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Review article

Quantum dots – Nano-sized probes for the exploration of cellular and intracellular targeting

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Abstract

Nanoparticles emerged as promising tool in drug targeting, since, after appropriate modification, they are able to deliver their payload to specific sites, like tissues, cells, or even certain cellular organelles. In this context, the delivery of nanoparticles from the circulation into the target cells represents a crucial step. Here, model drug delivery systems such as quantum dots are ideal candidates to elucidate this process in more detail, since they provide outstanding features like a small and uniform size, unique optical properties for most sensitive detection and modifiable surfaces. Recent progress in the surface chemistry of quantum dots expanded their use in biological applications, reduced their cytotoxicity and rendered quantum dots a powerful tool for the investigation of distinct cellular processes, like uptake, receptor trafficking and intracellular delivery. In this review, we will not only describe the ideal attributes of QDs for biological applications and imaging but also their distinct specific and non-specific pathways into the cells as well as their intracellular fate.

Keywords: Drug targeting; Quantum dot; Nanoparticle; Cellular uptake; Endocytosis; Cytotoxicity

1. Introduction

In contemporary drug therapy, most drugs are designed to bind to specific receptors or receptor subtypes. However, these drugs still lack selectivity for specific sites in the human body, namely specific cells, tissues or organs, since the receptors may be expressed at various sites of the body. In addition, modern drugs tend to have unfavorable pharmacokinetic properties. As a consequence, the dose of the drug has to be increased, which raises the cost of the

Abbreviations: 5HT, 5-hydroxytryptamine; AFM, atomic force microscopy; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; ANG II, angiotensin II; AT, angiotensin receptor; CCV, clathrin-coated vesicle; CHO, Chinese hamster ovary; CLSM, confocal laser scanning microscopy; CME, clathrin-mediated endocytosis; CPP, cell penetrating peptide; Da, dalton; DHLA, dihydrolipoic acid; DLS, dynamic light scattering; EDC, 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EPR, enhanced permeability and retention; FR, folate receptor; GPCR, G-protein coupled receptor; HEK, human embryonic kidney; Her2, human epidermal growth factor receptor 2; HIV, human immunodeficiency virus; HIV TAT, human immunodeficiency virus transactivator protein; IL-2, interleukin-2; LDL, low-density lipoprotein; MLS, mitochondrial localization sequence; MMP, matrix metalloprotease; MRI, magnetic resonance imaging; NGF, nerve growth factor; NHS, *N*-hydroxysuccinimide; NIR, near infrared; NLS, nuclear localization sequence; PEG, polyethylene glycol; PET, positron emission tomography; PSMA, prostate specific membrane antigen; PTD, protein transduction domain; QD, quantum dot; RES, reticulo endothelial system; ROS, reactive oxygen species; SERT, serotonin transporter protein; SNAC, serotonin-labeled CdSe nanocrystals; SPECT, single photon emission computerized tomography; SPION, superparamagnetic iron oxide nanoparticle; sulfo-SMCC, sulfosuccinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate; SV40, simian virus 40; TEM, transmission electron microscopy; Tf, transferrin; TfR, transferrin receptor; TOP, trioctylphosphine; TOPO, trioctylphosphine oxide; TRK, receptor tyrosine kinase.

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therapy as well as the risk of side-effects. Although the design of prodrugs can improve this situation we are far from a general solution.

Nanoparticles for site-specific drug delivery represent a more promising solution to this problem [1]. Under ideal circumstances, these drug carriers, mediated by a targeting sequence, should deliver their payload only to specific target cells, tissues or organs [2].

Moreover, nanoparticles are tools that can compensate for unfavorable physico-chemical properties of the drug and therefore may optimize the bioavailability and the biodistribution of the drug. However, after their administration in to the circulation such particles face several obstacles prior to reaching their destination. First, the particles have to escape the reticulo endothelial system (RES), which includes phagocytosing cells. These cells are mainly located in the liver, the spleen and the bone marrow and are specialized to rapidly remove "foreign" particles from the circulation. This recognition by the RES can be reduced by modifying the surface of the particles with "protein repulsive" molecules, such as polyethylene glycol (PEG) [3,4]. In addition, en route to their target the particles have to pass biological barriers like the endothelium and must penetrate tissues. Both processes require small, nanoparticulate drug delivery systems. Additionally, targeting sequences on the particle surfaces are needed to direct the drug carrier to specific cells [5]. Last but not least, when the nanoparticles have reached the target cell, the final question still remains unanswered: How can the drug be delivered into the cell, in case of an intracellular site of action?

To obtain detailed information on the particle design for optimized intracellular drug delivery, well-defined model colloids are necessary, since the pathways for nanoparticles into cells strongly depend on the properties of the colloid itself like its size. This is emphasized by the fact that the means of cellular uptake of a colloid already determine its fate inside the cell [6]. Another crucial parameter is the surface composition of the nanoparticles. As an example, protein repellant molecules on the surface of the colloid, like PEGs, can mask the particles. As a consequence, non-specific cellular uptake [7], can be reduced. This fact is crucial if specific targeting is desired. A last, obvious requirement for the model colloid is that it must be easily detectable, e.g. by fluorescence, in order to follow its cellular uptake and intracellular accumulation in certain organelles.

Unfortunately, conventional nanoparticles, like polymer-based ones, often possess quite broad size distributions, which limit the use of these particles for the examination of the cellular uptake and intracellular distribution. Moreover, the tagging of nanoparticles with fluorescent dyes may significantly change their physico-chemical properties.

Quantum dots (QDs), fluorescent, semiconductor nanoparticles, overcome these problems due to their small size, narrow size distribution, and unique optical properties. These features enable the investigation of various cellular uptake pathways and allow for easy detection within cells due to the bright fluorescence of the colloids. Furthermore, these nanoparticles can be modified with a number of targeting ligands. Taken together, these properties render QDs ideal model colloids to study the cellular uptake and intracellular targeting of nanoparticles.

In this review, we show that QDs are a favorable tool to answer particular questions in the realm of cellular nanoparticle delivery, like *what is the optimal size of a particle for endocytosis* [8], *how can non-specific uptake of particles be reduced by surface PEGylation* [9], or *which receptors can mediate specific uptake of colloids* [10,11]? Other approaches focus on how specific organelles can be targeted by QDs, once the particles have arrived inside a cell [12,13]. Dividing the complex field of drug targeting with nanoparticles into such distinct questions will promote the progress in this research area.

The review is structured as follows: after a short overview of the properties and advantages of QDs, we will show how these particles can be modified in order to use them for the analysis of specific cellular uptake. This will be followed by a presentation of the distinct pathways for cellular uptake and intracellular delivery of nanoparticles. In the next section, an overview of approaches of cellular uptake studies as well as of organelle targeting with QDs will be given. Finally, possibilities for further developments in intracellular drug targeting using QDs will be presented.

2. Non-invasive imaging using nanoparticles

For the development of drug targeting systems, non-invasive imaging technologies play a key role, since they allow one to follow the fate of nanoparticles inside biological systems, like cells, tissues or whole organisms.

Non-invasive imaging of nanoparticles includes a number of techniques, like positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and optical imaging. These techniques differ concerning their sensitivity, resolution complexity and demands on personnel and equipment (for more detailed information, see [14,19]).

In order to investigate the interactions between nanoparticles and biological systems, particles of well defined size and narrow size distribution are required, as presented above. Three widely used systems provide these properties: colloidal gold [14], superparamagnetic iron oxide particles (SPIONs) [15] and semiconductor nanocrystals (QDs) [10]. However, these particles show great differences in their detectability, which is the second, important demand on a model colloid. All of these particles can be detected by transmission electron microscopy, however, this technique requires the fixation of the observed material, which does not allow for studies of dynamic processes in living cells. SPIONs can additionally be detected by MRI. This technique proves especially favorable in deep-tissue and *in vivo* imaging. Unfortunately, the resolution of this tech-

Table 1
Comparison of imaging technologies for different types of nanocrystals

	Transmission electron microscopy (TEM)	Magnetic resonance imaging (MRI)	Fluorescence laser scanning microscopy (CLSM)
Applications	*In vitro* imaging (cell fixation required)	Deep-tissue *in vivo* imaging [16]	Intracellular imaging, *in vivo* imaging [17]
Resolution	Visualization of sub-cellular distribution [18]	Sensitivity and resolution (10–100 μm) not for sub-cellular processes [19]	High resolution (to the single molecule level) [11,20]
Imaged nanoparticles	Gold, superparamagnetic iron oxide, Quantum dots	Superparamagnetic iron oxide	Quantum dots

nique is not suitable for the investigation of processes on the cellular level (see Table 1).

QDs, on the other hand, offer some advantages in imaging cell-particle interactions. They can be detected easily by (confocal) fluorescence microscopy, which is preferred for the observation of distinct cellular processes, like endocytosis and intracellular trafficking. The high resolution, sensitivity and versatility of fluorescence detection render QDs the most suitable of these three systems for the described application.

3. Properties of QDs for optical imaging

QDs – small (1–10 nm) semiconductor nanocrystals – have attracted increasing attention due to their unique optical properties as compared to conventional organic fluorescent dyes. The exceptional brightness of QDs stems from a combination of efficient light absorption, quantified as a large extinction coefficient, and a high quantum yield (up to 85% [21]), which is the ratio of the number of photons emitted to the number of photons absorbed. Together with their high stability with regard to photobleaching as compared to conventional fluorophores, QDs enable highly sensitive detection and long observation times in fluorescence microscopy [22]. What is more, QDs have a large Stokes shift, which is the difference between the wavelength of absorbed and emitted light. They also exhibit narrow emission spectra [23] as compared to conventional organic fluorescent dyes. As a consequence, the fluorescence signal of QDs can easily be separated from the light of the excitation source. In addition, these unique optical properties allow for multi-color imaging without crosstalk between different detection channels in fluorescence microscopes. Additionally, QDs of different emission maxima can be excited by one single wavelength [24], which eliminates the need for numerous excitation sources in the instrumental setup. The relatively long fluorescence lifetime (20–50 ns [25]) enables time-resolved detection of the QD-fluorescence which significantly increases the signal-to-background ratio (by a factor of 15) relative to cell autofluorescence [26].

The range of the fluorescence maxima of QDs is determined by their elemental composition and extends over the whole visible spectrum (e.g. CdS and CdSe) to the near infrared (NIR; e.g. CdTe). Furthermore, within the emission range dictated by the elemental composition, the emission maximum of QDs can be tuned precisely by adjusting the size of the QDs. Smaller QDs emit light with shorter wavelengths than larger particles. For example, the emission maxima of CdSe nanocrystals of 3 and 7 nm in diameter are approximately 550 and 650 nm, respectively [27]. The so-called QD cores are typically capped with a shell of another semiconductor, mostly ZnS [21,28,29] (Fig. 1). These shell QDs provide enhanced quantum yields as compared to the bare cores [30]. An interesting phenomenon is the so-called "blinking", an on–off behaviour of the luminescence signal of the QDs. This phenomenon is caused by charge trapping and untrapping at surface defects during excitation and results in an alternation of bright and dark states during which no photons are emitted [31]. Blinking can be a limiting factor for detection but can also represent a useful fingerprint, for example to follow trafficking of receptors labeled with QDs [20].

Recent developments in QD technology also expanded the potential for *in vivo* imaging, especially of small animals. Important advances in this field have been achieved with the use of NIR emitting QDs [32] since the penetration of NIR light into tissues is significantly higher than light of shorter wavelengths, which is strongly scattered and absorbed by the tissue [33,34]. An increase in detectability can also be achieved by combining the unique optical

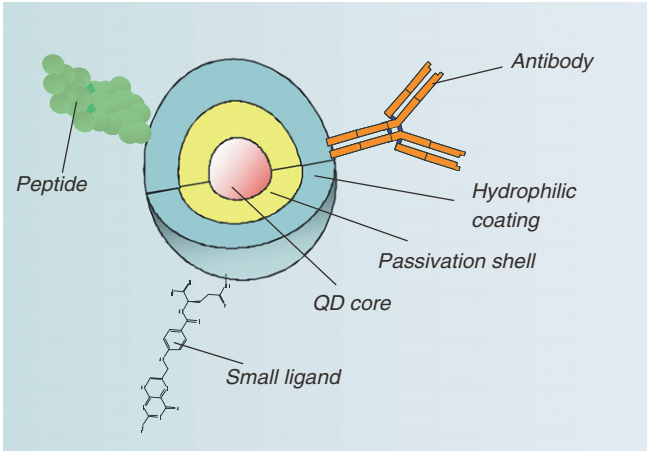

Fig. 1. Scheme of a QD for biological application. The nanocrystal core (e.g. CdSe) is passivated by another semiconductor shell (e.g. ZnS). The QD-surface is covered by a hydrophilic coating which enables conjugation to biological active compounds (e.g. antibodies, peptides or small ligands, here depicted for folic acid).

properties of the QDs with MRI traceability [35–37], which further enhances the possibility of tissue imaging. An appealing alternative for *in-vivo* experiments are so-called self-illuminating QDs, which allow for much better detection in tissues. Here, no excitation source is needed when using the phenomenon of bioluminescence resonance energy transfer (BRET) as a bioluminescent molecule coupled to QDs serves to excite the nanoparticle. As a consequence, the sensitivity of detection and the signal-to-background ratio *in vivo* are enhanced [38].

4. Surface modifications and bioconjugation of QDs

Unmodified QDs cannot be used for cellular studies since, after most of the QD synthesis strategies, the particles are water-insoluble. Therefore, to make use of their unique properties, the particles have to be modified on the surface. Additionally, the particles have to be conjugated with cell-specific biomolecules like proteins or peptides, in order to investigate specific uptake mechanisms and intracellular targeting.

QDs are mostly synthesized in non-polar organic solvents and are solubilized in these solvents by hydrophobic surface ligands like aliphatic phosphines and phosphine oxides, fatty acids and aliphatic amines. For solubilization in aqueous buffers, the hydrophobic QD-surface needs to be modified by a hydrophilic coating (Fig. 1). This coating additionally is responsible for hindering aggregation of the QDs, e.g. by electrostatic stabilization or by steric repulsion between the particles mediated by a polymer coating. The hydrophobic surface ligands can be exchanged for thiol-containing molecules [10,39], oligomeric phosphines [40], dendrons [41], or peptides [42,43]. Other approaches encapsulate the QDs in a silica shell [44] or use hydrophobic interactions between the QDs and amphiphilic polymers [45,46] or phospholipids [47] for modification (for a more detailed review, see [48]).

For QDs to be used for the investigation of distinct cellular uptake processes, the particles have to be conjugated to biomolecules like peptides or proteins that can target certain structures on the cell surface, such as receptors. Therefore, the surface of the QD colloid must provide functional groups or other reactive sites that allow for a coupling of various biomolecules (Fig. 1).

There are several possibilities for the attachment of biomolecules to QDs. A commonly used strategy is the use of QDs containing streptavidin because they can easily be linked to biotin-tagged biomolecules [11,20,24,49] (Fig. 2a). Yet, since streptavidin is quite bulky (~60 kDa) and can bind four biotin molecules, steric and size aspects have to be considered. Hence, small covalent linkages are often preferred when the particle size of the QD bioconjugate is critical for the application, for example when intracellular pores have to be crossed or cellular transport proteins are targeted. Such covalent bonds can be formed by carboxylic acids, for instance provided by a mercapto acid coating of the QDs. The mercapto groups can bind to the QD surface, while the carboxylic acid functions can form stable amide bonds with amines of various biomolecules in the presence of coupling reagents, such as carbodiimides and *N*-hydroxysuccinimides (NHS) [10,50] (Fig. 2b). Stable, covalent bonds can also be formed between maleimides and thiols, resulting in a thioether between QDs and biomolecules [47,49,51] (Fig. 2c). Heterobifunctional crosslinkers, such as sulfosuccinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (sulfo-SMCC), can be used to couple thiol-containing biomolecules with amine-coated QDs [47], or vice versa [52]. Thiol groups in proteins or peptides are provided by cysteine residues or, after thiolation, e.g. by iminothiolane [53]. Func-

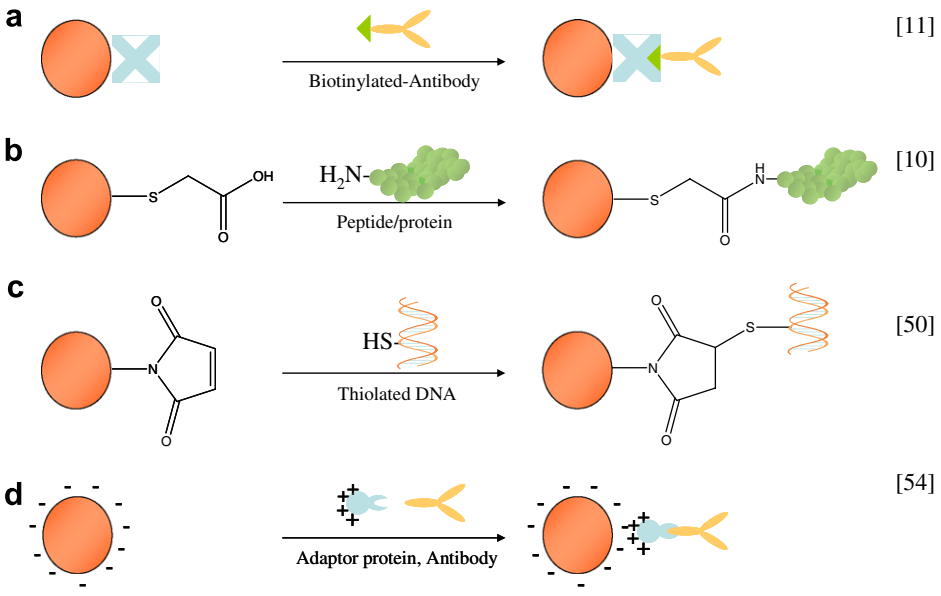

Fig. 2. Examples of the bioconjugation of differently functionalized QDs to biomolecules by various strategies.

tionalization of QDs stabilized with thiols can also be achieved by thiol exchange with biomolecules containing a sulfhydryl group [53]. After incubation, equilibrium of the thiols on the QD surface is achieved resulting in partial substitution of the initial coating molecules by the biomolecules. In addition, electrostatic interactions between the QD-surface and peptides or proteins (Fig. 2d) can be used for coating and linkage [54,55] (for reviews, see [56,57]).

5. Cytotoxicity of QDs

Since QDs are supposed to be used for studies involving living cells and organisms, the biocompatibility of the particles has to be characterized. As compared to other nanoparticles like gold or iron oxide nanoparticles, which have been used for several decades and proved to be biocompatible [58,59], QDs are new materials and have not been completely characterized with regard to their toxicity. Most of the QDs used for biological applications contain the heavy metal cadmium, known to cause toxic effects when in contact with cells. Using hepatocytes, Derfus et al. showed that oxidation of Cd on the QD surface and subsequent Cd^{2+} release, e.g. mediated by oxygen or UV light, is one possible mechanism responsible for QD cytotoxicity [60]. Consequently, steps have been taken to protect the QD core, as described above, by a shell of much lower toxicity, in most cases ZnS, which resulted in a significant reduction of cytotoxic effects [60,61]. As this layer also improves the optical properties, core-shell systems have become standard in most biological applications. But the introduction of capping layers was still not sufficient to solve the problem of cytotoxicity completely. Various other factors, like the aggregation of particles on the cell surface [62] and even the stabilizing QD surface ligands, have been shown to impair cell viability [63]. Hence, choosing an appropriate surface coating has also been shown to be a critical parameter, since simple coatings, like thiol-containing carboxylic acids, present only a minor diffusion barrier for Cd^{2+}. Additionally, thiol-coated QDs are known for their poor colloidal stability [44,64], and therefore, the introduction of inert and stable, macromolecular or cross-linked surface coatings could be an important improvement. This concept has been realized by several groups. For instance, Dubertret et al. integrated QDs in PEG-containing phospholipid micelles and microinjected them into early-stage *Xenopus* embryos [47]. After injection of 2×10^9 QDs per cell the development of the embryos was normal. However, at higher concentrations ($>5 \times 10^9$ QDs per cell) abnormalities (cell size, cell death, cell movement and axis elongation) became apparent. Other coatings, like silica encapsulation or polymer coatings [62], can also serve as fairly non-toxic modifications. Finally, the recent replacement of the toxic elements of the semiconductors by more biocompatible ones is a next step towards low-toxic QDs [61,69].

In addition to the toxic effects of the elemental building blocks, cell death can be induced by the formation of reactive oxygen species (ROS) [70–72], since QDs can transfer absorbed optical energy to oxygen molecules. Free radicals can cause damage to DNA [73] and other cellular components and as a consequence induce apoptosis and necrosis.

Surprisingly, in most of the studies involving QDs in live cells, no significant influence on cell viability, morphology, function or development was observed with the used concentrations (ranging from nM to μM) [24,51,54,65–68]. So far, little data on the toxicity of QDs, especially for *in vivo* applications, are available. Therefore, toxicity remains the main limitation for the use of QDs. Several questions remain unanswered, such as the clearance of the particles from the circulation and tissues and if there are long-term effects on gene expression. Initial gene arrays after the incubation of fibroblasts with silanized, PEGylated QDs, however, indicate only low cytotoxicity when the QDs have an appropriate surface modification [74].

6. Cellular particle uptake – a decisive step for intracellular particle delivery

When nanoparticles in general or QDs as model colloids have to be delivered into specific cells, two aspects have to be considered: first, specific cell-surface structures have to be targeted and, second, an appropriate pathway into the cell must be found.

As numerous factors influence the uptake and the intracellular delivery of particles, like the size and the surface charge [75], the particle design requires a profound understanding of the cellular mechanisms. Additionally, there are many ways leading inside a cell but the pathway chosen for delivery itself can already determine the intracellular fate of the colloid [76]. After clathrin dependent uptake, for instance, particles are trapped in endosomes and can further be degraded in lysosomes or be exocytosed [7]. Hence, for a better understanding of these processes, the most widely used cellular uptake mechanisms for delivery of nanoparticles will be described in the following section. Thereafter, concrete approaches of non-specific and specific cell targeting with QDs will be presented.

6.1. Gateways for cellular uptake and QD delivery

For intracellular delivery of nanoparticles, it is helpful to take a closer look at the uptake mechanisms of the cells which are subsumed under the global term *endocytosis*. By this process, macromolecules and small particles are transported across the plasma membrane. Cells use these pathways for the supply with nutrients, for intercellular communication and for immune response as well as for other essential functions, but these highly developed routes can also be exploited to deliver other kinds of freights, like drug-loaded nanoparticles or QDs. According to the mechanism of endocytosis, this process can be subdivided into two major categories: *phagocytosis*, which is the uptake of large particles, and *pinocytosis*, which is the uptake of fluid and solutes (Fig. 3; for review, see [76]). While the first mechanism is only typical of specialized mammalian cells,

like macrophages, monocytes and neutrophils, pinocytosis can be found in every cell. Pinocytosis can be further classified into in least four mechanisms: macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis and clathrin- and caveolae-independent endocytosis (Fig. 3). Macropinocytosis represents an efficient route for non-selective endocytosis of solute macromolecules. The formation of macropinosomes (diameter up to 5 μm) is started by actin-driven ruffling of the cell membrane induced by growth factors or other signals. The membrane protrusions engulf large volumes of extracellular fluid [78]. Micropinocytosis is preferred for the uptake of smaller particles. The different uptake mechanisms result in differently sized vesicles and can be classified into clathrin – (~120 nm), caveolin – (~60 nm) or clathrin – and caveolin-independent endocytosis (~90 nm). Clathrin-mediated endocytosis (specified later) is the most important mechanism for receptor-mediated uptake, occurs in all mammalian cells and plays an important physiological role, for example in mediating the uptake of low-density lipoprotein (LDL). Caveolae, small flask-shaped invaginations in the plasma membrane can most notably be found in endothelial cells, smooth muscle cells and adipocytes. Their physiological role is still being discussed (e.g. cholesterol uptake, solute transport and tumor suppression) [79]. Pathogens, e.g. the SV40 virus [80], are also able to enter cells via caveolae, which do not fuse with lysosomes after endocytosis, enabling their entry into the cell without impairing their activity in the acidic environment of lysosomes. This fact makes endocytosis via caveolae interesting for nanoparticle delivery, since in contrast to other mechanisms, the particles are neither entrapped in endosomes nor degraded in lysosomes and therefore can directly head for their intracellular targets. However, it is more complicated as caveolae uptake can also result in delivery to the Golgi complex and endoplasmic reticulum as well as in discharge of the particles by transcytosis [81].

Clathrin- and caveolin-independent endocytosis is only described in a few examples, e.g. for the recovery of membrane proteins in neurons or the internalization of the interleukin-2 (IL-2) receptor on lymphocytes [76]. It is still unclear how this ill-defined means of endocytosis can be selectively targeted by nanoparticles. QD-based colloids could help to investigate these ways of uptake due to their small size, narrow size distribution and easy detectability.

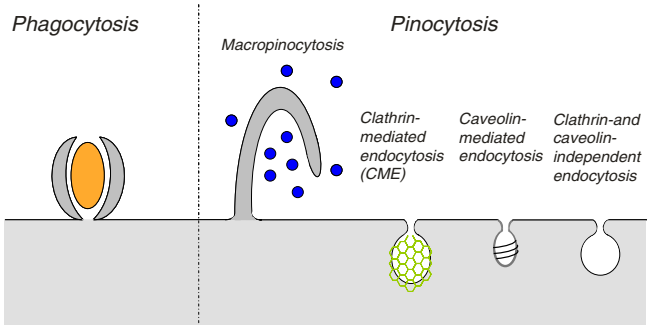

Fig. 3. Survey of different endocytic pathways. The pathways can be classified by the mechanism of vesicle formation as well as the nature and size of the cargo. Adopted from [76].

6.2. Receptor-mediated endocytosis

One pathway for active and selective transport of nanoparticulate systems into the cell is exploited in many applications – the receptor-mediated endocytosis [82–84]. The binding of ligands that are specific for certain receptors allows for a 1000-fold increase of the intracellular concentration of macromolecules, rendering this form of uptake very effective. The most frequent form of receptor-mediated endocytosis is clathrin-mediated endocytosis (CME) [77] (Fig. 4). This process begins with the binding of a ligand to a specific cell surface receptor, followed by clustering of the ligand–receptor complexes in *coated pits*, which are formed by the assembly of cytosolic coat proteins, mainly clathrin. The coated pits then invaginate and pinch off of the plasma membrane, aided by dynamin, to form intracellular clathrin-coated vesicles (CCVs). After depolymerization of clathrin, the endosomes are acidified due to proton influx, which leads to late endosomes. In this state, the ligands dissociate from the receptors and are subsequently either released from the endosome or degraded after the fusion of the late endosomes with lysosomes [7,76,85]. A crucial prerogative for intracellular particle delivery is the endosomal release of the particles, otherwise the particles will be degraded. A possible solution to this problem can be the use of pH-sensitive polymeric building blocks for the nanoparticle design [86–89].

QDs delivered into cells by CME were for example connected to transferrin, growth factors or folic acid. Details of these approaches will be described later.

6.3. Protein transduction domains

Another clathrin-independent pathway into cells that attracted growing attention during the last decade is mediated by protein transduction domains (PTDs). This cell delivery system enables the transport of certain peptides and proteins [90], liposomes [91,92] and nanoparticles [93,94] across the cell membrane. QDs have also been successfully delivered via PTD, examples of which will be given later.

In the literature, the term *cell penetrating peptides* (CPPs) is also used for both peptides and proteins of this class. PTDs are available to deliver cargo molecules into nearly every cell type. Additionally, PTDs are able to cross the blood–brain barrier and can even enter intracellular sites and the nucleus, though the exact mechanism for the cellular uptake has not been completely resolved [95,96].

An important example is the TAT PTD [97], derived from the TAT protein from the human immunodeficiency virus (HIV-1), which is able to deliver proteins into cells. Other PTDs, like the third α-helix of *Antennapedia* homeo-

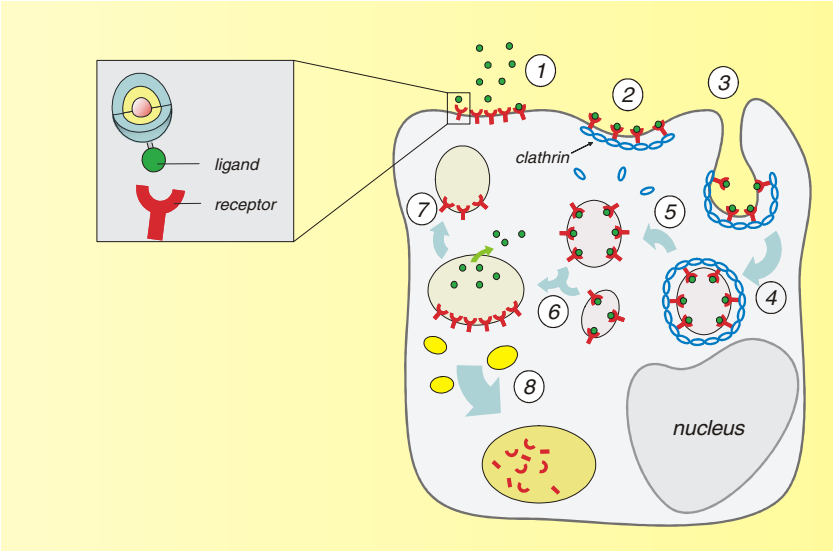

Fig. 4. Clathrin-mediated endocytosis. (1) Ligand binds to a specific cell surface receptor. (2) Invagination of cell membrane, clustering of the ligand–receptor complexes and (3) formation of clathrin-coated pit. After pinching off of the cell membrane the ligand–receptor complexes are sequestered in clathrin-coated vesicles (4). Clathrin depolymerizes and proton influx acidifies the early endosomes (pH ~ 6) (5). Several early endosomes can fuse to build late endosomes (pH ~ 5–6) from which the receptors can be recycled after release of the ligand (7) or fuse with lysosomes (pH ~ 5–5.5) (8) leading to degradation. Adopted from [6].

domain and VP22 protein from herpes simplex virus, can also serve as vector for protein delivery *in vivo* [95].

Morris et al. enhanced the possibilities of peptide transfection by engineering the peptide carrier Pep-1. This 21-residue peptide consists of two domains which are separated by a spacer. A tryptophan-rich domain allows for efficient cell membrane translocation and the hydrophilic lysine-rich domain, derived from the simian virus 40 (SV-40) T large antigen nuclear localization sequence (NLS), facilitates solubility and intracellular delivery [98].

Contemporary research deals with the question of whether PTD-mediated delivery, in contrast to CME, circumvents the endosomes [95]. This would enable more effective intracellular delivery. However, this form of uptake is non-specific, which may also be desirable if a collective of cells has to be targeted effectively, especially *in vitro*. The lack of selectivity, however, may be a limiting factor for drug targeting *in vivo*.

7. Non-specific particle uptake

Non-specific uptake of QDs, which is not mediated by certain targeting moieties, can be desirable for simply labeling a collective of cells, for example for *in vivo* applications in which cells are needed to be identified within an application. The following approaches demonstrate how the unique properties of QDs can be used for effective whole-cell labeling.

Phagocytosis, a form of non-specific uptake, was exploited for the investigation of cell motility and migration of epithelial cancer cells (phagokinetic tracks). After the deposition of small, non-PEGylated and silica-capped QDs on collagen-coated substrates and seeding of cells, QDs were internalized by phagocytosis [99]. The change in fluorescence intensity and thus the migration of the cells was observed by confocal microscopy. By the use of QDs problems of other previous approaches could be overcome. Conventional fluorophores suffered from photobleaching, while large, submicrometer gold particles would probably interfere with cell motility as more particles are ingested. The phagokinetic tracks are a powerful tool for the classification of cancer cells [65]. In another approach, phagocytosis of albumin-coated QDs by peritoneal mouse macrophages was used for immune cell tracing. The QDs were rapidly incorporated by the macrophages and then applied to the peritoneum of mice. After hapten injection, accumulation of the QDs on the inflammation site could be observed after tissue resection, detected by a handheld UV-lamp [100]. QDs emitting light in the NIR were able to label sentinel lymph nodes for imaging during resection. After intradermal injection, non-specific uptake by the RES was exploited for mapping and subsequent resection of sentinel lymph nodes guided by *in vivo* imaging [32].

The process of non-specific uptake was further investigated by Osaki et al., who proved that endocytosis is also an extremely size-dependent process. They investigated endocytosis by means of amphiphilic sugar-coated QDs (15 nm, determined by dynamic light scattering – DLS). Based upon fluorescence microscopy, they suggest an optimum of particle incorporation at ~50 nm (shown for an artificial 'glycovirus' consisting of plasmid DNA and the amphiphilic sugar), while the smaller sugar-coated QDs and larger aggregates of the glycovirus (>100 nm) were taken up at a smaller degree [8]. However, this influence

of the size effects on endocytosis has to be further investigated for more detailed information. Non-specific uptake of QDs can also be triggered by translocation peptides [12,101–104], cationic liposomes [51,68] and dendrimers [12], or enhanced physically by electroporation [13]. These methods, together with microinjection [47], are often used for intracellular delivery and subsequent organelle tracking (this will be addressed later).

8. Reduction of non-specific binding and uptake

In applications where specific cell-particle interactions have to be investigated, non-specific interactions have to be reduced. Otherwise, targeting of specific cells cannot be achieved, as every cell, even the non-targeted ones, would be labelled. A solution can be to mask the surfaces of the QDs with PEG (stealth effect) [3,4], which significantly reduces non-specific particle uptake, e.g. by the RES [3].

To this end, Chang et al. showed that surface modification with PEG remarkably reduced non-specific QD uptake by the human breast cancer cell line SK-BR-3 [105]. To this end, carboxylate-terminated QDs were used without modification or coupled to PEGs of different molecular weights (750–6000 Da). Photoluminescence measurements after incubation with the differently modified QDs and after washing of the cells revealed significantly reduced non-specific uptake of PEG-coated particles as compared to the QDs without PEG (approximately by a factor of 10 after 2 h of incubation). Quite interestingly, an increase in the molecular weight of the PEG only slightly reduced the uptake. Non-specific uptake was also shown to increase at prolonged incubation times and higher concentrations, despite the PEG coating of the QDs.

Similar effects were observed in another study if non-specific binding of QDs to cells [106] when QDs of different degrees of PEGylation were used. Here, non-specific binding studies using different cell lines revealed that this effect is also strongly dependent upon the cell type. Non-specific binding to HEK cells was the highest, while 3T3 cells only slightly interacted with QDs. In addition, effective reduction of non-specific binding can also be achieved by a partial coating of the QDs. Here, reduction of PEG bound to the carboxyl-modified QDs to one-third still sufficiently reduced non-specific binding. This partial PEGylation provides additional binding sites for biomolecules on the surface of the QDs. Ballou et al. demonstrated that the circulating half-lives of QDs in mice can be considerably improved by PEGylation and increase with longer molecular weights from 5 minutes for non-PEGylated QDs up to 71 minutes for QDs modified with 5 kDa PEG [17]. However, phagocytosis and subsequent particle removal by the RES still remains a problem [107,108].

Gao et al. investigated passive targeting of tumor tissue by PEGylated polymer-coated QDs *in vivo* exploiting the enhanced permeability and retention (EPR) of tumors [67]. Since the leaky tumor vasculature is permeable for macromolecules and tumors lack an effective lymphatic drainage system, macromolecule or nanoparticle accumulation in these tissues is enhanced [109]. The QDs were injected into mice implanted with prostate cancer cells. After this, the distribution of the QDs was observed by *in vivo* fluorescence imaging. Passive targeting was observed 6 h after injection of 6.0 nmol of the QDs. However, when compared to active tumor targeting, which was also achieved in this study after the QDs were linked to an antibody against PSMA (a prostate cancer marker) and applied in a dose of 0.4 nmol, passive targeting showed a lower accumulation in the tumor. Additionally, the accumulation, mediated by the antibody, was significantly faster (2 h). These findings show that active targeting of specific cells and tissues, here using an antibody, leads to more effective particle delivery to the target.

9. Specific cell-particle interactions

9.1. Cell surface antigens

In first approaches QDs were applied as fluorescent label of specific cells for analytical purposes. Many of them made use of antibodies [20,24,55,67,110], favored because of the fact that antibodies against numerous cell-surface proteins are commercially available. In this technique, a primary antibody specific for a cell-specific protein is usually applied. Subsequently, this antibody is bound by a secondary antibody labelled with a QD. The advantage of this method is that it is quite flexible, since the unit consisting of QDs and secondary antibodies can be used to bind to several primary antibodies.

However, it is obvious that large complexes consisting of primary and secondary antibodies and QDs can be omitted from uptake pathways that exclude larger particles. Therefore, antibodies are more suitable for diagnostics and in labeling.

Wu et al. used QDs conjugated to antibodies to label a cell surface cancer marker, the epidermal growth factor receptor Her2, on SK-BR-3 cells. The biotinylated secondary antibodies were conjugated to streptavidin-tagged polyacrylic acid-coated QDs. It was also possible to label intracellular structures, actin and microtubules, but this required fixation of the cells since the QD-antibody conjugates are not taken up into living cells [111].

An alternative to antibodies is presented by Howarth et al. They genetically attached an acceptor peptide to AMPA receptors (glutamate activated ion channels) of neurons [112]. Furthermore, *Escherichia coli* biotin ligase was added to perform biotinylation of the acceptor peptide on the receptor. In a last step, the modified receptor was labelled with streptavidin-coated QDs. This allowed for monitoring of the trafficking of the QD-tagged receptors in the neurons by fluorescence microscopy. The advantage of this labeling strategy is that customized chemistry for each ligand and antibody is not required.

9.2. The transferrin receptor

Serum-transferrin (Tf), an 80-kDa glycoprotein, plays a crucial role in the maintenance of iron in the organism since it carries Fe^{3+}-ions from the sites of intake, the mucosa cells, into the systemic circulation to the cells and tissues. The transferrin receptor (TfR), a homodimeric membrane receptor of 90 kDa per subunit, is internalized after Tf binding, followed by endosomal release of iron and recycling of the Tf-TfR complex. As the TfR is highly expressed in rapidly dividing cells like tumor cells, it provides a target for specific particle delivery [82].

Several early attempts to prove specific uptake were made with transferrin-conjugated QDs [10,113–115]. In an early report, Chan and Nie demonstrated the use of QDs for bioimaging by coupling mercaptoacetic acid-coated QDs to Tf using EDC [10]. Fluorescence microscopy indicated specific uptake of Tf-QDs by HeLa cells, compared to non-conjugated QDs. The colloid was further improved by cross-linking the mercapto acid coating with lysine yielding higher stability in a large pH-range (regarding the quantum yields) and high salt concentrations (regarding the monodispersity of particle size distribution). The monodispersity of the particles has been nicely shown using the phenomenon of blinking, which is characteristic for single QDs. Bioconjugation to Tf and subsequent uptake was achieved as described earlier [113]. Two other approaches further confirmed Tf-mediated endocytosis of QDs by atomic force microscopy (AFM) and two-photon excitation CLSM [114,115].

9.3. Tyrosine kinases

In the family of transmembrane receptor tyrosine kinases (TRKs), growth factor receptors, especially the epidermal growth factor receptors (EGFRs), are of special interest concerning cancer targeting, since they are implicated in the development and progression of many human solid tumors [116]. There are numerous approaches investigating interactions of QDs with EGFRs [11,115,117,118]. Lidke et al. applied streptavidin-coated QDs for cellular uptake studies at the erbB1 receptor, one of four homologous receptor subtypes [11]. The receptor was expressed in CHO cells together with green fluorescent protein (GFP) as fusion protein. After binding of biotinylated EGF to the receptor, the streptavidin–QDs were added and accumulated at the cell membrane due to the strong streptavidin–biotin binding as observed by confocal microscopy. The ligand–receptor complex quickly internalized at 37 °C, while at 4 °C endocytosis could be blocked (Fig. 5). After endocytosis, which was furthermore demonstrated to be clathrin-dependent by colocalization of transferrin-Alexa 633, fusion of the QD-containing vesicles could be observed. Moreover, the erbB1 was shown to be active after binding of EGF-linked QDs by staining with Cy5-anti-activated erbB1. These results indicate that the small QDs did not impair the biological function of the attached growth factor and the receptor trafficking. Incubation of the EGF–QDs with A431 cells, which express the erbB1 constitutively, unveiled a rapid movement of the ligand–receptor complexes along filopodia, thin extensions of the cell body, by a retrograde transport to the cell body.

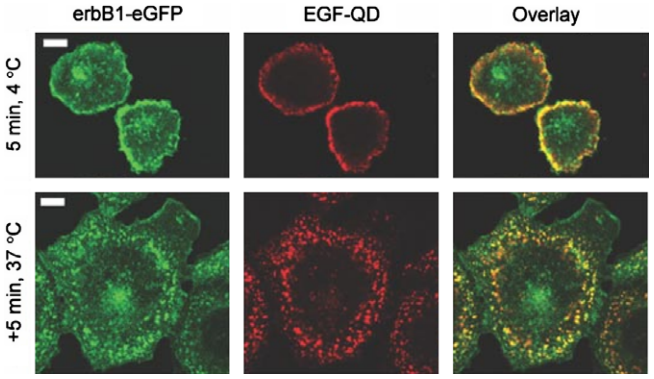

Fig. 5. Binding of EGF-QDs to EGFRs and endocytosis at erB1-eGFP expressing CHO cells. The EGFRs are marked by GFP, QDs show red fluorescence. Upper panels: after addition of biotin–EGF to the cells, QDs bind to the EGFRs and show colocalization with the receptor on the plasma membrane. Endocytosis is inhibited at 4 °C. Lower panels: at 37 °C endocytosis takes place. The ligand–receptor complexes are visible inside the cell in endocytic vesicles. Scale bars: 20 μm (Reprinted from [11] with permission from Macmillan Publishers Ltd: Nature Biotechnology, © 2004, www.nature.com/nbt/). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Similarly, the nerve growth factor receptor TrkA was targeted by QDs conjugated to anti-TrkA as well as by QDs attached to nerve growth factor (NGF), the naturally occurring ligand [119]. After binding of the QD-bioconjugates to the receptor on PC12 nerve cells, rapid internalization was observed by confocal microscopy. The shuttling of the endosomal QD–receptor complexes inside the cells was shown to be associated with microtubules. After addition of nocodazole, a microtubule inhibitor, the transport was suppressed.

9.4. Integrins

Integrins are a family of α,β-heterodimeric receptors that mediate dynamic linkages between extracellular adhesion molecules and the intracellular actin cytoskeleton [120]. Several integrins play an important role in angiogenesis and metastasis and are therefore upregulated in numerous tumor cell types. Integrins bind to the arginine–glycine–aspartic acid (RGD) peptide sequence present in many extracellular matrix proteins. This sequence binds, for example, to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins [121], which are overexpressed at the vasculature of tumors [122]. Therefore, these integrins are selective targets for cancer targeting. QDs attached to antibodies specific for the α_v subunit as well as QDs bound to RGD were able to bind to the nerve cell line SK-N-SH [110]. NIR-emitting,

PEG-coated QDs connected to cyclic RGD were targeted to integrin-positive cells *in vivo* [108]. At increasing receptor expression levels, depending on the respective tumor cell lines, staining of the cells became more visible, while the effect could be blocked by excess free RGD and did not occur when the blank QDs were applied. In the same study, these RGD-decorated QDs were also able to stain frozen tumor tissue *in vitro*, in contrast to QDs without RGD. Application to tumor bearing mice resulted in staining of the tumor vasculature, visualized by *in vivo* imaging (Fig. 6), but also in non-selective accumulation in the RES, mainly in liver, lymph nodes and bone marrow, though the particles were PEG-coated. This approach shows that *in vivo* targeting with QDs is feasible in principle, but the selectivity of targeted colloids still warrants improvement.

Another approach proved specific uptake of QDs coated with phospholipids containing the paramagnetic and MRI-traceable element gadolinium [36]. Therefore, the colloid was not only fluorescent, due to the QD, but also detectable by MRI. Thiolated RGD was connected to the colloid via maleimide linkage. The maleimide function was introduced by partial incorporation of maleimide-derivatized PEG-phospholipids into the coating. After incubation, HUVEC cells ($\alpha_v\beta_3$ positive) showed increased uptake of the QDs compared to control (bare QDs without RGD), visualized by fluorescence microscopy. However, non-specific uptake of the bare QDs was also observed.

9.5. The folate receptor

The folate receptor (FR) represents an adequate target for intracellular nanoparticle delivery since it is overexpressed in many cancer cell lines [123] due to the increased need of cancer cells for folic acid as this coenzyme is essential for the synthesis of amino acids as well as nucleic acids. Since folic acid can be easily connected to functionalized nanoparticles by carbodiimide/NHS chemistry and because of its ready availability, several approaches have been pursued to exploit FRs for drug targeting [124,125]. After preliminary results presented by Chan et al. [126], Bharali et al. showed uptake of QDs by KB cells, a FR positive epithelial cancer cell line, when mercaptoacetic acid-coated InP–ZnS QDs decorated by folic acid were applied. The QD uptake was proven by confocal microscopy. However their control-experiments showed non-specific particle uptake by FR-negative cells (A549 cells), which reduces the selectivity of the uptake of the QDs by FRs [69]. This problem of non-specific particle uptake is already known and can be reduced by introducing PEG into the coating of the QDs, as mentioned before [105,106]. Hence, further experiments are necessary to ensure specific uptake after optimization of the QD surface.

9.6. Phage displayed peptides

Screening of phage displayed peptide libraries yielded a number of peptides that are selective for specific tumor tissues and have great potential for the development of novel cancer-targeted drugs and could replace bulky antibodies in the future [127,128]. With this technique, genetic engineering is used to insert randomized oligonucleotide fragments into the phage DNA. The oligonucleotides are then expressed as peptides on the surface of the phage. The resulting phage displayed peptide libraries are subsequently screened for the binding affinity of the peptides to certain receptors (*biopanning*).

Akerman et al. applied three pre-scanned peptides from phage display, each connected to QDs [53]. These isolated peptides had already been proven to bind to specific sites in mice *in vivo* and were homing to membrane dipeptidase on endothelial cells in lung blood vessels, tumor blood vessels or lymphatic vessels and tumor cells, respectively [127]. After modification with iminothiolane to introduce a thiol group into the peptides, the compounds were bound to mercaptoacetic acid-coated QDs via thiol exchange. Thiolated PEG was also attached for passivation of the QDs. Following injection into mice, the QDs were found in the respective tissues using fluorescent microscopy depending on the peptide attached. Tissue samples of liver and spleen revealed reduced uptake of PEGylated QDs by the RES compared to non-PEGylated ones.

In another approach, a peptide identified using phage display was used to target streptavidin-QDs, to a lung carcinoma cell line. The colloid was internalized by the cells, whereas QDs attached to a tetrameric control peptide

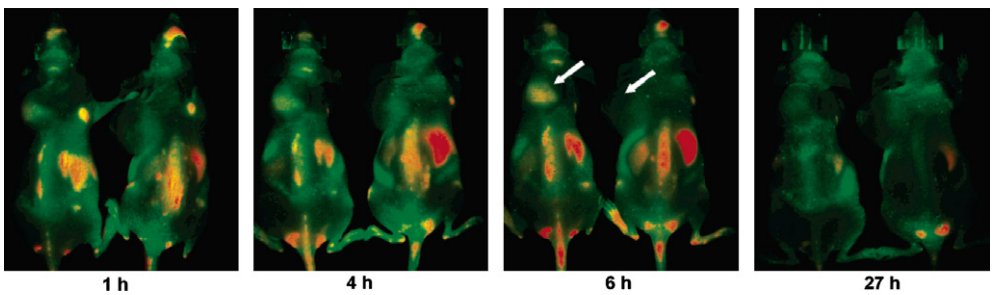

Fig. 6. *In vivo* imaging of tumor bearing mice (left shoulder, arrows) after injection of RGD–QD (left sides) and QDs without RGD (right sides). After 6h, localization of RGD–QDs in the tumor is visible. Strong fluorescence in liver bone marrow and lymph nodes indicates uptake by the RES (Reprinted with permission from [108]. © 2006 American Chemical Society.)

remained extracellular [129]. However, future studies have to prove the selectivity of the peptide for tumor cells only. Similar results were obtained by Kim et al. for another peptide [130]. They showed QD uptake and also transport of the QD-peptide–receptor complex into the endoplasmatic reticulum which was fluorescently labeled with glibenclamide.

9.7. Other targets

The largest cell surface receptor family consist of the G-protein coupled receptors (GPCRs) [131]. These receptors modulate various kinds of physiological processes and are involved in a number of diseases. Therefore, GPCRs represent the largest class of therapeutic targets [132]. As the localization of many of these receptors has been investigated intensively and as numerous ligands for these receptors are known, they might also be interesting for drug targeting. After binding of a ligand, which can be a biogenic amine or a peptide, the ligand–receptor complexes are usually internalized, mediated by clathrin. Therefore, this mechanism should also provide a gateway for selective uptake of particles tagged to ligands selective for certain GPCRs.

An early approach using ligand-conjugated QDs was presented by Rosenthal et al. [133]. In this publication, serotonin molecules were covalently bound to TOPO-coated CdSe QDs, referred to as serotonin-labeled CdSe nanocrystals (SNACs). The biogenic amine serotonin is recognized by serotonin transporter proteins (SERTs) which are responsible for the termination of neurotransmitter signal by clearing these molecules from extracellular spaces after release. The SNACs were able to label cell surface SERTs in transfected HEK cells but with reduced binding affinity, compared to free serotonin. However, electrophysiological experiments on *Xenopus* oocytes expressing the human serotonin receptor $5HT_3$ suggest that the steric hindrance of the relatively large QDs can impair the biological activity of the small neurotransmitter. This point is subject to further improvements for specific substances binding to serotonin and dopamine transporters [134,135].

In a later approach by Mason et al. it was possible to label cell surface GPCRs, in this case for a peptidic ligand [136] offering a more favorable size ratio between the QDs and the ligand. Mercaptoacetic acid-coated QDs were covalently EDC-coupled with PEG and angiotensin II (ANG II), an octapeptide binding to angiotensin receptors (AT). These QD bioconjugates can bind to AT 1 receptors expressed in transfected CHO cells. Binding did not take place either when excess ANG II was applied or when AT 1-negative CHO cells were incubated. Similar results were presented by Young and Rozengurt for streptavidin-functionalized QDs conjugated to biotinylated ANG II. Agonist-induced internalization was observed with fluorescence microscopy which could be inhibited at 4 °C, indicating uptake via endocytosis [137].

10. Intracellular delivery

As mentioned above, many targets of therapeutical relevance are located inside the cell, like the nucleus or the mitochondria, which requires intracellular delivery of nanoparticles [138]. Recent studies meet this requirement by the investigation of QD-based intracellular targeting strategies.

The nucleus, for example, plays a dominant role in gene therapy, since most of the genetic information of a cell is located there. In principle, nanoparticles are able to enter the nucleus by diffusion through the nuclear pores. Since these pores are in most cases too small for most of the particles (up to 26 nm) [139], active transport via nuclear localization sequences (NLS) seems more promising for targeting this organelle. Similarly, mitochondria can be addressed with mitochondrial localization sequences (MLS). These organelles play an important role in a number of pathophysiological dysfunctions, like neurodegenerative and cardiovascular diseases and cancer [140,141]. This fact renders mitochondria interesting targets for drug therapy.

Before QDs are able to find intracellular targets, the particles first have to enter the cell. Therefore, the complex problem of intracellular delivery has been divided into two parts. The first one, as described above, deals with the cellular uptake of colloids. CME is promising, due to its selective uptake of colloids, but also poses the problem that the particles remain sequestered in endosomal vesicles, preventing intracellular tracking. Therefore, to study the intracellular targeting processes, other delivery methods are used, usually non-specific ones, for direct delivery of the particles into the cytosol. A next step would be to combine specific uptake with intracellular delivery when both of these processes are better understood and when the colloids are further developed. The following approaches embark on this strategy in which the QDs are delivered into the cytosol. Subsequently, the QDs are able to first address the respective organelles.

In order to facilitate further studies of intracellular targeting of organelles with QDs, Derfus et al. investigated several methods to find an appropriate method to deliver PEG-coated QDs into the cytosol of cells [12]. Quantification of cellular uptake using HeLa cells, determined by flow cytometry, revealed that cationic liposomes (Lipofectamine™) yielded the highest delivery efficiency, followed by dendrimers (Superfect™) and translocation peptides (Chariot™). To prove whether green-light-emitting QDs remain trapped in vesicles, which would disturb later organelle tracking, a second fraction of red-light-emitting QDs coupled to EGF was simultaneously incubated and analyzed by fluorescence microscopy. Since EGF–QDs were shown to enter cells via CME, they were used as an endosome marker. The approach showed only minor colocalization of the both QD-fractions in vesicles after Lipofectamine-delivery, indicating that QDs can escape the endosomes (Fig. 7a). However, in this delivery method particles formed aggregates of several hundred nanometers.

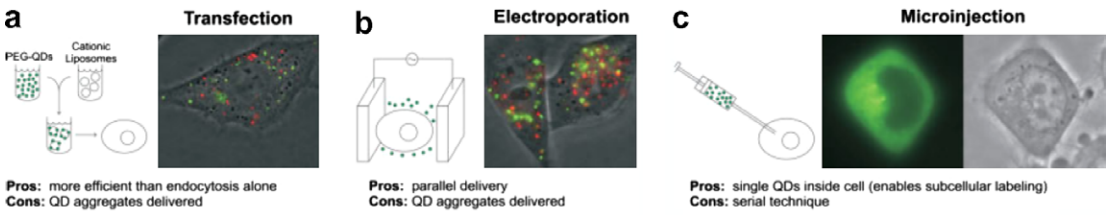

Fig. 7. Depiction of several delivery methods. Transfection via cationic liposomes (a) and electroporation (b). Both methods allow for parallel delivery in the cells simultaneously but result in aggregation of the QDs (green spots). Microinjection into single cells (c) yields in an aggregate-free dispersion of the QDs inside the cell (Reprinted with permission from [12]. © 2004 Wiley-VCH Verlag GmbH & Co. KGaA.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Electroporation showed similar effects (Fig. 7b); only microinjection prevented aggregation (Fig. 7c). Aggregation of the particles in the cytosol has to be avoided since larger aggregates would prevent trafficking to the nucleus or mitochondria. Additionally, nuclear localization sequences (NLS) – the SV40 T antigen – and mitochondrial localization sequences (MLS), consisting of the targeting presequence from human cytochrome oxidase subunit VIII (COX8), were adsorbed on the QD surface. These sequences are small peptides specific for transporter proteins at the respective organelle and should prove the feasibility of labeling these organelles. After microinjection of the NLS- and MLS-conjugated QDs into the cytosol, the particles were delivered into the nuclei and the mitochondria, respectively. However, control experiments with random peptides, necessary to attribute this intracellular delivery solely to the activity of the localization sequences, are missing from this study.

Similar results were obtained by Chen et al. for silica-coated QDs connected to SV40 T antigen via streptavidin–biotin linkage [13]. Here, QDs were delivered into the cytosol of HeLa cells via electroporation. Subsequently, QDs were delivered into the nucleus, mediated by the NLS. Controls with random peptides were not localized inside the nucleus, which indicates that the uptake was dependent upon the NLS. Silica QDs were chosen as they are fairly non-cytotoxic and small enough (10–15 nm) to pass through the nuclear pores. A colonigenic assay revealed no significant change of the surviving cell colony numbers after transfection compared to control. After 5 days and cell division, QDs were visible in the nuclei of the newly divided cells by fluorescence microscopy.

The peptide carrier Pep-1 was successfully connected to QDs in several studies and proved its potential for cellular delivery of QDs. Rozenhak et al. delivered streptavidin-coated QDs non-covalently linked to Pep-1 into mammalian cells [104]. The QD-Pep-1 complexes were visible in the cytoplasm as aggregates. Besides nuclear targeting, which was achieved via additional attachment of biotinylated SV-40 T-antigen, mitochondrial targeting was also possible. For this purpose, the GH3 domain of the Grim protein, an inducer of mitochondrial apoptosis, was coupled to the QDs before Pep-1 was attached. After incubation with cells, membrane blebbing and nuclear condensation was observed as a consequence of apoptosis and an indirect sign of mitochondrial targeting.

In another study, an oligoarginine CPP based on the HIV-1 TAT-protein and containing a terminal polyhistidine tag was applied. By this sequence the peptide could be attached to QDs capped with dihydrolipoic acid (DHLA) via metal-affinity interactions between the polyhistidine and the Zn atoms on the QD surface [142]. The colloid was able to be internalized into HEK293T/17 and COS-1 cells in a significantly higher manner than a control group without CPP. After uptake, colocalization with Alexa-labeled transferrin, an endocytic marker, showed localization of the QD-peptide conjugates in endosomes. Since uptake could be inhibited at 4 °C, endocytic uptake was suggested, though the exact mechanism was not further characterized.

The TAT-protein coupled to QDs, coated with tiopronin (*N*-2-mercaptopropionylglycine) by EDC, mediated uptake into fibroblast and even nuclear targeting. However, no quantitative investigation was conducted [143]. TAT could also mediate brain targeting of QDs after injection into living mice [144]. The QDs were visible in the brain after its resection by the use of a handheld UV-lamp.

11. Conclusion and outlook

Nanoparticles as colloidal drug carriers seem to be promising tools for targeting specific cells, tissues or organs. These drug carriers have not only to head for specific cell types but should also address distinct cellular uptake processes to deliver their payload *into* cells. In order to investigate the pathways for nanoparticles into specific cells, model colloids of defined size and shape, which in addition have to be easy to detect, are a powerful tool.

As presented above, the use of QDs for the investigation of cellular uptake and intracellular delivery of nanoparticles offers new possibilities in order to study sub-cellular processes, due to the small, uniform size and the unique optical properties of the QDs. The approaches demonstrated that the pathway into the cell should be considered carefully since it also determines the intracellular fate of the colloid. CME was shown effective for targeting and uptake into cells, but the endosomal release of the colloid after uptake can be a limiting factor for intracellular targeting.

Other promising pathways are the caveolae-mediated uptake [145] and uptake mediated by CPPs, which circumvent the clathrin pathway. However, there is still a need for

more information on the distinct mechanisms of this uptake, as the examples of the SV40 virus (caveolae-dependent [80] and caveolae-independent [146]) and the TAT peptide (macropinocytosis [147] and caveolae [148]) show. A better understanding of such processes would obviously aid in the design of targeted nanoparticles for numerous applications.

Finally, new concepts can exploit specific pathophysiological situations, e.g. when an enzyme is upregulated, and activate particle delivery at the affected site on demand. A first promising approach was presented by Zhang et al. In this study, non-specific, transduction peptide-driven uptake of QDs was rendered cell-selective by linkage to a negatively charged, transduction-inhibiting peptide, which was additionally a substrate for matrix metalloproteases (MMPs). After cleavage of the negatively charged peptide by MMPs, the colloid could enter the target cells [149]. Since MMPs are upregulated in many tumors and in arthritic tissues, this approach should be selective for these targets. This example shows that the design of effective, cell-targeting colloids probably requires several targeting strategies to be combined on one particle. Exploiting differently expressed enzymes or other forms of regulation specific for certain (patho)physiological events inside cells to 'switch on' colloids on the intracellular level could be an issue for the future development of advanced colloids.

In the field of nanomedicine [150], QDs can make a worthy contribution to the development of new diagnostic and delivery systems as they offer unique optical properties for highly sensitive detection, are well defined in size and shape and can be modified with various targeting principles.

Acknowledgements

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