Supporting Information for

Silica nanoparticles inhibit arsenic uptake into rice suspension cells via improving pectin synthesis and the mechanical force of the cell wall

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Content

S1. Supplemental Methods

S 1.1. Method for synthesis and characterization of Si NPs

The synthesis of the Si NPs was used the method of sol-gel.[1] 1 mL volume of tetraethylorthosilicate (TEOS) and 400 μL of octadecyltrimethoxysilane were mixed adequately using the magnetic heating stirrer. And then ammonia (1 mL) and ultrapure water (2 mL) were added dropwise into the mixture solution and continually stirred at 80°C for 12 h. Subsequently, the precipitate was collected by centrifugation. The samples were dripped onto the copper net and then dried at room temperature. The morphological of the samples were performed using transmission electron microscope (TECNAI 10, Philips, Netherlands). The particle size distribution (PSD) and zeta potential of the sample was measured using Malvern Zetasizer Nano ZS (Worcestershire, UK).

S1.2 Cell viability assay

The viability of rice cells was assayed according to the established protocols described.[2] Firstly, 1 μL of fluorescein diacetate (FDA) and propidium iodide (PI) was added into the glass bottle containing 98 mL ultra-high purity water. The FDA-PI solution was obtained by continually stirring. In this experiment, the rice cells were cultured 24h in the absence or presence of Si NPs (0.1 or 1.0 mM). After the addition of As (10, 40 or 80 μM), and the rice cells were incubated in the cell culture medium for an additional 24 h. Then, the rice cells was obtained by centrifugation with ultra-high purity water at 3000 rpm for 10 min and and suspended in the solution of phosphate-buffered saline (PBS). 1.0 mL FDA-PI solution was added into the cell culture medium and incubated at room temperature in the dark for 10 min. After staining, the samples were thoroughly washed in ultrapure water and observed with a fluorescence microscope (Zeiss, Germany). The survival percentage of rice cells was analyzed using CELLQuest software.

S1.3 Measurement of reactive oxygen species and mitochondrial transmembrane

potential

The rice cells was incubated in the absence or presence of Si NPs for 24h. Then the solution of As was added into the cell culture medium and the rice cells continued to incubate 24h in the shaker at 28 °C . Subsequently, the rice cells were transferred into cell culture dish, then were stained with 10 μM of rhodamine123 or DCFH-DA at 37 °C for 30 min in the dark. After staining, the treated rice cells was thoroughly washed in ultrapure water and immediately detected by flow cytometry (Becton Dickinson, CA, USA). The fluorescence intensity of the samples was analyzed using CELLQuest software.[3]

S1.4 Cell wall extraction

The obtained rice cells were washed by centrifuged with ultrapure water five times at 1700 rpm for 5 min. Then the cell materials were ground to a fine powder with Teflon mortar in liquid nitrogen. Subsequently, the samples were added into sodium dodecyl sulphate (SDS) buffer (1% SDS, 50 μM Tris-HCl, pH = 7.2), incubated in an 80° C water bath for 15 min and centrifuged at 2000 rpm for 5 min in order to remove all SDS. The obtained cell wall material was washed with a series of solution (3 times with 80 % ethanol, twice with 50 % ethanol and 3 times with water). In order to extract the pectin from the cell wall material, cell wall (1.0 g) was reacted with 50 mL trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (50mM, pH = 6.5) at 25°C for 6 h. Then the obtained solution was filtered by a nylon filter and the residue was extracted in 50 mL sodium carbonate (0.05 M) with an addition of 20mM sodium borohydride for approximately 20 h at 1°C. Finally, pectin was obtained and vacuum dried in vacuum freeze drier.[4]

S1.5 Analysis of the pectin content in the rice cells

The samples for measurement of pectin content were prepared according to the reported literature (Fukuda and Komamine, 1982). Galacturonic acid (GalA) was used as a calibration standard and the pectin content was calculated according to the GalA equivalents.

S2. Supplemental Results

S2-1. Results of characterization of Si NPs

As Figure S1 shown, we can observe that the obtained Si NPs appeared as spherical particles with good monodispersity. The distribution of Si NPs was recorded using the PSD analysis. The results showed that the mean sizes of the Si NPs were 97.8 ± 2.8 nm. The result of the PSD analysis of the Si NPs was also in agreement with the TEM images. In addition, the zeta potential of Si NPs were highly negative in magnitude with value of -38.5 ± 0.6 mV.

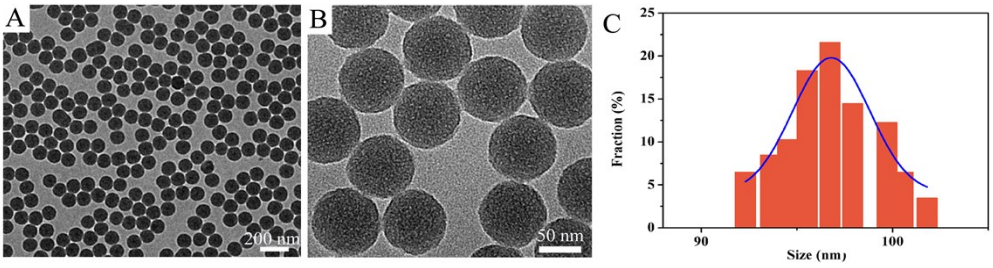

Figure S1. Transmission electron microscopy images of Si NPs (A and B); The particle size distributions of the Si NPs (C).

S3. Supplemental Tables

Table S1. The list of components in the modified N_6 medium

Component	Concentration(mg/L)	Component	Concentration(mg/L)
KNO_3	2830	Na_2EDTA	37.25
KH_2PO_4	400	2,4-D	2.0
$(NH_4)_2SO_4$	463	myo-Inositol	100
$MgSO_4 \cdot 7H_2O$	185	Thiamine Hydrochlori	0.5
$CaCl_2 \cdot 2H_2O$	166	Proline	500
KI	0.8	Casein Enzyniatic	600
H_3BO_3	1.6	Sucrose	30,000

Table S2. Primers for RT-PCR analysis of the genes.

Gene	Forward primer 5'→3'	Reverse primer 5'→3'
Lsi1	CGGTGGATGTGATCGGAACCA	CGTCGAACTTGTTGCTCGCCA
Lsi2	ATCTGGGACTTCATGGCCC	ACGTTTGATGCGAGGTTGG
OsNIP1;1	GGACTAGTATGGCAGGAGGTGACAACAA	GGACTAGTTTAGGTGGAGGAGTTCATCC
OsNIP3;3	GAAGATCTATGGAAGGGCACAAGAGTGG	GGACTAGTCTACAGCTTAATTGCAACAT
Actin	GACTCTGGTGATGGTGTCAGC	GGCTGGAAGAGGACCTCAGG

References

1. S. K. Park , K. Do Kim, H. T. Kim, Preparation of silica nanoparticles: determination of the optimal synthesis conditions for small and uniform particles, *Colloid Surface A*, 2002, **197**, 7-17.
2. J. Ma, X. Q. Zhang, W. J. Zhang, L. J. Wang, Multifunctionality of silicified nanoshells at cell interfaces of *Oryza sativa*, *ACS Sustain Chem Eng*, 2016, **4**, 6792-6799.
3. Q. Saquib, A. A. Al-Khedhairy, M. A. Siddiqui, F. M. Abou-Tarboush, A. Azam, J. Musarrat,

Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells, *Toxicol in Vitro* 2012, **26**, 351-361.

4. M. Szymanska-Chargot, A. Zdunek, Use of FT-IR spectra and PCA to the bulk characterization of cell wall residues of fruits and vegetables along a fraction process, *Food Biophysics*, 2013, **8**, 29-42.