Imogolite Synthesized in Presence of As(III) Induces Low Cell Toxicity and Hemolysis, in Vitro, Potential Stabilization of Arsenite Present in Aqueous Systems

Edgardo Rojas-Mancilla, † Alexis Oyarce, ‡ Leonor Alvarado-Soto, V César Echeverría, § Karen Manquía-Cerda, ¶ Nicolás Arancibia-Miranda, ‡§∥⊥ and Rodrigo Ramírez-Tagle*∥⊥

‡ Departamento de Ciencias Químicas y Biológicas and ‡ Escuela de Tecnología Médica, Universidad Bernardo O Higgins, General Gana 1702, Santiago 8370854, Chile
§ Facultad de Medicina, Universidad de Atacama, Copayapu 485, 1531772 Copiapo, Chile
¶ Facultad de Química and Biología, Universidad de Santiago de Chile, USACH, Casilla 40, C.P. 33, Santiago 9170022, Chile
⊥ Center for the Development of Nanoscience and Nanotechnology, CEDENNA, Santiago 9170124, Chile
∥ Facultad de Ingeniería, Ciencia y Tecnología, Universidad Bernardo O Higgins, Avenida Viel 1497, Santiago 8370993, Chile
⊥ Química del Maipo Ltda., Viña Pelvin, Parcela 23, Lote 10, Peñaflor, Chile

ABSTRACT: Imogolite is a nanotubular aluminosilicate that has low toxicity in biological systems and due to its morphological and surface properties has a growing interest in environmental applications and biomedical areas. Its synthesis is highly sensitive to the presence of other ions, being able to inhibit or retard the process of imogolite formation, which could change the cytotoxic response of this substrate, something scarcely reported in the literature. In this context, the presence of arsenate during the synthesis of imogolite caused significant changes in the dimensions and surface behavior of these nanotubes. Cell viability was evaluated on EA.hy926 and HepG2 cells by (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay at 24 h. Meanwhile, the potential effects on human red blood cells, namely, hemolysis and morphological changes, were determined at 0 and 24 h. The range of % As tested of the nanotube showed cell toxicity similar to the control condition. Similarly, the As-based nanotubes induced hemolysis similar to controls and slight morphological changes of red blood cells at 0 and 24 h of exposition. These results indicate that As-based imogolite-like nanotubes are not toxic nor hemolytic and can be potentially used in processes like water purification.

1. INTRODUCTION

Within the world of nanotechnology, nanotubes are considered key pieces for the construction of new functional materials,1 with enormous development potential in areas, such as electronics, biotechnology, biomedicine, sensing, separations, energy storage/management, and catalysis.2 However, the risks toward humans and ecosystems are still scarcely known, which limits the measures of protection to the population.3

This type of nanotubular structures can have a pathological response in humans, strongly conditioned by physicochemical properties, the state of aggregation, and, in particular, the relationship between their diameter and length; it is known as high-aspect ratio nanotubes (HARNs) exceeding the value of 3. Its exposure can be considered risky, causing inflammation, fibrosis, and several pathologies, including mesothelioma, because the body’s response is a progressive oxidative stress, which causes cell death.3,4 The most emblematic case is what happened with asbestos; however, with the incursion of nanotechnologies, other actors are found present in the environment and can affect in a similar way; such is the case of carbon nanotubes1 and silver nanowires,5 where multiple investigations have shown some similarities to what was observed with the asbestos. In this context, the emergence of these nanostructures has led to permutation in different areas of science, highlighting their study on environmental issues, where their physicochemical and morphological characteristics have been possessed as excellent pollutant removal agents, such as Cd, Pb, Hg, and mainly As.6 An excellent example of the versatility of this type of structure is the imogolite, a nanotubular aluminosilicate, which is found in the inorganic phase of soils of volcanic origin and that was synthesized from dilute solutions of Al and Si. Its stoichiometry is (OH)₃Al₂O₃SiOH, and it is characterized by a hollow cylindrical shape with an average outer and inner diameter of 2.0 and 1.0 nm, respectively, whereas its length ranges from 100 nm to several micrometers. Its marked surface differ-

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entiation, with isolated silanol groups (≡Si–OH) that make up its internal surface and aluminols (≡Al–OH y ≡Al–OH) predominant on the external surface, make it possible to classify it as a material whose surface charge depends on the pH, due to the rapid ionization that occurs in these groups. These morphological and surface properties are critical to defining the toxicity of this type of structure.

The process of synthesis of imogolite is highly sensitive to variables such as the concentration of reactants, temperature, and the presence of ions different from those usually used, which mainly causes the synthesis to have slower kinetics or generate structural defects. In this context, the synthesis process of imogolite allows modifying its composition in situ, thus obtaining different iso-structures that have different dimensions and surface behavior. The main iso-structures developed are those that consider the replacement of Al and Si atoms, by elements such as germanium (Ge) and iron (Fe). The stoichiometry of these analogous structures of imogolite is of the type (OH)₂Alₓ₋ₙXₙSi₁₋ₙY·OH (where X = Ge and Fe(III), 0 < n ≤ 2), where total or partial incorporation of these elements modifies the dimensions of these nanotubes, which have larger diameters and smaller lengths in comparison with the imogolite, which would potentially impact the HARN values.

The production of metal-based inorganic nanotubes and hybrid materials could have diverse technological applications, including fabrication of cellular scaffolds. However, questions have been raised concerning potential toxicological effects of these nanomaterials toward humans. In recent studies, three types of Al–Ge nanoparticles with the same chemical composition but various sizes and Shapes showed that the size effect on the amplitude of the genotoxic effects is less apparent when number instead of mass concentrations are considered, although the influence of the aspect ratio on the toxicity mechanism remains clearly marked, whereas another study shows that imogolite induces less toxic effects than single-walled or multiwalled carbon nanotubes in different cell models, suggesting that imogolite-like nanotubes could be safely administered in animal models.

Recently, Avellan et al. showed that lacunar nanotubes of the Ge-imogolites and Ge-imogolites doped with Fe (0.95% wt) inhibited or favored the bacterial growth of Pseudomonas brassicacearum, respectively. These results show that small changes in the composition of this nanotube could mean significant variations in its toxicity. In this context, it is proposed to synthesize imogolite in the presence of arsenite (As(III)), due to its similarities in the pKa values and geometric parameters with Si(OH)₄. This oxy anion is predominant in groundwater, being highly mobile and with a greater toxicity than that of arsenate (As(V)), because in the natural conditions of pH this is neutral (pKa = 9.2), being able to diffuse in mammalian organisms (including humans) and accumulate mainly in liver, kidney, lung, and spleen. The mechanism involves the formation of a stable lipoic acid linked to the enzyme, joining the sulhydrl groups (SH) complex. For the most part, As(III) poisoning is explained by the inhibition of those enzymes that require lipoic acid as coenzyme, either pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and α-ketoacid dehydrogenase.

In the present study, we seek to determine if the structure of the imogolite is influenced by the presence of As(III), which could be an inducer of vacancies or doping of this oxy anion and how these changes alter the potential cytotoxicity and hemolytic properties of imogolite exposing human cells and human blood cells to increasing doses of As-based imogolite-like nanotubes.

2. METHOD

2.1. Synthesis of Imogolite and As-Containing Imogolite (AlSiAs). Imogolite (Imo) and As-containing imogolite (AlSiAs) were synthesized adapting the existing protocol described by Arancibia-Miranda et al., 2011. Tetraethyl orthosilicate and sodium arsenite were added dropwise to a stirred solution of 5 mM Al(NO₃)₃·9H₂O solution until the Al/(Si + Oxy) ratio was 2, with a total arsenite percentage of 10–50% (hereafter called AlSiAs₉, AlSiAs₁₀, AlSiAs₂₅, and AlSiAs₅₀), and this mixture was allowed to stand for 45 min with vigorous stirring. Then, a 1 × 10⁻³ M NaOH solution was added at a rate of 0.5 mL/min until an Al/(Si + Oxy)/OH ratio of 2:1:4 was obtained. Growth of the nanotubes was allowed at 95 °C for 7 days, and the resulting suspension was dialyzed against ultrapure water. This aging solution was dialyzed or centrifuged.

2.2. Microscopy Analysis. The samples were observed, with a Philips Tecnai 12 BioTwee transmission electron microscope using an 80 kV acceleration potential, on carbon substrates prepared as follows: A drop of imogolite and AlSiAs suspended in water was transferred onto the face of a freshly cleaved sheet of mica allowing solvent evaporation. A thin layer of carbon was deposited on the surface by vacuum evaporation. The carbon/product film was separated from the mica sheet by flotation on distilled water and subsequently transferred to a perforated Cu support grid.

2.3. Electrophoretic Migration (EM). The isoelectric point (IEP) was determined by measuring the electrophoretic mobility of particles on a Zeta Meter 4.0 apparatus. About 30 mg of each sample was suspended in 200 mL of a solution with ionic strength 1.0 mM (NaCl), and the EM was determined as a function of pH. The IEP was obtained from the EM versus pH graph as the pH at which EM = 0.

2.4. Surface Area (S₄₃ₓ). The specific surface area (S₄₃ₓ) of imogolite and both nanocomposites was measured by the N₂ method from the Brunauer–Emmett–Teller (BET) theory using an automatic analyzer (Quantachrome Nova Station A).

2.5. Chemical Composition. The chemical composition of the final Imo-As-ite products after freeze-drying was analyzed by inductive coupled plasma atomic emission spectroscopy using a Perkin Elmer Optima 2000 equipment. The samples were ground and dissolved in a mix of a 10% nitric acid and hydrofluoric acid solutions 24 h before the measurement. For the best accuracy, each sample was measured seven times and one of the calibration standards was checked every seven samples.

2.6. Cell Culture. Human hepatoctellar carcinoma HepG2 cells (American Type Culture Collection HB-8065) were grown in monolayer culture in Dulbecco’s modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) (Gibco, NY) and 1% antibiotic–antimycotic (Gibco, NY) at 37 °C in a humidified 5% CO₂ Incubator. The HUVEC-derived endothelial cell line, (EA.hy926) was kindly provided by C-J Edgell (Edgell, McDonald, & Graham, 1983) and was grown in DMEM-low glucose (GIBCO), supplemented with 10% heat-inactivated FBS and 2 mmol/L and 50 U/mL penicillin/streptomycin (Sigma). All cell cultures were grown at 37 °C in a 5%: 95% CO₂/air atmosphere.
2.7. Cell Viability Assay. Cells were seeded in a 96-well plate at an initial density of $5 \times 10^3$ cells/well. Twenty-four hours later, the cells were treated with control (Milli-Q water) or various concentrations of AlSiAs for 24 h. The (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) method was used as we previously described. The absorbance of the dissolved formazan crystals was measured at 540 nm using a microplate reader (Tecan infinite f50, Grodig, Austria).

2.8. Preparation of Human Red Blood Cells. Red blood cells were obtained and prepared following the protocol previously published. Briefly, healthy adults were invited to donate a small volume of blood. All donors signed an informed consent explaining the risks of venipuncture and the use of red blood cells only in the hemolysis study. The protocol was evaluated and approved by a local bioethics committee. Blood was obtained through venipuncture and anticoagulated. Immediately, blood samples were centrifuged at 3500 RPM for 5 min. The buffy coat was removed, and then a portion of the red blood cells was separated and washed with phosphate buffered saline (PBS). The washed cells were used immediately after isolation.

2.9. Analysis of Cell Morphology. HepG2 and EA.hy926 cells cultured in 6-well plates were exposed to different concentrations of imogolite-As for 24 h. The cells were then washed twice with PBS and immediately observed under a microscopy EVOS FLoid cell (Life Technologies, Carlsbad, CA). The morphology of red blood cells incubated with AlSiAs50 was evaluated under a microscope. Both cultured cells and red blood cells were visualized and photographed under a microscope (Motic AE-31). Red blood cells’ sizes were determined using ImageJ software (NIH). Finally, cell morphology of red blood cells was carefully evaluated, for detection of echinocytosis, crenation, microcytosis, macrocytosis, spherocytosis, and poikilocytosis, among others.

2.10. Analysis of Hemolysis. Hemolysis of red blood cells exposed to growing concentrations of the AlSiAs50 ($10^{-3}$–1 mg/mL). These concentrations were selected to cover a wide range, on a logarithmic scale. Red blood cells diluted at 1%, were treated with increasing concentrations of imogolite-As in PBS. Furthermore, controls of hemolysis were prepared, mixing red blood cells with distilled water or PBS, corresponding to 100 or 0% of hemolysis, respectively. A tube containing 1 μM imogolite was also incorporated. Hemolysis protocol was performed as described before. After a brief period of incubation and centrifugation, the supernatant was separated and absorbance measured at 540 nm. Measurement of absorbance was performed at 0 and 24 h.

2.11. Data Analysis. All results are presented as the mean ± standard error of mean (SEM), comparisons were made by the Kruskal–Wallis test followed by Dunn’s multiple comparisons test using GraphPad Prism 8.0 and $p < 0.05$ considered significant.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Imogolite and As-Doped Imogolite. Figure 1 shows the transmission electron microscopy (TEM) images obtained from the samples (imogolite, AlSiAs5, AlSiAs10, and AlSiAs25).

The presence of As increased the by-product concentration, with two different types of substrates, spherical morphology (proto-imogolite) and needle-shaped, which would indicate that the process of obtaining AlSiAs is not entirely clean because arsenic would have an inhibitory or retarding role in the formation of the precursors responsible for the formation of nanotubes.

The morphology of the samples of AlSiAs and imogolite was evaluated using TEM. The presence of arsenite during the synthesis of imogolite generated modifications in the dimensions of this nanotube, mainly in the length, wherein a significant decrease was observed in comparison with the
imogolite prepared under standard conditions of synthesis, reaching values of an average length of <90 nm para AlSiAs_{50}, a behavior similar to that reported for Ge-imogolite. An ascending tendency was observed in the values of the diameter of the imogolite, with the increase of the concentration of As(III), which could suggest that the arsenite would have a role in the structure of the formed precursor, which finally evolves in to the nanotube. The diameter trend follows the sequence 2.30 = 2.30 < 2.32 = 2.32 nm for imogolite, AlSiAs_{10}, AlSiAs_{25}, and AlSiAs_{50}, respectively. In addition, there is an increase in the presence of precursor structures (proto-imogolite). These characteristics are under a thorough study and will be presented in a future report.

The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsenite increased. The presence of arsenite in the synthesis caused changes in the chemical composition of the samples (Table 1), observing that the Al/Si ratio was higher than that of the imogolite (Al/Si = 2.0:1.8) as the concentration of arsen...
Figure 5. AlSiAs nanotubes are not hemolytic and induce low morphological changes in human red blood cells following 1 day of exposition. (A) Representative pictures of red blood cells exposed to water (control of hemolysis), PBS (control of no hemolysis), AlSiAs50 nanotubes (1 mg/mL), and imogolite (1 mg/mL) after 24 h of incubation are shown. Exposition to the AlSiAs nanotubes did not change red blood cells’ morphology. (B) Membrane integrity was maintained, measured by hemolysis, similar to PBS control. All experiments show mean ± standard deviation from at least three independent experiments. (C) The size of red blood cells was preserved. All conditions are shown as the mean ± SEM from at least 50 red blood cells in every condition and from three independent experiments. All-treated groups were not significantly different, compared with control condition (PBS). Scale bar: 10 μm. PBS: phosphate buffered saline; Imo: standard imogolite, Imo-As: AlSiAs50.

These results reveal that the AlSiAs nanotubes are not cytotoxic and induce slight changes in cell morphology, without significant hemolysis at low and high doses, supporting the idea that the nanotube could be further studied in animal models.

4. CONCLUSIONS

The results obtained in this study indicate that the presence of arsenite in the synthesis of imogolite causes significant changes in its morphology and surface behavior, possibly associated with the adsorption or structural sites that this oxy anion would occupy. This would affect parameters, such as Al/Si ratio, largely and, slightly, the diameter.

In conclusion, AlSiAs nanotubes are shown to be safe for human cells and become a promising component for applying in water treatment for As elimination.

■ AUTHOR INFORMATION

Corresponding Authors
*E-mail: nicolas.arancibia@usach.cl (N.A.-M.).
*E-mail: rodrigoramireztagle@gmail.com (R.R.-T.).

ORCID

Nicolas Arancibia-Miranda: 0000-0002-0142-6922
Rodrigo Ramirez-Tagle: 0000-0003-0694-1808

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) De Volder, M. F. L.; Tawfick, S. H.; Baughman, R. H.; Hart, A. J. Carbon Nanotubes: Present and Future Commercial Applications. Science 2013, 335—359.
(2) Sheikhpour, M.; Golbabaie, A.; Kasaean, A. Carbon Nanotubes: A Review of Novel Strategies for Cancer Diagnosis and Treatment. Mater. Sci. Eng., C 2017, DOI: 10.1016/j.msec.2017.02.132.
(3) Harik, V. M. Geometry of Carbon Nanotubes and Mechanisms of Phagocytosis and Toxic Effects. Toxicol. Lett. 2017, 273, 69—85.
(4) van den Brule, S.; Beckers, E.; Chaurand, P.; Liu, W.; Ilboua, S.; Palmi,等.; Wilmayshina, F.; Yakoubo, Y.; Avellan, A.; Levard, C.; et al. Nanometer-Long Ge-Imogolite Nanotubes Cause Sustained Lung Inflammation and Fibrosis in Rats. Part. Fibre Toxicol. 2014, No. 67.
(5) Lee, W. S.; Chang, C. H.; Liao, I. C.; Lee, M. L.; Chen, S. L.; Shiang, T. The Longitudinal Effect of Concept Map Teaching on Critical Thinking of Nursing Students. Nurse Educ. Today 2013, 33, 1219—1223.
(6) Sarkar, B.; Mandal, S.; Tsang, Y. F.; Kumar, P.; Kim, K. H.; Ok, Y. S. Designer Carbon Nanotubes for Contaminant Removal in Water and Wastewater: A Critical Review. Sci. Total Environ. 2018, 561—581.
(7) Farmer, V. C.; Adams, M. J.; Fraser, A. R.; Palmieri, F. Synthetic Imogolite: Properties, Synthesis and Possible Applications. Clay Miner. 2006, 459.
(8) Ishikawa, K.; Abe, S.; Yawake, Y.; Suzuki, M.; Watari, F. Osteoblastic Cellular Responses to Aluminosilicate Nanotubes, Imogolite Using Sau-2 and MC3T3-E1 Cells. J. Ceram. Soc. Jpn. 1010, 118, 516—520.
(9) Hu, Y. J.; Fine, D. H.; Tasciotti, E.; Bouamrani, A.; Ferrari, M. Nanodevices in Diagnostics. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2011, 11.
(10) Nel, A. E.; Mller, L.; Veldegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. Health and Safety Implications of Engineered Nanomaterials. Part. Fibre Toxicol. 2009, 6, 543—557.
(11) Nel, A.; Xia, T.; Meng, H.; Wang, X.; Lin, S.; Ji, Z.; Zhang, H. Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening. Acc. Chem. Res. 2013, 607.
(12) Liu, X.; Sun, J. In Silico Nanoparticle Induce Apoptosis in Human Endothelial Cells via Reactive Oxygen Species, 2010 3rd International Nanoelectronics Conference (INEC); IEEE, 2010; pp 824—825.
(13) Rotoli, B. M.; Guidi, P.; Bonelli, B.; Bernardeschi, M.; Bianchi, M. G.; Esposito, S.; Frenzilli, G.; Luchessi, P.; Iglovikov, V.; Scarcelli, V.; et al. Imogolite: An Aluminosilicate Nanotube Endowed with Low Cytotoxicity and Genotoxicity. Chem. Res. Toxicol. 2014, 1142.
(14) Kumar, P. R.; Chaudhari, S.; Khilar, K. C.; Mahajan, S. P. Removal of Arsenic from Water by Electrocoagulation. Chemosphere 2004, 1245.
(15) Arancibia-Miranda, N.; Escuday, M.; Molina, M.; Garcia-Gonzlez, M. T. Use of Isoelectric Point and PH to Evaluate the Synthesis of a Nanotubular Aluminosilicate. J. Non-Cryst. Solids 2011, 357, 1750—1756.
(16) Edgell, C.; McDonald, C. C.; Graham, J. B. Permeation cell line expressing human factor VIII-related antigen established by hybridization. Proc. Natl. Acad. Sci. U. S. A. 1983, 80, 3734—3737.
(17) Rojas-Mancilla, E.; Oyarce, A.; Verdugo, V.; Morales-Verdejo, C.; Echeverría, C.; Velásquez, F.; Chnaiderman, J.; Valiente-Echeverría, F.; Ramirez-Tagle, R. The \([\text{Mo}_{6}\text{Cl}_{14}]^{2-}\) Cluster Is Biologically Secure and Has Anti-Rotavirus Activity in Vitro. *Molecules* **2017**, *22*(7), 1108.

(18) Rojas-Mancilla, E.; Oyarce, A.; Verdugo, V.; Zheng, Z.; Ramirez-Tagle, R. The Cluster \([\text{Re}_{6}\text{Se}_{8}\text{I}_{6}]^{3-}\) Induces Low Hemolysis of Human Erythrocytes in Vitro: Protective Effect of Albumin. *Int. J. Mol. Sci.* **2015**, *16*, 1728–1735.

(19) Ishikawa, K.; Akasaka, T.; Yawaka, Y.; Watari, F. High Functional Expression of Osteoblasts on Imogolite, Aluminosilicate Nanotubes. *J. Biomed. Nanotechnol.* **2010**, *6*, 59–65.