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Evaluation on Cytotoxicity and Genotoxicity of the Exfoliated Silicate Nanoclay

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ABSTRACT The concern about toxicity for nanosilicate platelets (NSP) derived from natural montmorillonite clay is addressed. The NSP nanoclay was isolated from polyamine−salt exfoliation of the layered silicate clay into randomized individual plates, possessing multiple ionic charges on the surface of silicate plates with an average geometric dimension of ca. 80 × 80 × 1 nm^3. The material had been previously shown to be effective for antimicrobial and tendency for adhering onto the biomaterial surface based on the direct observation by using scanning electron microscope. The material safety on genotoxic effect was investigated by using three different test systems: the Comet assay test on Chinese Hamster Ovary (CHO) cells in vitro, micronucleus (MN) assay in vivo and the *Salmonella* gene mutation assay on strain TA98, TA100, TA102, TA1535 and TA1537. The Comet assay showed no DNA damage after 24 h of incubation with NSP of 1000 μg/mL. The MN test indicated no significant micronucleus induction in the CHO cells at the concentrations tested. With all five strains of *Salmonella typhimurium*, none of mutations was found. Furthermore, cytotoxicity of the same material was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release, showing a low cytotoxicity on CHO cells below 1000 μg/mL after 12 h incubation period and a dose-dependent effect after 24 h incubation. For feeding to rats, the acute oral toxicity was shown a low lethal dose (LD_{50}) or greater than 5700 mg/kg body weight for both male and female Sprague-Dawley rats. Overall, the study has demonstrated the safety of the NSP for potential uses in biomedical areas.

KEYWORDS: nanosilicate platelets • cytotoxicity • genotoxicity • nanomaterials • acute oral toxicity

1. INTRODUCTION

Inorganic materials in nanometer scale can exhibit different physical properties (1−3), giving rise to new applications in the areas of biomedicines (4), drug-delivery agents (5, 6), and biosensors (7, 8). However, a general concern remains that these new nanomaterials ought to be evaluated for their safety to the environment (9, 10). Recently, numerous reports have revealed the cytotoxicity of carbon nanomaterials such as carbon black, C60, and carbon nanotubes (CNT) for their possible dermal absorption (11−15). The cytotoxicity of metal nanoparticles such as silver (16), gold (17−19), and iron oxide (20) may damage the cell membrane through oxidative stress or interacting with proteins and enzymes. Other metal oxides such as TiO_2 (21, 22), ZnO (23, 24), and CeO_2 (24) have been associated with the mechanism of oxidative stress or reactive oxygen species (ROS) leading to high toxicity. However, the detailed mechanism of nanoparticle-induced genotoxicity is not yet fully understood.

Among the developed nanomaterials including spherical Ag nanoparticles, TiO_2, and tubular CNT, the natural silicate minerals such as sodium montmorillonite (Na^+-MMT) are considered to be safe materials. Recent developments on the layered silicate clays have broadened their practical applications beyond the conventional uses as nanocomposites (1), adsorbents (25), and catalysts (26). New applications for clays have advanced to many biorelated applications, including drug-delivery systems (27), hemostatic agents (28), and antibacterial agent (29, 30). Naturally abundant smectite clays such as MMT with multilayered structure with encapsulated organic compounds may be applied as a drug carrier for pharmaceutical purposes (27). It was concluded that MMT alone could be considered to be safe for a myriad of applications (27). In another study, Baker et al. (28) reported that the changes in clay structure and surface property may alter their response to blood-clotting. The clays may be uses as alternatives to the currently utilized hemostatic agents. Other studies have also shown that clays may interact with or mitigate the pesticide toxicity (31, 32). Advances of using clays in biorelated fields prompt us to conduct a toxicology study for the exfoliated silicate plates.

The exfoliated silicate plates are prepared from the natural clays by processing the layered structure into individual nanosilicate platelets (NSP) with a thickness of 1 nm. The process involved the uses of polyamine-salts as the exfoliating agent (33) and the isolation of the randomized silicates from the originally aggregated form of layered primary structure. In the primary units, MMT possesses a multilayered structure with an average of 8−10 lamellar plates in each stack. The exfoliation actually converted the

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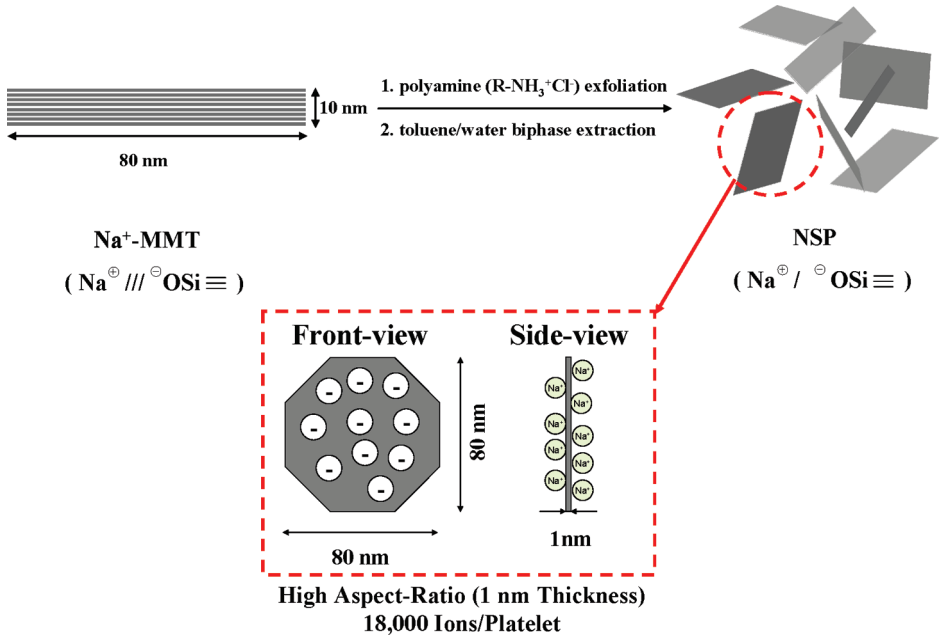

FIGURE 1. Preparation and Physical Properties of the NSP Material from the Exfoliation of Layered Na^+-MMT in Water.

layered structure of silicate plates into the randomized plates with an average dimension of ca. 80 × 80 × 1 nm^3. Moreover, the originally buried ionic surface charges (≡SiO^-) are completely exposed (34, 35). With the ionic properties, the randomized plates were shown to have a changeable zeta potential under various pH conditions (36). The newly developed NSP and their derivatives had been demonstrated to be effective for antimicrobial uses (37, 38). In this study, we focused on its cytotoxicity by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole] and LDH (lactate dehydrogenase) assays. The mutagenic activity was evaluated by the Ames test in *Salmonella typhimurium* strains (39, 40). Genotoxicity was assessed by in vitro Comet assay (41) using mammalian cells, and in vivo micronucleus assay. Potential acute oral toxicity in rats by three oral doses of NSP was evaluated.

2. MATERIALS AND METHODS

2.1. Materials Preparation of NSP. The nanosilicate platelets (NSP) with an average dimension of 80 × 80 × 1 nm^3 in polygonal shape were prepared from Na^+-MMT, as described in the previous reports (33−36). The Na^+-MMT, supplied by Nanocor, is a Na^+ form of layered smectite clay with a cationic exchange capacity (CEC) of 120 mequiv/100 g.

2.2. Cell Culture and Preparation. Chinese Hamster Ovary cells (CHO cells) cultured in Ham's Nutrient Mixture F-12K (Kaughn's modification) at pH 7.3 were supplemented with 10 % (v/v) fetal bovine serum (FBS), 100 unit/mL penicillin, 100 μg/mL streptomycin, grown at 37°C under 5 % CO_2. Upon reaching confluence, cells were cleaved by 0.05 % trypsin and replated onto 10 cm dishes. For experiments, proper cell numbers compatible with each dish size were allowed to grow for 1 day before treatments.

2.3. Scanning Electron Microscopy of CHO Cell Incubated with NSP. CHO cells were seeded on the coverslip glass in a 24-well plate for 24 h and the culture medium was then replaced with fresh medium containing 10 and 100 μg/mL NSP. After 24 h incubation, CHO cells left on the glass were treated with 2.5 % glutaraldehyde at 4°C for overnight. The samples were then dehydrated with alcohol and examined by FE-SEM (JSM-6700F, JEOL, Japan).

2.4. Genotoxicity Assay. 2.4.1. *Ames Test.* NSP was evaluated for mutagenicity by the Ames *Salmonella*/microsome mutagenicity assay using the plate incorporation method (31, 32). *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and TA102 strains were purchased from Culture Collection and Research Center, Food Industry Research and Development Institution of Taiwan. Rat liver S9 fraction of 3-MC (3-methylcholanthrene) was prepared by the procedures of 9000 g centrifugation for 30 min at 4°C and used for the treatment in Ames test (42). The test substance formed a homogeneous suspension at 0.625, 1.25, 2.5, 5, and 10 mg/mL. A plating aliquot of 0.1 mL was used. The dose levels of 62.5, 125, 250, 500, and 1000 μg per plate incubated with 0.5 mL of the S9 mix for 1 h at 37 °C were added to 2 mL of molten top agar including 0.1 mL of an overnight culture of histidine-requiring strains and spread over a minimum agar plate. The His^+-revertant colonies were counted after 48 h of incubation at 37°C.

2.4.2. *Micronucleus Assay In vivo.* ICR male and female mice of 8 week old and approximate weight of 25 g were acquired from Institutional Animal Care and Use Committee of National Taiwan University College of Medicine (IACUC, NTUCM). All mice were acclimatized for 1 week before treatment. The mice were fed with NSP at the concentrations of 20, 200, and 500 mg/kg body weight. The negative control group received double-distilled water while the positive control group received intraperitoneal (i.p.) injection of mitomycin C at the dose of 1 mg/kg. Peripheral-blood cells were collected after 24 and 48 h. Micronucleus formation of polychromatic erythrocytes in the cells was counted under microscope by Giemsa staining.

2.4.3. *Comet Assay In vitro.* The Comet assay was performed by following the standard procedures reported by Tice et al (41). The CHO cells were cultured in 6 cm culture plates at a density of 5 × 10^5 cells/plate for 24 h and treated with different amounts of NSP at 62.5, 125, 250, 500, and 1000 μg/mL. A negative control (with double-distilled water) and a positive control (with H_2O_2 100 μM addition) were performed for comparisons. After incubation for 24 h at the conditions of 37°C and under 5 % CO_2, the cells were trypsinized. The cell suspensions (1 × 10^5 cells/mL, 10 μL) were mixed with 1 % of

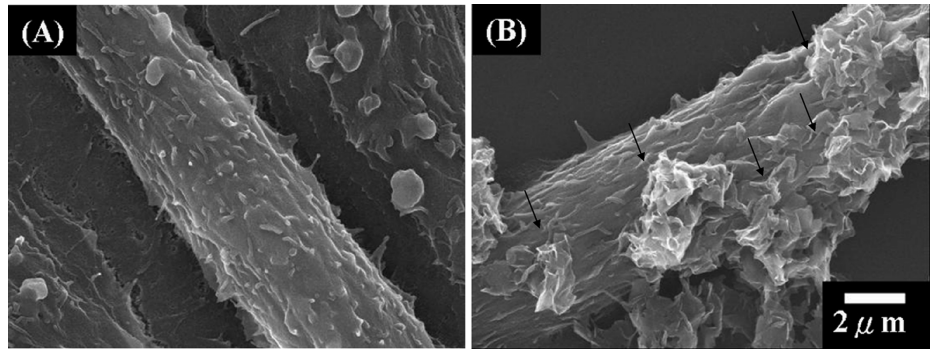

FIGURE 2. FE-SEM images showing the different morphologies of (A) CHO cells and (B) CHO cells with large clusters of NSP aggregates (indicated by arrows) at micrometer−meter size after 24 h incubation with the addition of NSP (100 μg/mL in medium).

Table 1. Mutagenic Activity of NSP on *Salmonella typhimurium* with and without S9

	revertant colonies (CFU/plate)									
	TA98		TA100		TA102		T1535		TA1537	
dosage (g/plate)	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
NC[b]	23 ± 2[a]	53 ± 9	166 ± 13	228 ± 6	227 ± 10	307 ± 20	16 ± 4	16 ± 3	7 ± 2	11 ± 3
62.5	24 ± 2	51 ± 6	149 ± 13	223 ± 7	197 ± 36	303 ± 4	13 ± 5	13 ± 1	8 ± 3	14 ± 3
125	23 ± 6	52 ± 4	151 ± 2	210 ± 9	201 ± 11	295 ± 4	19 ± 3	13 ± 2	7 ± 6	14 ± 3
250	22 ± 3	32 ± 23	141 ± 9	218 ± 20	209 ± 20	235 ± 68	16 ± 2	17 ± 2	9 ± 2	13 ± 2
500	21 ± 4	53 ± 4	147 ± 2	214 ± 14	191 ± 10	294 ± 5	18 ± 4	15 ± 2	4 ± 1	12 ± 2
1000	23 ± 2	48 ± 3	152 ± 16	225 ± 13	201 ± 8	270 ± 10	15 ± 2	15 ± 3	7 ± 1	12 ± 3
PC[c]	2717 ± 50[d]	219 ± 13[d]	3280 ± 20[d]	3400 ± 20[d]	634 ± 5[d]	745 ± 3[d]	3122 ± 32[d]	84 ± 5[d]	218 ± 11[d]	40 ± 3[d]

[a] Each value is the mean ± SD for three independent experiments. [b] The negative control: distilled water. [c] The positive control: without S9, 4-nitro-*o*-phenylenediamine (1 μg/plate) for TA98, sodium azide (8 μg/plate) for TA100 and TA1535; mitomycin C (1 μg/plate) for TA102 and 9-aminoacridine (50 μg/plate) for TA1537; with S9, benzo[a]pyrene (1 μg/plate) for TA98 and TA102, and 2-aminoanthracene (4 μg/plate) for TA100, TA1535, and TA 1537. [d] Significantly different from the negative control (student's *t*-test; $P < 0.001$).

low melting agarose (150 μL) at 37 °C and rapidly spread onto glass slides coated with 1.5 % of normal melting point agarose. The agarose was allowed to solidification by lowering the temperature to 4 °C for 20 min. The cells were lysed by immersing the slides in a freshly prepared solution (5 M NaCl, 0.5 M EDTA, 1 M Tris-HCl, 1 % Triton X-100, 10 % DMSO, pH 10.0) at 4 °C for 40 min. The slides were immersed in an alkaline solution (0.3 M NaOH, 1 mM EDTA, pH > 13) at room temperature. After 30 min, the slides were placed on a horizontal gel electrophoresis box containing freshly prepared alkaline electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH > 13) at 4°C for 30 min before performing electrophoresis. Electrophoresis was carried out at 13 V (1 V/cm) and 300 mA in an ice bath at 4 °C for 20 min. The sample slides were then rinsed by double-distilled water and immersed in 70 % ethanol for 5 min. After the slide was air dried, 50 μL of diluted propidium iodide (PI) solution was applied to each circle of dried agarose and covered with a coverslip. The samples were immediately examined by using a fluorescent microscope. Under the observation, scores ranging from 0 (undamaged) to 4 (maximally damaged) were estimated according to the tail intensity (size and shape) for 100 randomly selected cells.

2.5. Cytotoxicity Assay. 2.5.1. *MTT Viability Assay.* CHO cells were split into a 24-well culture plates at a density of 5×10^4 cells/well in l mL culture medium and allowed to attach for 24 h before treatment. After the medium was replaced by NSP (62.5, 125, 250, 500, and 1000 μg/mL), the cells were incubated at 37 °C under 5 % CO_2 for 3, 12, and 24 h. MTT solution (200 μL of 500 μg/mL) was added to each well to give a final concentration of 100 μg/well and then incubated at 37°C for 2 h. The supernatant was removed and dimethyl sulfoxide was added to each well to dissolve the formazan crystals. Afterward, the solution from each well was transferred to a 96-well plate. The optical density was read on an ELISA reader at 570 nm on the basis of linear absorbance to the number of living cells in culture.

2.5.2. *LDH Assay.* CHO cells were seeded at 5×10^4 cells/well in 24-well plates. After 24 h, the medium was removed and replaced with F-12K medium containing NSP at concentration of 62.5, 125, 250, 500 and 1,000 μg/mL. After 24 h incubation at 37°C, cells were precipitated by centrifugation at 1500 rpm for 5 min at room temperature. An aliquot of 50 μL supernatant was transferred into a 96-well plate and Lactate dehydrogenase (LDH) leakage from the cells was determined by a commercially available kit provided from Promega (CytoTox 96 non-radioactive cytotoxicity assay procedure). The cytotoxicity was calculated according to the percentage of cell death normalized by the control levels.

2.6. Fourteen-Day Oral Toxicity Study in Male and Female SD Rats. Sprague-Dawley rats that were 6 weeks old or weighed approximately 150 g were acquired from BioLASCO Taiwan Co., Ltd. (IACUC, NTUCM). The animals were subjected to quarantine and acclimated for a week before the treatment. The NSP water solutions at 50 and 25 mg/mL dilution were fed to rats at the feed dose of 60 mL/kg. A total 24 rats in each sex were divided into control (distill-water injection), low dose (1500 mg/kg), intermediate dose (3000 mg/kg), and high dose (5,700 mg/kg) groups. Following 14 days of observation, all animals were sacrificed and necropsied. According to the mortality and the toxicity symptom, the LD_{50} was evaluated.

2.7. Statistical Analysis. Each experiment was replicated three times and data are presented as mean ± SEM (standard error of mean). Statistical analysis of the data was carried out using Sigma Plot. Differences were analyzed with the student's *t*-test and considered significant at the *P*-value <0.05.

Table 2. Frequencies of Micronucleated Polychromatic Erythrocytes (MNPCEs) after Treating with NSP

		frequencies of MNPCEs[a]	
ICR mice	dose (mg/kg)	24 h	48 h
male	negative control	1.50 ± 0.32[c]	1.45 ± 0.27
	20	1.45 ± 0.35	1.45 ± 0.47
	200	1.50 ± 0.39	1.45 ± 0.39
	500	1.55 ± 0.42	1.50 ± 0.42
	positive control[b]	5.65 ± 1.12[d]	30.5 ± 2.45[d]
female	negative control	1.50 ± 0.32	1.55 ± 0.27
	20	1.44 ± 0.45	1.55 ± 0.42
	200	1.50 ± 0.42	1.55 ± 0.32
	500	1.55 ± 0.35	1.50 ± 0.52
	positive control	5.55 ± 0.91[d]	30.90 ± 2.92[d]

[a] Two-thousand polychromatic erythrocytes were analyzed per animal, for a total of 20 000 cells per group. [b] Positive control group received i.p. injection of 1 mg/kg of mitomycin C. [c] Data collected by mean ± SD for three independent experiments. [d] Significantly different from the negative control (student's *t*-tests; $P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Physical Affinity of NSP on Cell and Morphology by SEM. The NSP material was prepared (33−36) from the MMT primary stacks and characterized as thin silicate plates of ca. 80 × 80 × 1 nm^3 with surface ionic charges of ca. 18 000 ions per plate, as described in Figure 1. From the inherent MMT structure, each plate unit has a generic composition of 2:1 layered silicate/aluminum oxides with two tetrahedral sheets sandwiching an edge-shared octahedral sheet. The AFM analysis indicated that the water-dispersed NSP was actually in a lamellar bilayer or two-plate unit. Under the controlled evaporation, the NSP could further self-pile into lengthy rod-like aggregates in micrometer length (29), indicating its strong tendency for plate surface−surface adherence. The measurement of zeta potentials demonstrated its ionic characteristics showing an isoelectric point at pH 6.4 (36).

The direct interaction of NSP onto the surface of CHO cells was examined by using a SEM microscope, after the treatment with NSP of 100 μg/mL concentration for 24 h. The spindle shape and smooth surface of the normal cell (Figure 2A) was found to have the silicate aggregates on the cell surface, as shown in Figure 2B. The appearance of NSP aggregates depending on the exposing duration and concentration is attributed to the intensive interaction between the anionic nature of the cell surface and the positively charged NSP.

3.2. In vitro Genotoxicity Assay of NSP. A genotoxicity test was performed on the effect of NSP exposure to evaluate its possible damage to the genome. Three independent tests including gene mutation in bacteria by Ames test, chromosomal damage as micronucleus induction in vivo, and DNA damage by the Comet assay using mammalian cells were performed.

The Ames microbial revertant assay involved *Salmonella typhimurium* strains of TA98, TA100, TA1535, and TA1537 and TA102 and their treatments with NSP at doses of 62.5, 125, 250, 500, and 1000 μg per plate. Growth inhibition was

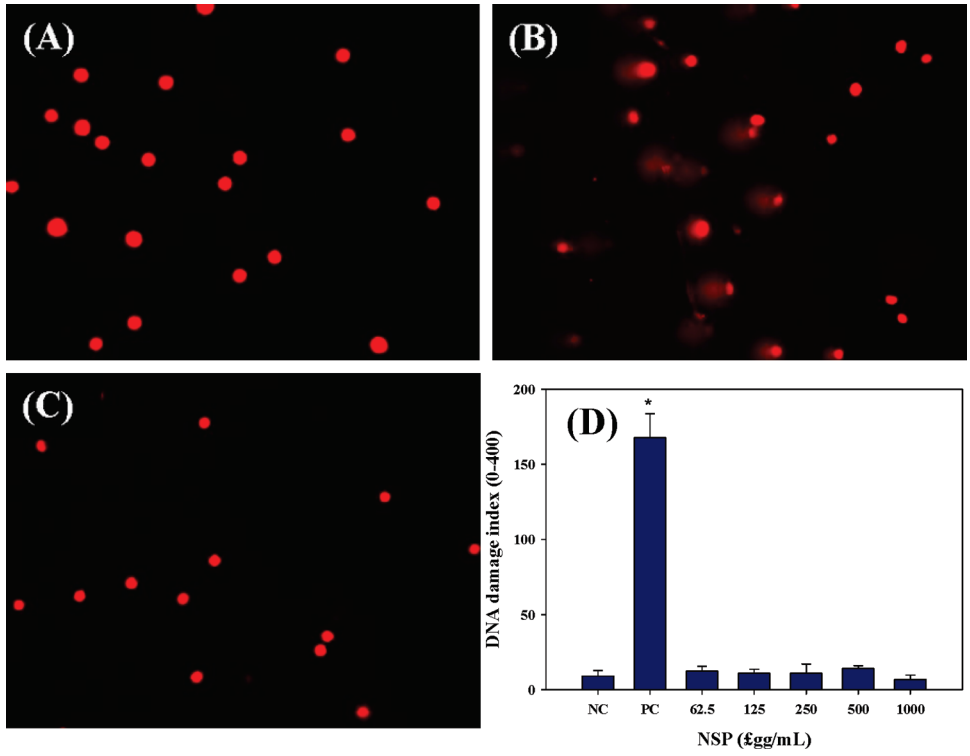

FIGURE 3. Detection of the possible NSP damage on DNA of CHO cells by the Comet assay (A) negative control (NC, by distilled water), (B) positive control (PC, by 100 μM H_2O_2), (C) exposure to NSP (by 1000 μg/mL), and (D) column graph showing the average comet tail lengths after being incubated with different concentrations of NSP, NC, and PC for 24 h. Data were shown with the mean ± SD from at least 200 cells Comet tail counts for each sample. All data were represented by averaging four independent experiments. * indicates significantly different from the negative control (student's *t*- test; $P < 0.001$).

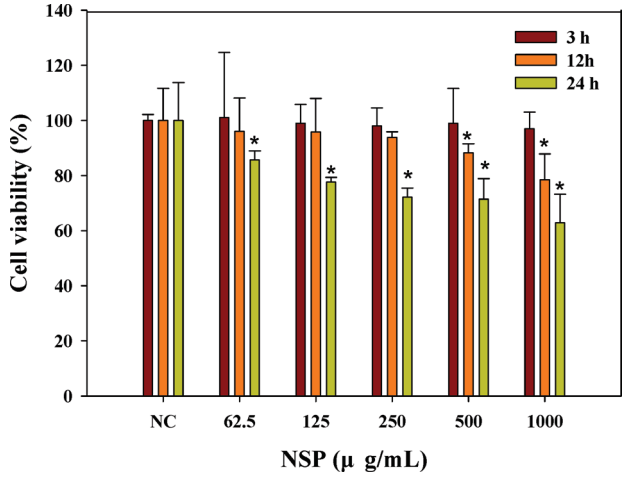

FIGURE 4. Cytotoxicity on CHO cells after 3, 12, and 24 h of incubation with NSP using the MTT assay. The percentage of survival cells was relative to the negative control cells and by the mean ± SD of four independent experiments. *Significantly different from the negative control (student's *t*-test; $P < 0.05$).

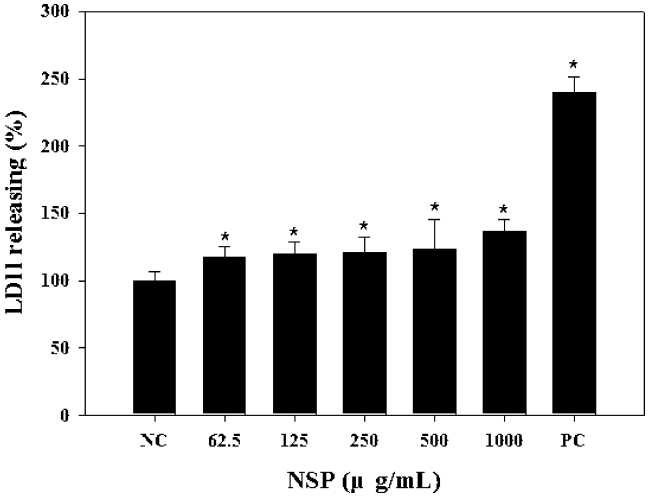

FIGURE 5. Effect of NSP on LDH leakage from CHO cells: the percentage of LDH release was calculated by dividing the amount of activity in the medium by the total activity (medium and cell lysate) after subtraction from the negative control. The data were expressed by averaging four independent experiments. * indicates significantly different from the negative control (student's *t*- test; $P < 0.05$).

observed in all tested strains with or without S9. The positive control is defined as the number of affected colonies at least two folds of the negative control. As summarized in Table 1, none of growth inhibition was observed for the five strains with or without S9 addition to NSP. The mutation frequencies remained the same. The addition of the microsomal fraction S9 (the rat liver microsomal enzyme system) is to ensure the observation of the possible mechanism involving organic matter that may activate mutagenic activity through the metabolic pathway. It is concluded that NSP has no effect on the mutagenicity in five different *Salmonella typhimurium* strains.

In addition, the micronucleus assay was performed to examine the possible chromosomal breakage and mitotic spindle damage in producing micronuclei during cell division, as shown in Table 2 for three doses of NSP (20, 200, and 500 mg/kg body weight). Peripheral-blood cells were collected after treating with NSP for 24 and 48 h and evaluated for micronucleated polychromatic erythrocytes (MNPCEs). The results showed no increase in the frequency of micronuclei. The frequency of expected MNPCEs increase in animals receiving an intra-peritoneal injection with mitomycin C was significantly different from that of the negative control, a clear indication of no chromosomal damages after the NSP treatment.

Extensive and dose-dependent damage to DNA was observed after treatment of the CHO cells with NSP, as shown by the Comet assay evaluated using electrophoresis tracing. In Figure 3, the DNA images for the CHO cells treated with NSP at various concentrations of 62.5, 125, 250, 500, and 1000 μg/mL are demonstrated. The results were compared to the positive control with H_2O_2 100 μM, showing the comet tails due to the DNA damage, and the negative control experiment as shown the spherical shape of DNA. The DNA damage index (ratio of tails to total comet bodies) is illustrated in the diagram of Figure 3D, indicating no significant DNA damage for the NSP exposure.

3.3. In vitro Cytotoxicity Assay of NSP. The NSP was examined for its cytotoxicity on CHO cells by the standard MTT and LDH assays. The MTT assay reveals the metabolic and mitochondrial activity of the NSP-treated cells. The results indicated a slight decrease in cell viability for the NSP-exposed CHO cells at high concentration and exposure time (Figure 4). However, the NSP has a low toxicity below concentrations of 1000 μg/mL for 70 % cell viability in the period of 12 h. The exposure up to the concentration of 1 mg/mL for 24 h showed a 40 % loss of viability on CHO cells.

Table 3. Body Weight and Food Consumption of Male and Female Rats Treated with NSP

		body weight (g)			food consumption (g)	
SD rats	dose (mg/kg)	day 0	day 7	day 14	day 7	day 14
male	0	154.8 ± 7.4[a]	211.2 ± 10.0	290.2 ± 15.5	328.7 ± 16.6	373.0 ± 16.7
	1500	154.7 ± 9.2	211.3 ± 11.2	291.2 ± 16.2	328.0 ± 22.1	375.7 ± 13.0
	3000	155.2 ± 9.4	210.2 ± 10.1	291.0 ± 14.4	321.3 ± 21.5	377.0 ± 18.7
	5700	155.3 ± 9.5	210.3 ± 10.4	291.0 ± 11.6	322.3 ± 12.1	375.0 ± 23.9
female	0	149.2 ± 10.6	183.8 ± 10.7	205.2 ± 10.5	226.7 ± 16.8	276.0 ± 17.3
	1500	149.8 ± 11.0	184.0 ± 11.6	206.5 ± 11.4	233.3 ± 19.0	279.0 ± 18.3
	3000	150.3 ± 12.2	184.5 ± 11.8	208.2 ± 10.8	231.3 ± 12.1	272.3 ± 16.5
	5700	150.0 ± 10.2	184.8 ± 10.4	208.8 ± 10.2	229.7 ± 18.2	275.0 ± 11.1

[a] Each value was represented by the mean ± SD of six repetitive experiments.

The half maximal inhibitory concentration (IC_{50}) value at 24 h for CHO cells was more than 1000 μg/mL.

For the LDH assay, the release of enzyme lactate dehydrogenase into cell culture medium was measured quantitatively. Cell membrane leakage was reflected in the elevated LDH levels in cell culture medium after exposure to NSP solution for 24 h. In Figure 5, the LDH levels of CHO cells were increased from 0 to 40 % when exposing at 62.5 to l000 μg/mL concentration. By comparing with other inorganic particles (43−45), NSP induces low damage on CHO cells in both assays.

3.4. Fourteen-Day Oral Toxicity Study in Male and Female SD Rats. Acute oral toxicity of LD_{50} was performed for three doses of 1500, 3000, and 5700 mg/kg. The treatment with NSP did not result in any mortalities, clinical signs or macroscopic changes for both male and female SD rats (data not shown here). There were no significant changes in the body weight or food consumption of the treated group compared to the control group (Table 3). The LD_{50} acute oral toxicity of NSP was more than 5700 mg/kg for the SD rats.

4. CONCLUSION

The exfoliated NSP nanoclay was prepared from the exfoliation of the natural silicate clay and evaluated for its toxicity. The cytotoxicity of testing on CHO cells showed the value of IC_{50} larger than 1000 μg/mL by the MTT assay. The NSP failed to affect the mortality of the SD rats by oral feeding at the dose level of 5700 mg/kg. In vitro and in vivo safety evaluations of NSP indicated none of mutagenic effect in three different genetic toxicity test systems. From the direct SEM observation, the NSP could accumulate and adhere on cell surface but showing no obvious changes in cell morphology. However, the presence of NSP may alter cells in permeability as evidenced by releasing LDH. The investigation on this nanoclay for its mechanism of interacting with living biomaterials and potential biomedical uses will be pursued.

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