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Physicochemical Properties Affecting the Potential *in vitro* Cytotoxicity of Inorganic Layered Nanoparticles

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Abstract

Inorganic layered nanoparticles, also known as anionic nanoclays, have great potential as delivery carriers, since they could efficiently intercalate anionic molecules into the gallery spaces and release the intercalated molecules in a controlled manner. They also exhibit low toxicity compared to other widely studied inorganic nanoparticles for biological purposes. In this review, we summarize and describe physicochemical factors influencing the cytotoxicity of layered nanoparticles in cultured cell lines for providing information on their optimum characteristics with minimized adverse effects.

Keywords: Inorganic layered nanoparticles, Chemical composition, Particle size, Chemical stability, Cytotoxicity

Introduction

Inorganic nanoparticles have recently attracted a great deal of attention as carriers for effectively and selectively delivering bioactive molecules or drugs into cells. Among them, anionic nanoclays typically stand for layered metal hydroxide (LMH) nanoparticles with a hydrotalcite-like structure, contained positively charged metal hydroxide layers in stacked composition, where their interlayer spaces are filled with charge-compensating anions and water molecules (Figure 1)[1]. The general formula for LMHs are expressed as $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{-}(A^{m-})_{x/m}$, nH_2O, where M^{n+} represent metal cations (M^{2+}=Mg^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+},…, M^{3+}=Al^{3+}, Fe^{3+},…) which compose the main layer with the positive charge, and A^{m-} are interlayer anions (A^{m-}=CO_3^{2-}, NO_3^{-}, Cl^{-}, SO_4^{2-}, and etc.), located at the center of octahedral OH^{-} ions. Thus, LMHs could serve as host materials to intercalate a variety of anionic molecules into the interlayer spaces. Many studies demonstrated that LMHs could efficiently deliver drugs or bioactive molecules into cells with strong correlations for their anion intercalation capacity, pH-dependent solubility, and controlled-release properties[2,3]. Moreover, recent study showed that the energy-dependent endocytic pathway, clathrin-mediated endocytosis plays an important role in the internalization of LMHs[4,5]. Hence, LMHs promoted very fascinating properties as delivery carriers, since LMHs could be easily and actively taken up by cells, giving rise to enhance delivery efficiency and drug efficacy as well[6,7]. On the other hand, easy uptake of LMHs by the human body raises concerns about their toxicity, especially in highly exposed conditions.

Toxicological effects of LMHs on human cell lines were recently investigated, although little information is actually available on the toxicity of LMHs in animal models, hence, their mechanism of toxicity remains to be determined. Comparative cytotoxicity study of inorganic nanoparticles showed that LMHs exhibited low toxicity in comparison with other inorganic nanoparticles such as carbon nanotube, iron oxide, and silica, indicating their great potentials for biological applications[8-10]. Study on the toxicity of nanoparticles would be essential to provide information about their dose-response relationships in biological systems.

In this review, we describe physicochemical properties affecting the cellular toxicity of LMHs in cultured cell lines: chemical composition, particle size, and chemical stability. Understanding the physicochemical characteristics of nanoparticles with respect to the cellular response would be useful to minimize their adverse effects, and to expand their biological and pharmaceutical applications.

Chemical Composition

The chemical composition of LMHs would be of importance for the toxic properties of nanoparticles, because their intrinsic chemical characteristics could influence the biological systems with different toxic effects, not as nanoparticles[11]. As mentioned above, LMHs are composed of positively charged metal hydroxide sheets with interlayer anions. The most

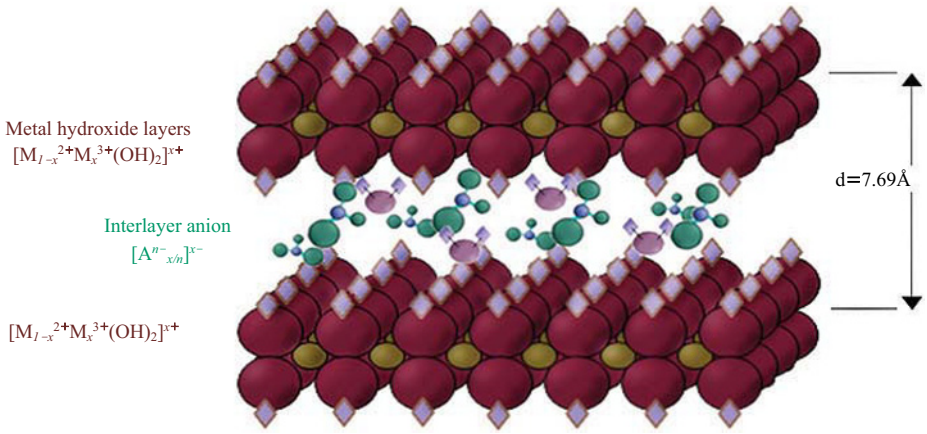

Figure 1. Schematic structure of anionic nanoclays, LMH nanoparticles.

widely applied divalent cations are Mg^{2+} or Zn^{2+}, and Al^{3+} is generally used as trivalent cations to form the layers. Thus, LMHs with two different formulas of $Mg_{0.68}Al_{0.32}(OH)_2(CO_3)_{0.16} \cdot 0.1H_2O$ (MgAl-LMH) and $Zn_{0.68}Al_{0.32}(OH)_2(CO_3)_{0.16} \cdot 0.1H_2O$ (ZnAl-LMH) were prepared by coprecipitation method[8,12]. The toxicity of each LMH was measured by the lactate dehydrogenase (LDH) leakage assay, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and hemolysis test[8]. The result showed that ZnAl-LMH released slightly higher concentrations of LDH into the extracellular medium than MgAl-LMH at high concentration (250-500 μg/mL). And the former (ZnAl-LMH) caused more hemolysis than the latter (MgAl-LMH) after long incubation time of 11-24 h, although the hemolytic effect of each LMH was negligible (less than 2%). It is note-worthy here that there was no significant difference in particle sizes; both MgAl-LMH and ZnAl-LMH were determined to be about 200 nm. In addition, the actual dissolved concentrations of Mg^{2+} or Zn^{2+} ions from the two LMHs were not that different under the experimental conditions. Hence, the result may be explained by the high cellular toxicity of Zn^{2+} ions compared to Mg^{2+} ions[13]. Recent research on the comparative cytotoxicity of manufactured nanoparticles with similar diameter and various elemental compositions also demonstrated clearly that copper- and zinc-based nanoparticles appeared to be the more toxic than titania, alumina, ceria, zirconia, and tungsten carbide-based nanoparticles[14]. These studies highlight the correlation between chemical compositions of nanoparticles and their cytotoxicity.

Particle Size

The most critical factor to determine the toxicity of nanoparticles could be the size itself. Many studies demonstrated that nanoparticles, so-called ultrafine particles, exhibited higher toxicity than micro-sized particles, since they possessed large surface area, high chemical reactivity, and easy cell penetration capacity[15-17]. LMHs of four different sizes of 50, 100, 200, and 350 nm were prepared under the hydrothermal condition to obtain homogeneous particle sizes, and then, the cytotoxicity of each LMH was evaluated by measuring the released levels of LDH or pro-inflammatory mediators like interleukin (IL)-8 into the medium[9]. The smallest particles tested, 50 nm, induced higher release of LDH as well as IL-8 than larger particles (100, 200, and 350 nm), suggesting the size-dependent toxicity of LMH nanoparticles in cultured cell lines. Nano-sized copper, silica, and zinc particles were also reported to have high toxic effects compared to micro-sized copper, silica, and zinc particles, respectively[18-20]. However, it is not clear whether a wide range of nanoparticles with various chemical compositions are more toxic than micro-sized particles. In the case of titanium dioxide, it was recently reported that its micrometer particles caused more DNA damage than the nanoparticles, and the size-dependent toxicity of iron oxide was not found[21]. More extended case study on the relationship between particle size of nanoparticles and their harmful effects should be performed to ascertain the toxicity of nano-sized particles.

Chemical Stability

The toxicity of nanoparticles could be strongly related to their chemical and structural stabilities under physiological conditions. In the case of LMH nanoparticles, one of the important key factors, affecting their stability, is the type of interlayer anions. The two most widely used forms of LMHs, carbonate form (LMH-CO_3) and chloride one (LMH-Cl), were evaluated for the cytotoxicity[22]. LMH-CO_3 is generally recognized as the most stable form of LMH, because

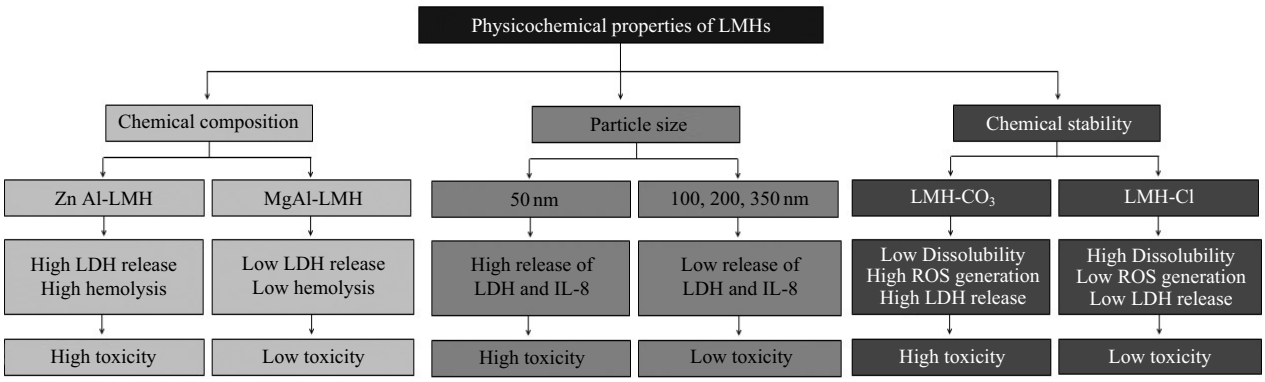

Figure 2. Relation between the physicochemical properties of LMH nanoparticles and their cytotoxicity determined *in vitro*.

divalent carbonate ions reveal high affinity for the positively charged LMH layers[22]. Moreover, natural abundance of CO_3^{-2} is also associated with the chemical stability of LMH-CO_3, since carbonate ions are easily generated abundantly by the spontaneous interconversion between water soluble carbon dioxide (CO_2) and carbonic acid (H_2CO_3). On the other hand, LMH-Cl is often applied as a precursor for biological purpose due to low toxicity of chloride anions intercalated into the gallery spaces. The stability of each LMH was evaluated in terms of its dissolubility in simulated body fluid (pH 7.4) and lysosomal condition (pH 4.5), respectively[22]. The partial dissolution rate of LMH layers into Mg^{2+} ions in the supernatant was measured with inductively coupled plasma atomic emission spectroscopy (ICP-AES). The result showed that LMH-Cl was more easily dissolved under both simulated body fluid and lysosomal conditions than LMH-CO_3, indicating high stability of the latter (LMH-CO_3) compared to the former (LMH-Cl). Interestingly, LMH-CO_3 produced more remarkable reactive oxygen species (ROS) and induced more LDH leakage than LMH-Cl, suggesting high toxicity of LMH-CO_3 compared to LMH-Cl. This result could be associated with the dissolution property of each LMH, suggesting the correlation between the high stability of LMH-CO_3 and its elevated cytotoxicity. It is, therefore, concluded that the non-degradable form of LMH exhibits more cytotoxic effects than biologically and easily degradable one. However, low chemical and structural stability of some nanoparticles could cause increased toxicological effects, for example, when metallic nanoparticles such as $Ce^{4+}O_2$, $Mn_3^{2+/3+}O_4$, and $Fe_2^{3+}O_3$, are dissolved, and their relativities could change the reduction or oxidation reactions in biological conditions[23].

The strong relationships between the physicochemical properties (chemical composition, particle size, and chemical stability) of LMH nanoparticles and their cytotoxicity are summarized in Figure 2.

Conclusions

Nanomaterials will open a new era for pharmaceutical and medical applications such as target specific delivery and precise diagnostic systems in the near future. The physicochemical properties of nanoparticles should be well defined and characterized to find their optimum composition, size, shape, surface charge, stability, and etc. and subsequently to obtain high efficiency. It is indisputable, but the physicochemical properties of nanoparticles also affect the toxicity. We have pointed out that the cytotoxicity of LMH nanoparticles could be strongly dependent on their chemical composition, particle size, and structural stability. Of course, as of now, these studies would be limited to *in vitro* cell lines tested. More extended *in vivo* case studies on the relations between physicochemical characteristics of nanoparticles and their adverse effects should be investigated, therefore, crucially developing nanotechnology safely and ascertaining the toxicity of nano-sized materials.

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