membrane fusion and release of the virus genome and viral proteins into the host cell cytoplasm (Fig. 13). Mutations in the sequence of the hemagglutinin receptor binding domain confer specificity for binding to different types of sialic acids. This helps to regulate species-specific tropism of the influenza family of viruses [223]. Virus-mimetic nanomaterials such as therapeutic nanoparticles can benefit from the hemagglutinin-mediated entry mechanism of influenza A virus to improve the ability of nanoparticles to be recognized by target cells, with higher specificity [224].

Caveolae-mediated pathway

Caveolae are lipid rafts that produce flask-shaped plasma membrane invaginations enriched in cholesterol, phospholipids and sphingolipids [225]. The major components of caveolae are caveolins

Table 3
Effects of nanoparticle geometry, size and functional groups on endocytosis.

Nanomaterials	Shape	Size (nm)	Functional groups or charge	Remarks	Ref.
Aldehyde-based polymer	Spheres, nanorods, nanowires, and vesicles	Spheres: 70, Nanorods: 19 (diameter); length:140, Vesicles: 137 (diameter)	Doxorubicin	Internalization of vesicles by HeLa cells was faster than that of nanorods. Spheres are the slowest to be internalized among the studied nanoparticles.	[180]
Carboxylated polystyrene	Spheres	40 and 150	Carboxyl group	Polystrene nanoparticles with smaller size were endocytosed mainly through the clathrin-dependent pathway. Those with a larger size were uptaken by caveolae-mediated endocytosis	[128]
Poly(β-amino ester)	Spheres	200	Positive charge	Caveolae-mediated endocytosis was the major route of nanoparticle uptake Endocytosis mechanism varied with the polymer end-group and molecular weight	[181]
Poly(ethylene glycol) diacrylate with tunable elastic moduli (0.255–3000 kPa)	Spheres	200	Surface charge (~ −35 mV)	Hard nanoparticles were phagocytosed more profusely (> 3.5 times) by macrophages compared with soft nanoparticles The hard nanoparticles remained less in circulation because of their rapid clearance by phagocytic immune cells	[161]
Graphene	Sheets	200, 550, 1000	With positively-charged ($-NH_3^+$) and negatively-charged ($-OSO_3^-$) functionalities	Neutral nanosheets did not have significant uptake. Both positively-charged and negatively-charged graphene nanosheets demonstrated energy-dependent uptake of ~ 90% of the graphene nanosheets was through energy-dependent endocytic pathways	[125]
Silver	Spheres	5, 20, 50, 100	Neutral	The size of Ag nanoparticles affected both the efficiency of cellular uptake and the type of endocytosis employed for the uptake	[182]
Gold	Rods	70–120 Aspect ratio: 2–4	Positive charge Poly(diallyldimethyl ammonium chloride)	Longer rods have less internalization compared to smaller ones	[114]
Gold	Rods	55	Positive charge Poly(diallyldimethyl ammonium chloride)	Enhanced cellular internalization due to positive charge	[136]
Gold	Spheres	15, 30, 60, 100	–	60 nm nanoparticles had the highest cellular uptake and clearance rates. However, nanoparticle accumulation within the tumor was similar for the 15, 30 and 60 nm nanoparticles	[183]
Gold	Stars, rods, triangle	70–90	Methylpolyethylene glycol	Gold triangles were uptaken into RAW264.7 cells the most efficiently, followed by gold nanorods and nanostars The gold nanostars that contained multiple branches of different lengths have to overcome a higher membrane bending energy barrier, resulting in their lower cellular uptake	[138]
Gold	Spheres	2	Positive charge	Changing the surface charge from zwitterionic to positive charge in a weakly acidic pH such as that present in the tumor microenvironment improved internalization of gold nanoparticles by HeLa and HMEC-1 cells	[143]
Silica	Sphere	20–50	Neutral	20 nm and 50 nm silica nanoparticles show different modes of cellular uptake, with the smaller nanoparticles capable of trafficking into the cells in an endocytosis-independent manner	[131]

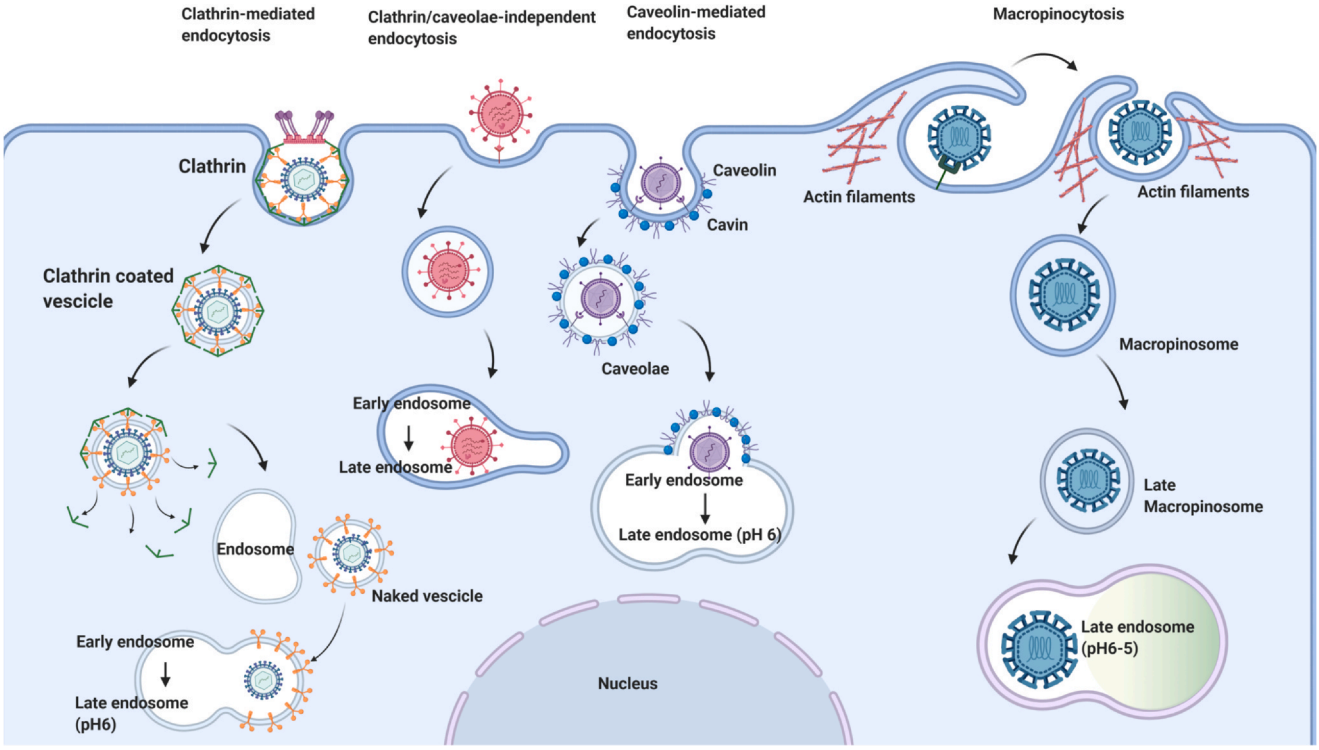

Fig. 12. Mechanisms of virus internalization by host cells. Viruses enter host cells by several endocytic mechanisms upon binding of a receptor on the cell surface: clathrin-mediated endocytosis, caveolin-mediated endocytosis or clathrin/caveolin-independent endocytosis and macropinocytosis. Following endocytosis, viruses are directed to the early endosome. During endosome maturation, the pH of endocytic vesicles decreases. This results in viral membrane fusion, endosomal escape or membrane disruption, with the release of viral genome.

and cavins (refer to Section 3). Binding of the virus to their host cell surface receptors results in dynamin-2 mediated caveosome formation, endocytosis of the cargo and its transport to the early endosomes [226].

Many enveloped and non-enveloped viruses such as SV40 and other polyomaviruses utilize caveolae-mediated endocytosis to infect host cells. Entry of the non-enveloped SV40 virus is mediated by its binding to major histocompatibility complex class I (MHC-I) molecules that are ubiquitous on the host cell surface. The SV40–MHC-I complexes then migrate to caveolin pits, where the MHC-I molecules are released and subsequently degraded. The pentamers of the major SV40 capsid protein subsequently bind ganglioside GM1 molecules, which represent the endocytic receptor for the virus. Binding of SV40 particles to GM1 induces the caveolin-1 mediated invaginations that eventually produce caveolae and trigger virus endocytosis (Fig. 14) [193,194]. Polyomaviruses use different sialylated glycans as receptors for cell entry. Experimental complex formation between different polyomavirus VP1 proteins and sialylated glycan receptors showed that VP1 proteins are highly specific for their respective glycan receptors. This affinity modulates virus uptake, tropism and pathogenesis [227].

Many enveloped viruses utilize caveolae-mediated endocytosis as an alternative route for cell entry. The route of entry of the ZIKA virus, an arthropod-borne virus causing neurological disorders and microcephaly in human infants, has recently been characterized in glioblastoma cells [195]. Although the majority of the ZIKA virus employed clathrin vesicles to reach the cytosol of host cells, virus infection was dramatically reduced by siRNA knockdown or

Table 4
Examples of viruses and their endocytosis mechanism.

Virus	Family	Receptor	Mechanism	Ref.
Vesicular stomatitis virus	Rhabdoviridae	Low-density lipoprotein receptors (LDL-R)	Clathrin-mediated	[189]
Dengue Virus	Flaviviridae	GAG, DC-SIGN and other C-Type lectin receptors	Clathrin-mediated	[190,191]
Influenza A virus	Myxoviridae	Sialic acid containing receptors	Clathrin-mediated, Macropinocytosis	[192]
Simian Virus 40	Poliomaviridae	GM1 ganglioside	Caveolae-mediated	[193,194]
ZIKA virus	Flaviviridae	DC-SIGN and other C-type lectin receptors, TIM and TAM receptors	Clathrin-mediated, Caveolae-mediated	[195,196]
Mayaro virus	Alphaviridae	Matrix Remodeling Associated 8 (MXRA8)	Clathrin-mediated, Caveolae-mediated	[197]
HCoV-OC43	Betacoronaviridae	Sialoglycan-based receptors	Caveolae-mediated	[198]
African swine fever virus	Asfarviridae	CD163	Clathrin-mediated, Macropinocytosis	[199,200]
Ebolavirus	Filoviridae	DC-SIGN and other C-type lectin receptors	Macropinocytosis	[201]
Vaccinia virus	Poxviridae	Heparan sulfate Proteoglycan	Macropinocytosis	[202]
Human immunodeficiency virus-1	Retroviridae	CD4 and CCR5or CXCR4	Clathrin-mediated Macropinocytosis	[203,204]
Human papilloma virus-16	Papillomaviridae	α6 integrin, Heparan sulfate proteoglycans	Clathrin-mediated, Caveolae-mediated, Non-canonical	[205,206]
Lymphocytic Choriomeningitis virus	Arenaviridae	α-dystroglycan	Non-canonical	[207]
Lassa arenavirus	Arenaviridae	α-dystroglycan	Non-canonical	[207]
Adeno-associated viruses	Parvoviridae	Heparan sulfate Proteoglycans	Non-canonical, Macropinocytosis	[208,209]
Sars-CoV-2	Betacoronaviridae	Angiotensin-converting enzyme *2*; CD147	Clathrin-mediated	[210,211]

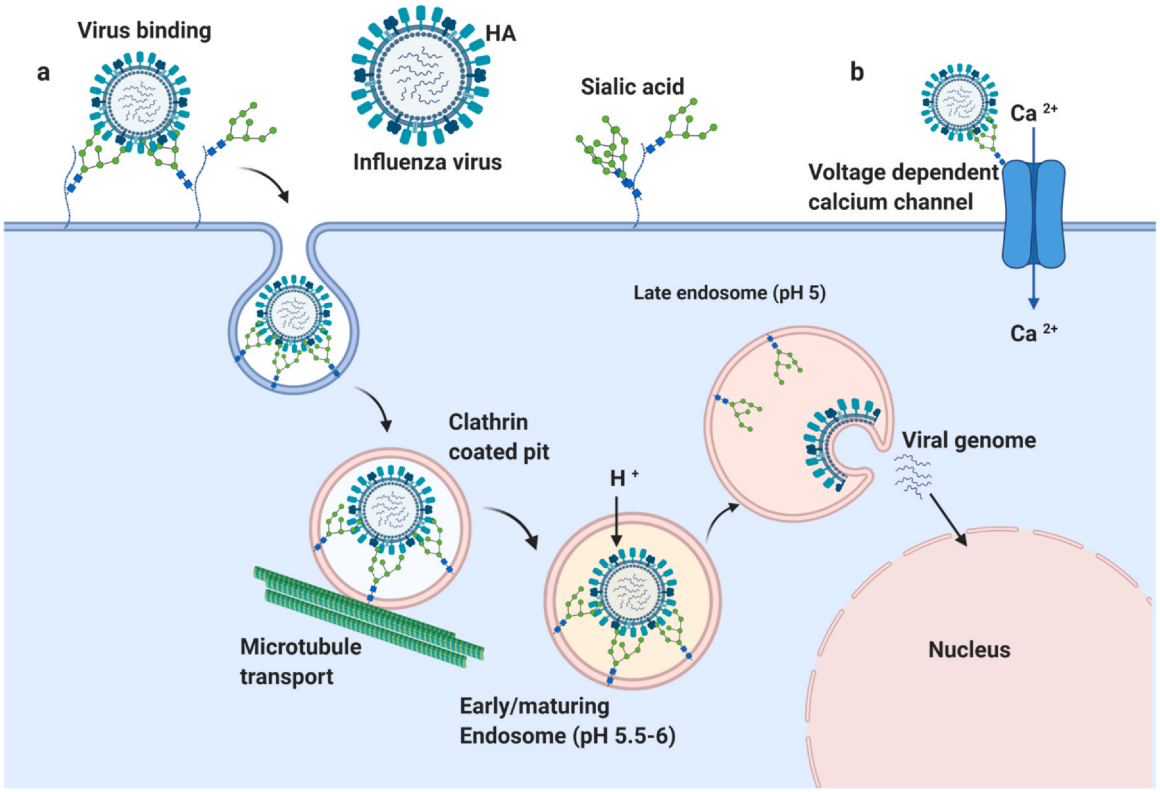

Fig. 13. Influenza A virus (IAV) endocytosis mechanism mediated by hemagglutinin proteins (HA). (a) Viral hemagglutinin proteins (HA1, HA2) of the virus coat bind to sialic acid-containing receptors on the cell surface. (b) HAs can also bind to the sialylated Ca^{2+} channels to induce intracellular Ca^{2+} fluxes. The virus is then internalized and transported to the early endosome via a specific endocytosis mechanism. Endosome maturation induces HAl to change from its pre-fusion to a post-fusion conformation. This causes fusion of the IAV membrane with the endosomal membrane. Subsequent virus uncoating releases the viral genome into the cytosol [192].

dominant-negative mutation of caveolin-1. This suggests the involvement of a caveolae-mediated pathway. The ZIKA infection pathway showed subsequent transport through Rab5-positive and Rab7-positive vesicles. This observation is indicative of the participation of early and late endosomes in the life cycle of the ZIKA virus within the host cell [195]. C-type lectin receptors recognize ZIKA viruses through their E surface proteins. Flaviviruses such as ZIKA can also enter a host cell through receptors that do not bind to E protein. Negatively-charged lipids on the viral membrane interact with lipid receptors such as T-cell immunoglobulin mucin (TIM) and tyrosine kinase receptors TAM (**T**YRO3, **A**XL and **M**ER) on the host cell surface to mediate endocytosis of the ZIKA virus [196].

The enveloped alphavirus, Mayaro virus, is the causative agent of a mosquito-borne febrile illness with highly debilitating arthralgia. Although its preferential route of endocytosis is mediated by clathrin, the virus also uses cholesterol-enriched caveolae-derived vesicles as an alternative route for cell entry. Mayaro viruses are highly dependent on cholesterol for cell infection. Caveolin-coated vesicles containing Mayaro viruses showed co-localization with the early endosome marker Rab5; the observation indicates that endocytosed viruses are rapidly delivered into early endosomes where membrane fusion occurs between the virus and the endosome [197].

Studies on the HIV-1 have identified multiple ways in which caveolin-1 is involved in HIV-1 infection and life cycle. A role for the HIV retrovirus protein “negative regulatory factor” (Nef) is the alteration of cholesterol metabolism, as was demonstrated in macrophages. There is enhanced cholesterol transport to the cell membrane and increase in caveolin-1 during HIV infection. The enhanced level of caveolin-1 subsequently promotes cholesterol efflux, blocks the fusion steps of virus infectivity and enables the viral particles to persist inside the infected cells [228].

Human coronavirus OC43 (hCoV-OC43) is a betacoronavirus responsible for diseases of the respiratory tracts in humans. This enveloped, positive-sense, single-stranded RNA virus uses sialoglycan-based receptors containing 9-*O*-acetylated sialic acid as entry portal for internalization [198]. hCoV-OC43 have been reported to utilize caveolin-1-dependent endocytosis for host cell infection. Formation of caveolae vesicles containing the betacoronaviruses is mediated by dynamin. Incubation of cells with the nystatin inhibitor, or depletion of caveolin-1 by RNA interference technology abrogated hCoV-OC43 cell infection. Those findings indicate that virus entry is caveolae-mediated [229]. Nasal epithelial cells, which represent the gateway of entry for many coronaviruses (including the novel SARS-CoV-2 betacoronavirus), express high levels of dynamin, the endocytic marker for CME and the caveolae-mediated endocytosis. Thus, it is not surprising that these pathways are involved in the entry of coronaviruses [12].

Macropinocytosis

Macropinocytosis is a transient, actin-dependent cellular process used by viruses larger than 150–200 nm, such as the African swine fever virus and the vaccinia virus [199]. The entry of a virus by macropinocytosis starts with contact between the virus and the cell membrane of the host, which may occur via receptor binding or by simple contact and adsorption to proteoglycans or other extracellular matrix components. Attachment of the virus to the cell membrane triggers the intracellular signals necessary to initiate transduction of the proteins involved in the formation of macropinosomes. The signaling pathway involves activation of multiple kinases, GTPases and other factors that in turn affect cell morphology, induce actin depolymerization, membrane blebbing and ruffles, as well as actin-dependent formation of membrane

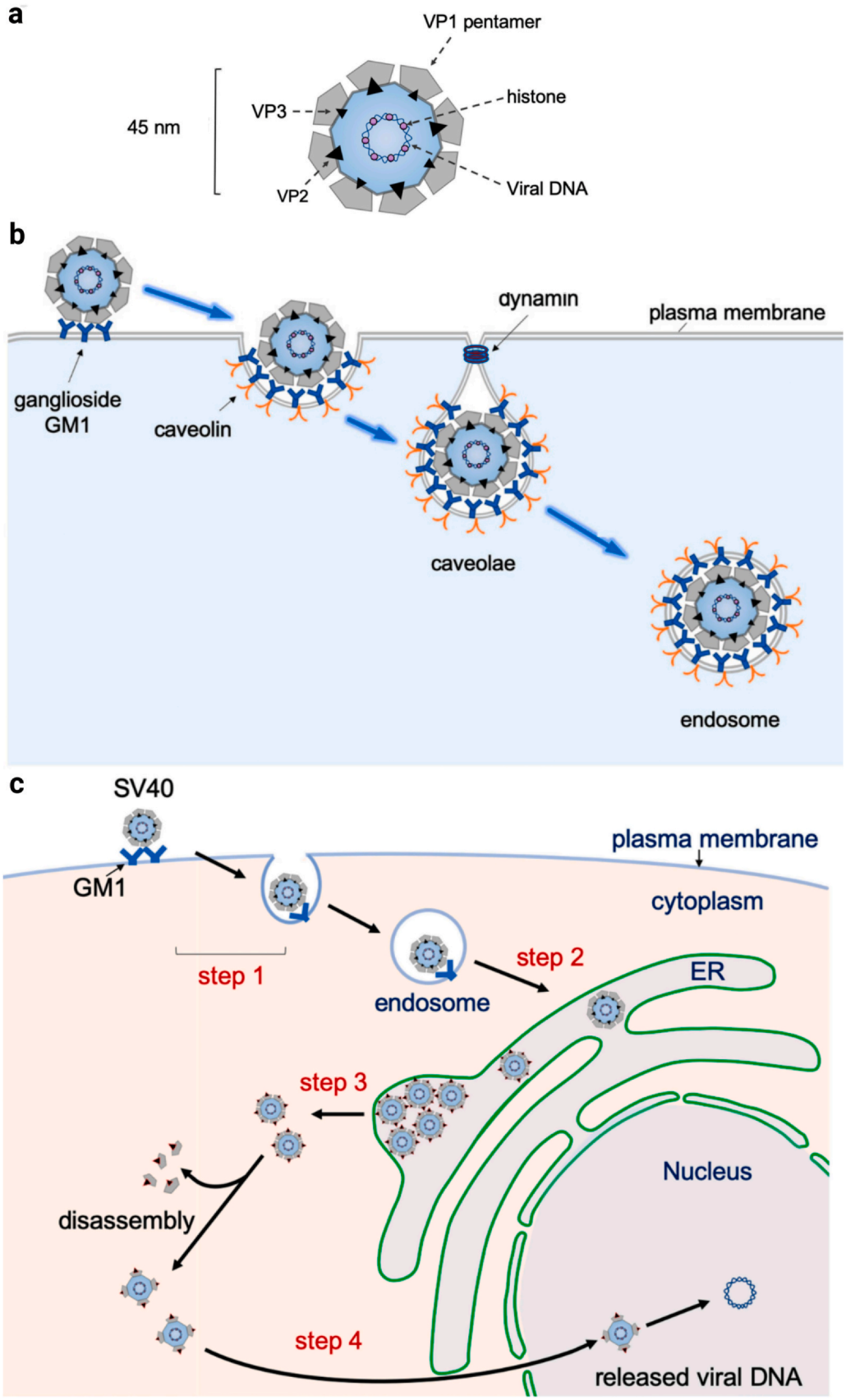

Fig. 14. Entry of simian vacuolating 40 (SV40) into a host cell by caveolae-dependent endocytosis. a. Schematic of the SV40 virus, showing the major structural protein VP1 assembled into pentamers. b. SV40 entry is mediated by its binding to the ganglioside GM1 on the host cell membrane. This binding triggers a signaling cascade that results in caveolae-mediated endocytosis and virus internalization into the endosome. c. The endosome carries the virus to the endoplasmic reticulum (ER). The virus escapes from the ER into the cytosol where it is disassembled. The disassembled virus components release their viral genome to the nucleus. This enables transcription and replication of the SV40 virus (reprinted from [193] with permission from MDPI under a Creative Commons Attribution-NonCommercial 4.0 International License).

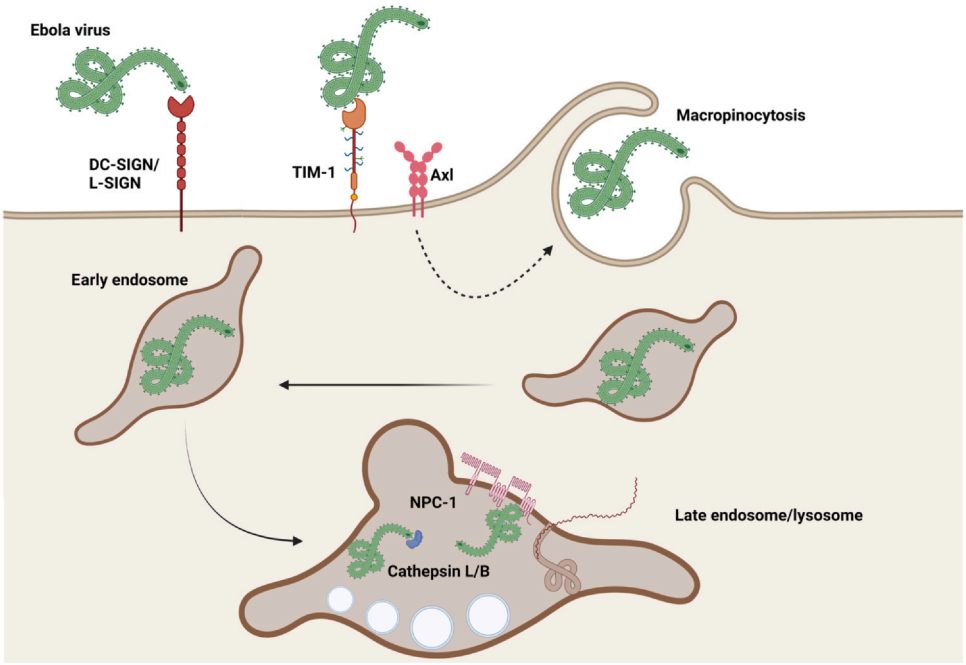

Fig. 15. Ebola virus entry by macropinocytosis. The Ebola virus binds to different molecules on the host cell membrane by means of its surface spike glycoproteins, including the C-type lectin receptors DC-SIGN and L-SIGN. TIM-1 and AXL1 receptors act as coreceptors. Following host cell membrane attachment, the Ebola virus is internalized by macropinocytosis. During endosome maturation to late endosome/lysosome, the glycoprotein GP1 is proteolytically cleaved by cathepsins L and B to its fusogenic form. Cleaved GP1 interacts with the endosomal receptor NPC1. These events result in the fusion of the membranes of the endosome and the lysosome, which releases the viral genome into the cytosol [233].

protrusions [230]. These transient changes permit the uptake of extracellular liquid and internalization of large parts of plasma membrane with surface-bound viruses [231]. The virus-containing macropinosomes are then transferred through the cytosol for fusion with late endosomes or lysosomes.

Viruses larger than the invaginations observed in CME or caveolae-mediated endocytosis employ preferentially this mechanism for cell entry, as observed for many filoviruses, poxviruses, paramyxovirus, adenovirus and HIV-1 [203,232]. One well-studied example is the filovirus Ebola (Fig. 15). Ebola viruses are very variable in length, ranging from 600 to 1400 nm long. Hence, viral entry excludes the involvement of clathrin- or caveolin-coated vesicles. The internalization of Ebola virus is dependent on the presence of cholesterol-enriched lipid raft microdomains and lipid raft formation. Ebola virus binds to the host cell surface using its viral spike glycoprotein. After internalization, the virus is trafficked through early and late endosomes to lysosomes where membrane fusion and genome release occur [201].

Unusual endocytic pathways

In addition to the aforementioned well-characterized pathways, some viruses utilize other mechanisms for internalization into host cells. These entry mechanisms are generally independent of clathrin and caveolae, and maybe dynamin-dependent or dynamin-independent. The evolution of such unusual endocytic pathways may have been dictated by the particular needs of various virus species to develop more productive infections, such as the need to bypass the endosomes and be transported to the low pH lysosomes to promote the fusogenic activity of the viral glycoproteins for virus uncoating. Non-canonical endocytosis pathways are used by several viruses. For example, human papilloma virus-16 (HPV-16) entry into epithelial cells involves a mechanism that is clathrin-, caveolin-, lipid raft-, flotillin-, cholesterol- and dynamin-independent, but shows similarities to macropinocytosis. Similar to classical macropinocytosis, HPV-16 endocytosis involves actin cytoskeleton reorganization and tyrosine kinase signaling. Nevertheless, this form of endocytosis is independent of classical Rho-like GTPase signaling [234]. Binding of HPV-16 to host cells occurs through interaction with heparan sulfate proteoglycans on the plasma membrane of epithelial cells. The viral particles are then transferred to a secondary uptake receptor complex including the tetraspanin CD151 and annexin A2/S100A10 heterotetramer as organizers of the HPV-16 entry platform and as a secondary receptor to mediate endocytosis and intracellular trafficking of HPV-16 through endosomes [205,235]. Some of the features of this unusual pathway have also been reported as one of the alternate pathways described for the entry of influenza A virus [236].

Lymphocytic choriomeningitis virus and Lassa fever arenavirus, the causative agents of acute hemorrhagic fever endemic in many West African countries, have been reported to enter host cells via an unknown clathrin-, caveolin- and dynamin-independent pathway. Upon alpha-dystroglycan binding, arenaviruses are able to enter host cells via multivesicular bodies and are rapidly delivered to the late endosomes where fusion occurs, bypassing the early endosomes. Unlike other viruses, the entry of lymphocytic choriomeningitis virus does not seem to induce evident changes in cell morphology or changes in actin, suggesting an unusual, yet-to-be-discovered endocytic mechanism for the entry of arenaviruses in their host cells [207].

Glycosylphosphatidylinositol-anchored protein-enriched endosomal compartments (GEEC) are less commonly used by viruses to enter host cells. Endocytosis through GEEC involves lipid rafts but not clathrin, caveolin or dynamin. The uptake of adeno-associated viruses was prevented by inhibiting Arf1, Cdc42 and GRAF1, factors that mediate the formation of clathrin-independent carriers (CLIC) involved in the GEEC pathway [208]. Nevertheless, other studies suggest that macropinocytosis is used by adeno-associated viruses as a mechanism for virus uptake in certain cell types [209]. Although the most commonly described entry route is clathrin-mediated, the Dengue virus has been reported to penetrate host cells via a non-canonical endocytic pathway mediated by dynamin but independent of clathrin, caveolae or lipid rafts [237].

Different pathways of endocytosis: identification and analysis

Endocytosis pathways have unique diagnostic criteria. The size of the endocytic vesicle, for example, is highly variable. Sizes including ~ 100 nm spherical, ~ 60–80 nm tubular (maybe several hundred nm long), ~ 100 nm tubular and > 200 nm tubular have been reported in the literature for CME in different cell types [28]. This variability enables large nanoparticles and rod-like viruses to enter the internal milieu of a cell via actin elongation of a clathrin-coated pit [238].

The fast endophilin-mediated endocytosis (FEME) pathway is dynamin-dependent and independent of clathrin. The FEME pathway produces endocytic carriers after ligand binding to specific receptors [239]. It relies on interactions between the SRC homology 3 (SH3) domain of dependent receptors and endophilin (e.g. G-protein-associated receptors) or indirect communication through intermediate proteins (CIN85 and CBl) [239]. These interactions stabilize endophilin that is localized to the leading edge of migrating cells through the phosphatidylinositol (3,4)-bisphosphate-binding protein lamellipodin. The FEME pathway can form carriers in a fraction of a second the created tubular carriers are 60–80 nm diameter and several hundred nm long [239]. The CLIC/GEEC pathway is entirely independent of dynamin or clathrin. However, it is similar to the FEME pathway in that both are located at the anterior edge of migratory cells and include tubular and ring-shaped pleomorphic carriers. Unlike FEME that is stimulated by receptor-ligand interaction, the CLIC/GEEC pathway occurs continuously. This unique feature of the CLIC/PEEC pathway results in the uptake of abundant surface proteins such as the hyaluronic acid receptor (CD44) and the glycosylphosphatidylinositol-anchored protein [239–241].

Phagocytosis involves the uptake of smaller particles, including nanoparticles [242]. Membrane extension and phagosome formation depend on the actin cytoskeleton, which eventually becomes polymeric at the base of the cup and polymerizes along with the particle at the tip of the lamellae. Phagocytosis involves a series of small GTPases, including cell division control protein 32 homolog (Cdc42), Rac, and RhoA, that work with effectors such as Wiskott-Aldrich syndrome protein (WASP) and Arp2/3 complex to produce a branched actin network [243].

Caveolae morphology is a valuable benchmark for their identification. A caveola comprises an onion-shaped pit (~ 60 nm in diameter) with a narrow neck attached to the plasma membrane. Caveolae are generated by caveolins and cavins that work in converting with accessory proteins such as Eps15 homology domain-containing 2 (EHD2), pacsin/syndapins and tyrosine-protein kinase transmembrane receptor (ROR1). Loss of caveolae occurs when the expression of caveolin-1, caveolin-3 or cavin1 is ablated [225].

Understanding the uptake mechanism of viruses is important for the development of effective and functional biotherapeutics. Knowledge of virus entry mechanisms into cells has inspired scientists to synthesize efficient and productive nanomaterials. Viruses are naturally-occurring nanoparticles that are capable of defying host protection mechanisms and entering the cells to exert their functions. Inspired by viruses, researchers have developed increasingly efficient nanomaterials in terms of size, shape and surface functionalization, to improve targeting to the host cell, maximize cargo entry and achieve more precise delivery to the destined subcellular compartments. For example, some encapsulated viruses enter host cells through direct fusion with the plasma membrane, a pathway that is exploited by different engineered nanoparticle systems [244]. Likewise, nanorods are inspired by rod-like viruses, the entry of which is mediated by CME through actin elongation of the clathrin-coated vesicles [245]. It has been shown that both viruses and nanoparticles of specific sizes can induce the formation of an appropriate curvature of the cell membrane. This process is necessary for recruiting the proteins involved in the CME pathways. In the case of viruses, the concomitant presence of signals mediated by their interaction with cell surface receptors trigger endocytosis and their internalization [246]. Highly-efficient viral infection that occurs through receptor-mediated endocytosis provides the blueprint for the biomimetic design of therapeutic nanoparticles that are engineered for specific entry through binding to specific cell surface receptors (e.g. via the hemagglutinin receptor or the C-type lectin receptor). The mechanisms of viral infection of host cells have also inspired scientists to develop positively-charged nanoparticles that can expedite endocytosis via absorption to the cell membrane [245]. The study of virus endocytosis mechanisms can also aid in developing advanced engineered nanomaterials that can escape the surveillance of host immune cells, thereby avoiding rapid clearance by the mononuclear phagocytic cells and prolonging circulation time of these novel nanomaterials in the bloodstream [247]. Although differences exist between the uptake and internalization pathways employed by viruses and nanomaterials, naturally-occurring viruses still represent an inexhaustible source of inspiration for researchers in the field of biomedicine.

Nanobiovectors (e.g. viruses) are usually recognized by cellular receptors on the plasma membrane [248]. Abiotic engineered nanomaterials (e.g. metal nanoparticles) are internalized by cells using multiple cellular mechanisms. Abiotic nanocompounds possess different sizes, compositions, architectures, surface charges, hydrophobicity, roughness and elasticity. Hence, there are different ways for nanoparticles to be recognized by cells through a selective endocytosis mechanism [1]. For example, the efficacy of entry of nanoparticles with various dimensions into cells is of the order: 50 nm > 200 nm > 500 nm > 1000 nm [249]. The geometric shape also affects the number of nanoparticles that can attach to the cell surface receptors, This is an essential characteristic for counteracting blood flow [250]. Nanorods can bind to many cell surface receptors because of their long axis structure, compared to spherical nanoparticles [251]. Nanoparticle shape can result in breaking of the curvature energy landscape, which, in turn, determines the endocytic pathway for their intake [134]. Cell uptake study of three gold nanoparticles with the same size (~ 50 nm) but different architectures (stars, rods and triangles) showed that the gold nanotriangles are preferentially uptaken by RAW264.7 macrophage cells [251]. In contrast, gold nanorods and gold nanostars were preferentially uptaken by CME. The surface charge of nanoparticles also affect their endocytosis efficacy.

Although abiotic engineered nanomaterials may enter cells by direct fusion with the plasma membrane, they are more often uptaken via endocytosis. Physical properties such as the size, surface engineering (e.g. expression of ligand for a certain cell receptor) or the target cell type can be instructional on the endocytosis pathway employed by the nanoparticles. For instance, large nanostructures with a size range from 500 nm to a few micrometers can enter cells via macropinocytosis, whereas smaller nanoparticles often rely on CME [26]. Irrespective of the cell type, surface functionalization of the nanomaterials (both before and after entering the human body) can affect the route of endocytosis. For instance, coating of nanoparticles with a bioactive agent can change their surface charge and size, which may alter the endocytic route. In addition, serum proteins play an essential role during nanoparticle endocytosis in vivo because these proteins are rapidly adsorbed on the nanoparticle surface to produce a protein corona. These proteins, like vitronectin, can bind to specific receptors on the cell surface and cause particles to accumulate before they reach the cell. This makes the nanoparticles much larger than when they are outside the body (in water or phosphate-buffered saline) [252,253]. This is an example illustrating that it may not be possible in many cases to predict the cell uptake pathway. Fig. 16 represents an overview of the different pathways through which viruses and nanomaterials can enter cells, depending on their shape, aspect ratio and surface functionality.

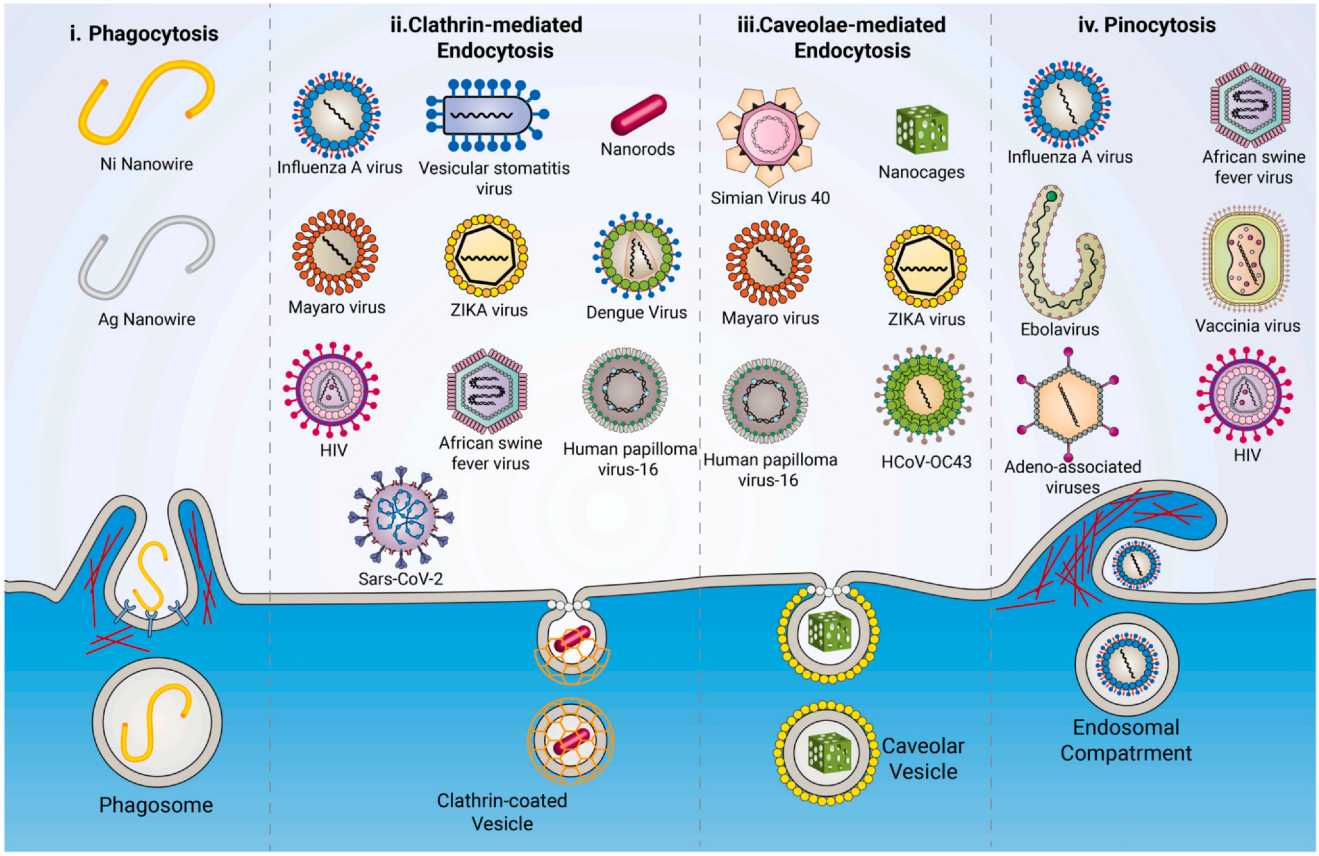

Fig. 16. Schematic portraying the different pathways employed by viruses and nanomaterials for cell entry, based on their shape, aspect ratio and surface functionality.

Scientists have devoted a lot of effort on defining endocytosis pathways. Many of those studies utilize chemical inhibitors such as drugs that interfere with actin polymerization during macropinocytosis or phagocytosis (e.g. cytochalasin D [254] or amiloride, which inhibit Na^+/H^+ exchange at the cell surface, blocking micropinocytosis). Others employ inhibitors of CME such as the endosome acidification blocker, chloroquine [255]. Library screening of chemical compounds to identify selective inhibitors of endocytic processes has led to the identification of dynasore, a pharmacological inhibitor of the guanosine triphosphatase (GTPase) activity of dynamin, a GTPase protein that is essential for membrane fission during CME [256]. Pitstop 2, another potent inhibitor of CME as well as clathrin-independent endocytosis, was discovered using a similar screening method [257].

Uptake studies often utilize a fluorescently-labeled cargo that can be analyzed using confocal fluorescence microscopy or flow cytometry. However, these techniques sometimes generate false information because of the inability to discriminate between internalized material and merely membrane-bound material [258]. Thus, a number of precautions must be used to discriminate the different internalization pathways. Examples include the use of fluorescent click sensors [259], pH-based sensors or quencher conjugates [258]. In addition, genetic approaches such as gene silencing or knockout of proteins involved in the different internalization pathways (e.g. Caveolin-1, Cavin1, Dynamin-2 or Clathrin) are often employed to discriminate the different routes of cargo internalization [260].

Endocytic routes are novel pathways that are still in their infancy of study and need to be investigated further. However, many of the current study approaches have limitations and challenges. It is difficult to identify cell uptake pathways due to the lack of standardized procedures. Moreover, the complexity of the endocytosis process, which involves many biomolecules, feedback loops and signaling cascades [49], is still not fully understood.

Inhibition of endocytic trafficking of nanomaterials and viruses

Researchers routinely employ specific and non-specific pharmacological/chemical inhibitors of endocytosis pathways to study the entry of cargoes (i.e. proteins, viruses or nanomaterials) into the cell as well as to discern the major endocytic pathway based on its differential sensitivity to the chemical blockers. Many compounds have been identified as selective blockers of the molecular components required for a specific form of endocytosis [255]. The possibility of rapid and acute administration, as well as the reversibility of the blocking are attractive characteristics. Nevertheless, endocytosis blockers should be employed with caution. First of all, the efficacy of the administered dose to the cells should be carefully determined for each cell type. This is because the cytotoxicity of endocytosis inhibitors is strongly cell type-dependent [255]. Another salient issue that has to be considered is that the non-specific effects of such inhibitors on other endocytic pathways cannot be excluded. For example, chlorpromazine and potassium depletion inhibits CME by sequestering clathrin and adapter proteins from the plasma membrane to the intracellular organelles. However, these two drugs were found to have other unexpected roles in some types of cells in inhibiting clathrin-independent endocytosis [261]. Similarly, several inhibitors employed to selectively block macropinocytosis also inhibit the clathrin-independent/dynamin-dependent cell entry pathway [239]. The dynamin blocker Dynasore (a GTPase inhibitor) has been shown to interfere with the actin cytoskeleton and cholesterol homeostasis, thereby disrupting the lipid raft organization [256]. All these off-target side effects should be meticulously considered in determining the role of a particular form of endocytosis in

the cells. The combined use of genetic approaches, RNA interference and selective new generation pharmacologic agents should be synergistically employed to inhibit endocytic trafficking of nanomaterials [260].

Understanding the cellular mechanisms that mediate the entry of viruses into their host cells can be of great help in identifying pharmacological targets useful for blocking viral infections. Identification of new drugs capable of specifically inhibiting the entry of a virus is a very expensive and time-consuming process. For this reason, re-purposing of existing drugs is a strategy that can accelerate the development of pharmacological therapies [262].

The new coronavirus SARS-CoV-2 is a respiratory virus belonging to the large family of coronaviruses and is the causative agent of the recent COVID-19 pandemic. To date, the entry mechanisms and the implication of the endocytic pathway of the new emerging SARS-CoV-2 have not been totally substantiated. Similar to SARS-CoV, the virus responsible for the original Severe Acute Respiratory Syndrome (SARS), SARS-CoV-2 predominantly binds its surface Spike proteins to the epithelial Angiotensin-Converting Enzyme II (ACE2) receptor for cellular entry. This is followed by the endocytosis of the viral particles (Fig. 17). This route of entry includes cleavage of the spike protein by the transmembrane protease serine 2 (TMPRSS2) into two functional subunits S1 and S2. The S1 subunit is responsible for the binding of ACE2 via its receptor-binding domain, while the S2 subunit is involved in the fusion of the viral envelope with the cell membrane [210].

Recently neuropilin-1 was proposed as a cell surface co-receptor for SARS-CoV-2 infection, interacting with the spike S1 subunit. Inhibition of neuropilin-1 by RNA interference, or using a monoclonal antibody against neuropilin-1, impairs the interaction of the plasma membrane with SARS-Cov-2 S1, reducing viral entry in the host cells [263,264].

Well-known drugs that are capable of blocking the endocytosis process are currently being evaluated for blockade of SARS-CoV-2 entry into lung epithelial cells. Some of these drugs have not yet been approved for human use [265], but are on the list of essential medicine published by the World Health Organization, making them easily accessible anywhere in the world [12]. Chloroquine (CQ) and hydroxychloroquine (HCQ) are ancient, clinically available anti-malaria drugs. Their re-purposing was proposed for the treatment and prophylaxis of ZIKA virus infections [266]. During the new COVID-19 pandemic, the scientific community is actively exploring the use of CQ and HCQ to combat SARS-CoV-2 infections. Both drugs are lysosomotropic drugs that block viral fusion events by increasing the pH in vacuoles, which adversely affect endolysosomal trafficking. In addition, both CQ and HCQ interfere with the glycosylation of ACE2, impairing binding of the viral spike proteins to host cell surface receptors [267,268]. Several in vitro [269,270] and preclinical [271,272] studies on the use of CQ and HCQ in blocking SARS-CoC-2 infection have been conducted. Although in vitro studies have clearly demonstrated the antiviral activity of these compounds, subsequent clinical studies failed to confirm the benefits of the use of these drugs in COVID-19 treatment [267,273].

Many viruses, including filoviruses and coronaviruses, require proteolytic processing of their surface glycoproteins to penetrate host cells [274]. Endosomal-lysosomal protease inhibitors such as the serine protease inhibitor Camostat have been recently proposed for the treatment of SARS-CoV and middle east respiratory syndrome-related coronavirus (MERS-COV) infections [275]. Serine proteases are involved in the cleavage of the spike protein into S1 and S2 subunits. Recently, Camostat has been reported to be hopeful in blocking the entry of SARS-CoV-2 by inhibiting the TMPRSS2 protein. The latter is indispensable for proteolytic processing of the SARS-CoV-2 spike protein. These pieces of evidence suggest a possible new target for anti-COVID-19 therapeutic interventions [276].

Cyclophilin A (CyPA) is an intracellular protein belonging to the PPIase family. It is involved in the replication of RNA viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV),

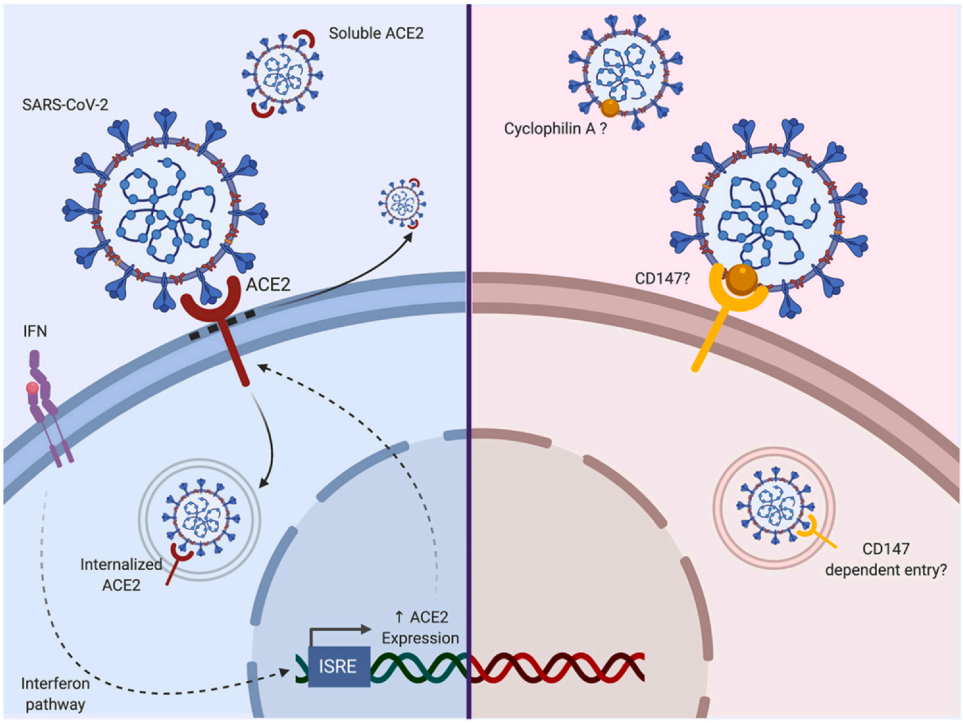

Fig. 17. The two proposed routes of entry for SARS-CoV-2 to date. Angiotensin-converting-enzyme 2 (ACE2), which has been described as an interferon-stimulated gene (ISG), is the predominant route of entry for SARS-CoV-2. In addition, CD147 is purported as a potential second route of entry. Based on a previous study with SARS-CoV, interaction with cyclophilin A is also possible. The blue background corresponds to cells expressing ACE2, whereas the red background represents cells expressing CD147. Solid arrows correspond to a direct activity involving ACE2. Dotted arrows correspond to an indirect promoting activity.
Reprinted from [210] with permission from MDPI under a Creative Commons Attribution-NonCommercial 4.0 International License.

hepatitis C virus (HCV) and coronaviruses such as SARS-CoV and SARS-CoV-2. Host cyclophilin A is a potential drug target for coronaviruses and cyclosporine A (CsA) can act as CyPA inhibitor. Cyclosporine A binding to CyPA changes it conformation, blocks viral replication and prevents its from binding with host cell surface receptor (see below) [277].

Cyclophilin A is involved not only in virus replication but also in virus infection. After interaction with the viral proteins, CyPA is incorporated on the surface of virus particles released from the host cells. The involvement of the highly glycosylated transmembrane protein CD147 as a third receptor for the SARS-CoV-2 virus entry has recently been reported [278]. SARS-CoV-2 can interact with CD147 via binding with CyPA, increasing virus adhesion to host cells. This important finding suggests that CyPA inhibitors such as CsA may be used for blocking this route of SARS-CoV-2 entry [210]. Because of its immunosuppressive effects, the use of CsA can cause adverse side effects. For this reason, non-immunosuppressive analogs based on chemical modifications of CsA are currently being evaluated in many clinical trials. These CsA derivatives can preserve the inhibitory effect of the drug but without inducing immunosuppression in humans [277].

Drugs blocking CME have been shown to be hopeful in blocking entry of SARS-CoV and MERS-CoV viruses. Although it has not been conclusively proven, evidence suggests that SARS-CoV-2 may also utilize a clathrin-mediated mechanism for endocytosis into host cells [211]**.** Targeting CME using blocking agents such as chlorpromazine, a drug yet to be approved by the US Food and Drug Administration but used extensively in the treatment of psychoses, may be a promising strategy for fighting SARS-CoV-2 infections [279]**.**

Nanotherapy may also be exploited to disable virus particles such as SARS-CoV-2, by deactivating their surface proteins. For example, polymeric nanoparticles may be surface functionalized by decorating them with biomimetic ACE2 proteins. In this scenario, they can as decoys by attacking the SARS-CoV-2 viruses and attaching to the viruses via those biomimetic ACE2 proteins to deactivate the viruses.

Concluding remarks, challenges and future perspectives

Despite their remarkable achievements, there are pivotal hurdles to be overcome prior to the routine use of nanomaterials for biomedical applications. One of these challenges is a clear comprehension of the endocytosis mechanisms involved in the cellular uptake of nanomaterials with different characteristics. Different parameters affect the endocytosis of nanoparticles. The major factors include size, architecture and surface functionality of the nanomaterials. Other variables such as topography [280], morphology [281], colloidal stability, density and powder kinetics also have to be considered [103]. Even if the aforementioned factors have been addressed for a specific type of nanomaterial, there are additional characteristics that should be contemplated. For example, the concentration of decorated substances on the nanomaterials (i.e. ligand density), as well as polydispersity of nanoparticles (i.e. size distribution ratio) are other important features. This is because cells are not exposed to one particle or monodisperse particles. Nanoparticles possess a size range and may have different degrees of functionalization.

It is important to reiterate that the effect of one variable of nanoparticle characteristics cannot be generalized for other nanoparticles. As an example, the influence of surface charge is different for nanosheets and nanorods. Enhancing the surface charge in one nanostructure may enhance cellular uptake while internalization may be reduced with another nanostructure. The quantity of cellular uptake of positively-charged graphene nanosheets is independent of their size, whereas the internalization efficacy of negatively-charged graphene nanosheets is dependent on their size [125]. The same trend, however, does not apply to gold nanorods.

A vaguely explored parameter that should also be comprehended in detail is the adverse effects of nanomaterials, especially metallic nanoparticles, after they are internalized into host cells via endocytosis. Metallic nanoparticles that are uptaken contain a significant amount of that metal element. Processing of these nanoparticles within acidic lysosomes may result in intracellular accumulation and even overloading of the particular metal ion. Because such an ion internalization process does not involve the cell's metal ion transporters, it may provoke excessive oxidative stresses that damage the cells' DNA, lipids and proteins. Thus, parameters such as dose-dependent impact of metal nanoparticle uptake, oxidative stress response, DNA damage response and the expression of apoptosis-associated genes should be stringently assessed when nanoparticle endocytosis is studied [282].

Another significant parameter is the type of cell lines used to evaluate the cellular uptake of nanoparticles *ex vivo*. For instance, normal and tumor cell lines possess different characteristics that induce different behavior for a specific nanoparticle. In addition, these cell lines possess different microenvironments compared to the tissues that surround cells in vivo. This microenvironment (e.g. normal tissue with pH 7.2–7.4 and tumor tissue with pH 6.0–6.8) also may affect the intrinsic properties of nanoparticles such as surface charge. In addition to the microenvironment, another parameter that should be taken into account when performing in vivo investigations is protein adsorption on the surface of the nanoparticles within the circulation. The adsorbed serum proteins in this protein corona may adversely influence how nanoparticles enter cells, such as the time required for internalization [4,283].

The last, but certainly not least challenge, is the inconsistency between in vitro and in vivo experimental results. Bluntly expressed, the positive results obtained from in vitro studies may not be demonstrable in vivo studies. Thus, it is crucial to construct an in vitro system that mimics the in vivo microenvironments well, or at least develop some theoretical models that enable the translation of in vitro results into in vivo application strategies [284].

Viruses can be valuable tools in the development of nanotechnologies. They are nature-derived nanoscaffolds with unique properties and that can be modified relatively easily in terms of structure and surface charge. Virus-like particles are examples of self-assembled nanocarriers incorporating viral structural proteins which mimic the physicochemical properties of live infectious viruses. These virus-like particles enter host cells using the same endocytosis mechanisms employed by natural pathogens. Thus, they are used in nanotechnology as drug and gene delivery systems, imaging agents and as vaccines against infectious diseases [285]. Understanding the mechanisms of endocytosis may provide avid opportunities to control the fate of virus-like particles and other nanomaterials in human cells, and to improve the efficacy of nanoparticle uptake. Knowledge on the cell surface receptors utilized by viruses in receptor-mediated endocytosis pathways may be exploited for targeting nanocarriers in specific cell subpopulations. Likewise, such information may be employed for designing nanoparticles with augmented rates of endocytosis for optimized delivery of therapeutic drugs to specific target cells. Studies on the escape mechanisms developed by viruses to avoid the degradative environment of the endolysosomal compartments will be extremely useful in enhancing the pharmacokinetics and the stability of therapeutic nanoparticles [213]. In addition, laboratory-synthesized molecules that mimic the ligands of specific cellular receptors may be used to localize nanoparticles in endosomal/phagosomal compartments, to activate antigen-presenting immune cells such as dendritic cells and macrophages to trigger adaptive immune responses [286].

A profound understanding of endocytosis mechanisms and intracellular ingress pathways used by viruses should help in developing drugs that block the entry of viruses or determine their fate during their trafficking within the host cell. As in the case of the recent SARS-CoV-2 pandemic, strategies for blocking host cell infection may focus on various stages of the viral cycle. Drugs that are capable of blocking the interaction of the virus with its host cell receptor, such as the ACE inhibitors and angiotensin receptor blockers (e.g. captopril, ramipril and losartan) and guanidino-based serine protease inhibitors (e.g. camostat and nafamostat) have been used for emergency treatment of severely-infected Covid-19 human subjects [287]. Drugs that interfere with CME and/or caveolae-mediated endocytosis, drugs that prevent endosome maturation, as well as drugs that block cleavage and activation of viral proteins involved in the fusion of the viral capsid with the host cell membrane, are valuable tools in development of broad-spectrum pharmaceutics for combating the emergence and re-emergence of virus-based epidemics and pandemics [288,289].

CRediT authorship contribution statement

Pooyan Makvandi: Conceptualization, Writing – original draft, review & editing, Comments to the draft. **Meiling Chen:** Literature investigation, Writing – original draft. **Rossella Sartorius:** Literature investigation, Writing – original draft. **Ali Zarrabi**: Literature investigation, Writing – original draft, review & editing. **Milad Ashrafizadeh:** Literature investigation, Writing – original draft. **Farnaz Dabbagh Moghaddam:** Literature investigation, Writing – original draft. **Jingzhi Ma:** Comments to the draft. **Virgilio Mattoli:** Writing – review & editing. **Franklin R. Tay:** Conceptualization, Writing – review & editing, Comments to the draft. All authors discussed and commentated on the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] F. Dabbagh Moghaddam, I. Akbarzadeh, E. Marzbankia, M. Farid, L. khaledi, A.H. Reihani, M. Javidfar, P. Mortazavi, Delivery of melittin-loaded niosomes for breast cancer treatment: an in vitro and in vivo evaluation of anti-cancer effect, Cancer Nanotech. 12 (2021) 14, https://doi.org/10.1186/s12645-021-00085-9

[2] J. Wolfram, M. Ferrari, Clinical cancer nanomedicine, Nano Today 25 (2019) 85–98, https://doi.org/10.1016/j.nantod.2019.02.005

[3] T. Wang, J. Bai, X. Jiang, G.U. Nienhaus, Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry, ACS Nano 6 (2012) 1251–1259, https://doi.org/10.1021/nn203892h

[4] P. Shadmani, B. Mehrafrooz, A. Montazeri, R. Naghdabadi, Protein corona impact on nanoparticle-cell interactions: toward an energy-based model of endocytosis, J. Phys. Condens. Matter 32 (2020) 115101, https://doi.org/10.1088/1361-648X/ab5a14

[5] A. Jhaveri, V. Torchilin, Intracellular delivery of nanocarriers and targeting to subcellular organelles, Expert Opin. Drug Deliv. 13 (2016) 49–70, https://doi.org/10.1517/17425247.2015.1086745

[6] A.R. Voltan, K.M. Alarcon, A.M. Fusco-Almeida, C.P. Soares, M.J.S. Mendes-Giannini, M. Chorilli, Highlights in endocytosis of nanostructured systems, Curr. Med. Chem. 24 (2017) 1909–1929, https://doi.org/10.2174/0929867324666170214111205

[7] X. Ma, N. Gong, L. Zhong, J. Sun, X.J. Liang, Future of nanotherapeutics: targeting the cellular sub-organelles, Biomaterials 97 (2016) 10–21, https://doi.org/10.1016/j.biomaterials.2016.04.026

[8] E. Blanco, H. Shen, M. Ferrari, Principles of nanoparticle design for overcoming biological barriers to drug delivery, Nat. Biotechnol. 33 (2015) 941–951, https://doi.org/10.1038/nbt.3330

[9] M. Lopes, N. Shrestha, A. Correia, M.A. Shahbazi, B. Sarmento, J. Hirvonen, F. Veiga, R. Seiça, A. Ribeiro, H.A. Santos, Dual chitosan/albumin-coated alginate/dextran sulfate nanoparticles for enhanced oral delivery of insulin, J. Control. Release 232 (2016) 29–41, https://doi.org/10.1016/j.jconrel.2016.04.012

[10] M. Li, M. Zhao, Y. Fu, Y. Li, T. Gong, Z. Zhang, X. Sun, Enhanced intranasal delivery of mRNA vaccine by overcoming the nasal epithelial barrier via intra-and paracellular pathways, J. Control. Release 228 (2016) 9–19, https://doi.org/10.1016/j.jconrel.2016.02.043

[11] J. Shang, Y. Wan, C. Luo, G. Ye, Q. Geng, A. Auerbach, F. Li, Cell entry mechanisms of SARS-CoV-2, Proc. Natl. Acad. Sci. 117 (2020) 11727–11734.

[12] O.O. Glebov, Understanding SARS-CoV-2 endocytosis for COVID-19 drug repurposing, FEBS J. 287 (2020) 3664–3671, https://doi.org/10.1111/febs.15369

[13] B. Dragnea, Virus-based devices: prospects for allopoiesis, ACS Nano 11 (2017) 3433–3437, https://doi.org/10.1021/acsnano.7b01761

[14] A.V. Nicola, H.C. Aguilar, J. Mercer, B. Ryckman, C.M. Wiethoff, Virus entry by endocytosis, Adv. Virol. 2013 (2013) 803–833, https://doi.org/10.1155/2013/469538

[15] E. Ketter, G. Randall, Virus impact on lipids and membranes, Annu. Rev. Virol. 6 (2019) 319–340, https://doi.org/10.1146/annurev-virology-092818-015748

[16] A. El-Sayed, H. Harashima, Endocytosis of gene delivery vectors: from clathrin-dependent to lipid raft-mediated endocytosis, Mol. Ther. 21 (2013) 1118–1130, https://doi.org/10.1038/mt.2013.54

[17] P. Cossart, A. Helenius, Endocytosis of viruses and bacteria, Cold Spring Harb. Perspect. Biol. 6 (2014) a016972, https://doi.org/10.1101/cshperspect.a016972

[18] A. Bernheim, R. Sorek, Viruses cooperate to defeat bacteria, Nature 559 (2018) 482–484, https://doi.org/10.1038/d41586-018-05762-1

[19] E.A. Latomanski, H.J. Newton, Taming the triskelion: bacterial manipulation of clathrin, Microbiol. Mol. Biol. Rev. 83 (2019), https://doi.org/10.1128/mmbr.00058-18

[20] J. Deng, C. Gao, Recent advances in interactions of designed nanoparticles and cells with respect to cellular uptake, intracellular fate, degradation and cytotoxicity, Nanotechnology 27 (2016) 412002, https://doi.org/10.1088/0957-4484/27/41/412002

[21] C. yu Wang, P. Makvandi, E.N. Zare, F.R. Tay, L. na Niu, Advances in antimicrobial organic and inorganic nanocompounds in biomedicine, Adv. Ther. 3 (2020) 2000024, https://doi.org/10.1002/adtp.202000024

[22] E.N. Zare, R. Jamaledin, P. Naserzadeh, E. Afjeh-Dana, B. Ashtari, M. Hosseinzadeh, R. Vecchione, A. Wu, F.R. Tay, A. Borzacchiello, P. Makvandi, Metal-based nanostructures/PLGA nanocomposites: antimicrobial activity, cytotoxicity, and their biomedical applications, ACS Appl. Mater. Interfaces 12 (2020) 3279–3300, https://doi.org/10.1021/acsami.9b19435

[23] P. Makvandi, Metal-based nanomaterials in biomedical applications: antimicrobial activity and cytotoxicity aspects, Adv. Funct. Mater. 30 (2020) 1910021, https://doi.org/10.1002/adfm.201910021

[24] N. Oh, J.H. Park, Endocytosis and exocytosis of nanoparticles in mammalian cells, Int. J. Nanomed. 9 (2014) 51–63, https://doi.org/10.2147/IJN.S26592

[25] D. Manzanares, V. Ceña, Endocytosis: the nanoparticle and submicron nanocompounds gateway into the cell, Pharmaceutics 12 (2020) 371, https://doi.org/10.3390/pharmaceutics12040371

[26] S. Behzadi, V. Serpooshan, W. Tao, M.A. Hamaly, M.Y. Alkawareek, E.C. Dreaden, D. Brown, A.M. Alkilany, O.C. Farokhzad, M. Mahmoudi, Cellular uptake of nanoparticles: journey inside the cell, Chem. Soc. Rev. 46 (2017) 4218–4244, https://doi.org/10.1039/c6cs00636a

[27] P. Foroozandeh, A.A. Aziz, Insight into cellular uptake and intracellular trafficking of nanoparticles, Nanoscale Res. Lett. 13 (2018) 339, https://doi.org/10.1186/s11671-018-2728-6

[28] M. Kaksonen, A. Roux, Mechanisms of clathrin-mediated endocytosis, Nat. Rev. Mol. Cell Biol. 19 (2018) 313–326.

[29] J.P. Ferguson, S.D. Huber, N.M. Willy, E. Aygün, S. Goker, T. Atabey, C. Kural, Mechanoregulation of clathrin-mediated endocytosis, J. Cell Sci. 130 (2017) 3631–3636, https://doi.org/10.1242/jcs.205930

[30] J.E. Hassinger, G. Oster, D.G. Drubin, P. Rangamani, Design principles for robust vesiculation in clathrin-mediated endocytosis, Proc. Natl. Acad. Sci. USA 114 (2017) E1118–E1127, https://doi.org/10.1073/pnas.1617705114

[31] M. Mettlen, P.-H. Chen, S. Srinivasan, G. Danuser, S.L. Schmid, Regulation of clathrin-mediated endocytosis, Annu. Rev. Biochem. 87 (2018) 871–896.

[32] S.M. Smith, M. Baker, M. Halebian, C.J. Smith, Weak molecular interactions in clathrin-mediated endocytosis, Front. Mol. Biosci. 4 (2017) 72, https://doi.org/10.3389/fmolb.2017.00072

[33] M.M. Lacy, D. Baddeley, J. Berro, Single-molecule turnover dynamics of actin and membrane coat proteins in clathrin-mediated endocytosis, eLife 8 (2019) e52355, https://doi.org/10.1101/617746

[34] V. Haucke, M.M. Kozlov, Membrane remodeling in clathrin-mediated endocytosis, J. Cell Sci. 131 (2018), https://doi.org/10.1242/jcs.216812

[35] R. Kalia, A. Frost, Open and cut: allosteric motion and membrane fission by dynamin superfamily proteins, Mol. Biol. Cell 30 (2019) 2097–2104, https://doi.org/10.1091/mbc.E16-10-0709

[36] I. Mani, K.N. Pandey, Emerging concepts of receptor endocytosis and concurrent intracellular signaling: mechanisms of guanylyl cyclase/natriuretic peptide receptor-a activation and trafficking, Cell Signal. 60 (2019) 17–30, https://doi.org/10.1016/j.cellsig.2019.03.022

[37] Y. Boullegui, R. Ben Younes, R. Oueslati, D. Sheehan, Redox proteomic insights into involvement of clathrin-mediated endocytosis in silver nanoparticles toxicity to Mytilus galloprovincialis, PLOS One 13 (2018) 0205765, https://doi.org/10.1371/journal.pone.0205765

[38] E. Huerta-García, M.D.P. Ramos-Godinez, A. López-Saavedra, E. Alfaro-Moreno, N.P. Gómez-Crisóstomo, Z. Colín-Val, H. Sánchez-Barrera, R. López-Marure, Internalization of titanium dioxide nanoparticles is mediated by actin-dependent reorganization and clathrin- and dynamin-mediated endocytosis in H9c2 rat cardiomyoblasts, Chem. Res. Toxicol. 32 (2019) 578–588, https://doi.org/10.1021/acs.chemrestox.8b00284

[39] C. Lamaze, N. Tardif, M. Dewulf, S. Vassilopoulos, C.M. Blouin, The caveolae dress code: structure and signaling, Curr. Opin. Cell Biol. 47 (2017) 117–125, https://doi.org/10.1016/j.ceb.2017.02.014

[40] I. Levental, K.R. Levental, F.A. Heberle, Lipid rafts: controversies resolved, mysteries remain, Trends Cell Biol. 30 (2020) 341–353, https://doi.org/10.1016/j.tcb.2020.01.009

[41] N. Chaudhary, G.A. Gomez, M.T. Howes, H.P. Lo, K.A. McMahon, J.A. Rae, N.L. Schieber, M.M. Hill, K. Gaus, A.S. Yap, R.G. Parton, Endocytic crosstalk: cavins, caveolins, and caveolae regulate clathrin-independent endocytosis, PLOS Biol. 12 (2014) 1001832, https://doi.org/10.1371/journal.pbio.1001832

[42] R.G. Parton, B.M. Collins, Unraveling the architecture of caveolae, Proc. Natl. Acad. Sci. USA 113 (2016) 14170–14172, https://doi.org/10.1073/pnas.1617954113

[43] O. Kovtun, V.A. Tillu, N. Ariotti, R.G. Parton, B.M. Collins, Cavin family proteins and the assembly of caveolae, J. Cell Sci. 128 (2015) 1269–1278, https://doi.org/10.1242/jcs.167866

[44] M. Hubert, E. Larsson, R. Lundmark, Keeping in touch with the membrane; protein- and lipid-mediated confinement of caveolae to the cell surface, Biochem. Soc. Trans. 48 (2020) 155–163, https://doi.org/10.1042/BST20190386

[45] R.G. Parton, V.A. Tillu, B.M. Collins, Caveolae, Curr. Biol. 28 (2018) R402–R405.

[46] M. Hoernke, J. Mohan, E. Larsson, J. Blomberg, D. Kahra, S. Westenhoff, C. Schwieger, R. Lundmark, EHD2 restrains dynamics of caveolae by an ATP dependent, membrane-bound, open conformation, Proc. Natl. Acad. Sci. USA 114 (2017) E4360–E4369, https://doi.org/10.1073/pnas.1614066114

[47] C. Hinze, E. Boucrot, Local actin polymerization during endocytic carrier formation, Biochem. Soc. Trans. 46 (2018) 565–576, https://doi.org/10.1042/BST20170355

[48] E.S. Kornilova, Receptor-mediated endocytosis and cytoskeleton, Biochem 79 (2014) 865–878, https://doi.org/10.1134/S0006297914090041

[49] A.P.A. Ferreira, E. Boucrot, Mechanisms of carrier formation during clathrin-independent endocytosis, Trends Cell Biol. 28 (2018) 188–200, https://doi.org/10.1016/j.tcb.2017.11.004

[50] F. Zhang, H. Guo, J. Zhang, Q. Chen, Q. Fang, Identification of the caveolae/raft-mediated endocytosis as the primary entry pathway for aquareovirus, Virology 513 (2018) 195–207, https://doi.org/10.1016/j.virol.2017.09.019

[51] J. Staring, M. Raaben, T.R. Brummelkamp, Viral escape from endosomes and host detection at a glance, J. Cell Sci. 131 (2018) jcs216259, https://doi.org/10.1242/jcs.216259

[52] K. Sandvig, S. Kavaliauskiene, T. Skotland, Clathrin-independent endocytosis: an increasing degree of complexity, Histochem. Cell Biol. 150 (2018) 107–118, https://doi.org/10.1007/s00418-018-1678-5

[53] J.S. King, R.R. Kay, The origins and evolution of macropinocytosis, Philos. Trans. R. Soc. Lond. B Biol. Sci. 374 (2019) 20180158, https://doi.org/10.1098/rstb.2018.0158

[54] M. Gray, R.J. Botelho, Phagocytosis: hungry, hungry cells, Methods in Molecular Biology, Springer, 2017, pp. 1–16, https://doi.org/10.1007/978-1-4939-6581-6_1

[55] C. Melcarne, B. Lemaitre, E. Kurant, Phagocytosis in Drosophila: from molecules and cellular machinery to physiology, Insect Biochem. Mol. Biol. 109 (2019) 1–12, https://doi.org/10.1016/j.ibmb.2019.04.002

[56] S.R. Barger, N.S. Reilly, M.S. Shutova, Q. Li, P. Maiuri, J.M. Heddleston, M.S. Mooseker, R.A. Flavell, T. Svitkina, P.W. Oakes, M. Krendel, N.C. Gauthier, Membrane-cytoskeletal crosstalk mediated by myosin-I regulates adhesion turnover during phagocytosis, Nat. Commun. 10 (2019) 1–18, https://doi.org/10.1038/s41467-019-09104-1

[57] C. Rosales, E. Uribe-Querol, Phagocytosis: a fundamental process in immunity, Biomed. Res. Int. (2017) (2017), https://doi.org/10.1155/2017/9042851

[58] E. Uribe-Quero, C. Rosales, Control of phagocytosis by microbial pathogens, Front. Immunol. 8 (2017) 1368, https://doi.org/10.3389/fimmu.2017.01368

[59] E. Uribe-Querol, C. Rosales, Phagocytosis: our current understanding of a universal biological process, Front. Immunol. 11 (2020) 1066, https://doi.org/10.3389/fimmu.2020.01066

[60] C.M. Beddoes, C.P. Case, W.H. Briscoe, Understanding nanoparticle cellular entry: a physicochemical perspective, Adv. Colloid Interface Sci. 218 (2015) 48–68, https://doi.org/10.1016/j.cis.2015.01.007

[61] C.T. Ng, F.M.A. Tang, J.J. en Li, C. Ong, L.L.Y. Yung, B.H. Bay, Clathrin-mediated endocytosis of gold nanoparticles in vitro, Anat. Rec. 298 (2015) 418–427, https://doi.org/10.1002/ar.23051

[62] J.Y. Yhee, H.Y. Yoon, H. Kim, S. Jeon, P. Hergert, J. Im, J. Panyam, K. Kim, R.S. Nho, The effects of collagen-rich extracellular matrix on the intracellular delivery of glycol chitosan nanoparticles in human lung fibroblasts, Int. J. Nanomed. 12 (2017) 6089–6105, https://doi.org/10.2147/IJN.S138129

[63] Y.K. Lee, S.W. Kim, J.Y. Park, W.C. Kang, Y.J. Kang, D. Khang, Suppression of human arthritis synovial fibroblasts inflammation using dexamethasone-carbon nanotubes via increasing caveolin-dependent endocytosis and recovering mitochondrial membrane potential, Int. J. Nanomed. 12 (2017) 5761–5779, https://doi.org/10.2147/IJN.S142122

[64] A.L. Mescher, Basic Histology UEIRA'S, n.d., Chapter 4, pp. 72–82.

[65] B. He, L. Yuan, W. Dai, W. Gao, H. Zhang, X. Wang, W. Fang, Q. Zhang, Dynamic bio-adhesion of polymer nanoparticles on MDCK epithelial cells and its impact on bio-membranes, endocytosis and paracytosis, Nanoscale 8 (2016) 6129–6145, https://doi.org/10.1039/c5nr08858e

[66] A. Milewska, P. Nowak, K. Owczarek, A. Szczepanski, M. Zarebski, A. Hoang, K. Berniak, J. Wojarski, S. Zeglen, Z. Baster, Z. Rajfur, K. Pyrc, Entry of human coronavirus NL63 into the cell, J. Virol. 92 (2018), https://doi.org/10.1128/jvi.01933-17

[67] A. Szczepanski, K. Owczarek, A. Milewska, Z. Baster, Z. Rajfur, J.A. Mitchell, K. Pyrc, Canine respiratory coronavirus employs caveolin-1-mediated pathway for internalization to HRT-18G cells, Vet. Res. 49 (2018) 55, https://doi.org/10.1186/s13567-018-0551-9

[68] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Z. Xiang, Z. Mu, X. Chen, J. Chen, K. Hu, Q. Jin, J. Wang, Z. Qian, Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, Nat. Commun. 11 (2020) 1–12, https://doi.org/10.1038/s41467-020-15562-9

[69] E. Xiaofei, P. Meraner, P. Lu, J.M. Perreira, A.M. Aker, W.M. McDougall, R. Zhuge, G.C. Chan, R.M. Gerstein, P. Caposio, A.D. Yurochko, A.L. Brass, T.F. Kowalik, OR14I1 is a receptor for the human cytomegalovirus pentameric complex and defines viral epithelial cell tropism, Proc. Natl. Acad. Sci. USA 116 (2019) 7043–7052, https://doi.org/10.1073/pnas.1814850116

[70] C.I. Vardavas, K. Nikitara, COVID-19 and smoking: a systematic review of the evidence, Tob. Induc. Dis. 18 (2020) 20.

[71] A. Pizzorno, B. Padey, T. Julien, S. Trouillet-Assant, A. Traversier, E. Errazuriz-Cerda, J. Fouret, J. Dubois, A. Gaymard, F.X. Lescure, V. Dulière, P. Brun, S. Constant, J. Poissy, B. Lina, Y. Yazdanpanah, O. Terrier, M. Rosa-Calatrava, Characterization and treatment of SARS-CoV-2 in nasal and bronchial human airway epithelia, BioRxiv (2020), https://doi.org/10.1101/2020.03.31.017889

[72] A.O. Docea, A. Tsatsakis, D. Albulescu, O. Cristea, O. Zlatian, M. Vinceti, S.A. Moschos, D. Tsoukalas, M. Goumenou, N. Drakoulis, J.M. Dumanov1, V.A. Tutelyan, G.G. Onischenko, M. Aschner, D.A. Spandidos, D. Calina, A new threat from an old enemy: re-emergence of coronavirus (review), Int. J. Mol. Med. 45 (2020) 1631–1643, https://doi.org/10.3892/ijmm.2020.4555

[73] W. Wei, Y. Huang, D. Li, H.F. Gou, W. Wang, Improved therapeutic potential of MSCs by genetic modification, Gene Ther. 25 (2018) 538–547, https://doi.org/10.1038/s41434-018-0041-8

[74] H.V. Quang, C.C. Chang, P. Song, E.M. Hauge, J. Kjems, Caveolae-mediated mesenchymal stem cell labelling by PSS-coated PLGA PFOB nano-contrast agent for MRI, Theranostics 8 (2018) 2657–2671, https://doi.org/10.7150/thno.23206

[75] P. Nold, R. Hartmann, N. Feliu, K. Kantner, M. Gamal, B. Pelaz, J. Hühn, X. Sun, P. Jungebluth, P. Pino, H. Hackstein, P. Macchiarini, W.J. Parak, C. Brendel, Optimizing conditions for labeling of mesenchymal stromal cells (MSCs) with gold nanoparticles: a prerequisite for in vivo tracking of MSCs, J. Nanobiotechnol. 15 (2017) 1–11, https://doi.org/10.1186/s12951-017-0258-5

[76] L. Saulite, D. Dapkute, K. Pleiko, I. Popena, S. Steponkiene, R. Rotomskis, U. Riekstina, Nano-engineered skin mesenchymal stem cells: potential vehicles for tumour-targeted quantum-dot delivery, Beilstein J. Nanotechnol. 8 (2017) 1218–1230, https://doi.org/10.3762/bjnano.8.123

[77] L. Miao, P.L. Liu, Stem cell therapy for ischemic heart disease, Chin. J. Tissue Eng. Res. 20 (2016) 906–912, https://doi.org/10.3969/j.issn.2095-4344.2016.06.021

[78] V. Hartenstein, P. Martinez, Phagocytosis in cellular defense and nutrition: a food-centered approach to the evolution of macrophages, Cell Tissue Res. 377 (2019) 527–547, https://doi.org/10.1007/s00441-019-03096-6

[79] H. Li, K. Tatematsu, M. Somiya, M. Iijima, S. Kuroda, Development of a macrophage-targeting and phagocytosis-inducing bio-nanocapsule-based nanocarrier for drug delivery, Acta Biomater. 73 (2018) 412–423, https://doi.org/10.1016/j.actbio.2018.04.023

[80] B. Kim, H.B. Pang, J. Kang, J.H. Park, E. Ruoslahti, M.J. Sailor, Immunogene therapy with fusogenic nanoparticles modulates macrophage response to Staphylococcus aureus, Nat. Commun. 9 (2018) 1–13, https://doi.org/10.1038/s41467-018-04390-7

[81] N. Luo, D. Ni, H. Yue, W. Wei, G. Ma, Surface-engineered graphene navigate divergent biological outcomes toward macrophages, ACS Appl. Mater. Interfaces 7 (2015) 5239–5247, https://doi.org/10.1021/am5084607

[82] J. Gilleron, W. Querbes, A. Zeigerer, A. Borodovsky, G. Marsico, U. Schubert, K. Manygoats, S. Seifert, C. Andree, M. Stöter, H. Epstein-Barash, L. Zhang, V. Koteliansky, K. Fitzgerald, E. Fava, M. Bickle, Y. Kalaidzidis, A. Akinc, M. Maier, M. Zerial, Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape, Nat. Biotechnol. 31 (2013) 638–646, https://doi.org/10.1038/nbt.2612

[83] D.A. Kuhn, D. Vanhecke, B. Michen, F. Blank, P. Gehr, A. Petri-Fink, B. Rothen-Rutishauser, Different endocytotic uptake mechanisms for nanoparticles in epithelial cells and macrophages, Beilstein J. Nanotechnol. 5 (2014) 1625–1636, https://doi.org/10.3762/bjnano.5.174

[84] I. Fiorentino, R. Gualtieri, V. Barbato, V. Mollo, S. Braun, A. Angrisani, M. Turano, M. Furia, P.A. Netti, D. Guarnieri, S. Fusco, R. Talevi, Energy independent uptake and release of polystyrene nanoparticles in primary mammalian cell cultures, Exp. Cell Res. 330 (2015) 240–247, https://doi.org/10.1016/j.yexcr.2014.09.017

[85] D.M. Monti, D. Guarnieri, G. Napolitano, R. Piccoli, P. Netti, S. Fusco, A. Arciello, Biocompatibility, uptake and endocytosis pathways of polystyrene nanoparticles in primary human renal epithelial cells, J. Biotechnol. 193 (2015) 3–10, https://doi.org/10.1016/j.jbiotec.2014.11.004

[86] H.J. Johnston, M. Semmler-Behnke, D.M. Brown, W. Kreyling, L. Tran, V. Stone, Evaluating the uptake and intracellular fate of polystyrene nanoparticles by primary and hepatocyte cell lines in vitro, Toxicol. Appl. Pharmacol. 242 (2010) 66–78, https://doi.org/10.1016/j.taap.2009.09.015

[87] T. dos Santos, J. Varela, I. Lynch, A. Salvati, K.A. Dawson, Effects of transport inhibitors on the cellular uptake of carboxylated polystyrene nanoparticles in different cell lines, PLOS One 6 (2011) 24438, https://doi.org/10.1371/journal.pone.0024438

[88] Y. Xiao, S.P. Forry, X. Gao, R.D. Holbrook, W.G. Telford, A. Tona, Dynamics and mechanisms of quantum dot nanoparticle cellular uptake, J. Nanobiotechnol. 8 (2010) 1–9, https://doi.org/10.1186/1477-3155-8-13

[89] H. Duan, S. Nie, Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings, J. Am. Chem. Soc. 129 (2007) 3333–3336, https://doi.org/10.1021/ja068158s

[90] K.L. Douglas, C.A. Piccirillo, M. Tabrizian, Cell line-dependent internalization pathways and intracellular trafficking determine transfection efficiency of nanoparticle vectors, Eur. J. Pharm. Biopharm. 68 (2008) 676–687, https://doi.org/10.1016/j.ejpb.2007.09.002

[91] K. Sansanaphongpricha, P. Sonthithai, P. Kaewkong, B. Thavornyutikarn, S. Bamrungsap, W. Kosorn, T. Thinbanmai, N. Saengkrit, Hyaluronic acid-coated gold nanorods enhancing BMP-2 peptide delivery for chondrogenesis, Nanotechnology 31 (2020) 435101, https://doi.org/10.1088/1361-6528/aba46d

[92] M.H. Fernandes, M.M. Alves, M. Cebotarenco, I.A.C. Ribeiro, L. Grenho, P.S. Gomes, M.J. Carmezim, C.F. Santos, Citrate zinc hydroxyapatite nanorods with enhanced cytocompatibility and osteogenesis for bone regeneration, Mater. Sci. Eng. C Mater. Biol. Appl. 115 (2020) 111147, https://doi.org/10.1016/j.msec.2020.111147

[93] W. Zhang, M. Yu, Z. Xi, D. Nie, Z. Dai, J. Wang, K. Qian, H. Weng, Y. Gan, L. Xu, Cancer cell membrane-camouflaged nanorods with endoplasmic reticulum targeting for improved antitumor therapy, ACS Appl. Mater. Interfaces 11 (2019) 46614–46625, https://doi.org/10.1021/acsami.9b18388

[94] Y. Xiao, W. Xu, Y. Komohara, Y. Fujiwara, H. Hirose, S. Futaki, T. Niidome, Effect of surface modifications on cellular uptake of gold nanorods in human primary cells and established cell lines, ACS Omega 5 (2020) 32744–32752, https://doi.org/10.1021/acsomega.0c05162

[95] D. Jhala, H. Rather, D. Kedaria, J. Shah, S. Singh, R. Vasita, Biomimetic polycaprolactone-chitosan nanofibrous substrate influenced cell cycle and ECM secretion affect cellular uptake of nanoclusters, Bioact. Mater. 4 (2019) 79–86, https://doi.org/10.1016/j.bioactmat.2018.12.004

[96] Y. Chen, L. Ju, M. Rushdi, C. Ge, C. Zhu, Receptor-mediated cell mechanosensing, Mol. Biol. Cell 28 (2017) 3134–3155, https://doi.org/10.1091/mbc.E17-04-0228

[97] X. Yi, H. Gao, Kinetics of receptor-mediated endocytosis of elastic nanoparticles, Nanoscale 9 (2017) 454–463, https://doi.org/10.1039/c6nr07179a

[98] A.W.M. Haining, T.J. Lieberthal, A. Del Río Hernández, Talin: a mechanosensitive molecule in health and disease, FASEB J. 30 (2016) 2073–2085, https://doi.org/10.1096/fj.201500080R

[99] M. Yazdimamaghani, Z.B. Barber, S.P. Hadipour Moghaddam, H. Ghandehari, Influence of silica nanoparticle density and flow conditions on sedimentation, cell uptake, and cytotoxicity, Mol. Pharm. 15 (2018) 2372–2383, https://doi.org/10.1021/acs.molpharmaceut.8b00213

[100] M. Björnmalm, M. Faria, X. Chen, J. Cui, F. Caruso, Dynamic flow impacts cell-particle interactions: sedimentation and particle shape effects, Langmuir 32 (2016) 10995–11001, https://doi.org/10.1021/acs.langmuir.6b03216

[101] H. Wang, L. Wu, B.M. Reinhard, Scavenger receptor mediated endocytosis of silver nanoparticles into J774A.1 macrophages is heterogeneous, ACS Nano 6 (2012) 7122–7132, https://doi.org/10.1021/nn302186n

[102] D. Suresh, A. Zambre, N. Chanda, T.J. Hoffman, C.J. Smith, J.D. Robertson, R. Kannan, Bombesin peptide conjugated gold nanocages internalize via clathrin mediated endocytosis, Bioconjug. Chem. 25 (2014) 1565–1579, https://doi.org/10.1021/bc500295s

[103] C. Kinnear, L. Rodriguez-Lorenzo, M.J.D. Clift, B. Goris, S. Bals, B. Rothen-Rutishauser, A. Petri-Fink, Decoupling the shape parameter to assess gold nanorod uptake by mammalian cells, Nanoscale 8 (2016) 16416–16426, https://doi.org/10.1039/c6nr03543d

[104] M. Ahir, S. Bhattacharya, S. Karmakar, A. Mukhopadhyay, S. Mukherjee, S. Ghosh, S. Chattopadhyay, P. Patra, A. Adhikary, Tailored-CuO-nanowire decorated with folic acid mediated coupling of the mitochondrial-ROS generation and miR425-PTEN axis in furnishing potent anti-cancer activity in human triple negative breast carcinoma cells, Biomaterials 76 (2016) 115–132, https://doi.org/10.1016/j.biomaterials.2015.10.044

[105] M. Alaraby, A. Hernández, R. Marcos, Systematic in vivo study of NiO nanowires and nanospheres: biodegradation, uptake and biological impacts, Nanotoxicology 12 (2018) 1027–1044, https://doi.org/10.1080/17435390.2018.1513091

[106] S. Bao, S. Huang, Y. Liu, Y. Hu, W. Wang, M. Ji, H. Li, N.X. Zhang, C. Song, S. Duan, Gold nanocages with dual modality for image-guided therapeutics, Nanoscale 9 (2017) 7284–7296, https://doi.org/10.1039/c7nr01350g

[107] M.M. Song, W.J. Song, H. Bi, J. Wang, W.L. Wu, J. Sun, M. Yu, Cytotoxicity and cellular uptake of iron nanowires, Biomaterials 31 (2010) 1509–1517, https://doi.org/10.1016/j.biomaterials.2009.11.034

[108] P.M. Favi, M.M. Valencia, P.R. Elliott, A. Restrepo, M. Gao, H. Huang, J.J. Pavon, T.J. Webster, Shape and surface chemistry effects on the cytotoxicity and cellular uptake of metallic nanorods and nanospheres, J. Biomed. Mater. Res. Part A 103 (2015) 3940–3955, https://doi.org/10.1002/jbm.a.35518

[109] N. Ma, F.G. Wu, X. Zhang, Y.W. Jiang, H.R. Jia, H.Y. Wang, Y.H. Li, P. Liu, N. Gu, Z. Chen, Shape-dependent radiosensitization effect of gold nanostructures in cancer radiotherapy: comparison of gold nanoparticles, nanospikes, and nanorods, ACS Appl. Mater. Interfaces 9 (2017) 13037–13048, https://doi.org/10.1021/acsami.7b01112

[110] L. Au, Q. Zhang, C.M. Cobley, M. Gidding, A.G. Schwartz, J. Chen, Y. Xia, Quantifying the cellular uptake of antibody-conjugated Au nanocages by two-photon microscopy and inductively coupled plasma mass spectrometry, ACS Nano 4 (2010) 35–42, https://doi.org/10.1021/nn901392m

[111] T. Sun, Y. Wang, Y. Wang, J. Xu, X. Zhao, S. Vangveravong, R.H. Mach, Y. Xia, Using sv119-gold nanocage conjugates to eradicate cancer stem cells through a combination of photothermal and chemo therapies, Adv. Healthc. Mater. 3 (2014) 1283–1291, https://doi.org/10.1002/adhm.201400026

[112] S. Chen, A.E. Goode, S. Sweeney, I.G. Theodorou, A.J. Thorley, P. Ruenraroengsak, Y. Chang, A. Gow, S. Schwander, J. Skepper, J. Zhang, M.S. Shaffer, K.F. Chung, T.D. Tetley, M.P. Ryan, A.E. Porter, Sulfidation of silver nanowires inside human alveolar epithelial cells: a potential detoxification mechanism, Nanoscale 5 (2013) 9839–9847, https://doi.org/10.1039/c3nr03205a

[113] S. Sweeney, I.G. Theodorou, M. Zambianchi, S. Chen, A. Gow, S. Schwander, J. Zhang, K.F. Chung, M.S.P. Shaffer, M.P. Ryan, A.E. Porter, T.D. Tetley, Silver nanowire interactions with primary human alveolar type-II epithelial cell secretions: contrasting bioreactivity with human alveolar type-I and type-II epithelial cells, Nanoscale 7 (2015) 10398–10409, https://doi.org/10.1039/c5nr01496d

[114] Y. Qiu, Y. Liu, L. Wang, L. Xu, R. Bai, Y. Ji, X. Wu, Y. Zhao, Y. Li, C. Chen, Surface chemistry and aspect ratio mediated cellular uptake of Au nanorods, Biomaterials 31 (2010) 7606–7619, https://doi.org/10.1016/j.biomaterials.2010.06.051

[115] J. Zhao, Hyaluronic acid-modified and TPCA-1-loaded gold nanocages alleviate inflammation, Pharmaceutics 11 (2019) 1–9, https://doi.org/10.3390/pharmaceutics11030143

[116] L. Xu, Y. Dai, Z. Wang, J. Zhao, F. Li, J.C. White, B. Xing, Graphene quantum dots in alveolar macrophage: uptake-exocytosis, accumulation in nuclei, nuclear responses and DNA cleavage 06 biological sciences 0601 biochemistry and cell biology, Part. Fibre Toxicol. 15 (2018) 1–17, https://doi.org/10.1186/s12989-018-0279-8

[117] M.K. Kumawat, M. Thakur, R.B. Gurung, R. Srivastava, Graphene quantum dots for cell proliferation, nucleus imaging, and photoluminescent sensing applications, Sci. Rep. 7 (2017) 1–16, https://doi.org/10.1038/s41598-017-16025-w

[118] Y.J. Lu, P.Y. Lin, P.H. Huang, C.Y. Kuo, K.T. Shalumon, M.Y. Chen, J.P. Chen, Magnetic graphene oxide for dual targeted delivery of doxorubicin and photothermal therapy, Nanomaterials 8 (2018) 1–20, https://doi.org/10.3390/nano8040193

[119] J. Pi, L. Shen, H. Shen, E. Yang, W. Wang, R. Wang, D. Huang, B.S. Lee, C. Hu, C. Chen, H. Jin, J. Cai, G. Zeng, Z.W. Chen, Mannosylated graphene oxide as macrophage-targeted delivery system for enhanced intracellular M. tuberculosis killing efficiency, Mater. Sci. Eng. C Mater. Biol. Appl. 103 (2019) 109777, https://doi.org/10.1016/j.msec.2019.109777

[120] Y.J. Lu, Y.H. Wang, R.S. Sahu, J.P. Chen, B.S. Dash, P.J. Chung, H.W. Yang, E.Y. Chuang, T.L. Hwang, Mechanism of nanoformulated graphene oxide-mediated human neutrophil activation, ACS Appl. Mater. Interfaces 12 (2020) 40141–40152, https://doi.org/10.1021/acsami.0c12490

[121] L. Xu, J. Zhao, Z. Wang, Genotoxic response and damage recovery of macrophages to graphene quantum dots, Sci. Total Environ. 664 (2019) 536–545, https://doi.org/10.1016/j.scitotenv.2019.01.356

[122] J. Ding, X. Zhang, C. Chen, Y. Huang, X. Yu, X. Li, Ultra pH-sensitive polymeric nanovesicles co-deliver doxorubicin and navitoclax for synergetic therapy of endometrial carcinoma, Biomater. Sci. 8 (2020) 2264–2273, https://doi.org/10.1039/d0bm00112k

[123] J. Zhou, Y. Chau, Different oligoarginine modifications alter endocytic pathways and subcellular trafficking of polymeric nanoparticles, Biomater. Sci. 4 (2016) 1462–1472, https://doi.org/10.1039/c6bm00371k

[124] C.-Y. Tsai, S.-L. Lu, C.-W. Hu, C.-S. Yeh, G.-B. Lee, H.-Y. Lei, Size-dependent attenuation of TLR9 signaling by gold nanoparticles in macrophages, J. Immunol. 188 (2012) 68–76, https://doi.org/10.4049/jimmunol.1100344

[125] Z. Tu, K. Achazi, A. Schulz, R. Mülhaupt, S. Thierbach, E. Rühl, M. Adeli, R. Haag, Combination of surface charge and size controls the cellular uptake of functionalized graphene sheets, Adv. Funct. Mater. 27 (2017) 1–11, https://doi.org/10.1002/adfm.201701837

[126] X. Cai, Q. Yang, Q. Weng, S. Wang, pH sensitive doxorubicin-loaded nanoparticle based on Radix pseudostellariae protein-polysaccharide conjugate and its improvement on HepG2 cellular uptake of doxorubicin, Food Chem. Toxicol. 136 (2020) 111099, https://doi.org/10.1016/j.fct.2019.111099

[127] P. Panja, N.R. Jana, Arginine-terminated nanoparticles of < 10 nm size for direct membrane penetration and protein delivery for straight access to cytosol and nucleus, J. Phys. Chem. Lett. 11 (2020) 2363–2368, https://doi.org/10.1021/acs.jpclett.0c00176

[128] T. Wang, L. Wang, X. Li, X. Hu, Y. Han, Y. Luo, Z. Wang, Q. Li, A. Aldalbahi, L. Wang, S. Song, C. Fan, Y. Zhao, M. Wang, N. Chen, Size-dependent regulation of intracellular trafficking of polystyrene nanoparticle-based drug-delivery systems, ACS Appl. Mater. Interfaces 9 (2017) 18619–18625, https://doi.org/10.1021/acsami.7b05383

[129] N. Fatima, R. Gromnicova, J. Loughlin, B. Sharrack, D. Male, Gold nanocarriers for transport of oligonucleotides across brain endothelial cells, PLOS One 15 (2020) 0236611, https://doi.org/10.1371/journal.pone.0236611

[130] P. Joyce, H. Ulmefors, S. Maghrebi, S. Subramaniam, A. Wignall, S. Jõemetsa, F. Höök, C.A. Prestidge, Enhancing the cellular uptake and antibacterial activity of rifampicin through encapsulation in mesoporous silica nanoparticles, Nanomaterials 10 (2020) 815, https://doi.org/10.3390/nano10040815

[131] W. Kim, W.K. Kim, K. Lee, M.J. Son, M. Kwak, W.S. Chang, J.K. Min, N.W. Song, J. Lee, K.H. Bae, Corrigendum: a reliable approach for assessing size-dependent effects of silica nanoparticles on cellular internalization behavior and cytotoxic mechanisms (Int J Nanomedicine. 2019; 14: 7375–7387.), Int. J. Nanomed. 15 (2020) 10169–10170, https://doi.org/10.2147/IJN.S295273

[132] P. Makvandi, G.W. Ali, F. Della Sala, W.I. Abdel-Fattah, A. Borzacchiello, Hyaluronic acid/corn silk extract based injectable nanocomposite: a biomimetic antibacterial scaffold for bone tissue regeneration, Mater. Sci. Eng. C Mater. Biol. Appl. 107 (2020) 110195, https://doi.org/10.1016/j.msec.2019.110195
[133] C. Kinnear, T.L. Moore, L. Rodriguez-Lorenzo, B. Rothen-Rutishauser, A. Petri-Fink, Form follows function: nanoparticle shape and its implications for nanomedicine, Chem. Rev. 117 (2017) 11476–11521, https://doi.org/10.1021/acs.chemrev.7b00194
[134] C. Huang, Y. Zhang, H. Yuan, H. Gao, S. Zhang, Role of nanoparticle geometry in endocytosis: Laying down to stand up, Nano Lett. 13 (2013) 4546–4550, https://doi.org/10.1021/nl402628n
[135] B.D. Chithrani, A.A. Ghazani, W.C.W. Chan, Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells, Nano Lett. 6 (2006) 662–668, https://doi.org/10.1021/nl052396o
[136] B.D. Chithrani, W.C.W. Chan, Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes, Nano Lett. 7 (2007) 1542–1550, https://doi.org/10.1021/nl070363y
[137] L. Ding, C. Yao, X. Yin, C. Li, Y. Huang, M. Wu, B. Wang, X. Guo, Y. Wang, M. Wu, Size, shape, and protein corona determine cellular uptake and removal mechanisms of gold nanoparticles, Small 14 (2018) 1801451, https://doi.org/10.1002/smll.201801451
[138] X. Xie, J. Liao, X. Shao, Q. Li, Y. Lin, The effect of shape on cellular uptake of gold nanoparticles in the forms of stars, rods, and triangles, Sci. Rep. 7 (2017) 1–9, https://doi.org/10.1038/s41598-017-04229-z
[139] P.N. Yaron, B.D. Holt, P.A. Short, M. Lösche, M.F. Islam, K.N. Dahl, Single wall carbon nanotubes enter cells by endocytosis and not membrane penetration, J. Nanobiotechnol. 9 (2011) 45, https://doi.org/10.1186/1477-3155-9-45
[140] K. Maruyama, H. Haniu, N. Saito, Y. Matsuda, T. Tsukahara, S. Kobayashi, M. Tanaka, K. Aoki, S. Takanashi, M. Okamoto, H. Kato, Endocytosis of multi-walled carbon nanotubes in bronchial epithelial and mesothelial cells, Biomed. Res. Int. 2015 (2015) 793186, https://doi.org/10.1155/2015/793186
[141] X. Cui, B. Wan, Y. Yang, X. Ren, L.H. Guo, Length effects on the dynamic process of cellular uptake and exocytosis of single-walled carbon nanotubes in murine macrophage cells /631/80 /704/172 /82/29 /14/19 /14/34 /123 article, Sci. Rep. 7 (2017) 1–13, https://doi.org/10.1038/s41598-017-01746-9
[142] N. Yang, Y. Ding, Y. Zhang, B. Wang, X. Zhao, K. Cheng, Y. Huang, M. Taleb, J. Zhao, W.F. Dong, L. Zhang, G. Nie, Surface functionalization of polymeric nanoparticles with umbilical cord-derived mesenchymal stem cell membrane for tumor-targeted therapy, ACS Appl. Mater. Interfaces 10 (2018) 22963–22973, https://doi.org/10.1021/acsami.8b05363
[143] T. Mizuhara, K. Saha, D.F. Moyano, C.S. Kim, B. Yan, Y.K. Kim, V.M. Rotello, Acylsulfonamide-functionalized zwitterionic gold nanoparticles for enhanced cellular uptake at tumor pH, Angew. Chem. Int. Ed. 54 (2015) 6567–6570, https://doi.org/10.1002/anie.201411615
[144] A.M. Alkilany, C.J. Murphy, Toxicity and cellular uptake of gold nanoparticles: what we have learned so far? J. Nanopart. Res. 12 (2010) 2313–2333, https://doi.org/10.1007/s11051-010-9911-8
[145] S. Weinbaum, J.M. Tarbell, E.R. Damiano, The structure and function of the endothelial glycocalyx layer, Annu. Rev. Biomed. Eng. 9 (2007) 121–167, https://doi.org/10.1146/annurev.bioeng.9.060906.151959
[146] R. Gromnicova, M. Kaya, I.A. Romero, P. Williams, S. Satchell, B. Sharrack, D. Male, Transport of gold nanoparticles by vascular endothelium from different human tissues, PLOS One 11 (2016) 0161610, https://doi.org/10.1371/journal.pone.0161610
[147] W. Tao, X. Zeng, J. Wu, X. Zhu, X. Yu, X. Zhang, J. Zhang, G. Liu, L. Mei, Polydopamine-based surface modification of novel nanoparticle-aptamer bioconjugates for in vivo breast cancer targeting and enhanced therapeutic effects, Theranostics 6 (2016) 470–484, https://doi.org/10.7150/thno.14184
[148] M. Ishii, Y. Fukuoka, S. Deguchi, H. Otake, T. Tanino, N. Nagai, Energy-dependent endocytosis is involved in the absorption of indomethacin nanoparticles in the small intestine, Int. J. Mol. Sci. 20 (2019) 476, https://doi.org/10.3390/ijms20030476
[149] N. Nagai, F. Ogata, H. Otake, Y. Nakazawa, N. Kawasaki, Energy-dependent endocytosis is responsible for drug transcorneal penetration following the instillation of ophthalmic formulations containing indomethacin nanoparticles, Int. J. Nanomed. 14 (2019) 1213–1227, https://doi.org/10.2147/IJN.S196681
[150] B. Zhang, X. Feng, H. Yin, Z. Ge, Y. Wang, Z. Chu, H. Raabova, J. Vavra, P. Cigler, R. Liu, Y. Wang, Q. Li, Anchored but not internalized: shape dependent endocytosis of nanodiamond, Sci. Rep. 7 (2017) 46462, https://doi.org/10.1038/srep46462
[151] T. Fotticchia, R. Vecchione, P.L. Scognamiglio, D. Guarnieri, V. Calcagno, C. Di Natale, C. Attanasio, M. De Gregorio, C. Di Cicco, V. Quagliariello, N. Maurea, A. Barbieri, C. Arra, L. Raiola, R.V. Iaffaioli, P.A. Netti, Enhanced drug delivery into cell cytosol via glycoprotein H-derived peptide conjugated nanoemulsions, ACS Nano 11 (2017) 9802–9813, https://doi.org/10.1021/acsnano.7b03058
[152] T. Yan, J. He, R. Liu, Z. Liu, J. Cheng, Chitosan capped pH-responsive hollow mesoporous silica nanoparticles for targeted chemo-photo combination therapy, Carbohydr. Polym. 231 (2020) 115706, https://doi.org/10.1016/j.carbpol.2019.115706
[153] A.C. Anselmo, S. Mitragotri, Impact of particle elasticity on particle-based drug delivery systems, Adv. Drug Deliv. Rev. 108 (2017) 51–67, https://doi.org/10.1016/j.addr.2016.01.007
[154] S. Wang, H. Guo, Y. Li, X. Li, Penetration of nanoparticles across a lipid bilayer: effects of particle stiffness and surface hydrophobicity, Nanoscale 11 (2019) 4044–4052, https://doi.org/10.1039/c8nr09381d
[155] N. Tsuda, Atomic force microscope, J. Jpn. Soc. Precis. Eng. 57 (1991) 1159–1164, https://doi.org/10.2493/jjspe.57.1159
[156] S. Zauscher, Z. Parlak, Q. Tu, Handbook of Nanomaterials Properties, (2014), pp. 1023–1051, https://doi.org/10.1007/978-3-642-31107-9
[157] P. Guo, D. Liu, K. Subramanyam, B. Wang, J. Yang, J. Huang, D.T. Auguste, M.A. Moses, Nanoparticle elasticity directs tumor uptake, Nat. Commun. 9 (2018) 1–9, https://doi.org/10.1038/s41467-017-02588-9
[158] C. Yao, O.U. Akakuru, S.G. Stanciu, N. Hampp, Y. Jin, J. Zheng, G. Chen, F. Yang, A. Wu, Effect of elasticity on the phagocytosis of micro/nanoparticles, J. Mater. Chem. B 8 (2020) 2381–2392, https://doi.org/10.1039/c9tb02902h
[159] X. Banquy, F. Suarez, A. Argaw, J.M. Rabanel, P. Grutter, J.F. Bouchard, P. Hildgen, S. Giasson, Effect of mechanical properties of hydrogel nanoparticles on macrophage cell uptake, Soft Matter 5 (2009) 3984–3991, https://doi.org/10.1039/b821583a
[160] V.K. Switacz, S.K. Wypysek, R. Degen, J.J. Crassous, M. Spehr, W. Richtering, Influence of size and cross-linking density of microgels on cellular uptake and uptake kinetics, Biomacromolecules 21 (2020) 4532–4544, https://doi.org/10.1021/acs.biomac.0c00478
[161] A.C. Anselmo, M. Zhang, S. Kumar, D.R. Vogus, S. Menegatti, M.E. Helgeson, S. Mitragotri, Elasticity of nanoparticles influences their blood circulation, phagocytosis, endocytosis, and targeting, ACS Nano 9 (2015) 3169–3177, https://doi.org/10.1021/acsnano.5b00147
[162] J. Sun, L. Zhang, J. Wang, Q. Feng, D. Liu, Q. Yin, D. Xu, Y. Wei, B. Ding, X. Shi, X. Jiang, Tunable rigidity of (polymeric core)-(lipid shell) nanoparticles for regulated cellular uptake, Adv. Mater. 27 (2015) 1402–1407, https://doi.org/10.1002/adma.201404788
[163] R. Kurapati, T.W. Groth, A.M. Raichur, Recent developments in layer-by-layer technique for drug delivery applications, ACS Appl. Bio Mater. 2 (2019) 5512–5527, https://doi.org/10.1021/acsabm.9b00703
[164] S. Zhao, F. Caruso, L. Dahne, G. Decher, B.G. De Geest, J. Fan, N. Feliu, Y. Gogotsi, P.T. Hammond, M.C. Hersam, A. Khademhosseini, N. Kotov, S. Leporatti, Y. Li, F. Lisdat, L.M. Liz-Marzan, S. Moya, P. Mulvaney, A.L. Rogach, S. Roy, D.G. Shchukin, A.G. Skirtach, M.M. Stevens, G.B. Sukhorukov, P.S. Weiss, Z. Yue, D. Zhu, W.J. Parak, The future of layer-by-layer assembly: a tribute to ACS Nano Associate Editor Helmuth Möhwald, ACS Nano 13 (2019) 6151–6169, https://doi.org/10.1021/acsnano.9b03326
[165] S. Chen, D.J. McClements, L. Jian, Y. Han, L. Dai, L. Mao, Y. Gao, Core-shell biopolymer nanoparticles for co-delivery of curcumin and piperine: sequential electrostatic deposition of hyaluronic acid and chitosan shells on the zein core, ACS Appl. Mater. Interfaces 11 (2019) 38103–38115, https://doi.org/10.1021/acsami.9b11782
[166] R. Jenjob, T. Phakkeeree, D. Crespy, Core-shell particles for drug-delivery, bioimaging, sensing, and tissue engineering, Biomater. Sci. 8 (2020) 2756–2770, https://doi.org/10.1039/c9bm01872g
[167] M. Müllner, A.H.E. Müller, Cylindrical polymer brushes – anisotropic building blocks, unimolecular templates and particulate nanocarriers, Polymer 98 (2016) 389–401, https://doi.org/10.1016/j.polymer.2016.03.076 (Guildf).
[168] M.F. Bédard, A. Munoz-Javier, R. Mueller, P. Del Pino, A. Fery, W.J. Parak, A.G. Skirtach, G.B. Sukhorukov, On the mechanical stability of polymeric microcontainers functionalized with nanoparticles, Soft Matter 5 (2009) 148–155, https://doi.org/10.1039/b812553h
[169] H. Sun, E.H.H. Wong, Y. Yan, J. Cui, Q. Dai, J. Guo, G.G. Qiao, F. Caruso, The role of capsule stiffness on cellular processing, Chem. Sci. 6 (2015) 3505–3514, https://doi.org/10.1039/c5sc00416k
[170] R. Hartmann, M. Weidenbach, M. Neubauer, A. Fery, W.J. Parak, Stiffness-dependent in vitro uptake and lysosomal acidification of colloidal particles, Angew. Chem. Int. Ed. 54 (2015) 1365–1368, https://doi.org/10.1002/anie.201409693
[171] C. Zhang, J. Lu, F. Tian, L. Li, Y. Hou, Y. Wang, L. Sun, X. Shi, H. Lu, Regulation of the cellular uptake of nanoparticles by the orientation of helical polypeptides, Nano Res. 12 (2019) 889–896, https://doi.org/10.1007/s12274-019-2319-6
[172] X. Liu, F. Wu, Y. Tian, M. Wu, Q. Zhou, S. Jiang, Z. Niu, Size dependent cellular uptake of rod-like bionanoparticles with different aspect ratios, Sci. Rep. 6 (2016) 24567, https://doi.org/10.1038/srep24567
[173] M. Deserno, Elastic deformation of a fluid membrane upon colloid binding, Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 69 (2004) 031903, https://doi.org/10.1103/PhysRevE.69.031903
[174] S. Dasgupta, T. Auth, G. Gompper, Wrapping of ellipsoidal nano-particles by fluid membranes, Soft Matter 9 (2013) 5473–5482, https://doi.org/10.1039/c3sm50351h
[175] X. Yi, X. Shi, H. Gao, Cellular uptake of elastic nanoparticles, Phys. Rev. Lett. 107 (2011) 098101, https://doi.org/10.1103/PhysRevLett.107.098101
[176] A.H. Bahrami, Orientational changes and impaired internalization of ellipsoidal nanoparticles by vesicle membranes, Soft Matter 9 (2013) 8642–8646, https://doi.org/10.1039/c3sm50885d
[177] R. Vácha, F.J. Martinez-Veracoechea, D. Frenkel, Receptor-mediated endocytosis of nanoparticles of various shapes, Nano Lett. 11 (2011) 5391–5395, https://doi.org/10.1021/nl2030213
[178] S. Dasgupta, T. Auth, G. Gompper, Shape and orientation matter for the cellular uptake of nonspherical particles, Nano Lett. 14 (2014) 687–693, https://doi.org/10.1021/nl403949h
[179] P. Chen, Z. Xu, G. Zhu, X. Dai, L.T. Yan, Cellular uptake of active particles, Phys. Rev. Lett. 124 (2020) 198102, https://doi.org/10.1103/PhysRevLett.124.198102
[180] L. Qiu, C.R. Xu, F. Zhong, C.Y. Hong, C.Y. Pan, Fabrication of functional nano-objects through RAFT dispersion polymerization and influences of morphology

on drug delivery, ACS Appl. Mater. Interfaces 8 (2016) 18347–18359, https://doi.org/10.1021/acsami.6b04693
[181] J. Kim, J.C. Sunshine, J.J. Green, Differential polymer structure tunes mechanism of cellular uptake and transfection routes of poly(β-amino ester) polyplexes in human breast cancer cells, Bioconjug. Chem. 25 (2014) 43–51, https://doi.org/10.1021/bc4002322
[182] M. Wu, H. Guo, L. Liu, Y. Liu, L. Xie, Size-dependent cellular uptake and localization profiles of silver nanoparticles, Int. J. Nanomed. 14 (2019) 4247–4259, https://doi.org/10.2147/IJN.S201107
[183] M.S. Ehrenberg, A.E. Friedman, J.N. Finkelstein, G. Oberdörster, J.L. McGrath, The influence of protein adsorption on nanoparticle association with cultured endothelial cells, Biomaterials 30 (2009) 603–610, https://doi.org/10.1016/j.biomaterials.2008.09.050
[184] C.K. Navaratnarajah, A.R. Generous, I. Yousaf, R. Cattaneo, Receptor-mediated cell entry of paramyxoviruses: mechanisms, and consequences for tropism and pathogenesis, J. Biol. Chem. 295 (2020) 2771–2786, https://doi.org/10.1074/jbc.REV119.009961
[185] E. Zaitseva, E. Zaitsev, K. Melikov, A. Arakelyan, M. Marin, R. Villasmil, L.B. Margolis, G.B. Melikyan, L.V. Chernomordik, Fusion stage of HIV-1 entry depends on virus-induced cell surface exposure of phosphatidylserine, Cell Host Microbe 22 (2017) 99–110.e7, https://doi.org/10.1016/j.chom.2017.06.012
[186] M.S. Maginnis, Virus–receptor interactions: the key to cellular invasion, J. Mol. Biol. 430 (2018) 2590–2611, https://doi.org/10.1016/j.jmb.2018.06.024
[187] M. Schelhaas, Come in and take your coat off – how host cells provide endocytosis for virus entry, Cell. Microbiol. 12 (2010) 1378–1388, https://doi.org/10.1111/j.1462-5822.2010.01510.x
[188] J.J. Thottacherry, M. Sathe, C. Prabhakara, S. Mayor, Spoiled for choice: diverse endocytic pathways function at the cell surface, Annu. Rev. Cell Dev. Biol. 35 (2019) 55–84, https://doi.org/10.1146/annurev-cellbio-100617-062710
[189] J. Nikolic, L. Belot, H. Raux, P. Legrand, Y. Gaudin, A.A. Albertini, Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein, Nat. Commun. 9 (2018) 1–12, https://doi.org/10.1038/s41467-018-03432-4
[190] S.Y. Kim, B. Li, R.J. Linhardt, Pathogenesis and inhibition of flaviviruses from a carbohydrate perspective, Pharmaceuticals 10 (2017) 44, https://doi.org/10.3390/ph10020044
[191] P. Liu, M. Ridilla, P. Patel, L. Betts, E. Gallichotte, L. Shahidi, N.L. Thompson, K. Jacobson, Beyond attachment: roles of DC-SIGN in dengue virus infection, Traffic 18 (2017) 218–231, https://doi.org/10.1111/tra.12469
[192] H.S. Hwang, M. Chang, Y.A. Kim, Influenza–host interplay and strategies for universal vaccine development, Vaccines 8 (2020) 1–23, https://doi.org/10.3390/vaccines8030548
[193] Y.J. Chen, X. Liu, B. Tsai, SV40 hijacks cellular transport, membrane penetration, and disassembly machineries to promote infection, Viruses 11 (2019) 917, https://doi.org/10.3390/v11100917
[194] M.G. Toscano, P. de Haan, How Simian virus 40 hijacks the intracellular protein trafficking pathway to its own benefit … and ours, Front. Immunol. 9 (2018) 1160, https://doi.org/10.3389/fimmu.2018.01160
[195] M. Li, D. Zhang, C. Li, Z. Zheng, M. Fu, F. Ni, Y. Liu, T. Du, H. Wang, G.E. Griffin, M. Zhang, Q. Hu, Characterization of Zika virus endocytic pathways in human glioblastoma cells, Front. Microbiol. 11 (2020) 242, https://doi.org/10.3389/fmicb.2020.00242
[196] A. Agrelli, R.R. de Moura, S. Crovella, L.A.C. Brandão, ZIKA virus entry mechanisms in human cells, Infect. Genet. Evol. 69 (2019) 22–29, https://doi.org/10.1016/j.meegid.2019.01.018
[197] C.A.M. Carvalho, J.L. Silva, A.C. Oliveira, A.M.O. Gomes, On the entry of an emerging arbovirus into host cells: Mayaro virus takes the highway to the cytoplasm through fusion with early endosomes and caveolae- derived vesicles, PeerJ 5 (2017) 3245, https://doi.org/10.7717/peerj.3245
[198] R.J.G. Hulswit, Y. Lang, M.J.G. Bakkers, W. Li, Z. Li, A. Schouten, B. Ophorst, F.J.M. Van Kuppeveld, G.J. Boons, B.J. Bosch, E.G. Huizinga, R.J. De Groot, Human coronaviruses OC43 and HKU1 bind to 9-O-acetylated sialic acids via a conserved receptor-binding site in spike protein domain A, Proc. Natl. Acad. Sci. USA 116 (2019) 2681–2690, https://doi.org/10.1073/pnas.1809667116
[199] G. Andrés, African swine fever virus gets undressed: new insights on the entry pathway, J. Virol. 91 (2017), https://doi.org/10.1128/jvi.01906-16
[200] E.G. Sánchez, D. Pérez-Núñez, Y. Revilla, Mechanisms of entry and endosomal pathway of African swine fever virus, Vaccines 5 (2017) 42, https://doi.org/10.3390/vaccines5040042
[201] L. Falasca, C. Agrati, N. Petrosillo, A. Di Caro, M.R. Capobianchi, G. Ippolito, M. Piacentini, Molecular mechanisms of Ebola virus pathogenesis: focus on cell death, Cell Death Differ. 22 (2015) 1250–1259, https://doi.org/10.1038/cdd.2015.67
[202] F.I. Schmidt, C.K.E. Bleck, A. Helenius, J. Mercer, Vaccinia extracellular virions enter cells by macropinocytosis and acid-activated membrane rupture, EMBO J. 30 (2011) 3647–3661, https://doi.org/10.1038/emboj.2011.245
[203] M. Marin, Y. Kushnareva, C.S. Mason, S.K. Chanda, G.B. Melikyan, HIV-1 fusion with CD4+ T cells is promoted by proteins involved in endocytosis and intracellular membrane trafficking, Viruses 11 (2019) 100, https://doi.org/10.3390/v11020100
[204] L.-A. Gobeil, R. Lodge, M.J. Tremblay, Macropinocytosis-like HIV-1 internalization in macrophages is CCR5 dependent and leads to efficient but delayed degradation in endosomal compartments, J. Virol. 87 (2013) 735–745, https://doi.org/10.1128/jvi.01802-12
[205] S. Mikuličić, L. Florin, The endocytic trafficking pathway of oncogenic papillomaviruses, Papillomavirus Res. 7 (2019) 135–137, https://doi.org/10.1016/j.pvr.2019.03.004
[206] T. Letian, Z. Tianyu, Cellular receptor binding and entry of human papillomavirus, Virol. J. 7 (2010) 1–7, https://doi.org/10.1186/1743-422X-7-2
[207] J. Oppliger, G. Torriani, A. Herrador, S. Kunz, Lassa virus cell entry via dystroglycan involves an unusual pathway of macropinocytosis, J. Virol. 90 (2016) 6412–6429, https://doi.org/10.1128/jvi.00257-16
[208] M. Nonnenmacher, T. Weber, Adeno-associated virus 2 infection requires endocytosis through the CLIC/GEEC pathway, Cell Host Microbe 10 (2011) 563–576, https://doi.org/10.1016/j.chom.2011.10.014
[209] G.E. Berry, A. Asokan, Cellular transduction mechanisms of adeno-associated viral vectors, Curr. Opin. Virol. 21 (2016) 54–60, https://doi.org/10.1016/j.coviro.2016.08.001
[210] G. Lebeau, D. Vagner, É. Frumence, F. Ah-Pine, X. Guillot, E. Nobécourt, L. Raffray, P. Gasque, Deciphering SARS-CoV-2 virologic and immunologic features, Int. J. Mol. Sci. 21 (2020) 1–40, https://doi.org/10.3390/ijms21165932
[211] A. Bayati, R. Kumar, V. Francis, P. McPherson, SARS-CoV-2 uses clathrin-mediated endocytosis to gain access into cells, BioRxiv (2020) 2020.07.13.201509.
[212] S. Patel, J. Kim, M. Herrera, A. Mukherjee, A.V. Kabanov, G. Sahay, Brief update on endocytosis of nanomedicines, Adv. Drug Deliv. Rev. 144 (2019) 90–111, https://doi.org/10.1016/j.addr.2019.08.004
[213] A. Akinc, G. Battaglia, Exploiting endocytosis for nanomedicines, Cold Spring Harb. Perspect. Biol. 5 (2013) 016980, https://doi.org/10.1101/cshperspect.a016980
[214] J.D.S. Hanauer, B. Rengstl, D. Kleinlützum, J. Reul, A. Pfeiffer, T. Friedel, I.C. Schneider, S. Newrzela, M.L. Hansmann, C.J. Buchholz, A. Muik, CD30-targeted oncolytic viruses as novel therapeutic approach against classical Hodgkin lymphoma, Oncotarget 9 (2018) 12971–12981, https://doi.org/10.18632/oncotarget.24191
[215] A. Ammayappan, K.-W. Peng, S.J. Russell, Characteristics of oncolytic vesicular stomatitis virus displaying tumor-targeting ligands, J. Virol. 87 (2013) 13543–13555, https://doi.org/10.1128/jvi.02240-13
[216] M. Laureti, D. Narayanan, J. Rodriguez-Andres, J.K. Fazakerley, L. Kedzierski, Flavivirus receptors: diversity, identity, and cell entry, Front. Immunol. 9 (2018) 2180, https://doi.org/10.3389/fimmu.2018.02180
[217] B.A. Hackett, A. Yasunaga, D. Panda, M.A. Tartell, K.C. Hopkins, S.E. Hensley, S. Cherry, RNASEK is required for internalization of diverse acid-dependent viruses, Proc. Natl. Acad. Sci. USA 112 (2015) 7797–7802, https://doi.org/10.1073/pnas.1424098112
[218] B.A. Hackett, S. Cherry, Flavivirus internalization is regulated by a size-dependent endocytic pathway, Proc. Natl. Acad. Sci. USA 115 (2018) 4246–4251, https://doi.org/10.1073/pnas.1720032115
[219] J. Yu, C. Liang, S.L. Liu, Interferon-inducible LY6E protein promotes HIV-1 infection, J. Biol. Chem. 292 (2017) 4674–4685, https://doi.org/10.1074/jbc.M116.755819
[220] K.B. Mar, N.R. Rinkenberger, I.N. Boys, J.L. Eitson, M.B. McDougal, R.B. Richardson, J.W. Schoggins, LY6E mediates an evolutionarily conserved enhancement of virus infection by targeting a late entry step, Nat. Commun. 9 (2018) 1–14, https://doi.org/10.1038/s41467-018-06000-y
[221] C. Herrscher, F. Pastor, J. Burlaud-Gaillard, A. Dumans, F. Seigneuret, A. Moreau, R. Patient, S. Eymieux, H. de Rocquigny, C. Hourioux, P. Roingeard, E. Blanchard, Hepatitis B virus entry into HepG2-NTCP cells requires clathrin-mediated endocytosis, Cell. Microbiol. 22 (2020) 13205, https://doi.org/10.1111/cmi.13205
[222] Y. Fujioka, S. Nishide, T. Ose, T. Suzuki, I. Kato, H. Fukuhara, M. Fujioka, K. Horiuchi, A.O. Satoh, P. Nepal, S. Kashiwagi, J. Wang, M. Horiguchi, Y. Sato, S. Paudel, A. Nanbo, T. Miyazaki, H. Hasegawa, K. Maenaka, Y. Ohba, A sialylated voltage-dependent Ca2+ channel binds hemagglutinin and mediates influenza a virus entry into mammalian cells, Cell Host Microbe 23 (2018) 809–818.e5, https://doi.org/10.1016/j.chom.2018.04.015
[223] R.P. Vries, N. Tzarum, W. Peng, A.J. Thompson, I.N. Ambepitiya Wickramasinghe, A.T.T. Pena, M.J. van Breemen, K.M. Bouwman, X. Zhu, R. McBride, W. Yu, R.W. Sanders, M.H. Verheije, I.A. Wilson, J.C. Paulson, A single mutation in Taiwanese H6N1 influenza hemagglutinin switches binding to human-type receptors, EMBO Mol. Med. 9 (2017) 1314–1325, https://doi.org/10.15252/emmm.201707726
[224] S.M. Figueroa, A. Veser, K. Abstiens, D. Fleischmann, S. Beck, A. Goepferich, Influenza A virus mimetic nanoparticles trigger selective cell uptake, Proc. Natl. Acad. Sci. USA 116 (2019) 9831–9836, https://doi.org/10.1073/pnas.1902563116
[225] R.G. Parton, Caveolae: structure, function, and relationship to disease, Annu. Rev. Cell Dev. Biol. 34 (2018) 111–136, https://doi.org/10.1146/annurev-cellbio-100617-062737
[226] Y. Xing, Z. Wen, W. Gao, Z. Lin, J. Zhong, Y. Jiu, Multifaceted functions of host cell caveolae/caveolin-1 in virus infections, Viruses 12 (2020) 487, https://doi.org/10.3390/v12050487
[227] L.J. Ströh, T. Stehle, Glycan engagement by viruses: receptor switches and specificity, Annu. Rev. Virol. 1 (2014) 285–306, https://doi.org/10.1146/annurev-virology-031413-085417
[228] A. Mergia, The role of caveolin 1 in HIV infection and pathogenesis, Viruses 9 (2017) 129, https://doi.org/10.3390/v9060129
[229] K. Owczarek, A. Szczepanski, A. Milewska, Z. Baster, Z. Rajfur, M. Sarna, K. Pyrc, Early events during human coronavirus OC43 entry to the cell, Sci. Rep. 8 (2018) 1–12, https://doi.org/10.1038/s41598-018-25640-0
[230] J. Mercer, A. Helenius, Virus entry by macropinocytosis, Nat. Cell Biol. 11 (2009) 510–520, https://doi.org/10.1038/ncb0509-510

[231] J. Mercer, A. Helenius, Gulping rather than sipping: macropinocytosis as a way of virus entry, Curr. Opin. Microbiol. 15 (2012) 490–499, https://doi.org/10.1016/j.mib.2012.05.016
[232] H. Sobhy, A comparative review of viral entry and attachment during large and giant dsDNA virus infections, Arch. Virol. 162 (2017) 3567–3585, https://doi.org/10.1007/s00705-017-3497-8
[233] J. Grove, M. Marsh, The cell biology of receptor-mediated virus entry, J. Cell Biol. 195 (2011) 1071–1082, https://doi.org/10.1083/jcb.201108131
[234] M. Schelhaas, B. Shah, M. Holzer, P. Blattmann, L. Kühling, P.M. Day, J.T. Schiller, A. Helenius, Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis, PLOS Pathog. 8 (2012) 1002657, https://doi.org/10.1371/journal.ppat.1002657
[235] J.R. Taylor, D.J. Fernandez, S.M. Thornton, J.G. Skeate, K.P. Lühen, D.M. Da Silva, R. Langen, W.M. Kast, Heterotetrameric annexin A2/S100A10 (A2t) is essential for oncogenic human papillomavirus trafficking and capsid disassembly, and protects virions from lysosomal degradation, Sci. Rep. 8 (2018) 1–15, https://doi.org/10.1038/s41598-018-30051-2
[236] E. de Vries, D.M. Tscherne, M.J. Wienholts, V. Cobos-Jiménez, F. Scholte, A. García-Sastre, P.J.M. Rottier, C.A.M. de Haan, Dissection of the influenza a virus endocytic routes reveals macropinocytosis as an alternative entry pathway, PLOS Pathog. 7 (2011) 1001329, https://doi.org/10.1371/journal.ppat.1001329
[237] C. Cruz-Oliveira, J.M. Freire, T.M. Conceição, L.M. Higa, M.A.R.B. Castanho, A.T. Da Poian, Receptors and routes of dengue virus entry into the host cells, FEMS Microbiol. Rev. 39 (2015) 155–170, https://doi.org/10.1093/femsre/fuu004
[238] M. Ehrlich, W. Boll, A. Van Oijen, R. Hariharan, K. Chandran, M.L. Nibert, T. Kirchhausen, Endocytosis by random initiation and stabilization of clathrin-coated pits, Cell 118 (2004) 591–605, https://doi.org/10.1016/j.cell.2004.08.017
[239] E. Boucrot, A.P.A. Ferreira, L. Almeida-Souza, S. Debard, Y. Vallis, G. Howard, L. Bertot, N. Sauvonnet, H.T. McMahon, Endophilin marks and controls a clathrin-independent endocytic pathway, Nature 517 (2015) 460–465, https://doi.org/10.1038/nature14067
[240] M. Sathe, G. Muthukrishnan, J. Rae, A. Disanza, M. Thattai, G. Scita, R.G. Parton, S. Mayor, Small GTPases and BAR domain proteins regulate branched actin polymerisation for clathrin and dynamin-independent endocytosis, Nat. Commun. 9 (2018) 1–16, https://doi.org/10.1038/s41467-018-03955-w
[241] R. Lakshminarayan, C. Wunder, U. Becken, M.T. Howes, C. Benzing, S. Arumugam, S. Sales, N. Ariotti, V. Chambon, C. Lamaze, D. Loew, A. Shevchenko, K. Gaus, R.G. Parton, L. Johannes, Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers, Nat. Cell Biol. 16 (2014) 592–603, https://doi.org/10.1038/ncb2970
[242] M. Desjardins, G. Griffiths, Phagocytosis: latex leads the way, Curr. Opin. Cell Biol. 15 (2003) 498–503, https://doi.org/10.1016/S0955-0674(03)00083-8
[243] G.J. Doherty, H.T. McMahon, Mechanisms of endocytosis, Annu. Rev. Biochem. 78 (2009) 857–902, https://doi.org/10.1146/annurev.biochem.78.081307.110540
[244] R. Mout, M. Ray, T. Tay, K. Sasaki, G. Yesilbag Tonga, V.M. Rotello, General strategy for direct cytosolic protein delivery via protein-nanoparticle co-engineering, ACS Nano 11 (2017) 6416–6421, https://doi.org/10.1021/acsnano.7b02884
[245] Z. Li, L. Sun, Y. Zhang, A.P. Dove, R.K. O'Reilly, G. Chen, Shape effect of glycononanoparticles on macrophage cellular uptake and immune response, ACS Macro Lett. 5 (2016) 1059–1064, https://doi.org/10.1021/acsmacrolett.6b00419
[246] M. Fratini, T. Wiegand, C. Funaya, Z. Jiang, P.N.M. Shah, J.P. Spatz, E.A. Cavalcanti-Adam, S. Boulant, Surface immobilization of viruses and nanoparticles elucidates early events in clathrin-mediated endocytosis, ACS Infect. Dis. 4 (2018) 1585–1600, https://doi.org/10.1021/acsinfecdis.8b00134
[247] K.M. Tsoi, S.A. Macparland, X.Z. Ma, V.N. Spetzler, J. Echeverri, B. Ouyang, S.M. Fadel, E.A. Sykes, N. Goldaracena, J.M. Kaths, J.B. Conneely, B.A. Alman, M. Selzner, M.A. Ostrowski, O.A. Adeyi, A. Zilman, I.D. McGilvray, W.C.W. Chan, Mechanism of hard-nanomaterial clearance by the liver, Nat. Mater. 15 (2016) 1212–1221, https://doi.org/10.1038/nmat4718
[248] M.P. Stewart, R. Langer, K.F. Jensen, Intracellular delivery by membrane disruption: mechanisms, strategies, and concepts, Chem. Rev. 118 (2018) 7409–7531, https://doi.org/10.1021/acs.chemrev.7b00678
[249] A. Banerjee, J. Qi, R. Gogoi, J. Wong, S. Mitragotri, Role of nanoparticle size, shape and surface chemistry in oral drug delivery, J. Control. Release 238 (2016) 176–185, https://doi.org/10.1016/j.jconrel.2016.07.051
[250] J. Tan, S. Shah, A. Thomas, H.D. Ou-Yang, Y. Liu, The influence of size, shape and vessel geometry on nanoparticle distribution, Microfluid. Nanofluid. 14 (2013) 77–87, https://doi.org/10.1007/s10404-012-1024-5
[251] P. Makvandi, A. Zarepour, X. Zheng, T. Agarwal, M. Ghomi, R. Sartorius, E.N. Zare, A. Zarrabi, A. Wu, T.K. Maiti, B.R. Smith, R.S. Varma, F.R. Tay, V. Mattoli, Non-spherical nanostructures in nanomedicine: from noble metal nanorods to transition metal dichalcogenide nanosheets, Appl. Mater. Today 24 (2021) 101107, https://doi.org/10.1016/J.APMT.2021.101107
[252] J. Rejman, V. Oberle, I.S. Zuhorn, D. Hoekstra, Size-dependent internalization of particles via the pathways of clathrin-and caveolae-mediated endocytosis, Biochem. J. 377 (2004) 159–169, https://doi.org/10.1042/BJ20031253
[253] G. Caracciolo, F. Cardarelli, D. Pozzi, F. Salomone, G. Maccari, G. Bardi, A.L. Capriotti, C. Cavaliere, M. Papi, A. Laganà, Selective targeting capability acquired with a protein corona adsorbed on the surface of 1,2-dioleoyl-3-trimethylammonium propane/dna nanoparticles, ACS Appl. Mater. Interfaces 5 (2013) 13171–13179, https://doi.org/10.1021/am404171h
[254] K. Mortensen, L.I. Larsson, Effects of cytochalasin D on the actin cytoskeleton: association of neoformed actin aggregates with proteins involved in signaling and endocytosis, Cell. Mol. Life Sci. 60 (2003) 1007–1012, https://doi.org/10.1007/s00018-003-3022-x
[255] D. Dutta, J.G. Donaldson, Search for inhibitors of endocytosis, Cell. Logist. 2 (2012) 203–208, https://doi.org/10.4161/cl.23967
[256] G. Preta, J.G. Cronin, I.M. Sheldon, Dynasore – not just a dynamin inhibitor, Cell Commun. Signal. 13 (2015) 1–7, https://doi.org/10.1186/s12964-015-0102-1
[257] L. Von Kleist, W. Stahlschmidt, H. Bulut, K. Gromova, D. Puchkov, M.J. Robertson, K.A. MacGregor, N. Tomlin, A. Pechstein, N. Chau, M. Chircop, J. Sakoff, J.P. Von Kries, W. Saenger, H.G. Kräusslich, O. Shupliakov, P.J. Robinson, A. McCluskey, V. Haucke, Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition, Cell 146 (2011) 471–484, https://doi.org/10.1016/j.cell.2011.06.025
[258] L.I. FitzGerald, A.P.R. Johnston, It's what's on the inside that counts: techniques for investigating the uptake and recycling of nanoparticles and proteins in cells, J. Colloid Interface Sci. 587 (2021) 64–78, https://doi.org/10.1016/j.jcis.2020.11.076
[259] L.I. Selby, L. Aurelio, D. Yuen, B. Graham, A.P.R. Johnston, Quantifying cellular internalization with a fluorescent click sensor, ACS Sens. 3 (2018) 1182–1189, https://doi.org/10.1021/acssensors.8b00219
[260] J.J. Rennick, A.P.R. Johnston, R.G. Parton, Key principles and methods for studying the endocytosis of biological and nanoparticle therapeutics, Nat. Nanotechnol. 16 (2021) 266–276, https://doi.org/10.1038/s41565-021-00858-8
[261] D. Vercauteren, R.E. Vandenbroucke, A.T. Jones, J. Rejman, J. Demeester, S.C. De Smedt, N.N. Sanders, K. Braeckmans, The use of inhibitors to study endocytic pathways of gene carriers: optimization and pitfalls, Mol. Ther. 18 (2010) 561–569, https://doi.org/10.1038/mt.2009.281
[262] B. Mercorelli, G. Palù, A. Loregian, Drug repurposing for viral infectious diseases: how far are we? Trends Microbiol. 26 (2018) 865–876, https://doi.org/10.1016/j.tim.2018.04.004
[263] L. Cantuti-Castelvetri, R. Ojha, L.D. Pedro, M. Djannatian, J. Franz, S. Kuivanen, F. van der Meer, K. Kallio, T. Kaya, M. Anastasina, T. Smura, L. Levanov, L. Szirovicza, A. Tobi, H. Kallio-Kokko, P. Österlund, M. Joensuu, F.A. Meunier, S.J. Butcher, M.S. Winkler, B. Mollenhauer, A. Helenius, O. Gokce, T. Teesalu, J. Hepojoki, O. Vapalahti, C. Stadelmann, G. Balistreri, M. Simons, Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity, Science 370 (2020) 856–860, https://doi.org/10.1126/science.abd2985 (New York, N.Y.).
[264] J. Daly, B. Simonetti, C. Antón-Plágaro, M. Kavanagh Williamson, D. Shoemark, L. Simón-Gracia, K. Klein, M. Bauer, R. Hollandi, U. Greber, P. Horvath, R. Sessions, A. Helenius, J. Hiscox, T. Teesalu, D. Matthews, A. Davidson, P. Cullen, Y. Yamauchi, Neuropilin-1 is a host factor for SARS-CoV-2 infection, BioRxiv (2020), https://doi.org/10.1101/2020.06.05.134114
[265] R. Poduri, G. Joshi, G. Jagadeesh, Drugs targeting various stages of the SARS-CoV-2 life cycle: exploring promising drugs for the treatment of Covid-19, Cell Signal. 74 (2020) 109721, https://doi.org/10.1016/j.cellsig.2020.109721
[266] S.A. Shiryaev, P. Mesci, A. Pinto, I. Fernandes, N. Sheets, S. Shresta, C. Farhy, C.T. Huang, A.Y. Strongin, A.R. Muotri, A.V. Terskikh, Repurposing of the anti-malaria drug chloroquine for Zika virus treatment and prophylaxis, Sci. Rep. 7 (2017) 1–9, https://doi.org/10.1038/s41598-017-15467-6
[267] M.S. Khuroo, Chloroquine and hydroxychloroquine in coronavirus disease 2019 (COVID-19). Facts, fiction and the hype: a critical appraisal, Int. J. Antimicrob. Agents 56 (2020) 106101, https://doi.org/10.1016/j.ijantimicag.2020.106101
[268] S.A. Meo, D.C. Klonoff, J. Akram, Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19, Eur. Rev. Med. Pharmacol. Sci. 24 (2020) 4539–4547, https://doi.org/10.26355/eurrev_202004_21038
[269] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res. 30 (2020) 269–271, https://doi.org/10.1038/s41422-020-0282-0
[270] J. Liu, R. Cao, M. Xu, X. Wang, H. Zhang, H. Hu, Y. Li, Z. Hu, W. Zhong, M. Wang, Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro, Cell Discov. 6 (2020) 1–4, https://doi.org/10.1038/s41421-020-0156-0
[271] J. Gao, Z. Tian, X. Yang, Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies, Biosci. Trends 14 (2020) 72–73, https://doi.org/10.5582/BST.2020.01047
[272] C.G. Wambier, Focus on clinical outcomes of "hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial", Int. J. Antimicrob. Agents 57 (2021) 106175, https://doi.org/10.1016/j.ijantimicag.2020.106175
[273] S. Ibáñez, O. Martínez, F. Valenzuela, F. Silva, O. Valenzuela, Hydroxychloroquine and chloroquine in COVID-19: should they be used as standard therapy? Clin. Rheumatol. 39 (2020) 2461–2465, https://doi.org/10.1007/s10067-020-05202-4
[274] Y. Zhou, P. Vedantham, K. Lu, J. Agudelo, R. Carrion, J.W. Nunneley, D. Barnard, S. Pöhlmann, J.H. McKerrow, A.R. Renslo, G. Simmons, Protease inhibitors targeting coronavirus and filovirus entry, Antivir. Res. 116 (2015) 76–84, https://doi.org/10.1016/j.antiviral.2015.01.011
[275] N. Zhou, T. Pan, J. Zhang, Q. Li, X. Zhang, C. Bai, F. Huang, T. Peng, J. Zhang, C. Liu, L. Tao, H. Zhang, Glycopeptide antibiotics potently inhibit cathepsin l in the late endosome/lysosome and block the entry of ebola virus, middle east respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV), J. Biol. Chem. 291 (2016) 9218–9232, https://doi.org/10.1074/jbc.M116.716100

[276] A.L.Z. Djomkam, C.O. Olwal, T.B. Sala, L. Paemka, Commentary: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Front. Oncol. 10 (2020) 1448, https://doi.org/10.3389/fonc.2020.01448
[277] C. Liu, A. von Brunn, D. Zhu, Cyclophilin A and CD147: novel therapeutic targets for the treatment of COVID-19, Med. Drug Discov. 7 (2020) 100056, https://doi.org/10.1016/j.medidd.2020.100056
[278] K. Wang, W. Chen, Y.-S. Zhou, J.-Q. Lian, Z. Zhang, P. Du, L. Gong, Y. Zhang, H.-Y. Cui, J.-J. Geng, B. Wang, X.-X. Sun, C.-F. Wang, X. Yang, P. Lin, Y.-Q. Deng, D. Wei, X.-M. Yang, Y.-M. Zhu, K. Zhang, Z.-H. Zheng, J.-L. Miao, T. Guo, Y. Shi, J. Zhang, L. Fu, Q.-Y. Wang, H. Bian, P. Zhu, Z.-N. Chen, SARS-CoV-2 invades host cells via a novel route: CD147-spike protein, BioRxiv (2020), https://doi.org/10.1101/2020.03.14.988345 2020.03.14.988345.
[279] N. Yang, H.M. Shen, Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19, Int. J. Biol. Sci. 16 (2020) 1724–1731, https://doi.org/10.7150/ijbs.45498
[280] C. Huang, T. Ozdemir, L.C. Xu, P.J. Butler, C.A. Siedlecki, J.L. Brown, S. Zhang, The role of substrate topography on the cellular uptake of nanoparticles, J. Biomed. Mater. Res. Part B Appl. Biomater. 104 (2016) 488–495, https://doi.org/10.1002/jbm.b.33397
[281] A. Gentile, F. Ruffino, M.G. Grimaldi, Complex-morphology metal-based nanostructures: fabrication, characterization, and applications, Nanomaterials 6 (2016), https://doi.org/10.3390/nano6060110 (Basel, Switzerland).
[282] B.M. Strauch, W. Hubele, A. Hartwig, Impact of endocytosis and lysosomal acidification on the toxicity of copper oxide nano-and microsized particles: uptake and gene expression related to oxidative stress and the DNA damage response, Nanomaterials 10 (2020) 679, https://doi.org/10.3390/nano10040679
[283] S.P. Mutalik, A. Pandey, S. Mutalik, Nanoarchitectronics: a versatile tool for deciphering nanoparticle interaction with cellular proteins, nucleic acids and phospholipids at biological interfaces, Int. J. Biol. Macromol. 151 (2020) 136–158, https://doi.org/10.1016/j.ijbiomac.2020.02.150
[284] Y. Liu, B. Workalemahu, X. Jiang, Open-label phase I clinical trial of Ad5-EBOV in Africans in China, Hum. Vaccines Immunother. 13 (2017) 2078–2085, https://doi.org/10.1002/smll.201701815
[285] K.J. Koudelka, A.S. Pitek, M. Manchester, N.F. Steinmetz, Virus-based nanoparticles as versatile nanomachines, Annu. Rev. Virol. 2 (2015) 379–401, https://doi.org/10.1146/annurev-virology-100114-055141
[286] T. Frenz, E. Grabski, V. Durán, C. Hozsa, A. Stępczyńska, M. Furch, R.K. Gieseler, U. Kalinke, Antigen presenting cell-selective drug delivery by glycan-decorated nanocarriers, Eur. J. Pharm. Biopharm. 95 (2015) 13–17, https://doi.org/10.1016/j.ejpb.2015.02.008
[287] R.A. Al-Horani, S. Kar, K.F. Aliter, Potential anti-covid-19 therapeutics that block the early stage of the viral life cycle: structures, mechanisms, and clinical trials, Int. J. Mol. Sci. 21 (2020) 1–41, https://doi.org/10.3390/ijms21155224
[288] X. Wang, P. Zou, F. Wu, L. Lu, S. Jiang, Development of small-molecule viral inhibitors targeting various stages of the life cycle of emerging and re-emerging viruses, Front. Med. 11 (2017) 449–461, https://doi.org/10.1007/s11684-017-0589-5
[289] G. Chauhan, M.J. Madou, S. Kalra, V. Chopra, D. Ghosh, S.O. Martinez-Chapa, Nanotechnology for COVID-19: therapeutics and vaccine research, ACS Nano 14 (2020) 7760–7782, https://doi.org/10.1021/acsnano.0c04006