DE GRUYTER BioNanoMat 2016; 17(1-2): 33–41

Muharrem Seleci, Didem Ag Seleci, Rebecca Joncyzk, Frank Stahl*, Cornelia Blume and Thomas Scheper

Smart multifunctional nanoparticles in nanomedicine

DOI 10.1515/bnm-2015-0030
Received December 31, 2015; accepted April 8, 2016; previously published online April 28, 2016

Abstract: Recent advances in nanotechnology caused a growing interest using nanomaterials in medicine to solve a number of issues associated with therapeutic agents. The fabricated nanomaterials with unique physical and chemical properties have been investigated for both diagnostic and therapeutic applications. Therapeutic agents have been combined with the nanoparticles to minimize systemic toxicity, increase their solubility, prolong the circulation half-life, reduce their immunogenicity and improve their distribution. Multifunctional nanoparticles have shown great promise in targeted imaging and therapy. In this review, we summarized the physical parameters of nanoparticles for construction of "smart" multifunctional nanoparticles and their various surface engineering strategies. Outlook and questions for the further researches were discussed.

Keywords: drug delivery system; medical applications; nanomaterials; nanomedicine; nanoparticles; targeting therapy.

Introduction

Nanotechnology and nanobiotechnology have gained much momentum in recent years. The term nanotechnology mostly refers to the fabrication of new materials with at least one dimension in a size range between 1 and 100 nanometres (nm), that is one billionth (or 10^{-9}) of a meter. It is already used in a variety of products across various industries such as agriculture, cosmetics, electronics, textiles, recycling, energy, chemicals, as well as healthcare. Nanobiotechnology is defined by science's growing ability to work at the molecular level, atom by atom, combining biological materials and the rules of physics, chemistry, and genetics to fabricate synthetic structures such as biosensors, nanosized microchips and even tissue analogs for growing skin, bones, muscle, and other organs of the body [1, 2]. The development of a wide range of nanoscale technologies currently enables new scientific approaches in disease diagnosis, treatment, monitoring and prevention. These technological innovations of nanotechnology within medicine are referred to as "nanomedicine" by the National Institutes of Health in USA [3].

Nanoparticles with ~100 nm have been widely used to improve the drug accumulation, internalization and therapeutic efficacy. As shown in Figure 1, the physicochemical and biological properties of the nanoparticles can also be finely adjusted by tailoring their chemical properties, sizes, shapes, structures, morphologies, and surface properties [4]. The conjugation chemistry strategy of the drug molecule and surface modification is very important for drug delivery. Drug molecules may be adsorbed or attached covalently to the surface of nanocarrier. Besides, the interior core can also entrap drug molecules. The surface coating of nanocarriers with molecules, for instance, hydrophilic and/or hydrophobic polymers [e.g. polyethylene glycol (PEG) and poly(ε-caprolactone) (PCL)] or surface modifications with targeting ligands such as antibodies, aptamers, peptides or small molecules determine the mechanism of uptake for the nanocarriers by the cells. Nanoparticles can be modified by active and passive targeting to enhance the concentration of the drug molecule inside the specific area. Once the drug loaded nanocarriers reach the diseased tissue, the therapeutic agent is released through changes in physiological environment such as temperature, pH, osmolarity, or via an enzymatic activity [5].

Delivering of therapeutic compound to the target site is a major problem in the treatment of many diseases. A conventional application of drugs may have limited effectiveness, poor biodistribution and lack of selectivity [6]. It has been established that nanocarriers can become concentrated preferentially in tumors or at inflammatory

***Corresponding author: Frank Stahl,** Institute of Technical Chemistry, Leibniz University of Hannover, 30167 Hannover, Germany, Phone: +(49) 511 762 2968,
E-mail: stahl@iftc.uni-hannover.de
Muharrem Seleci, Didem Ag Seleci, Rebecca Joncyzk, Cornelia Blume and Thomas Scheper: Institute of Technical Chemistry, Leibniz University of Hannover, 30167 Hannover, Germany

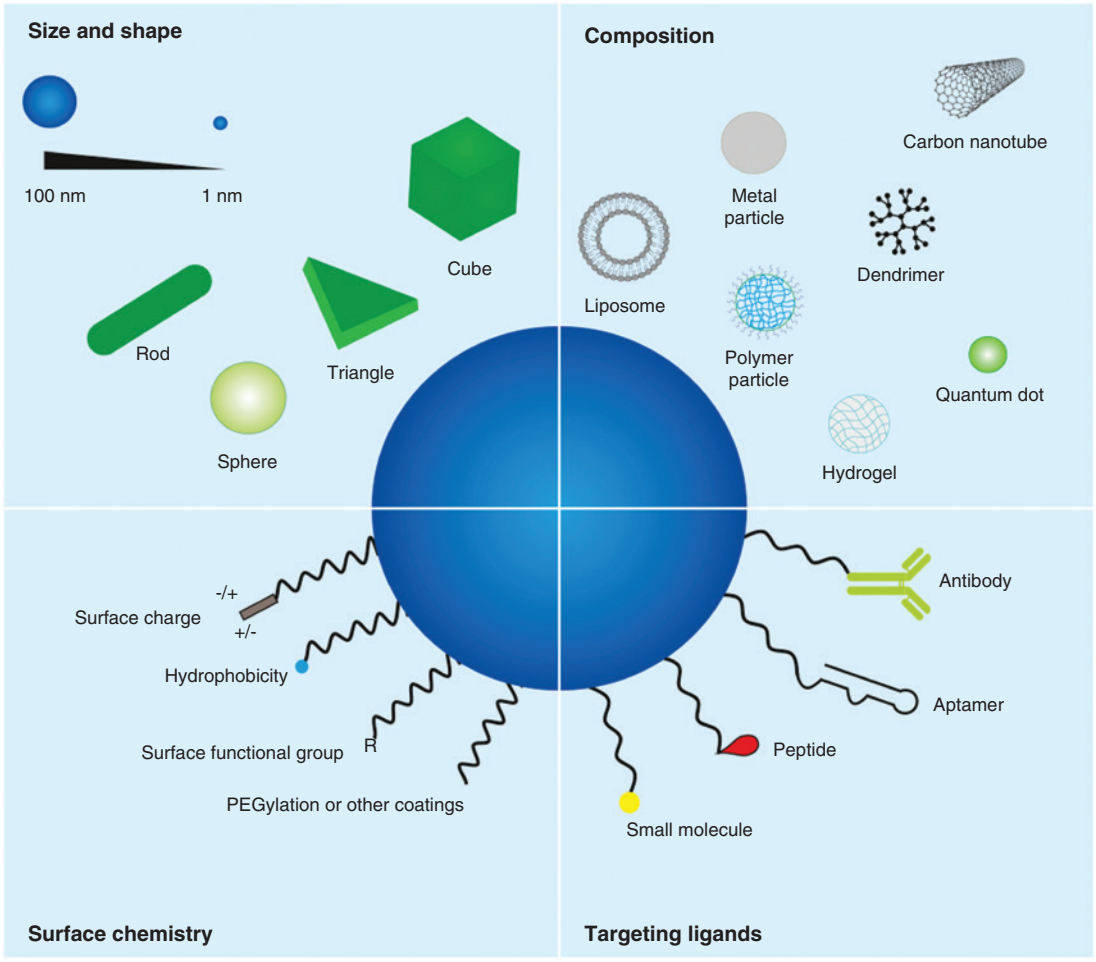

Figure 1: Design of nanoparticles for drug delivery.
Multifunctional nanoparticles can be generated from the different materials composition with different properties and functionalities. Various strategies are used to combine therapeutic agents and imaging probes with the particles.

sites, and this is due to antigen sampling by virtue of the enhanced permeability and retention (EPR) effect of the vasculature [7]. The current treatment options for most solid tumors are surgical intervention combined with chemotherapy or radiation therapy. However, today's therapy in its very general and systemic application form damages healthy tissues and causes unwarranted toxicity to the patient [8, 9]. In recent years, nanoparticle based delivery systems have been exploited in various medical applications. Their attractive features are that they are made from biocompatible, well-characterized and easily functionalized materials [10]. On the basis of these materials' properties, nanomaterials exhibit a highly differential targeting and uptake efficiency in a cell- or tissue-specific manner. Furthermore, the drug molecule on the nanocarrier is protected from harsh conditions before it can reach the target. In contrast to conventional drug delivery, using nanomaterials a prolonged and controlled drug release can be achieved [11, 12]. Thus, nanomedicine represents an innovative field with enormous potential for treatment by combination of smart nanoparticles with small molecules carrying a wide range of functions.

Liposomes, polymeric nanoparticles, dendrimers, metal nanoparticles, and quantum dots are versatile molecules with a variety of biomedical uses, such as diagnostic assays [13, 14], radiotherapy enhancement [15, 16], as well as drug and gene delivery [17, 18]. Liposomes are artificially prepared vesicles composed of lipid bilayers. They are biocompatible and their size can be varied in broad ranges (50–500 nm). Both hydrophobic and hydrophilic drugs can be encapsulated in the hydrophobic lipid bilayer and hydrophilic aqueous core, respectively [19]. An alternative approach to liposome is the use of niosomes which are composed mainly of non-ionic surfactants [20]. Polymeric nanoparticles made of natural polymers (e.g. chitosan, collagen) and synthetic polymers

[e.g. poly(lactide-coglycolide) (PLGA) and (PCL)] are colloidal solid platforms for controlled and sustained release of drug molecules and gene delivery. Natural polymers are less toxic and more biodegradable than synthetic polymers. The majority of these compounds are synthesized through a spontaneous self-assembly process using block polymers of two or more polymeric chains with different hydrophilicity [21]. Dendrimers are synthesized from branched monomers by stepwise a repetitive reaction sequence and it is possible to control of their structural and chemical properties, including size, shape and number of branches. Poly(amidoamine) (PAMAM) and poly-L-lysine are largely used dendrimers in drug and gene delivery. Low polydispersity and biocompatibility are the main problems using these nanoparticles as a tool in nanomedicine [22]. Semiconductor quantum dots are utilized as fluorescent probes for imaging and labeling of the molecules due to their unique photophysical characteristics such as broad excitation and tunable emission. Nevertheless, the main components of the quantum dots are hazardous heavy metals such as cadmium and selenium, which are highly toxic in living organisms. The heavy metal core can be coated with shell and a further surface coating can be carried out to make quantum dot more biocompatible [23]. Researchers are interested to study tracking of these nanoparticles and to investigate their physical and biological properties for their cellular uptake and delivery.

Physical properties and applications of nanoparticle based drug carriers

Size and shape

The physicochemical properties of nanoparticles, size, shape, and surface chemistry play a critical role in determining tissue penetration, cellular delivery, and therapeutic efficacy. Nanoparticles with varying size and shape are taken up to the cells at different rates. Cell membrane is composed of lipids, carbohydrates and proteins that mediate cellular functions. The nanoparticle surface can interact with these molecules and may activate the cell's uptake mechanisms. Nanoparticle cell interactions can be remarkably different from that of small or large sized particles at nanoscale [24]. As nanoparticles with a smaller size have larger surface areas available to adhere and interact with cell membranes, the decrease of nanoparticle size may lead to an increase in mobility and interaction with cell membranes, which can result in enhanced cellular uptake of nanoparticles [25]. Besides, because of their small size, nanoparticles are often not recognized as a foreign agent by macrophages and consequently do not enter macrophages through membrane pores to be led to the digestion apparatus for such microparticles, the reticuloendothelial system [26]. Donkor and Tang showed that, the cellular and nuclear internalization of 30 nm sized single-walled carbon nanotubes (SWCNT) was higher than 50 nm SWCNT [27]. In a subsequent study, Jiang et al. reported that relatively small sizes of 2, 4 and 6 nm core gold nanoparticles (AuNP) were sufficient to induce dramatic changes in nanoparticle internalization efficiency and mechanism [28].

The shape of nanoparticles also plays a direct role for their function in biological systems [29]. Li and coworkers investigated the influence of nanoparticle shape on the cellular uptake. To clarify its on cellular uptake of the nanoparticles, sphere, rod, cube, and disk shaped polymer-coated nanoparticles were compared. Based on a detailed free energy analysis, the effect of the nanoparticle shape was found to be mainly induced by the different membrane bending energies during endocytosis. The spherical nanoparticles needed to overcome a minimal membrane bending energy barrier, compared with non-spherical particles. The spherical nanoparticles thus demonstrated the fastest internalization rate, followed by cubic-, then rod- and disk-like nanoparticles [30].

Even though nanoparticles show a certain size and shape after synthesis, they might induce aggregation into larger clumps during the in vitro and in vivo applications. The formation of these aggregates complicates the interpretation of the results [31]. Besides, although both size and shape are essential features, surface charge and functionality are also significant for the interaction of nanoparticles with the physiological system.

Surface chemistry

In addition to their size and shape, the surface chemistry of nanoparticles is an important factor for their interaction with the biological environment. Nanoparticles should ideally have a hydrophilic surface to escape macrophage capture [32]. Generally, that can be achieved by coating the surface of nanoparticles with a hydrophilic polymer such as PEG, which has favorable inherent physicochemical properties and is biocompatible (e.g. it possesses high flexibility, but a low toxicity and immunogenicity). For this purpose, nanoparticles can also be fabricated from block copolymers with hydrophilic and hydrophobic parts

[33, 34]. A surface modification of nanoparticles with PEG was found to reduce nanoparticle accumulation in off-target organs such as liver and spleen. PEG-coated nanoparticles have a high solubility in a number of solvents and exhibit a reduced adsorption of blood proteins, leading to prolonged circulation half-life compared to non-PEGylated nanoparticles [35–37]. In this way, it reduces the aggregation. PEG chains can be functionalized with alcohols, carboxylic acids amines and thiols to conjugate small molecules or targeting ligands. Yoon et al. demonstrated that tumor-targeting ability of hyaluronic acid nanoparticles (HANPs) could be optimized by chemical conjugation of amine-functionalized PEG, which might help escaping the unintended accumulation in liver [38].

The charge of nanoparticle's surface is commonly characterized using the zeta potential, which variably reflects the electrostatic potential of particles and is influenced by the composition of the particles as well as by the medium in which the nanoparticles are suspended [3]. Particles with a zeta potential more positive than +30 mV or more negative than –30 mV are normally considered to be physically stable [39, 40]. The negative membrane of cells interact differently with positively/negatively charged nanoparticles, whereby positively charged nanoparticles are generally known to be more easily internalized than neutral and negatively charged nanoparticles [41, 42]. Remarkably, some nanoparticles such as polymeric nanocomplexes and AuNP, which have the same size range, but different surface charges yielded marked discrepancies in both distribution and uptake efficiency. The particles also showed different levels of toxicity depending on their surface charges [43–46].

Targeting ligands

Nanoparticle surfaces are often bioconjugated with small molecules and/or targeting ligands to enable both in vitro and in vivo cell specific targeting [47]. Proteins and peptides, carbohydrates, vitamins, aptamers, antibodies, and antibody fragments are the mostly used molecules that bind specifically to an overexpressed target on the cell surface [48]. Immunogenicity, stability, and difficulties in site-specific conjugations with nanoparticles are the major obstacles in targeting ligands. Monoclonal antibodies (mAbs) are widely preferred targeting ligands because of their availability to research and their high affinity and specificity for molecular targets [49, 50]. Several mAbs have been used in clinics for cancer therapy, such as bevacizumab (against colorectal cancer) and trastuzumab (against breast cancer) [51]. However, they are large, complex molecules and may cause immunogenicity.

Aptamers are small synthetic nucleic acid oligomers that can bind to targets with high sensitivity and high specificity. Aptamers are selected through an in vitro process called systematic evolution of ligands by exponential enrichment (SELEX) to be most specific for a particular target [52]. They have potential advantages as targeting ligands. Aptamers can be synthesized with a specific functional moiety, such as a carboxylate, amino or sulfhydryl at only one end of the nucleic acid sequence of the aptamer. They are small in size and non-immunogenic [50, 53]. Peptides are an attractive alternative as targeting molecule due to several advantages, including a smaller size, a lower immunogenicity, a higher tissue penetration capability, a higher stability and a relatively easy production process. Phage-displayed peptide libraries are a valuable screening resource for identification of the peptides that target a specific receptor [54, 55]. The most widely used peptide in targeted delivery is the integrin targeted arginine-glycine-aspartic acid (RGD) peptide. This peptide has been extensively studied by combining various nanoparticles [56]. A promising approach for overcoming the barrier of the cell membrane in drug delivery are cell penetrating peptides (CPPs). Arginine rich CPPs such as transactivator of transcription (TAT), penetratin and polyarginine are often used for intracellular delivery of the cargo conjugates [57]. It has been hypothesized that the positively charged CPPs provide a strong electrostatic interaction with anionic species presented at the extracellular surface of cell membranes, including lipid head groups, proteins such as nucleolin, and proteoglycans like heparin sulfate [58, 59].

Passive and active targeting

Targeting of the nanoparticles is performed via two different strategies: passive and active targeting, as schematically shown in Figure 2. Nanoparticles can enhance the intracellular concentration of the drugs in cancer cells while avoiding unwarranted toxicity towards healthy cells. Malignant tumors release angiogenic growth factor proteins that stimulate new blood vessels or demand rerouting of existing vessels to supply them oxygen and nutrients [61, 62]. Tumor tissue is characterized by highly disorganized vascular architecture, irregular blood flow, reduced lymphatic drainage, and vessels are leaky [63, 64]. Because of the reduced lymphatic drainage, the permeating nanocarriers are not removed efficiently, and thus are retained in the tumor tissue [65]. These features provide an EPR effect, which constitutes an important mechanism

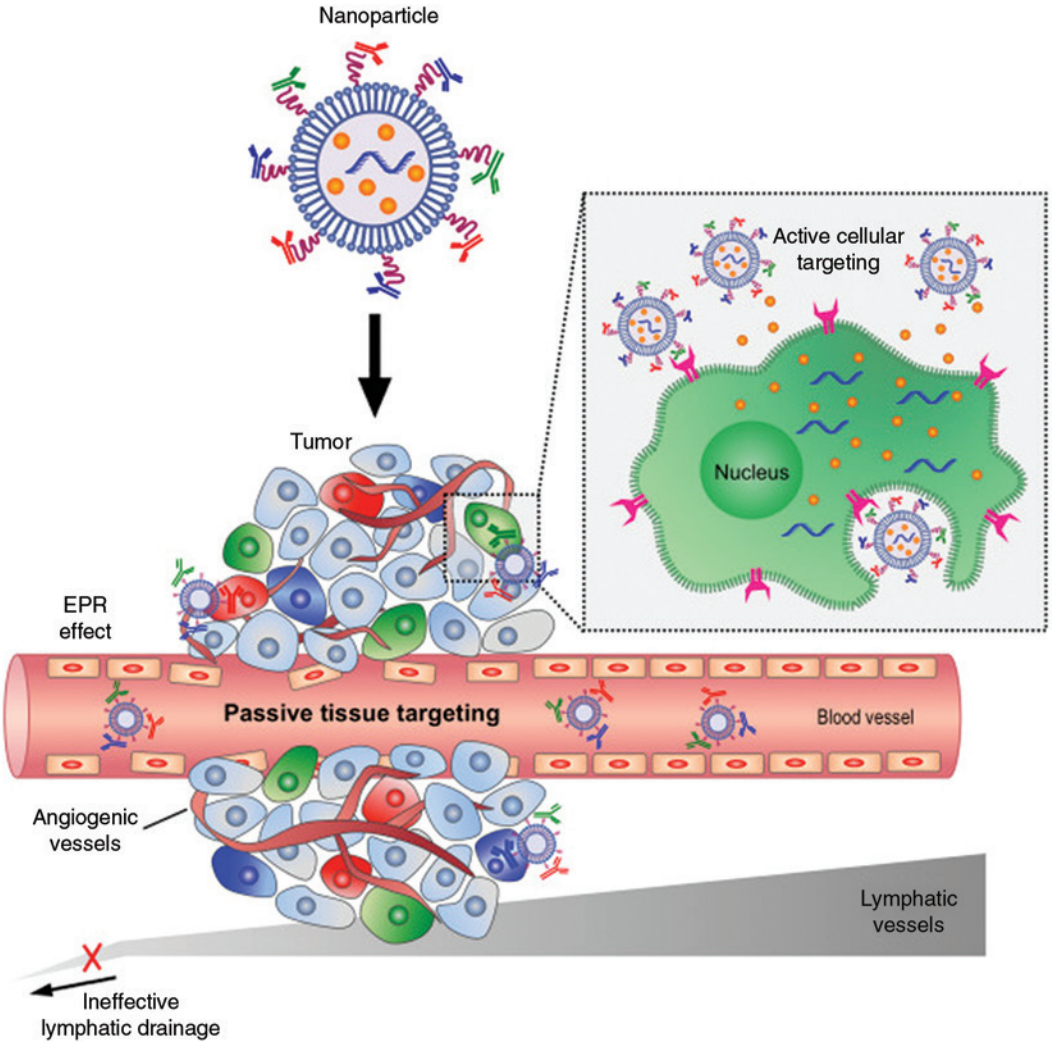

Figure 2: Active and passive targeting of drug loaded nanoparticles.
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for the passive targeting and the selective accumulation of nanoparticles in the tumor and also in its surrounding tissue [66]. Gabizon et al. showed that a PEGylated liposomal doxorubicin (Dox) formulation had a long circulation time in plasma, enhanced accumulation in murine tumors and a high therapeutic activity over free (unencapsulated) Dox [67].

The objective of the active targeting strategy is to overcome specificity limitations of drug conjugate by using targeting moieties. Nanoparticles are typically conjugated with targeting ligands that was described in the previous section, thereby allowing preferential accumulation of the drug within selected tissues or intracellular organelles [68]. Fabricated nanoparticles may enter the cells via different endocytic pathways such as receptor-mediated endocytosis depending on their size, shape, and surface properties (Figure 3).

Drug loading and release

High drug loading capacity is essential for a successful drug delivery system. The surface chemistry of the nanoparticles and the properties of the drug as well as some environmental factors such as release conditions have a strong influence on both drug loading and release. The loading of drug molecules into the nanoparticles can be performed by two methods. Within the incorporation method, the drug should be incorporated at the time of nanoparticle formation. In the adsorption/absorption method, the nanocarrier should be incubated with the concentrated drug solution [69]. Drug loading and entrapment efficiency is determined by the properties of the drug and the carrier molecule. Biomolecules, drugs or proteins show the greatest loading efficiency when they are loaded at or near their isoelectric point (pI),

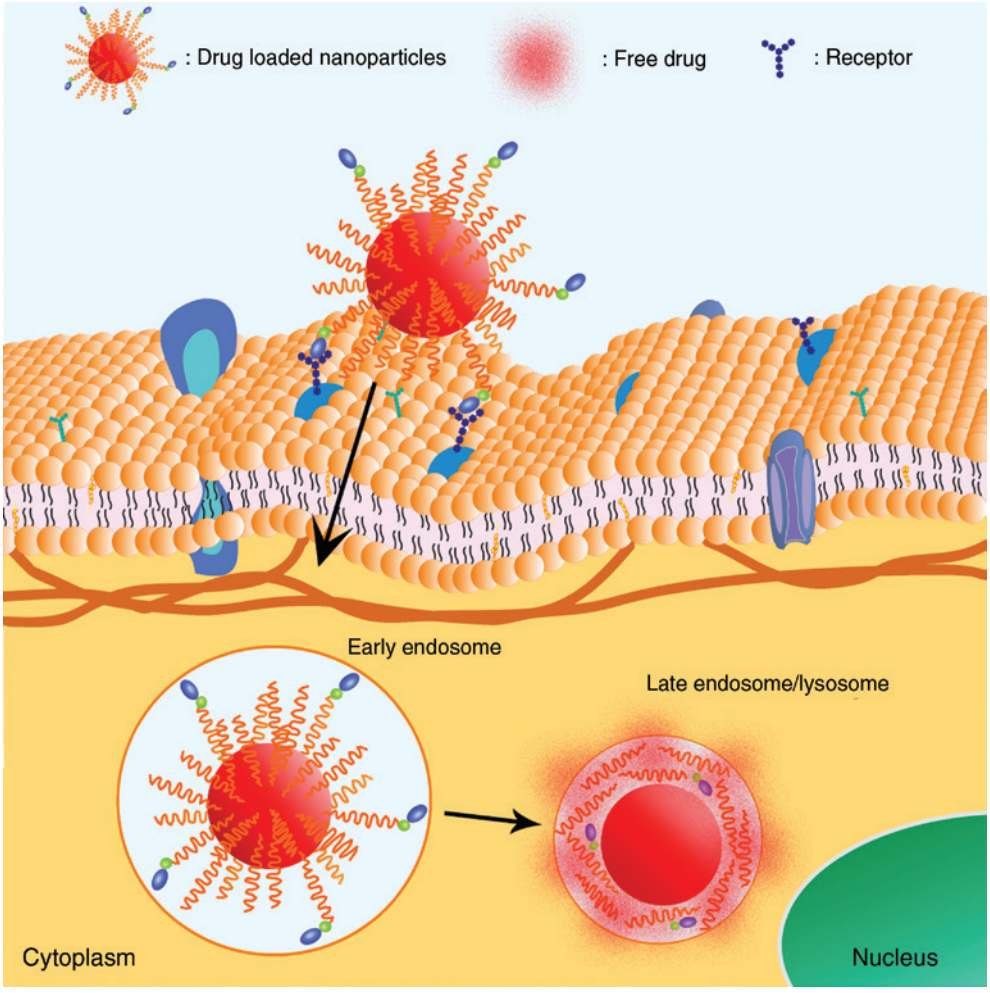

Figure 3: Schematic representation of the cytosolic delivery of drug loaded nanoparticles via receptor mediated endocytosis. The nanoparticles are engulfed in a vesicle, called early endosome, after receptor mediated cell association with nanoparticles. Endosomal escape of the nanoparticles leads to the cytosolic release of the encapsulated drug molecule.
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comprising a minimal solubility and a maximal absorption. Prior studies showed, that the use of ionic interactions between the drug and the matrix material can be an effective way to increase drug loading for small molecules [70, 71].

Summarizing, while designing a nanoparticle based drug delivery system, subsequent biodegradation and drug release should be considered. Loading the drug molecules inside or on the surface of nanoparticle carrier allows controlled release of the drug. This method bears many advantages such as improving biodistribution, reducing unwarranted side toxicity for healthy tissue and protecting the drug from physiological degradation compared to conventional free drugs administration [49]. Desorption of the drugs, drug diffusion from the nanoparticle matrix, matrix erosion or degradation of nanoparticles determine the release rate of the drug [3, 69].

Conclusions

Utilization of nanoparticles as drug carriers offer promising improvements in drug delivery. The loading of drug molecules into or on top of the nanocarriers can improve the pharmacokinetic properties of the drug and can protect the drug against early degradation enabling targeted and controlled drug release. Thus, accumulation of the drugs in targeted size can be increased and in this way occurrence of undesired drug effects can be prevented in healthy tissues. Furthermore, multifunctional nanoparticles with simultaneously useful capabilities such as targeting and imaging contrast enhancement can be synthesized. These nanoparticles combine both diagnostic and therapeutic features within a single formulation and therefore are denoted as “theranostic” agents [72–74].

Despite the fact that there are several nanoparticle based drug delivery systems, being developed or being currently under preclinical and/or clinical evaluation, still only a few such nano-drugs are in use on the market [75]. This is due to the fact that some problems in clinical usage encountered such as low drug loading capacity and a low circulation half-life, leading to low release and diagnostic features. Furthermore, the still relatively low physical stability and scale up problems bring along limitations for therapeutic applications of nanoparticle based drug delivery systems. Besides, the studies show that ultrafine particles (UFPs; diameter <100 nm) are more toxic and induce more severe inflammation than larger particles in their effects [76]. Within the field of nanotoxicology, safe nanomaterials will be designed and new methods and techniques for analyzing these nanostructures in in vitro test platforms will be developed.

The greater part of the fabricated drug delivery systems work well in vitro, however many systems fail in in vivo testing because of excessive accumulation and toxicity in the kidneys and liver [77]. In the last decade, three-dimensional (3D) cell culture systems have drawn great attention because they often offer levels of cell differentiation and tissue organization not observed in conventional two-dimensional (2D) culture systems [78]. In recent years "organ-on-a-chip" systems are useful as an in vitro approach to test drugs and nanoparticles by mimicking the specific physiological environment found in certain organs. But both these technologies are relatively new and require further validation to predict clinical responses in human.

Consequently, smart multifunctional nanoparticles will be the most promising candidates as drug carriers in nanomedicine after identification on relevant targets and production of novel molecules. This molecules will enable the development of personalized drug delivery systems by improving the life quality and duration of the patients.

Acknowledgments: Konrad Adenauer Foundation is acknowledged for the financial support to Didem Ag Seleci. Rebecca Joncyzk thanks to Niedersächsische Krebsgesellschaft e.V. for funding support.

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