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Interaction of functionalized nanoparticles with cell membranes

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ABSTRACT

Interaction of nanoparticles with cell membranes is a very important parameter for the use of nanoparticles as carriers for many drugs, and also in imaging and phototherapy. Knowing their wide use in various therapeutic and diagnosis approaches, this paper aims, on the light of biophysics, to review the most recent findings regarding the interactions between functionalized nanoparticles and cellular membranes., Highlighting these aspects is essential in choosing the raw materials nanoparticles are made of, the therapeutic target to which it heading, and also for increasing their functionality and lower their immunogenicity.

1. INTRODUCTION

The use of nanotechnology in medicine and more specifically drug delivery is expanding. Pharmaceutical sciences are using nanoparticles to reduce toxicity and side effects associated with the administration of high amounts of many drugs, which are necessary in several diseases, such as cancer. The risk that is introduced by using nanoparticles for drug delivery is beyond the one posed by conventional hazards imposed by chemicals in classical delivery matrices. The toxicology of particulate matter differs from toxicology of substances as the composing chemical(s) may be or not soluble in biological matrices. Toxicology degree proved to be higher in the lungs, where the exposure is usually higher in current formulations. Because of their size, nanoparticles-based formulations open the potential for crossing various biological barriers, as cell membranes. Their nanosized dimensions also allow for access into various cellular compartments, for the example the nucleus. Many substances are now under investigation for the preparation of nanoparticles for drug delivery (nanoparticles – solid phases/liposomes/albumin/gelatine with antibiotics, extracts plants, antitumor drugs or diferent other drugs) [1].

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience. Nanotechnology applied to medicine brings many advances in the diagnosis and treatment of several diseases. Applications in the biomedical field include drug delivery, *in vitro* and *in vivo* diagnostics and production of improved biocompatible/bioactive materials for medical surfaces and prosthetic devices. In creating nanoparticles, very important, is their composition. Nanomaterials may either by of chemical origin (i.e. gold, silver, silica, magnetite) or of biological origin (i.e. dextran, lactic acid, chitosan, lipids, phospholipids). Their composition makes the difference in the interaction with cellular components [2 - 6].

One of the major challenges in drug delivery is to get the drug at the place it is needed in the body, while avoiding potential side effects to non diseased organs. This aspect is very important in cancer treatment, where the tumor may be localized as distinct metastases in various organs. Local drug delivery or drug targeting results in increased local drug concentrations and provides strategies for a more specific therapy. Their small sizes allow penetration of the cell membranes, binding and stabilization of proteins, and lysosomal escape after endocytosis [1]. Pharmaceutical development of nanometric size drug delivery systems consists of two or more components, one active pharmaceutical agent [2, 6] and its support made of nanoparticles [7, 8, 2, 9]. These nanosystems are called smart-drugs or theragnostics [10]. Nanoparticles proved to increase the effectiveness of the treatment of cancer cells, can improve the solubility of poorly soluble drugs in water, modify the pharmacokinetics of drugs and reduce their immunogenicity, while enhancing the bioavailability of drugs [44, 45].These characteristics motivated this study, aimed to add knowledge on the interaction between nanoparticles and cell membranes, which represents the first step in the management and development of novel therapeutic approaches.

2. CURRENT NANOMATERIALS WITH BIO-MEDICAL DESTINATION

Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. Their size-dependent properties make these materials superior and indispensable in many areas of human activity. They are use in many applications designed for biology or medicine as: fluorescent biological labels [66 – 68], drug and gene delivery [69, 70], bio detection of pathogens [71], detection of proteins [72], probing of DNA structure [73], tissue engineering [74, 75], tumor destruction via heating (hyperthermia) [76], separation and purification of biological molecules and cells (77), MRI contrast enhancement [78], phagokinetic studies [79]. There are many types of nanoparticles that can be functionalized with different substances, which serve as carriers. One type of wide used nanostructures are the poly (lactic-co-glycolic) acid nanoparticles (PLGA) impregnated with the LXR agonist N, N-dimethyl-3β-hydroxycholenamide (DMHC). They are used in the treatment of

bone defects by stimulating bone growth and healing. DMHC coupled with this drug delivery mechanism seems to be a therapeutic option with high potential for orthopedic surgery [11].

Other authors have developed a dual-functional nanoparticle drug delivery system loaded with β-sheet breaker peptide H102 (TQNP / H102) that are used for entry into the central nervous system (CNS) and subsequent location of Alzheimer's disease (AD) lesions in the brain, thus offering a highly-specific method for AD therapy [12].

For therapeutic dermatology and cosmetics, retinyl palmitate was studied when enclosed in **(**RP) loaded nanoemulsions (NE), liposomes (LPs) and solid lipid nanoparticles (SLNs). LPs treated skin offered significant higher retention than SLNs. Even though all developed nanocarriers were found to be biocompatible, according to histological studies, NE was the system that most disrupted the skin. These encouraging findings can guide the proper selection of topical carriers among the diversity of available lipid-based nanocarriers [13].

Gold nanoparticles (GNPs) proved considerable applications in biomedicine. Gold nanoparticles are metallic nanoparticles. Other examples of metallic nanoparticles include Ag, Ni, Pt, and TiO2 nanoparticles. Gold nanoparticles (1–150 nm) can be prepared with different forms [21, 22]. These gold nanoparticles are very useful because they can be prepared in various sizes and shapes, which give a very good biocompatibility and functionality [23]. They can be used to sensitize cells and tissues for treatment regimens [24], and to monitor and to guide surgical procedures [25 – 27]. DNA and various proteins, and many synthetic drugs were fused with gold nanoparticles and they have been used in the treatment of various tumors, including melanoma. Also, GNPs may be used as biosensors, and they may be detected by optical absorption, fluorescence, and electrical conductivity [28]. The use of the confocal reflectance microscope with antibody-conjugated GNPs has made the development of highly sensitive cancer imaging possible [29]. They are biocompatible and not toxic and do not cause any immune response [30, 31].

Yang H and coworkers suggest that GNPs biodistribution and potential toxic effects are based on their size (1.5, 4.5, 13, 30 and 70nm in diameter) in non-pregnant and pregnant mice at different gestational ages (E5. 5, 7.5, 9.5, 11.5 and 13.5). Kinetic studies showed that4.5nm GNPs were primarily excreted through urine within 5h, whereas 30nm GNPs had a more prolonged blood circulation time and induced mild emphysema-like changes in lungs of pregnant mice. Studies also concluded that the distribution of GNPs in mice depends on the size of these nanoparticles and not gestational age [14].

Kim and collaborators have shown in a recent study that silver nanoparticles induce growth inhibition of cultures of *Escherichia coli* and *Staphylococcus aureus*, the antimicrobial properties being due to the silver ions (Ag). Their results suggest that Ag nanoparticles can be used as effective growth inhibitors of various bacterial pathogens and may be used also for improving the surface of medical devices for a better antimicrobial control [82]. Other authors have made silver nanoparticles by reduction of [Ag (NH (3)) (2)] (+) complex cation by four saccharides, two monosaccharides (glucose and galactose) and two disaccharides (maltose and lactose). The reduction of [Ag(NH(3))(2)](+) by maltose produced silver particles with a narrow size distribution, which showed antimicrobial and bactericidal activity against Gram-positive and Gram-negative bacteria (for example multiresistant strains such as methicillin-resistant *Staphylococcus aureus*). Silver nanoparticles have an antibacterial activity that was found to be dependent on the size of silver particles. A low concentration of silver gave antibacterial performance [81].

Other nanoparticles are mesoporous silica nanoparticles (MSN). MSN have attracted growing interest in the last decades as an efficient drug delivery system [15–17]. Compared with conventional organic carriers, MSN have special properties, as a uniform mesoporous structure which allow high drugs loading and high functionality [18-20].

Superparamagnetic iron oxide nanoparticles (SPIONs) have a magnetic moment in a magnetic field [32], which makes them interesting for use in MRI and other biomedical applications [34]. They are capable of producing high contrast per unit of nanoparticles, and small quantities of SPIONs are enough for imaging therapy, therefore reducing the toxicity issues [32, 33]. SPIONs can convert the energy supplied by an externally applied alternating magnetic field into heat [35]. This generated heat can be used for the selective destruction of a tumor, which is composed by cells that are more sensitive to heating than normal cells [32, 35]. SPIONs surface can be optimized to increase biocompatibility and biodegradability which makes them useful in many biomedical applications [36].

Another type of nanoparticles are magnetic gold nanoparticles (Fe3O4@Au NPs), which are usually conjugated with an active drug. Studies reported Fe3O4@Au NPs functionalized with thiol-terminated polyethylene glycol (PEG), and then loaded with anti-cancer drug, doxorubicin (DOX). The maximum DOX-loading capacity proved to be 100 μg DOX/mg for Fe3O4@Au NPs. Studies performed *in vitro* on MCF-7 cell line show that DOX loaded Fe3O4@Au NPs (Fe3O4@Au-PEG-DOX) have more potent therapeutic effect than free DOX (43).

Yi-Cheng Huang and Rou-Ying Li reported another type of nanoparticles used as an efficient carrier, chitosan / fucoidan nanoparticles (CS / F NPs). CS/F NPs proved to be stable for 25 days in phosphate-buffered saline (pH 6.0–7.4). The developed CS/F NPs were evaluated for their potential to be used as carriers for antibiotics delivery (gentamicin), in order to be used in treating airway disorders such as cystic fibrosis [37], asthma [38], pulmonary infections [39], pulmonary hypertension [40] and lung cancers [41]. Studies revealed that CS / F NPs controlled the release of gentamicin with an initial burst effect followed by a slow drug release phase. Overall, the experimental results indicated promising features of CS / F NPs use in developing pulmonary drug delivery systems [42].

3. CELL MEMBRANE PENETRATION

When investigating the biological impact of functionalized nanoparticles one key aspect is their interaction with cell membranes. Cell membranes are basic structures composed of, phospholipid bilayers which contain a unique set of

associated proteins, depending on the cell type. The two basic categories of integral membrane proteins are: cross-membrane proteins (proteins that penetrate all or part of the phospholipid bilayer and peripheral proteins which interact with the hydrophobic core of the bilayer [46] (Fig. 1).

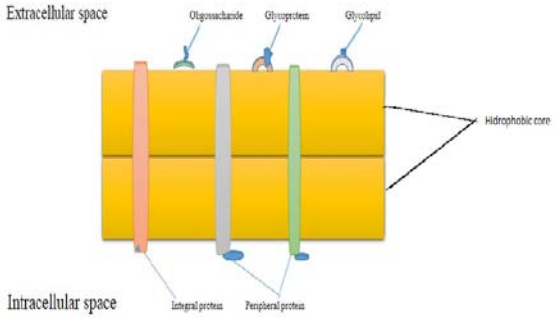

Figure 1. Schematic structure of the proteins in a biological membrane.

The phospholipid bilayer defines many of the physical attributes of the membrane (e.g. the fluidity at any temperature). Lipids are involved in cellular communication and they are arranged in a particular way: phosphatidylcholine outside, phosphatidylserine inside and glycolipids only on the external face [47, 48] (Fig. 2). Phospholipids are amphiphilic lipids characterized by the negative charge of the phosphate groups (at physiological pH) and non-polar fatty acids [49-51].

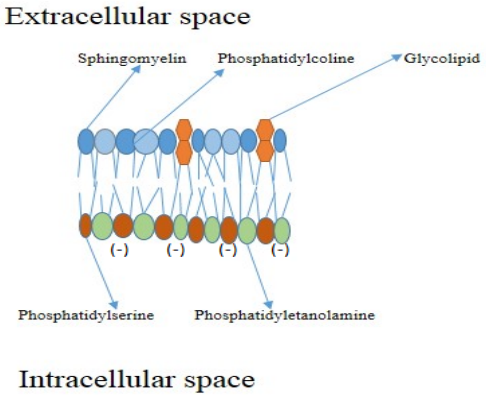

Figure 2. Glycolipids and main membrane phospholipids arrangement.

Lipid bilayer is a complex 3D network with a wide variety of physical characteristics that modulate cell signaling and function of the proteins. Lateral and transverse forces have a significant impact on the membrane and they may change rapid its shape by adding new constituents and eliminating the chemically modified ones. Recent studies have shown how differences in the structure of the double layer and the different parts of the bilayer membrane deformation can interact together to modify the activity of the transmembrane channels and peripheral membrane protein binding affinity. Lipid bilayer of the cell membrane may be compared with a passive film that blocks water flow and dissolved substances. The variety of lipids and the strictly controlled spatial organization defining the biophysical properties of the membrane, have an active role in the cell function.

Chemical compositions of both leaflets of the lipid bilayer are complex and very different from each other. For example, most of the anionic lipids are facing the cytoplasm of eukaryotic cells, while the glycosylated lipids are exposed to the extracellular environment. Differences in bilayer asymmetry between eukaryotic and prokaryotic membranes are essential for the activity of endogenous antimicrobial factors that lead to the specific destruction of bacterial membranes.

The consistency of the bilayer affects its mechanical strength and vice versa, the application of an external force on the membrane can alter its chemical composition [52 -54].

The lipid bilayer is a dynamic structure, where lipid molecules constantly change their location, permuted with the neighboring molecules in the same monolayer (translational motion in the monolayer = lateral diffusion); lipid molecules rotate rapidly around its axis (describing a conical surface) or migrate from the monolayer to other monolayer = motion "flip-flop" [55].

Flip-flop type movement is generally associated with asymmetric distribution of lipids in cell membranes. This asymmetry is crucial for many cellular functions and is involved in the mechanical stability of the cell membrane, controls the electrostatic interactions of membrane type and the functional role of membrane proteins [56].

This arrangement allows the cell membrane to be penetrated with different molecules / drugs. The penetration of membrane is controlled by some physico-chemical parameters, such as, basicity and lipophilicity. Since 77.5% of the drugs bear ionizable groups [57], they may have different charges depending on the position [63].

The ability of drugs to diffuse passively through biological membranes has long been known to be largely influenced by their lipophilicity [58]. Although a recent theory proposed that drug transport is only carrier-mediated and new transporters that possess specific transport characteristics will be discovered [59 - 67], it is more probable that passive and carrier-mediated processes coexist [60 - 62]. Recent experimental studies have shown the ability of tailoring the nanoparticle (NP)-cell interactions via the engineering of NP. Several studies have been done on the development of NPs used as drug delivery systems, however, NPs-cells interaction is not yet fully known. There are some studies that show the effects of NPs on the membrane surface pattern. There are two types of NPs sizes (stripy or patchy NP) which are responsible for an "insertion-rotation" penetration mechanism. Results indicate that stripy NPs and patchy NPs coated with narrow stripes or small patches can directly penetrate the cell membrane with a rotation. Observing spontaneous penetration of several NPs in a membrane vesicle, it was found that NPs lead changes in vesicle shape which can cause fluid loss and membrane rupture, implying the possible cytotoxicity of the NPs [64].

The cell uptake rate of nanoparticles (NPs) coated with mixed hydrophilic/hydrophobic ligands is known to be strongly influenced by the ligand pattern on the nanoparticle surface. Recent studies have used various combinations of hydrophilic and hydrophobic ligands bound on the surface of nanoparticles and their effect was analyzed during membrane translocation. The translocation of the NPs is facilitated by the constraint of their rotational degree of freedom by the anisotropic ligand, which blocks the free energy of the system from sinking to a deeper valley as the NP passes through the hydrophobic core of the bilayer. The forces required for almost instant penetration of these

patterned NPs across the bilayer are calculated and shown to be consistent with the free energy analysis. These findings are useful guidelines for the molecular design of patterned NPs for controllable cell penetrability [65].

4. NANOPARTICLES CELL UPTAKE

In order to reach their target most nanoparticles functionalized with various drugs, plant extracts or any other compounds used for various treatments have to cross the cell membrane.

For example, despite their high antimicrobial activity, silver nanoparticles proved to induce some severe changes of eukaryotic cells. In the interaction with erythrocytes (RBC), Ag nanoparticles induce changes that seem to be ireversibile. Studies revealed that these nanostructures may change the hem conformation of hemoglobin from the usual R-state (oxy-state) to the T-state (deoxy- states) [82]. The RBC membranes have glycoproteins rich in sialic acid that are responsible for the negatively charged surface. This electronegativity helps in preventing the interaction between RBCs and the other blood cells, and especially between each other [83]. The zeta potential is the electrostatic potential that exists at the shear plane of a particle; it is related to both surface charge and the particle's local environment. The electropositivity of Ag NPs makes them electrostatically attractive to RBC membranes. The negatively charged cell membrane is known to show a tendency to adsorb positively charged or neutral nanoparticles [84]. Nanoparticles adsorption on cell membrane involves electrostatic charges, van der Waals forces, steric interactions and/or surface charges. The nanoparticle uptake by cells comprises two steps: i) the binding of nanoparticles to the cell surface and, ii) the internalization of nanoparticles by specific endocytosis pathways [84]. RBCs are not expected to uptake nanoparticles by endocytosis but their entrance can be affected through the RBC membrane's ion transport channels [82].

The interaction of silver nanoparticles with cell membranes is very important. It has been shown that the charge on the silver ion is crucial for its antimicrobial activity; the mechanism is based upon electrostatic attraction between the negatively-charged cell membrane of a micro-organism and the positive charge of the Ag+ comprised within the Ag NPs [85]. Thiol-containing proteins in the cell membrane are likely to be one of the effective Ag+ ion binding sites [86]. The cysteine residues of NADH dehydrogenases that are present in NADH ubiquinonereductase complexes (Complex I) of *Escherichia coli* may also be possible sites for Ag+ ion binding [86, 87].

In the case of silica nanoparticles, studies revealed that for a better penetration and cytosolic dispersion they must be covered with lipids. This coated silica nanoparticles have a lower aggregative pattern and single NPs are highly absorbed [88].

PLGA nanoparticles may enter into bone cells and therefore can be used in various orthopedic treatments functionalized with various drugs [11]. PLGA microspheres fused with β-sheet breaker peptide H102 can enter CNS (central nervous system) cells and may be used in the treatment of diseases such as Alzheimer's [12]. Nanocarriers for retinyl palmitate (RP) loaded nanoemulsions (NE) staying, liposomes (LPs) and solid lipid nanoparticles (SLNs) can enter epithelial cells and are used in various skin treatments and cosmetics [13]. Gold nanoparticles functionalized with various drugs as well as gold magnetic nanoparticles (Fe3O4 @ Au NPs) penetrate tumor cells in melanocytes [28, 43]. Mesoporous silica nanoparticles (MSN) that are well suited to merge with drugs, enter through the cell membranes usually helped by wall lipids [88, 15- 17, 18-20]. Superparamagnetic iron oxide nanoparticles (SPIONs) used in MRI may specifically enter tumor cells [34, 36]. On the other hand, some nanosystems are able to specifically target some normal cells, as for example chitosan / fucoidan nanoparticles (CS / F NPs) may enter the cells of the lungs and are used in the pulmonary drug delivery system [42].

The plasma membrane is a selectively permeable membrane which defines the intracellular limit and maintains the essential part of the cell together. Small polar molecules such as O_2 and CO_2 diffuse easily into the lipid bilayer while polar molecules such as ions and larger nanomaterials are incapable to cross the membrane on their own. In nature, proteins, ions, and different nanometer size materials are transported through channels in the lipid bilayer formed by specialized transport proteins [89]. Most macromolecules at nanoscale and molecular level are internalized by endocytosis, as is the case of nanoparticles (Fig. 3) [90].

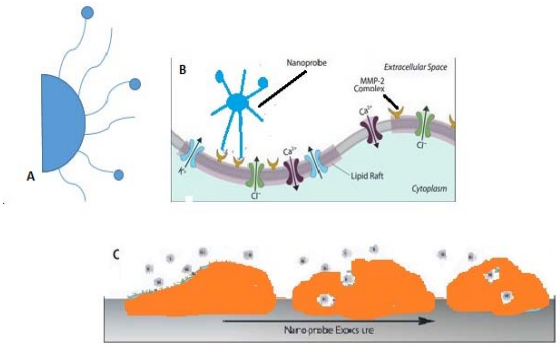

Figure 3. Schematic illustration of nanoparticles incorporation by endocytosis.

Cho *et al.*, recent study examined the role of surface charge in the internalization of gold nanoparticles [91]. In this study, it was noted that neutral and negatively charged nanoparticles were absorbed less on negatively charged membrane cells and therefore have low levels of internalization compared to the positive charged particles. Other studies have examined the cellular uptake of negatively charged nanoparticles with various base materials. Villanueva *et al.* have studied the uptake of iron oxide nanoparticles (size of aggregates) functionalized with carbohydrates on a human cervical carcinoma cells line (HeLa) [92]. It was observed that the surface absorption and toxicity of nanoparticles depends on the negative charge. Anionic nanoparticles of iron oxide (Fe_3O_4) with molecular surface coatings showed high nonspecific internalization, they strongly interacting with the plasma membrane as shown by magnetic complementary tests as magnetoforesis (MP) and electron spin resonance (ESR) [93]. The internalization of negatively charged nanoparticles is believed to occur through nonspecific binding particles clustering on the cationic sites on the plasma membrane

(which are relatively rare than negatively charged areas), which are then subjected to subsequent endocytosis [94].

To better understand the mechanisms of interaction of nanoparticles with cells, there have been several studies that have examined the interaction between nanoparticles and bilateral synthetic lipids and their nature. Holl *et al.* investigated the effect of positively charged dendrimers on bilateral lipids [95]. Studies revealed that positively charged dendrimers destabilized cell membranes and were able to form pores, of about15-40 nm in diameter for G7as observed by AFM studies [96].

Nanoparticles can be embedded in the cell by two major mechanisms pinocytosis and phagocytosis. Phagocytosis occurs for particles larger than 0,5 mm and involves a limited number of mammalian cells as macrophages, monocytes and neutrophils. Pinocytosis is a general uptake process that is divided in macropinocytosis or micropinocytosis. Non-selective uptake of macromolecules with a diameter greater than 0.2 pm involves macropinocytosis [97], while micropinocytosis (clathrin-mediated, caveolae / lipid, and clathrin / caveolae-mediated independent floats) occurs for smaller particles in all cell types. Therefore given the size regime of NPs used for current therapeutic purposes (10-200 nm), is more likely that they will enter cells predominantly by micropinocytosis. Although some essential progress have been made on the NPs cellular uptake, additional studies aiming to reveal the specific delivery pathway of different types of nanosystems are needed in order to facilitate their medical use.

4. CONCLUSIONS

In conclusion, using different types of nanoparticles functionalized with various drugs we bring important clues in most biomedical areas. These nanostructured systems not only reduce drug toxicity, but they also facilitate a better cellular penetration and a more controlled target delivery. This paper offers a current overview on the nanoparticles membrane penetration and reveals the gaps of knowledge regarding their specific cellular and membrane action.

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