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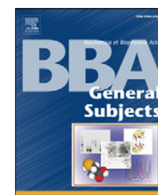

Physicochemical properties and in vitro intestinal permeability properties and intestinal cell toxicity of silica particles, performed in simulated gastrointestinal fluids

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ABSTRACT

Background: Amorphous silica particles with the primary dimensions of a few tens of nm, have been widely applied as additives in various fields including medicine and food. Especially, they have been widely applied in powders for making tablets and to coat tablets. However, their behavior and biological effects in the gastrointestinal tracts associated with oral administration remains unknown.
Methods: Amorphous silica particles with diameters of 50, 100, and 200 nm were incubated in the fasted-state and fed-state simulated gastric and intestinal fluids. The sizes, intracellular transport into Caco-2 cells (model cells for intestinal absorption), the Caco-2 monolayer membrane permeability, and the cytotoxicity against Caco-2 cells were then evaluated for the silica particles.
Results: Silica particles agglomerated in fed-state simultaneous intestinal fluids. The agglomeration and increased particles size inhibited the particles' absorption into the Caco-2 cells or particles' transport through the Caco-2 cells. The in vitro cytotoxicity of silica particles was not observed when the average size was larger than 100 nm, independent of the fluid and the concentration.
Conclusion: Our study indicated the effect of diet on the agglomeration of silica particles. The sizes of silica particles affected the particles' absorption into or transport through the Caco-2 cells, and cytotoxicity in vitro, depending on the various biological fluids.
General significance: The findings obtained from our study may offer valuable information to evaluate the behavior of silica particles in the gastrointestinal tracts or safety of medicines or foods containing these materials as additives.

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1. Introduction

Nanomaterials are materials that have at least one dimension in the nanoscale range (approximately 1 nm to 100 nm). Recently, nanomaterials have been applied in various fields, including medicine, cosmetics, and foods, because nanomaterials may have physical, chemical, or biological properties that are different from those of their bulk. While nanotechnology can exploit the improved and often novel properties of materials, there have been publications about concerns regarding the safety to humans and potential environmental impact of such materials [1–4].

In the medical field, nanomaterials have been used as drug carriers for drug delivery systems (DDS) [5–8].

Amorphous silica particles have been widely applied as additives for various purposes, for example, to improve the flowability used in powders for making tablets; they have also been applied as additives to coat tablets, to improve their hardness. In solid oral dosage form, silicates are often used as glidants. Glidants are substances that improve the flowability of cohesive powders and granules. Silicates are well suited for that purpose, because of their small particle size and large specific surface area. One of the most frequently used glidants is colloidal silica (e.g., Aerosil 200), which exhibits very small particle sizes in the nanometer range, and a large specific surface area of approximately 200 m^2/g [9]. Although the primary dimensions of these particles are a few tens of nm, they form aggregates of a few hundred nm. These novel materials have been the focus of medical developments in a number of areas, and many researchers have investigated their use not only as additives in tablets, but also as novel carriers for poorly-water-soluble drugs [10–12].

Amorphous silica particles have also been used in other fields, where they are applied directly to the human body as ingredients in cosmetics and toothpaste, or even as powdered food ingredients to prevent caking [13,14].

Abbreviations: DDS, drug delivery system; PDI, polydispersity index; TEM, transmission electron microscopy; FaSSGF, fasted-state simulated gastric fluids; FeSSGF, fed-state simulated gastric fluids; FaSSIF, fasted-state simulated intestinal fluids; FeSSIF, fed-state simulated intestinal fluids; DMEM, Dulbecco's modified Eagle's medium; PBS, Phosphate buffer saline; HBSS, Hanks' balanced salt solution; FBS, fetal bovine serum; TEER, transepithelial electrical resistance

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However, when amorphous silica particles were swabbed on skin, it was reported that the nano-sized materials penetrated through the skin, became distributed in the body, and induced unexpected toxicity [2]. It has been reported that the toxicity derived from amorphous silica particles depends on the particle size and the surface properties [15,16].

Generally, tablets are taken via oral administration. Using this administration route, the particles contained in the tablets do not remain at specific sites for a long period. However, if the particles are absorbed from the intestine and enter the blood circulation, it is important to consider whether the phenomena that occur when the drugs are administered intravenously would also occur in this case. In addition, no studies have been performed to investigate the toxicity associated with the oral administration of amorphous silica particles as additives in oral solid dosage forms; the evaluation of the physicochemical properties, intestinal permeability properties, and intestinal cell toxicity resulting from the oral administration of nano- or submicron-size amorphous silica particles is therefore essential to ensure the safety of solid oral dosage forms containing these materials.

In the present study, we evaluated the size, the absorption from the intestine, and the cytotoxicity using in vitro models of amorphous silica particles after oral administration. In particular, we investigated the effects of the size of the particles and the composition of the intestinal tract fluid on the intestinal permeability properties and the intestinal cell toxicity. In this study, we used amorphous silica particles with diameters of 50, 100, and 200 nm. In the oral administration of medicines, the physiological conditions in the intestinal tract are dramatically different in the fasted state and the fed state. It is known that these differences affect the absorption of drugs from the gastrointestinal tract. This study therefore investigated the changes in the size of amorphous silica particles in the fasted-state and fed-state simulated gastric and intestinal fluids.

As an in vitro model for intestinal absorption, we used the Caco-2 cell. Caco-2 cells grown as a monolayer become differentiated and polarized such that their morphological and functional phenotype resembles that of the enterocytes that line the small intestine [17,18]. Caco-2 cells express tight junctions, microvilli, and numerous enzymes and transporters that are characteristic of such enterocytes. The Caco-2 monolayer is widely used throughout the pharmaceutical industry as an in vitro model of the human small intestinal mucosa to predict the absorption of orally administered drugs. The correlation between the in vitro apparent permeability across Caco-2 monolayers and the in vivo fraction absorbed is well established [19]. Caco-2 cells have in fact been used as a model to investigate the possible harmful effect of silica nanoparticles in the gastrointestinal tract [20]. Furthermore, Caco-2 monolayers have been used to evaluate the intracellular uptake of nanosized-drug delivery systems [21,22].

In this study, the intracellular transport into Caco-2 cells, the Caco-2 monolayer membrane permeability, and the cytotoxicity against Caco-2 cells were then evaluated for the amorphous silica particles, in the fasted-state and fed-state simulated gastric and intestinal fluids.

2. Materials and methods

2.1. Silica particles

Suspensions of fluorescently labeled amorphous silica particles (nominal diameters as stated by suppliers: 50 nm, SP-50; 100 nm, SP-100; and 200 nm, SP-200) were obtained from Micromod Partikeltechnologie (Rostock, Germany). These were amorphous silica particles [16]. Silica suspensions were shaded and stored at 4 °C and diluted in various fluids before each experiment. The suspensions were sonicated for 10 min, and then vortexed for 1 min immediately prior to use. The silica particles were suspended in various fluids, and then incubated at 37 °C for 1 h before the measurements were performed. The mean particle size, the polydiversity index (PDI), and the ζ-potentials of the silica particles were measured using a Zetasizer Nano-ZS (Malvern Instruments, UK), with a concentration of 0.1 mg/mL in water (Table 1). The water used in this study was purified using Milli-Q system (Millipore, Tokyo, Japan). Fig. 1 shows transmission electron microscopy (TEM) images of the silica particles used in this study. The images were obtained using an H-9000 UHR instrument (Hitachi, Tokyo, Japan).

Table 1
The mean particle size, the polydiversity index (PDI), and the ζ-potentials of the silica particles used in this study.

Sample	Particle size (nm)	PDI	ζ-Potential
Silica			
SP-50	47.5 ± 4.0	0.14 ± 0.053	−43.2 ± 2.0
SP-100	99.0 ± 3.2	0.03 ± 0.030	−53.8 ± 1.4
SP-200	176 ± 6.4	0.16 ± 0.024	−51.8 ± 1.4

Samples were dissolved in MilliQ water with a concentration of 0.1 mg/mL.
Each value represents the mean ± S.D. ($n = 3$).

2.2. Cell culture

Caco-2 cells—human epithelial colorectal adenocarcinoma cells (American Type Culture Collection (ATCC), Manassas, VA, USA)—were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Brooklyn, NY, USA) supplemented with 10% FBS (Nichirei Biosciences, Tokyo, Japan), 100 U/ml penicillin/streptomycin (Life Technologies). Cells were grown in a humidified incubator at 37 °C under 5% CO_2, and the culture medium was changed every other day.

2.3. Composition of simulated gastric and intestinal fluids

The fasted-state simulated gastric fluids (FaSSGF) and the fed-state simulated gastric fluids (FeSSGF) were prepared according to a previous report [23]. The fasted-state simulated intestinal fluids (FaSSIF) and the fed-state simulated intestinal fluids (FeSSIF) were prepared according to the manufacturer's instructions (Celeste Co., Tokyo, Japan). An Ubbelohde-type viscometer was used for the viscosity measurements. The detailed components of each fluid are shown in Table 2.

2.4. Stability of fluorescence labeling

Fluorescently labeled silica particles were diluted to 1 mg/mL in various types of fluids (MilliQ water, D-MEM, PBS, FaSSIF, FeSSIF, FaSSGF, FeSSGF, 0.25% trypsin-ethylenediamine tetraacetic acid, and lysis buffer (1.0% Triton X-100 in HBSS)) and incubated at 37 °C for 6 h. After incubation, the particles were centrifuged (20,000 g, 30 min) and the precipitated silica particles were resuspended with the same volumes of fresh fluids as those before centrifugation. The fluorescent intensities of the suspension before and after centrifugation were measured at an excitation wavelength of 542 nm and an emission wavelength of 602 nm in a fluorescence spectrophotometer (F-7000; Hitachi High-Technologies, Tokyo, Japan). The percentage of the fluorescent dye retained in the silica particles after 6-h incubation was expressed as follows:

$$A/B \times 100(\%),$$

where A represents the fluorescence intensity of the silica particles resuspended with fresh fluid after centrifugation, and B represents the fluorescence intensity of the silica suspension before centrifugation.

2.5. Intracellular uptake study

Fluorescently labeled silica particles were used in this study to evaluate the intracellular uptake of silica particles. Caco-2 cells (1×10^6) were plated in a 6-well plate in medium containing 10% FBS and 100 U/mL penicillin/streptomycin. The fluorescently labeled silica particles were diluted to a concentration of 0.1 mg/mL in various types of fluids (D-MEM, PBS, FaSSIF, and FeSSIF), and then incubated at 37 °C for 1 h to mimic the intestinal conditions. In addition, to mimic the

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