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Cell membrane disruption induced by amorphous silica nanoparticles in erythrocytes, lymphocytes, malignant melanocytes, and macrophages

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ABSTRACT

It is of critical importance to examine carefully the potential adverse effects of engineered nanoparticles (NPs) on human health and environments. In the present study, we have investigated the disruption of cell membranes induced by amorphous silica NPs in erythrocytes, lymphocytes (Jurkat), malignant melanocytes (B16F10), and macrophages (J774.1); these four types of mammalian cells have distinctive characteristics in terms of nucleated/non-nucleated cells, adherent/non-adherent cells, endocytosis, and phagocytosis. The silica-induced membranolysis was examined by exposing these different cells to serum-free culture media containing the amorphous silica NPs of different diameters (28, 50, 55, 156, and 461 nm) under similar conditions. We investigated how the silica-induced membranolysis of the cells of different origins is influenced by the size and dose of the silica NPs. Additionally, the interaction forces of a silica microsphere with a living cell or a giant unilamellar vesicle composed of zwitterionic phosphatidylcholine lipids were measured by colloid-probe atomic force microcopy, whereby the affinities of silica surface for plasma membranes and protein-free phospholipid membranes were estimated. Possible mechanism of the silica-induced membranolysis was discussed.

1. Introduction

The recent advancement in nanotechnology enables us to produce a variety of functional nanoparticles (NPs) with unique physicochemical, optical, and/or electrical properties such as large specific surface area, high reactivity, tuned photoluminescence, and high electric conductivity, which differ from those of bulk materials of the same composition. The increasing use of these NPs in commercial and consumer products has given rise to heightened concern for their biological activity and environmental impact. It is therefore of critical importance to address the potential adverse effects of NPs on human health and environments.

In spite of the very simple chemical formula of silicon dioxide (SiO_2), silica exists in a very large number of different forms, which are characterized by crystallinities and specific physicochemical surface properties [1]. Since silica is a common mineral found naturally in sand and rock, silica exposure is usual in lots of occupations such as mines and quarries, where inhalation of crystalline silica dusts causes a lung disease (silicosis) [2–4]. Recently, the amorphous silica NPs possessing uniform size and shape as well as the nonspherical morphologies and/or the mesoporous structures have been produced for industrial and biomedical applications [5–9]. For this reason, the effects of silica on human health should be still extensively investigated in vitro and in vivo [10–12].

The impacts of silica on red blood cells (RBCs) have been investigated; consequently, the silica particles are found to exhibit hemolytic activity against washed RBCs in serum-free buffered saline within a few fours [13,14], where the hemolytic activity is indicative of the degree of membrane damage. The hemolytic activity of silica varies according to the structural forms of silica such as crystallinity [15] and porosity [5–9], and is significantly reduced by (pre)treatment of the silica particle surfaces with serum [13,16,17], some proteins [16–19], or some chemicals [5–7,14,16,17,20–22]. Nonetheless, the hemolytic activity of amorphous silica NPs with uniform size and shape other than crystalline silica dusts remains to be explored [7,23].

In addition to the hemolytic activity, the cytotoxic effects of silica NPs on various types of cell lines and primary cells (originated from different kinds of tissues and organs) have been examined in terms of various cellular responses such as disruption of cell membrane, viability of cells, inhibition of cell growth, transformation, and release of various factors (e.g. cytokines): the target cells

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include lymphocytes [24], macrophages [25–31], epithelial cells [28,32–37], endothelial cells [27,38–40], mesothelial cells [41], keratinocytes [33,42], fibroblasts [34,37], neuroblasts [24,36], hepatic cells [43], myoblasts [44], and kidney cells [45]. The testing strategy and the treatment conditions vary study by study, because the robust methodology has not been established for assessing the cytotoxicity of NPs. Indeed, the cytotoxicity outcome of NPs is influenced by assay conditions, such as the addition of serum or surfactants [28,37] and the procedure/medium for rinsing the cell surfaces before exposure to NPs (according to our experience): the former alters the surface properties of NPs and cells, and the latter determines whether the serum proteins added in the culture medium and/or the extracellular proteins secreted by cells are efficiently removed from the cell surfaces or not. For these reasons, reliable in vitro studies must contain the detailed information of the assay conditions, which generates consistent toxicity data on NPs. Unfortunately, many of the reported studies lack of such information; this is the case for the cytotoxicity studies of silica NPs.

Our main focus of the present study is placed on the rupture of cell membrane caused by amorphous silica NPs, which is considered as a short-time cellular response to high-dose silica NPs [46]. We have examined this silica-induced membranolysis by exposing four different types of mammalian cells to serum-free media containing the amorphous silica NPs of different diameters (28, 50, 55, 156, and 461 nm) under similar conditions, whereby the results of membranolysis for the different types of cells can be compared with each other. The target cells include erythrocytes (rabbit RBCs) as well as three cell lines of Jurkat (T lymphocytes), B16F10 (malignant melanocytes), and J774.1 (macrophages); the characteristics of these four types of cells significantly differ from each other, as summarized in Table 1. We have investigated how the silica-induced membranolysis of these cells of different origins is influenced by the size and dose of the silica NPs. In order to estimate the affinity of silica surface for plasma membranes, we have measured the interaction forces of a silica microsphere with a living cell or a giant unilamellar vesicle (GUV) composed of zwitterionic phosphatidylcholine lipids, using colloid-probe atomic force microcopy (AFM).

2. Materials and methods

2.1. Reagents and culture media

Culture media of RPMI 1640 (11875-093), phenol-red-free RPMI 1640 (11835-030), DMEM (21063-029, Dulbecco's modified Eagle medium with 25-mM D-glucose, 4-mM L-glutamine, and 25.03-mM HEPES buffer, without sodium pyruvate or phenol red), and DPBS (14190-094, Dulbecco's phosphate-buffered saline without calcium or magnesium) were purchased from Life Technologies (Carlsbad, CA, USA), and MEM (05900, Eagle's minimum essential medium with kanamycin, without L-glutamine or sodium bicarbonate) was from Nissui Pharmaceutical (Tokyo, Japan). Sodium chloride (NaCl), L-glutamine, and sodium bicarbonate were purchased from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA) was heat-inactivated in prior to use. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in chloroform (25 mg/mL = 31.8 mM) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Chloroform, acetone, ethanol (Kishida Chemical, Osaka, Japan), and methanol (Sigma–Aldrich, St. Louis, MO, USA) were used without further treatment: these reagents were of analytical grades. All water used in the experiments was purified using a system composed of Elix Advantage 3 (Millipore, Billerica, MA, USA) and WR600A (Yamato Scientific, Tokyo, Japan) to give a resistance of 18.2 MΩ cm and a total organic carbon of less than 20 ppb.

2.2. Red blood cells (RBCs)

The red blood cells (RBCs) were collected from rabbit blood preserved in Alsever's solution (0105-1; Nippon Biotest Laboratories, Tokyo, Japan). The RBCs were gently rinsed three times with DPBS through a series of centrifugation (at a gravitational field of 750 g for 5 min), aspiration of the supernatant, and redispersion of the remained cell pellet. Consequently, the RBC suspension of 7.5×10^9 cells/mL was obtained.

2.3. Cell lines and culture conditions

The human lymphocyte cell line of Jurkat (JCRB0147; JCRB Cell Bank, Osaka, Japan) was cultured in a complete medium composed of an RPMI 1640 medium, a 1% penicillin–streptomycin mixed solution (26253-84; Nacalai Tesque), and 10% FBS. The murine melanoma cell line (B16F10; ATCC CRL-6475) was cultured in a complete medium composed of MEM, 2-mM L-glutamine, and 10% FBS; additionally, sodium bicarbonate was used to adjust the pH to 7.4. The murine macrophage cell line of J774.1 (JCRB0018; JCRB Cell Bank) was cultured in a complete medium composed of an RPMI 1640 medium and 10% FBS. The non-adherent (Jurkat) and the adherent (B16F10 and J774.1) cells were cultivated statically in a flask with nontreated surface (1110-075; IWAKI, Tokyo, Japan) and in a flask with tissue culture treated surface (3110-075; IWAKI), respectively; every flask had a culture area of 75 cm^2 and contained 10 mL of the complete medium. These flasks were stored in an incubator, inside which a moist atmosphere of 5.0% CO_2 was kept at temperature of 37.0 °C. This ensured that the complete medium maintained the physiological pH of 7.4. The complete medium for each cell line was changed every 2 days. The cells were subcultured every 4 days, when they were grown at the concentration of about 1.0×10^6 cells/mL for Jurkat and at the surface density of 1.4×10^5 cells/cm^2 for B16F10 and 1.3×10^5 cells/cm^2 for J774.1.

2.4. Zeta potentials of cells

The electrophoretic mobility of the cells (RBC, Jurkat, B16F10, and J774.1) in 100-mM NaCl solution was measured at room temperature, using a homemade apparatus, where a rectangular silica-glass cell (10-mm × 1-mm cross section), a cell holder, and a pair of platinum electrodes for Micro-Electrophoresis Apparatus Mark II (Rank Brothers, Cambridge, UK) were employed. Before

Table 1
The properties of vesicles and mammalian cells employed in the present study.

Cell	Membrane proteins	Nucleated	Adherent	Endocytosis	Phagocytosis
DOPC vesicles	No	No	No	No	No
Erythrocyte (RBC)	Yes	No	No	No	No
Jurkat	Yes	Yes	No	Yes	No
B16F10	Yes	Yes	Yes	Yes	No
J774.1	Yes	Yes	Yes	Yes	Yes