ENDOCYTOSIS AND EXOCYTOSIS OF GOLD NANOPARTICLES

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Abstract. The therapeutic effect of the drug molecules in the body strongly depends on the half-life and the internalizing pathways in cells and tissues. At the same time, removal from the body of nanoparticles remains an important objective in the design of drug delivery In recent years, scientific reports have emphasized the main biophysical mechanisms of nanoparticles (NP) interaction with cellular membranes, depending on their size and surface chemistry. This review discusses the optimization of physicochemical properties of nanoparticles focusing on NP size effects in their interaction with living cells. This review highlights the role of NP size and modality of coating to prevent aggregation and agglomeration.

Key words: nanoparticles, endocytosis, membrane.

INTRODUCTION

Nanoparticles area of interest is very large, including medicine, electronics, environmental applications, food industry and cosmetics. Surface chemical modification of the nanoparticles has crucial effect on the interaction of nanoparticles with cells and tissues. Recently special attention was paid to control the shape and size of nanoparticles because all the catalytic, magnetic, electrical and optical properties of nanoparticles are influenced by their geometry and dimensionality.

Various nanoparticulate systems have been used to enhance the efficiency and selectivity of the targeted drug delivery methods because they act as drug vectors to the centers of therapeutic interest. Among many other metallic NPs, gold nanoparticles yielded for biomedical purposes have average size between 1–100 nm being usually suspended in a carrier fluid. Compared to bulk material nanometric particles show specific physical and chemical properties resulting from their surface to volume high ratio such as summarized in [15].

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Various methods of obtaining gold nanoparticles have been developed that allow to get various forms such as spherical particles, rods, stars or triangular. Recent studies revealed the importance of gold nanoparticle geometry, the most appreciated being spherical shape.

It has been proven that nanoparticles can be coated with adequate polymers to form convenient protected nanosystems. Otherwise NPs tend to agglomerate in clusters and impede their crossing through the cell membrane [16]. Ligands such as pharmaceutical molecules could be further embedded to polymer shell for drug delivery. Experimental studies have shown that NPs lose steering ability in complex biological environment [21]. Adsorption of macromolecules is external (e.g. opsonic proteins) to the NPs surface and blocks specific interactions between receptors in the cell membrane and ligands on the NP.

According to some studies it was shown that the absorption of NPs in cells can be controlled as well as the sensitivity of the coating polymers, the pH in the extracellular medium. The use of NPs coated with pH sensitive polymers showed that particles can be ingested by endocytosis in membranes under conditions of low and high pH value, while in the middle range of the pH, the process of endocytosis is blocked. For example, in a biological environment with low pH, the adsorption of polymers on the particle surface is very weak and cannot prevent strong attraction between membrane receptors and ligands on the surface of nanoparticles. Hence the polymer will detach from the surface of the NPs, which will be absorbed by the cell membrane. Further, it was found that receptor-ligand interactions as well as surface charge of NPs and membranes can also have important impacts on the endocytosis. In Fig. 1, the NP macrotransport processes are illustrated.

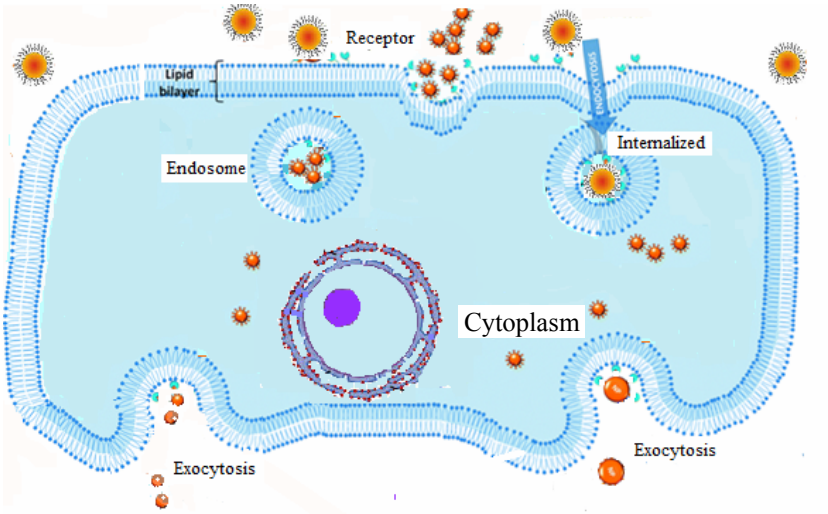

Fig. 1. Clathrin and caveolae-type endocytosis pathways are mediated by receptors and are the most important ways of functionalized nanoparticle internalization into cells.

Regardless of the mechanism of internalization, the interactions between cells and nanoparticles are determined by size, shape, surface charge and surface chemistry [33]; it seems also that cell-specific parameters (type of cell or cell cycle phase) have a great importance [19].

THE ROLE OF NANOPARTICLE SIZE IN ENDOCYTOSIS

The use of nanoparticles in drug transport was intensively studied in the last decades from the viewpoint of their possible toxicity [28]. According to the cytotoxicity studies, the main NP toxic consequences could be related to their size and/or size distribution [8, 7]. Reducing the size of NPs has led to better diffusion into the body, with higher penetration in certain tissues and more efficient internalization into cells, but increased toxic effects are also expected [24].

Recent studies have shown that toxicity can be related to the degree of interaction between nanoparticles [1]. Specifically, when the attractive Van der Waals forces between nanoparticles are higher than rejecting electrostatic forces, aggregates or clusters could form – as mentioned above – that interact differently with biological systems compared with nanoparticles that are not interacting with each other. This remark has led to the necessity of synthesizing NPs with narrower size distribution (very similar particle sizes). Synthesis techniques have been improved through more accurate control of reagents, which influences the size of NPs and control the pH value, which allows lower polydispersity of NPs in the colloidal suspension [12].

Monitoring the size of gold nanoparticles can be done by molecular absorption spectrophotometry (UV/Vis). Both absorption and scattering phenomena contribute to light intensity changes following the interaction with AuNP in aqueous suspension. Surface plasmon resonances (SRP) were shown to be present in gold thin layers as such at the AuNP. In Fig. 2 some of our research results in yielding AuNP from chloroauric acid reduction and coated with chitosan polymer can be seen.

SPR is an optical phenomenon resulting from the interaction between an electromagnetic wave and conduction electrons in AuNPs [12]. With UV-Vis technique one can record absorption band of resonant surface plasmons generated by nanoparticles in colloidal suspensions coated with polymers. Incident light induces coherent collective oscillation of conduction band electrons with positively charged metal core. These oscillations can be dipolar resonance with the incident light at a certain frequency depending on size and shape of NP.

The ratio of absorption and scattering depends on the size of NPs. For diameters smaller than 20 nm, charge density is rather homogeneous on nanoparticles surface and absorption is observed as dominant phenomenon. For example, small AuNPs, such as 13 nm diameter, have a maximum in the green

light range, generating an intense absorption band at 520 nm in the visible light spectrum. With increasing nanoparticle size, charge density becomes inhomogeneous, *i.e.* charge polarization occurs and thus there is a widening of the surface plasmon band and a shift towards the red band produced by a phase delay [4].

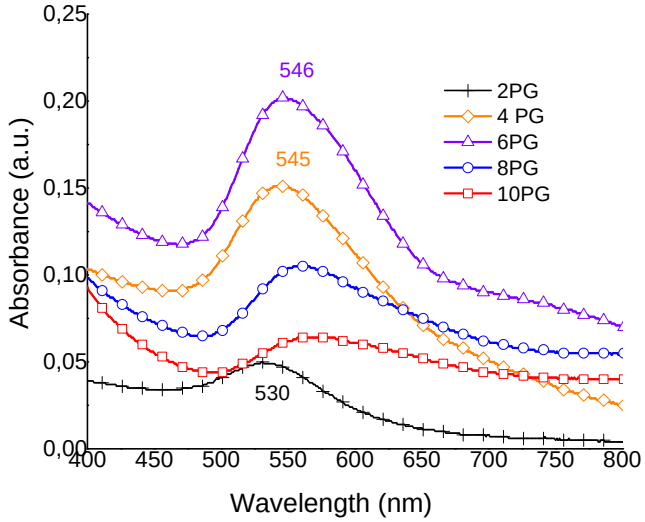

Fig. 2. Absorption spectra of several colloidal solutions with AuNPs (2,4,6,8,10 correspond to gold precursor concentrations) synthesized chitosan with high molecular weight. Position and half-width of the SPR absorption band can be correlated with the mean size of AuNPs (in press results).

Using transmission electron microscopy (TEM), the diameter of the nanoparticles can be accurately measured. This technique allows visualization and measurement of metallic core of core/shell nanosystems. In order to determine the size of colloidal NPs including their organic coating shell in suspension, dynamic light scattering (DLS) may be used to measure the size of both core and coating layer [11].

Endocytosis is a biological process used by cells to internalize (bio) molecules or particulate matter. NPs can be internalized into cells because of their size similar to biomolecules [3, 13]. NPs can induce the occurrence of clathrin or caveolin. Endocytosis of clathrin-mediated type involves a mechanism of small vesicle formation with a protein (clathrin) having the role of intracellular transport of the molecules. The presence of caveolin proteins leads to a local change in the morphology of the membrane, allowing molecule endocytosis. NPs size can affect the efficiency of absorption and kinetics, in the mechanism of internalization and subcellular distribution. It was observed that, for gold nanoparticles, the absorption in different cell lines depends on the size [19, 29, 35]. The maximum cell uptake was achieved in the range of 30–50 nm, which suggests that there is an optimal size

for the active uptake [35]. It was reported that 45 nm Au NPs entered the cells via clathrin-mediated endocytosis, while nanoparticles smaller than 13 nm came mostly through phagocytosis [20]. Receptors internalized by nanoparticles via endocytosis can be recycled back to the membrane. Also they can be degraded in lysosomes and endosome so that new receptors to be produced and spread in the cell membrane.

Nanoparticles suspended in the blood serum are coated by proteins and become electrically charged. Protein coated nanoparticles can be internalized into the cells using the path clathrin- and caveolae-mediated endocytosis [10, 27, 34, 36]. Nanoparticles with less than 10 nm sizes can translocated through the cell by micropinocytosis [30] by means of protein aggregates of the lipid bilayer of cell membranes specialized in carrying nano-sized materials [38]. Micropinocytosis is an endocytosis in which the small particles are brought to the cell, forming an invagination and being then suspended in small vesicles. Several studies have been aimed to understand the mechanism of internalization of AuNPs depending on size.

Cytotoxicity studies of AuNPs in HEp-2 cells indicated that 3 nm nanoparticles have the highest toxicity. Hep-2 cells exposure to different size gold nanoparticles for different time intervals (1, 2, 4, 12, and 24 hours) were followed by imaging using scanning electron microscope (SEM) and atomic force microscopy (AFM). SEM and AFM results showed that, after 1 hour of incubation, the gold nanoparticles between 3 and 10 nm entered the nucleus, while the particles of 25 to 50 nm were accumulated around the nucleus. With exposure time increasing, more NPs entered the cells and accumulated in the cytosol and nucleus, depending on their size [4, 8, 17]. By using confocal microscopy and quantitative analysis of the image, Nienhaus *et al.* [37] reported the uptake of various NPs systematically investigated in the range of 3.3–100 nm in live HeLa cells.

The reason of size dependence of AuNPs receptor-mediated endocytosis may be explained by theoretical models [39, 40]. Through mathematical modeling it was shown that 50–60 nm NPs are able to recruit enough receptors to trigger the active internalization by receptor-mediated endocytosis [16]. This model leads to the idea that endocytosis of nanoparticles is achieved by competitive contribution of two types of energy. Another type of energy in the form of free energy is required for translocation into the cell NPs. These two factors seem to determine how fast and how many NPs are taken up by the cell. The absorption of nanoparticles smaller than 40 nm occurs more likely in NP groups, but it is much slower due to longer diffusion times. Endocytosis of NPs with a diameter higher than 80 nm is less probable because the free energy of connection is limited by depletion of free residual receptors. Formation of membrane curvature (induced by these NPs) determines a number of membrane receptors to match a certain size NPs [29].

Pharmacokinetic analysis of NPs includes adsorption of biomolecules, their tissue distribution, metabolism and excretion changes [28]. Once entered inside a cell or tissue the coating material on the surface of NPs will probably be metabolized. Subsequently, NPs without shell may be removed from the body (Fig. 1).

THE ROLE OF NANOPARTICLE SIZE IN THE EXOCYTOSIS

Nanoparticles that have entered the body through different pathways are internalized in various organs such as the liver and spleen, where they could remain for a long time. Increasing organ storage time increases the risk of toxicity. The study of the mechanisms of cell exocytosis, indicated that this process may allow the elimination of nanoparticles from the cell, preventing the storage in the body. There are studies that have shown that there are different exocytosis types according to nanoparticles shape. Studies have shown that AuNPs bar shaped had a higher rate of exocytosis than spherical ones [7].

If nanoparticles with larger size had a higher rate of endosomal packaging and are more likely to be internalized by the cell, the smaller nanoparticles show a lower rate of endocytosis but a higher rate of exocytosis. Exocytosis of 14 nm AuNPs was much faster than the 74 nm AuNPs, as shown in [24]. They also observed that AuNPs coated with certain types of peptides increased the rate of exocytosis. In such case, the peptides caused endocytosis without interacting with membrane receptors [2]. On the other hand, it was observed that AuNPs cations were retained for longer inside the cell because of agglomeration, but after NP coating with polyethylene glycol PEG (PEGylation) they have been removed more quickly. AuNPs coverage with PEG diminishes their interaction with intracellular proteins. In addition, their exocytosis did not depend significantly on the size of nanoparticles (10–40 nm). These results suggest that systemic excretion and toxicity of nanoparticles eliminated by macrophages could be modulated by surface chemical engineering [22].

Recent information on the fate of nanoparticles, found in biological systems, is the less, being obtained by analysis of the relationship between the rate of exocytosis of AuNPs and physiological calcium ion concentration in the extracellular space. Evaluation of HT-29 cell membranes fluidity in the presence of internalized AuNPs showed how extracellular calcium concentration increase causes increased exocytosis of nanoparticles [26]. Other studies have shown that occurrence of complexes formed by interactions of coated AuNPs and proteins may lead to inhibition of exocytosis [6]. The removal phenomenon has been observed with other types of nanoparticles, too. For example, quantum dots coated with D-penicillamine, to improve the colloidal stability in biological solutions,

have been transported toward the cell periphery and exocytosed within 21 minutes after internalization [33].

CONCLUSIONS

Due to a huge variety of nanomaterials and complexity of biological samples it is difficult to draw general conclusions from available data in spite of the efforts made by multidisciplinary research studies. But some conclusions can be drawn about the role of size and shape of the nanoparticles. Smaller nanoparticles appear to enter and exit the cell more efficiently. Spherical nanoparticles can more efficiently be internalized into the cell than cylindrical ones. Optimal size for nanoparticle internalization ranges theoretically between 40–60 nm, as confirmed by most accurate investigations. Also we can say that exocytosis is not dependent only on the nanoparticle size but also on their ability of interaction with cellular structures and the extracellular fluids.

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