Materials Science and Engineering C 70 (2017) 889–896

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Review

Preferential binding of positive nanoparticles on cell membranes is due to electrostatic interactions: A too simplistic explanation that does not take into account the nanoparticle protein corona

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ARTICLE INFO

Article history:
Received 31 May 2016
Received in revised form 30 August 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Nanoparticle
Protein corona
Nanoparticle/cell interaction
Cellular uptake

ABSTRACT

The internalization of nanoparticles by cells (and more broadly the nanoparticle/cell interaction) is a crucial issue both for biomedical applications (for the design of nanocarriers with enhanced cellular uptake to reach their intracellular therapeutic targets) and in a nanosafety context (as the internalized dose is one of the key factors in cytotoxicity). Many parameters can influence the nanoparticle/cell interaction, among them, the nanoparticle physico-chemical features, and especially the surface charge. It is generally admitted that positive nanoparticles are more uptaken by cells than neutral or negative nanoparticles. It is supposedly due to favorable electrostatic interactions with negatively charged cell membrane. However, this theory seems too simplistic as it does not consider a fundamental element: the nanoparticle protein corona. Indeed, once introduced in a biological medium nanoparticles adsorb proteins at their surface, forming a new interface defining the nanoparticle "biological identity". This adds a new level of complexity in the interactions with biological systems that cannot be any more limited to electrostatic binding. These interactions will then influence cell behavior. Based on a literature review and on an example of our own experience the parameters involved in the nanoparticle protein corona formation as well as in the nanoparticle/cell interactions are discussed.

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http://dx.doi.org/10.1016/j.msec.2016.09.016
0928-4931/

1. Introduction

The knowledge of nanoparticle/cell interactions is of paramount importance in nanomedicine and nanotoxicology. Indeed, when nanoparticles are used for biomedical purposes, therapeutic and/or diagnostic agents must generally enter the cells to reach their targets. Furthermore, because of their size, high reactivity and large surface area nanoparticles can interact with cell components, potentially inducing side effects and toxicity. Therefore, the investigation of the underlying mechanism of cellular uptake is a crucial issue for the understanding of the biological fate of nanoparticles as well as potential adverse aspects [1].

Nanoparticle/cell interactions are largely influenced by the nanoparticle physico-chemical characteristics, for instance features such as size, shape, surface chemical functions, etc. seem to have a major impact on nanoparticle binding to cell membrane and the subsequent cellular uptake [2,3]. In most cases, the nanoparticle surface provides the driving forces (electrostatic, hydrophobic and hydrophilic (polar) forces) for the cellular internalization and decides the uptake pathway [4]. This latter will have a deep effect on cell response.

In particular, it is commonly admitted that positively charged nanoparticles interact more with cell membranes than neutral or negatively charged nanoparticles. This preferential binding is supposedly due to favorable electrostatic interactions, as cell membranes are negatively charged. However, and as already expressed in a previous opinion paper [5], this explanation seems too simplistic as it only considers the nanoparticle charge whereas many other parameters are involved. For example, the nanoparticle protein corona is now acknowledged to play a critical role. The aim of this review is to draw attention on this issue and further demonstrate with details that the above-mentioned theory is too reductive and that other parameters should not be neglected.

2. General considerations on cellular uptake of nanoparticles

Nanoparticles can be uptaken by cells through endocytosis. As reviewed by Wang et al. [6], endocytosis can be subdivided into phagocytosis (cell eating, mainly for large particles) and pinocytosis (cell drinking, rather for small particles, fluids and solutes). Phagocytosis primarily occurs in macrophages and polymorphonuclear neutrophils. In contrast, pinocytosis occurs in all types of cells through at least four distinct mechanisms: macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and clathrin- and caveolae independent endocytosis [6]. It is also worth noting that endocytosis can be either non-specific or receptor-mediated [7]. The uptake of nanoparticles by cells occurs through two steps: binding to the cell membrane and then internalization. The first one seems to be most affected by the physico-chemical characteristics of the particles and especially the surface charge [7,8].

As previously mentioned, it is commonly acknowledged that positively charged nanoparticles are more internalized by cells than neutral or negatively charged nanoparticles [1,2,4,7,9–15]. Some authors have even observed a strong correlation between the amount of positive charges and internalization into cells [2]. The accepted explanation lies in the fact that electrostatic interactions are favored with cell membrane that is negatively charged.

This observation seems to be a general tendency, observed with nanoparticles of various chemical bulk compositions (silica, gold, iron oxide, etc.) and in various cell types. Indeed, several studies aiming at getting insight into the nanoparticle uptake efficiency by cells compare cell internalization of nanoparticles of similar size and shape, differing only in their surface charge owed by different surface chemical coatings. For example, Kralj et al. [9] used silica-coated maghemite nanoparticles functionalized either with amine groups for positive surface charge or with carboxyl groups for negative surface charge and clearly showed that the positively charged nanoparticles were internalized into the cells to a much higher extent than the negative ones in two different cell lines. Similarly, Lankoff et al. [16] modified the surface of silica nanoparticles with vinyl or aminopropyl/vinyl groups and observed that the uptake of these latter by lymphocytes was more efficient than that of the former. The same conclusion was reached by Ge et al. [17] using magnetic iron oxide nanoparticles coated with chitosan (positive charge) or with dimercaptosuccinic acid (DMSA, negative charge) in a model of oral squamous carcinoma cell.

All these examples illustrate why positively charged nanoparticles are usually chosen as carriers for drug or gene delivery [1,2,15]. Consequently, nanoparticle surface functionalization by modifying surface charge is an efficient and easy way to alter cellular uptake rate [4,9,11,18].

At this point, we should specify some definitions as behind the term of charge many concepts could be hidden depending on the level of observation (macro- or micro-scale) and they should involve different levels of subtlety and complexity.

3. Nanoparticle charge and cell membrane charge, what are we precisely talking about?

Concerning nanoparticles, the term charge is confusing as it could refer to distinct and complex physical quantities. Indeed, when a nanoparticle is exposed to a fluid, a double electrical layer appears on its surface, consisting of two parallel layers of charges surrounding the particle, as illustrated in Fig. 1.

The first layer, called the Stern layer, corresponds to the primary electric surface potential (caused by protonation/deprotonation reactions on the surface) and ions from the bulk electrolyte strongly bound to its surface. Indeed, when immersed in an electrolyte, a nanoparticle develops a surface charge mainly associated with the hydroxylation of its surface and the specific ion adsorption due to chemical interactions. The second diffuse outer layer is composed of free ions attracted to the primary electric surface potential of the particle under the influence of electric attraction and thermal motion rather than being firmly anchored. The diffuse layer electrically screens the Stern layer. In other words, the surface charge of the nanoparticle interacting with dissolved ions in the bulk dispersant induces an electrical neutralization by accumulation of counterions. As the particle moves, a boundary exists between the ions in the diffuse layer moving with the nanoparticle and free ions that remain with the bulk dispersant. From a theoretical viewpoint, the electric potential is defined as the local electrical potential at the slipping plane that separates the mobile phase and the stationary layer of fluid attached to the particle. Thus the zeta potential is widely used in the literature for quantification of the magnitude of the nanoparticle charge. However, the zeta potential is rigorously not equal to the electric surface potential nor to the Stern potential because these are defined at different locations in the electrical double layer [19].

Regarding cell membrane, surface charge and membrane potential are often confused and a sharper distinction should be done between the two as they refer to different concepts. Indeed, surface charge corresponds to the distribution of charges in a surface whereas membrane potential is due to ion distribution between both sides of the membrane following the Nernst principle [20]. To better understand let's briefly come back to the structure of plasma membrane. As shown in Fig. 2 (left part), mammalian cell membranes consist of phospholipid bilayers where proteins are inserted. Lipids and proteins of the outer leaflet are generally glycosylated (with oligosaccharides oriented toward the extracellular environment). Consequently, the surface of the cell is covered by a carbohydrate coat, known as the glycocalyx, bearing negative charges [20].

On the other hand, membrane potential originates from the difference in ion concentrations between the cytoplasm and the extracellular compartment and the selective permeability of the plasma membrane for ions [20]. Many ions have a concentration gradient across the membrane but potassium (K^+) plays a major role because cell membrane is particularly permeable to this ion. As illustrated in the right part of Fig. 2,