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Tailor-made biofunctionalized nanoparticles using layer-by-layer technology

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ABSTRACT

Layer-by-layer (LBL) technique is a well-established method for the formation of nanofilms on planar surfaces, as well as on micro-sized colloidal particles. For surface functionalization of nanoparticles, it is a very recent area of research receiving great interest due to the advantage of obtaining fine control over the shell composition, charge and thickness, offering several applications in pharmaceutical and biomedical fields. This article provides an overview of the recent achievements in application of this technology on nanoparticles and their potential applications. Future research directions are also discussed.

Contents

1. Introduction

Nanotechnology, or more appropriately nanoscience, is a multidisciplinary branch of science that currently receives enormous efforts of scientists and researchers. Several definitions for nanotechnology were provided by researchers and institutions:

1. A practical definition of nanotechnology is "the design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property" (Koo et al., 2005).
2. The National Nanotechnology Initiative (NNI), on the other hand, defined nanotechnology as the "understanding and control of matter at dimensions of roughly 1–100 nm, where unique phenomena enable novel applications" (Bawa et al., 2005).

Both definitions are aiming towards the control of objects at the nanometer scale but in slightly different size ranges. Advances in nanoscience are spurring a revolution in the future of pharmaceutical and biomedical fields, in which the application of nanotechnology has provided new avenues for engineering materials with molecular precision allowing for fabricating nanoscale delivery devices that integrate molecular recognition and site-specific delivery. In addition to controlled drug release and drug targeting, nanodelivery systems could offer protection and improve the pharmacokinetics of easily degradable peptides and proteins, which often have short half-lives *in vivo*. A growing number of therapeutic compounds currently being developed by pharmaceutical companies are poorly water soluble leading to limited

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and/or erratic bioavailability. Nanoparticle formation has been exploited as a method to improve the bioavailability of these poorly water-soluble active pharmaceutical ingredients. In addition, nanotechnology applications in gene delivery and tissue engineering are of high interest (Bawarski et al., 2008).

The rapid development of nanotechnology and nanomaterials has led to a need for engineering the surface properties of nanoparticles for a variety of applications. Coating of nanoparticles changes the physicochemical parameters of the nanoparticles through altering the charge, functionalities and reactivity of the surface. The impetus for coating nanoparticles was not only to improve the colloidal stability in biological fluids and extend the half-life of the nanoparticulate formulations inside the body but also to tailor the surface to specific physical, optical, electronic, chemical and biomedical properties using wide varieties of coating materials. Almost all types of nanoparticles were subjected to coating, including lipid (Cui and Mumper, 2002), polymeric (Sheng et al., 2009) and inorganic (Aqil et al., 2008; Neumann et al., 2009) nanoparticles. The surface of nanoparticles was modified with biomolecules such as proteins (Cui and Mumper, 2002; Ogawara et al., 2004) and DNA (Neumann et al., 2009) as well as polymeric (Acar et al., 2005; Gu et al., 2007; Aqil et al., 2008; Sheng et al., 2009), inorganic (Mine et al., 2003; Dang et al., 2010) and lipid (Zhang et al., 2006; Li et al., 2008a) materials. The methods used for coating of nanoparticles are mainly based on chemical methods, thus differ from one case to another depending on the core chemistry and physical properties. The layer-by-layer (LBL) deposition of polyelectrolytes depending on electrostatic self-assembly was therefore a significant innovation allowing for the development of an easy standard method for the surface functionalization of a broad range of nanoparticles. A key advantage of this technique is the preparation of nanoparticles of core-independent but shell-dependant characteristics. This would result in nanoparticles of different sizes suitable for targeting different sites in the body depending on the site-specific needs. In addition, studies based on investigating the effect of size and surface chemistry of a single nanosystem would be thus feasible.

LBL deposition of polyelectrolytes on planar surfaces or micro- or submicroparticles has been the focus of several reviews (Caruso, 2001; Gittins and Caruso, 2001; Antipov and Sukhorukov, 2004; Shchukin and Sukhorukov, 2004; Johnston et al., 2006; Gil et al., 2008). However, coating of nanoparticles, of size less than or around 100 nm with polyelectrolytes using LBL technique is a more recent approach. The main objective of this review is thus to shed light on the potential of pharmaceutical and biomedical applications for core/shell nanoparticles, prepared by layer-by-layer technique, with special emphasis on using gold nanoparticles (AuNP) as the most often utilized template for subsequent layering. However, other nanoparticulate templates are also highlighted.

2. Layer-by-layer technique

LBL deposition is an established method for the fabrication of multicomposite ultrathin films on solid surfaces (Decher et al., 1992; Decher, 1997; Ariga et al., 2007). Typically, this technique is based on the use of polyelectrolytes of opposite charges assembled layer-wise on the surface of interest, thereby building up a layer system of tunable characteristics, in terms of composition, nanometer range thickness, surface charge, permeability, and elasticity. The most commonly used polyelectrolytes are poly(ethyleneimine), poly(allylamine) hydrochloride, poly(diallyldimethylammonium chloride), poly(styrenesulfonate), poly(vinylsulfate), and poly(acrylic acid). However, the great versatility of the technique has allowed for the use of a wide variety of materials, such as polymers, (nano)particles, lipids, proteins and dye molecules, not only depending on electrostatic forces but also on hydrogen bonding, covalent bonding, complementary base pairing and hydrophobic interactions (Wang et al., 2008). Subsequent application of LBL technology to coating of microparticles was then achieved without drastic changes in the deposition parameters (Sukhorukov et al., 1998; Peyratout and Dahne, 2004). This transfer of the technology to colloids of size, ranging from submicron to several micrometers, was considered a key success of the method. This was of special interest in the area of drug delivery, offering additional mechanisms for controlling drug release (Antipov et al., 2001; Qiu et al., 2001; Ye et al., 2006; Wang et al., 2007) and protection of the encapsulated protein and other bioactive agents from the surrounding environmental hazards (Li et al., 2008b). Furthermore, tailoring of the colloidal surface at the molecular level together with the possibility to remove the core material was beneficial for the formation of capsules with tunable permeability. These capsules are of value in drug delivery, biotechnology, medicine, etc. (Antipov and Sukhorukov, 2004). A schematic representation of LBL deposition of oppositely charged polyelectrolytes on colloidal surfaces is shown in Fig. 1.

3. Functionalization of nanoparticles using "layer-by-layer" technique

Despite the widespread use of LBL technique for coating colloidal particles, this has been limited to the size of colloids. Challenges associated with coating nanoparticles with polymeric shells include establishing appropriate experimental protocols for (i) avoiding aggregation of nanoparticles that are likely to occur due to crosslinking of the added polymer as a result of wrapping the polymer chains around a particle with high curvature, and (ii) efficient separation of excess polyelectrolytes from the coated nanoparticles that are less than 100 nm in diameter, which is more problematic for polymeric nanoparticles of low density (Caruso, 2004). The power of LBL technology to functionalize nanoparticles smaller than 100 nm while limiting aggregation and bridging of nanoparticles was first introduced in 1999 by Caruso et al. (1999).

Since then, coating of nanoparticles by LBL technology is an active area of research and is currently considered a hot topic with many potential applications in drug delivery, gene delivery and biomedical diagnosis. Such coatings not only stabilize the colloidal dispersion, a major challenge on coating nanoparticles using other methods, but also allowing modification and tailoring of particle properties. Furthermore, it is possible to incorporate drugs in these coatings (Schneider et al., 2009). LBL deposition is also known to avoid case-by-case optimization of functionalization of nanoparticles. Moreover, wrapping of flexible polyelectrolytes around charged nanoparticles is of considerable importance in biology, especially in the process of DNA replication, as this could be a model for DNA–histone complexes formed inside the cell nucleus (Netz and Joanny, 1999; Kunze and Netz, 2000).

This technology is known to work on a variety of surfaces differing in chemical and physical attributes, and also for drug, polymeric and inorganic nanoparticles. This is explained in more detail in the next sub-sections.

3.1. Drug nanoparticles

Interestingly, polyelectrolytes were successfully assembled directly on drug nanoparticles. By the LBL assembly of polyelectrolytes on drug nanoparticles, drug delivery systems with well-controlled drug release, modulated via layer thickness and composition, were successfully prepared for several anticancer drugs (dexamethasone, paclitaxel, and tamoxifen) (Zahr et al., 2005; Agarwal et al., 2008). It should be noted here that the formation of the first layer requires a charged surface in order to prevent

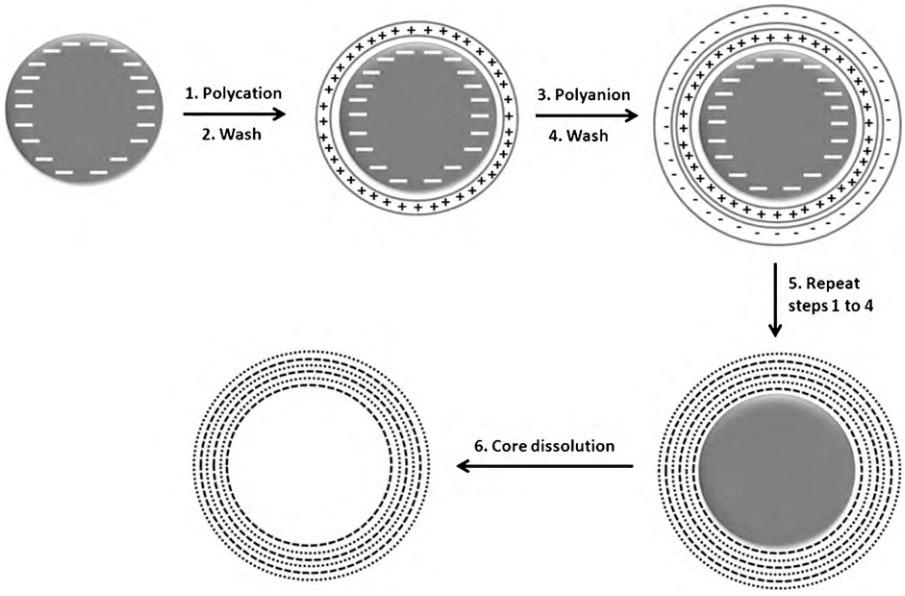

Fig. 1. (a) Schematic representation of LBL deposition of oppositely charged polyelectrolytes on a negatively charged colloidal substrate. Steps 1–4, including adsorption of a polycation then a polyanion and washing after each adsorption step, represent the basic build-up sequence for the development of the simplest film. Repetition of these steps result in a colloid coated with multilayers of polyelectrolytes. Finally, there is a possibility of dissolving the core resulting in capsule formation.

the partial desorption of the first layer. This technique has the benefit of not only modulating drug release but also has the power of solubilizing poorly water-soluble drugs. Using this novel approach, encapsulation efficiency higher than other known drug solubilization techniques was achieved (Agarwal et al., 2008). Moreover, there should be no more concern regarding either possible changes in drug activity *in vivo* or the possibility of high carrier-related side effects since the drug in its free form is present in the core and a comparably small quantity of the polymer is used (Agarwal et al., 2008).

3.2. Polymeric nanoparticles

The problem of undesirable burst drug release, associated with polymeric nanoparticulate delivery systems is considered to be one of the major problems confronting their development. Being easy, cost effective and practical than chemically modifying the surface of nanoparticles, LBL technique was adopted for coating procaine hydrochloride-loaded methacrylic acid-ethyl acrylate nanogel. It was claimed that the initial burst release was significantly reduced on using more than two polyelectrolyte layers and was further minimized with the introduction of each additional polyelectrolyte layer. This is despite the fact that an initial burst of the hydrophilic drug would be expected to be released into the aqueous coating solutions or even during the subsequent washing procedure. According to them, this was attributed to two main factors, the permeability of the nanogels which was altered by sequential layering and the increase in the diffusion length which the drug had to cross in order to be released (Tan et al., 2008).

3.3. Inorganic nanoparticles

In terms of physicochemical characteristics, inorganic nanoparticles posses a number of advantages over polymer capsules, namely, superior mechanical and thermal stabilities and a multitude of tunable optical properties making them intriguing and well suited templates for layering. In addition, for the preparation of polyelectrolyte capsules, core destruction is generally more problematic in case of polymeric colloids. Some polymeric core materials, e.g. melamine formaldehyde (Sukhorukov et al., 1998), are adsorbed on the shell during core dissolution leading to modification of the layer properties (Mohwald et al., 2003).

Magnetite nanoparticles were coated with sequential layers of temperature-responsive polymers (Yamamoto et al., 2008) and fluorescent polyelectrolytes (Sun et al., 2009) based on electrostatic deposition and the coated particles were magnetically separated. These prepared nanoparticles hold promise for potential biomedical applications, especially in cancer therapy and medical diagnosis. Recently, calcium phosphate nanoparticles were also surface-functionalized, with two photosensitizers, methylene blue and 5,10,15,20-tetrakis(3-hydroxyphenyl)porphyrin (mTHPP), incorporated in the shell layer, in the aim of preparing water dispersable model dyes for photodynamic therapy. Using this method of preparation, administration in alcoholic solution, known to cause a local pain to the patient is avoided and also a better tissue biocompatibility should be expected. The photodynamic activity was found to depend on a multitude of parameters, including the particle charge and the coating polymer (Schwiertz et al., 2009). Finally, biosensors based on a highly stable system of a core of a quantum dot sequentially coated with polyallylamine hydrochloride and polyvinylsulfonic acid were successfully prepared making them promising candidates for the investigation of cellular traffic on a single particle level (Jaffar et al., 2004; Fernandez-Arguelles et al., 2007).

3.3.1. Gold nanoparticles functionalized by layer-by-layer technique

AuNP are the most commonly used nanocore for LBL deposition of polyelectrolytes and is considered a good model for understanding the parameters controlling polymer multilayer formation on nanoparticles, exploiting the sensitivity of the nanoparticle surface Plasmon band caused by adsorbed species to follow multilayer growth (Mulvaney, 1996). The optical properties of AuNP also allow for the visible detection of successful preparation of nonaggregated AuNP. Further advantages of AuNP include their limited cytotoxicity, though literature shows contradictory data (Goodman et al., 2004; Connor et al., 2005; Shukla et al., 2005; Pan et al., 2007) and the ability to exhibit luminescence properties which allow for their detection in biological tissues (Huang et al., 2007; Pissuwan et al., 2008; Schneider et al., 2008). This is in addition to the ease of prepa-

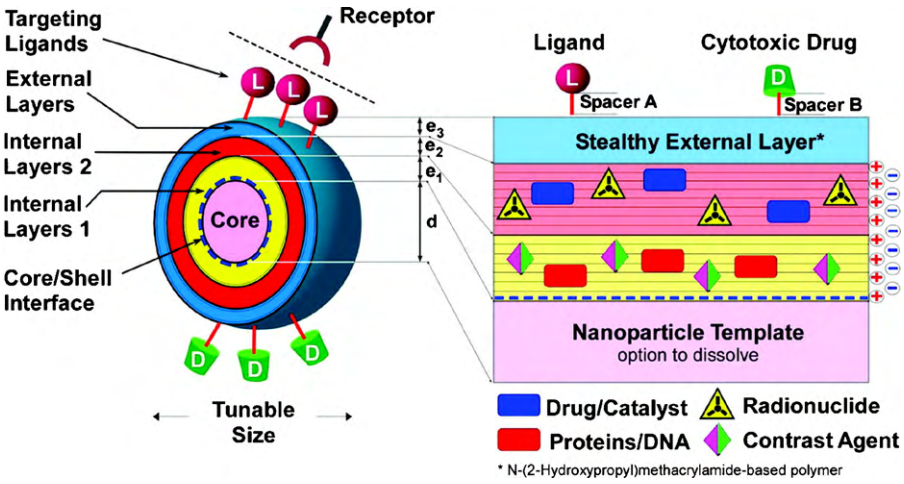

Fig. 2. Schematic representation of nanoparticles coated with multilayer shells as an ideal drug delivery system (Ref. taken from Schneider et al. (2009)).

ration of AuNP with variable sizes and shapes (Huang et al., 2007; Funston et al., 2009; Zhou et al., 2009). All these advantages offer a flexible model system which could drive further understanding of cellular uptake and interactions as well as for designing tailor-made carriers for drugs, genes, DNA, particularly if their mechanisms of interaction with the cells are elucidated.

LBL deposition to fabricate gold core/polyelectrolyte-shell nanoparticles was first reported by Gittins and Caruso (2000). The same working group then reported the successful coating of gold nanoparticles by an approach based on a combination of complexation and LBL deposition of polyelectrolytes. They prepared core–shell gold/titania nanoparticles by first coating gold particles, derivatized with sodium 10-mercaptodecanesulfonate (anionic), with three layers of polyelectrolytes; poly(dimethyldiallylammonium chloride) (PDADMAC) (cationic), poly(sodium-4-styrenesulfonate) (anionic) and then PDADMAC. After the coating of the gold cores a negatively charged titanium precursor was adsorbed onto the last PDADMAC layer. Afterwards the titanium precursor was hydrolyzed by temperature increase resulting in titania precipitation (Mayya et al., 2001).

This method of coating nanoparticles has been reported by Schneider and Decher (2004) as the sole possible method for functionalization of 13.5 nm sized AuNP. The resulting dispersions were highly stable and the stability would even increase on increasing the number of layers. More interestingly, AuNP as small as 7 nm (Mayya et al., 2003) or even smaller (Dorris et al., 2008) were also successfully derivatized by the self-assembly of oppositely charged polyelectrolytes. Even with small particles, core dissolution was possible to obtain nano-sized polyelectrolyte capsules (Schneider et al., 2006).

The parameters controlling polymeric multilayer formation on highly curved nanoparticles were thoroughly investigated for gold cores by Schneider and Decher (2008), and the findings of this research paper can be taken as a guide to accelerate the optimization of other systems. Parameters of outmost importance in terms of enhancing the stability of the dispersion preventing bridging flocculation were found to be polyelectrolyte concentration, the contour length of the polyelectrolyte chain and the ionic strength. Optimized conditions lead to small particle losses and a preservation of individual coated particles higher than 90% per adsorption cycle.

3.3.1.1. Benefits and potential applications of coated gold nanoparticles. AuNP have recently attracted significant research interest due to their great potential applications in many fields such as photothermal therapy, drug and gene delivery and biomedical diagnosis (Ghosh et al., 2008). A polymer-stabilized shell around AuNP is additionally attractive due to a versatile composition and functionalities of the polymer. Surface functionalization of AuNP using LBL technique has potential applications in various pharmaceutical and biomedical fields.

Drug delivery: Drug delivery is an interdisciplinary area of research that enables the administration of complex new drugs and even adds a critical value to the old drug generations (Alonso, 2004). The use of uncoated nanoparticles for drug delivery can mainly control drug release and bioavailability in a temporal manner. However, functionalizing nanoparticles offers the potential of cellular targeting, thus enabling spatial control of its release and bioavailability (Rothenfluh and Hubbell, 2009). Additional advantage of adding a functionality to the nanoparticles by coating with polymers, such as polyethylene glycol, is protecting the surface of nanoparticles from protein adsorption. This obsonisation occurs when nanoparticles come in contact with the biological media (Rocker et al., 2009). The identity and lifetime of these proteins on the particle would dramatically change the composition, size, charge and surface characteristics of the prepared nanoparticles in an uncontrolled manner. This has implications on the interaction of nanoparticles with cells and their *in vivo* biodistribution (Aggarwal et al., 2009; Lynch et al., 2009; Rocker et al., 2009).

Schneider et al. (2009) proposed an ideal design for a highly versatile nanoparticle-based core/shell system in which the shell, deposited by LBL technology, is engineered in a modular fashion allowing the incorporation of various functionalities that are mandatory for addressing different applications (Fig. 2). These functionalities may differ from one layer to another as indicated in the figure by internal layers 1 and internal layers 2. This "ideal" system should be characterized by adjustable and small size distribution, high stability in physiological media, low toxicity of the carrier system, protection against macrophage uptake, in addition to triggered drug activation and release at the specific site of action.

As a proof of concept, they formulated a gold core of size 10 nm. Gold cores have the advantage that they can be easily dissolved by KCN, resulting in the formation of nonaggregated functional nanocapsules (Schneider and Decher, 2004). This was first coated with five internal primer layers of poly(allylamine) and poly(styrenesulfonate) to create a defined polyamine surface that is independent of the core material, therefore this system could be applicable to other core materials. The stability of the particles was increased by further coating of the particles with a functional terpolymer (F-HPMA) composed of three different monomers; N-(2-hydroxypropyl)methacrylamide, N-methacryloyl-glycyl-glycyl thiazolidine-2-thione and N-

methacryloyl-glycyl-DL-phenylalanyl-leucyl-glycyl doxorubicin. The former monomer offered stealthing of the nanoparticles by offering a highly water solvated corona layer. The latter monomer is well known for incorporating a nontoxic prodrug form of doxorubicin into copolymers providing release of the active drug after endocytosis through the enzymatic degradation of the oligopeptide spacer. This example is intended for a potential application in cancer therapy, however the modular concept behind the LBL deposition allows it to be easily extended to other therapies (Schneider et al., 2009).

Lopez-Viota et al. (2009) have prepared AuNP coated with a single layer of creatine followed by another layer of human serum albumin, using LBL deposition technique. This system was assumed to cross the blood–brain barrier, based on low surface charge, as was shown by electrophoretic mobility measurement, and the albumin coat, known to induce transcytosis of the nanoparticles through the blood–brain barrier. Preliminary experiments not yet published by these authors, show measurable amounts of these nanoparticles in the brain of experimental animals. Accordingly, this was assumed as a novel drug carrier for brain delivery. These results are also considered promising in terms of extrapolation of the electrokinetic data for the design of suitable nanoparticulate drug delivery systems.

Nanoreactors/nanocontainers: AuNP have also been used for the LBL preparation of polyelectrolyte capsules, in which the gold core is efficiently removed upon exposure to potassium cyanide. The synthesis of hollow gold nanocapsules with well-defined tunable dimensions in terms of size diameter, wall thickness and surface functionalization and charge is an expanding area of research that holds potential application in drug delivery, in addition to other applications in gene delivery and biomedical diagnosis (Gittins and Caruso, 2000; Gittins and Caruso, 2001; Schneider and Decher, 2004; Schneider et al., 2006).

The resulting "Nanocontainer/Nanoreactor systems" correspond to a scaled-down version of larger hollow microcapsules reported earlier by Möhwald et al. (Sukhorukov et al., 1998; Donath et al., 2002; Shenoy et al., 2003) bringing the toolbox established by these authors to the nanoscale range. One example of polyelectrolyte nanocapsules loaded with functional group was prepared by Schneider and Decher (Schneider et al., 2006).

Gene delivery: In recent years significant efforts have been devoted to develop nanocarriers for gene delivery. These novel nanostructures should be tailored to offer more control on their *in vivo* stability, a key issue in designing a gene delivery system. In that respect, stable gold nanorods coated with bovine serum albumin and polyethyleneimine were prepared for application in gene delivery, however, the surface properties should be more tuned for better transfection of the genes (Takahashi et al., 2008). However, this preliminary work has opened up a new direction for others to follow for the development of core/shell AuNP for gene delivery.

Intracellular delivery of therapeutically relevant small interfering RNA molecules (siRNA) necessitates the use of small monodisperse nanocarrier with defined ζ potential and surface properties. The first proof of concept that nanoparticles can be modified with siRNA using LBL approach for cellular delivery was very recently reported (Elbakry et al., 2009). The technique offered a unique opportunity to fabricate well-defined and homogenously distributed nanocarriers coated with multilayers of siRNA and polyethylenimine. Polyelectrolyte concentration and ionic strength were regarded as important parameters to wrap the stiff rod-like siRNA molecule round the prepared AuNP, having a diameter of about 15 nm, in order to obtain a high yield and avoid aggregation. The surface properties, especially the surface charge, were shown to strongly affect interactions with the cells. This important study will help design new materials for siRNA delivery.

Biomedical diagnosis: Though it was often reported that LBL coated AuNP loaded with a fluorescent dye hold promise for utilization in biomedical diagnosis, to our knowledge this was only once physically investigated in literature by Schneider and Decher (2006), highlighting different problematic aspects as quenching of the fluorescence. Hence, this was not supported by *in vivo* data. They studied the contribution of the gold core on the quenching of fluorescence of the organic dyes: fluorescein isothiocyanate and lissamine rhodamine B sulfonyl chloride. The fluorescence was assessed before and after the gentle dissolution of the gold core by potassium cyanide. The gold core was found to quench the fluorescence to a certain extent. Nevertheless, the fluorescence of the core–shell particles remains sufficient for potential applications as diagnostic devices. The capsules obtained after gold core dissolution can form the basis of fluorescent sensors.

Cellular uptake and cytotoxicity: Though the field of nanomedicine has shown great advances leading to the development of various types of nanocarriers that found applications in drug delivery, gene delivery and medical diagnosis, still the studies on the interface between the physicochemical parameters of the nanocarriers and cell biology are in its infancy and a great deal of the interaction of nanocarriers with the cells, in addition to potential cellular toxicity, is still unknown. Hauck et al. (2008) were able to tune the cellular uptake of gold nanorods coated with polydiallyldimethylammonium chloride and poly(4-styrenesulfonic acid) through manipulation of the surface charge and functionalities of the final system. The coated system was shown to have a negligible impact on the cell function though the individual polyelectrolytes were reported to be themselves cytotoxic (Chanana et al., 2005). This was attributed to the individual behaviour of AuNP, shown previously to enter and leave the cells in vesicles thus exerting limited toxicity on the cellular compartments (Chithrani et al., 2006). Chanana et al. (2005) showed that the cytotoxic effect of polyelectrolytes-coated AuNP (15–18 nm) vary from low to strong not only depending on the surface charge but also on the number of applied layers. Considerable cellular uptake is an important advantage for these core/shell AuNP, however a single model to withdraw data on cellular biocompatibility is not yet available and this largely differs in a case-to-case manner.

4. Standpoint and outlook for future developments

Functionalization of AuNP or other nanoparticles using LBL technology is a rapidly developing topic with a promising future for designing delivery systems with tailored surface properties. However, application of this technology on nanoparticles less than 100 nm is difficult and still in its infancy, as evident from the small number of publications with only two main research groups contributing intensively to this field, Caruso and colleagues and Decher and colleagues.

In the future, more focus should be spotted on understanding the physicochemical parameters of the system and a core-size and shape dependent, however, core-composition independent, standard method for preparation. Investigations should go a step further by transferring the formulations to *in vivo* examinations to proceed from the level of postulations to the 'real' applications level. Studying the cellular behaviour and the effect on cellular function should also be an integral part of designing a delivery system.

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