

Supporting Information

Size-control of Arsenic Trioxide Nanocrystals Grown in Nanowells

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1. General methods

All the chemicals used in this work were purchased from Sigma-Aldrich unless stated otherwise. Atomic force microscopy (AFM) was performed on JEOL JSPM-5200 and scanning electron microscopy (SEM) was performed on a LEO Gemini 1525 sFEG SEM was used for scanning electron microscopy. A Hitachi H-8100 was used for transmission electron microscopy and selective area electron diffraction. A JEOL JEM-2100F FEG Fas TEM was used for energy dispersive X-ray spectroscopy. A Zetasizer Nano-ZS (Malvern Instruments) was used for dynamic light scattering and zeta potential measurements.

2. Fabrication of SU-8 Nanowells

To fabricate SU-8 (SU-8 2000.5, Microchem) nanowells, we diluted SU-8 in cyclopentanone in a 1:2 ratio, which was determined to be the optimal thickness of SU-8 for the growth of ATO crystals. The diluted SU-8 was spin-coated onto a piranha-etched glass substrate. The procedure for photolithography is as follows. After spin-coating of the diluted SU-8 on glass, SU-8 was baked at 95 °C for 1 min. The baked SU-8 with dot mask was exposed to UV light (365 nm) for 1.2 s and then the exposed photoresist was baked at 95°C for 1 min. Finally, the baked SU-8 substrate was developed in propylene glycol monomethyl ether acetate for 1 min.

3. Comparison of the Volumes of ATO Nanocrystals to Calculated Values

Table S1. Experimental and Calculated Values of ATO Nanoparticles

	Calculation (cm ³)	Experiment (cm ³)	Error (%)
50 mM	1.39 x 10 ⁻¹⁶	9.15(±1.65) x 10 ⁻¹⁷	57.0±28.3
100 mM	2.78 x 10 ⁻¹⁶	2.46(±0.54) x 10 ⁻¹⁶	18.7±26.1
150 mM	4.17 x 10 ⁻¹⁶	5.10(±0.78) x 10 ⁻¹⁶	16.3±12.8
200 mM	5.56 x 10 ⁻¹⁶	9.22(±1.05) x 10 ⁻¹⁶	38.9±7.0

4. Dynamic Light Scattering Measurement

	50 mM	100 mM	150 mM	200 mM
Average Diameter (nm)	57	86	129	177
Polydispersity Index (PDI)	0.227	0.095	0.031	0.042

5. Elemental Analysis of ATO Nanocrystals by Energy Dispersive X-ray Spectroscopy (EDS)

The EDS measurement showed that the elemental ratios of As: O: Na to be 40: 60: 0 (%) (Fig. S1). Because the result indicated that the nanocrystals did not contain sodium, we confirmed that NaOH, which was used for dissolving the ATO in water, was not included in the nanocrystals.

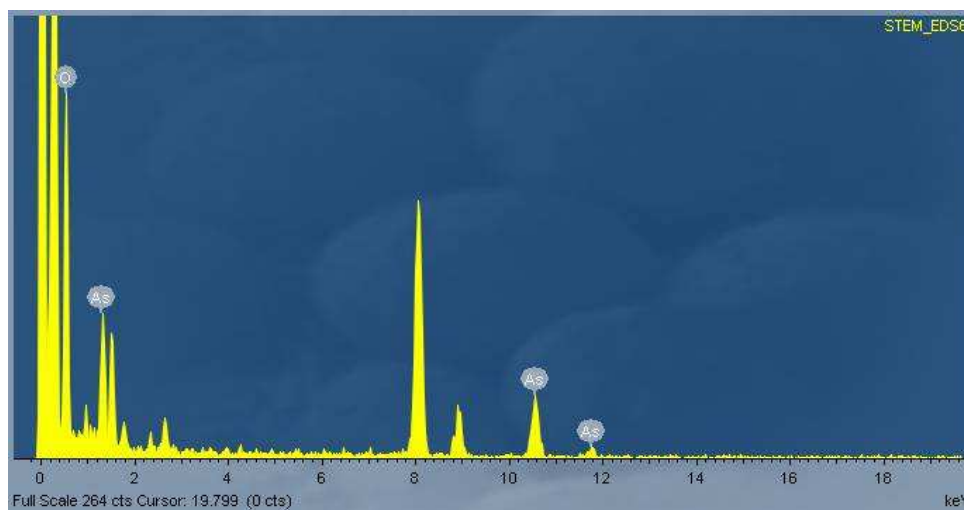


Figure S1. Energy dispersive x-ray spectra of an ATO nanocrystal.

6. Fabrication of SIL (Soft Interference Lithography) PDMS Nanowells

To scale up the density of ATO nanocrystals for cell studies, we fabricated PDMS nanowells on a 400-nm pitch, which has 25 times higher densities of nanowell arrays compared to SU-8 nanowells on a 2- μ m pitch (see section 2, above). First, arrays of photoresist posts (Shipley 1805) were patterned on Si

wafers using phase-shifting photolithography and an SIL PDMS photomask.¹ The photoresist post array (a one-time use master) had diameters of 230 nm and were spaced center-to-center by 400 nm. PDMS was then molded against the array of photoresist posts to form a PDMS nanowell template. A daughter master was made out of polyurethane (PU) by molding PU against the PDMS template. Hundreds of PDMS nanowell substrates could then be replicated from a single PU daughter master. The diameter and depth of the PDMS nanowells were measured to be 230 nm and 100 nm by SEM and AFM.

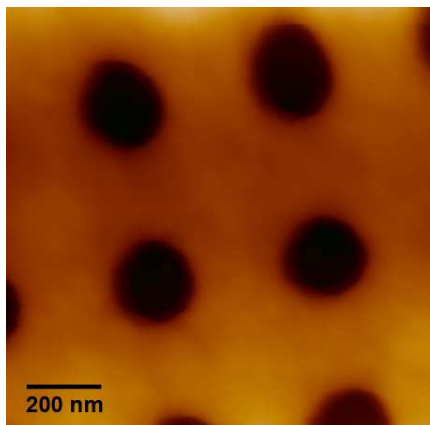


Figure S2. AFM image of PDMS nanowells.

7. ATO Nanocrystals used in Cell Study

ATO nanocrystals were grown in PDMS nanowells from 300 mM ATO solution. The released ATO nanoparticles were single crystalline. The average size was around 70 nm determined by TEM (**Fig. S3**), which was also supported by DLS measurement (average diameter = 72 nm, PDI = 0.267).

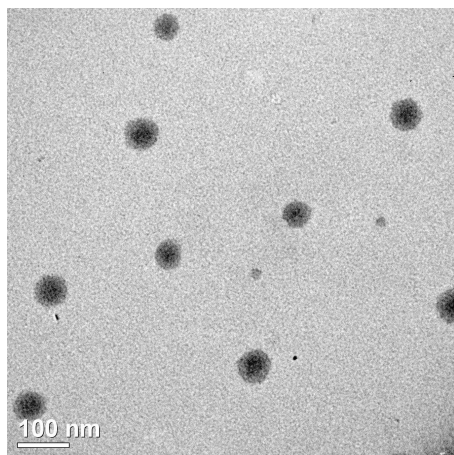


Figure S3. TEM image of ATO nanocrystals used for the cell study.

8. Cell Culture

K562 cells were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA; USA) supplemented with 4 mM L-glutamine (Invitrogen, Carlsbad, CA; USA) and 100 units/mL of penicillin/streptomycin (Invitrogen, Carlsbad, CA; USA). For MTS assays, 1,200 cells were plated into Tissue-Culture Treated Half Area 96 Well Plates (Greiner, Frickenhausen, Germany). A serial dilution of 150 μ M ATO and ATO nanocrystals dispersed into cell culture media was added to the cells. The concentration of arsenic in the ATO nanocrystal suspensions was determined by ICP-MS. After 24 h, the soluble tetrazolium salt, MTS (Promega, Madison, WI; USA) was added and cells were incubated for an additional 2 h. The absorbance of the wells was read at 492 nm (Synergy 2, Biotek, USA). The viability of cells was plotted versus the log concentration of elemental As, and a sigmoidal dose response was fit (Prism, Graphpad, USA).

9. Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

ICP-MS was used to measure the As content in ATO nanocrystals and free drug containing media. The ATO-containing media were dissolved in neat trace metal grade nitric acid (Fisher Scientific, Pittsburgh, PA) for 30 min. at room temperature. The acid solution was then diluted with ultra-pure laboratory grade water (Millipore, Billerica, MA). 2% v/v Triton X-100 (Acros Organics, Geel, Belgium) and 2% v/v glacial acetic acid (Mallinckrodt Baker, Inc, Phillipsburg, NJ). An internal standard mixture of Sc, Tb, Y, In, and Bi (CPI International, Santa Rosa, CA) was added to this solution. Standards between 0 and 90 ppb were made using a mixed element solution (CPI International). Arsenic standards and samples also contained 2% w/V trace metal grade nitric acid (Fisher Scientific, Pittsburgh, PA). All measurements were performed on an XSeries II ICP-MS instrument (Thermo Scientific, Waltham, MA). The concentration of the stock free drug was

determined to be $160 \pm 2.1 \mu\text{M}$ and the concentration of ATO nanocrystals was determined to be $341 \pm 14.6 \mu\text{M}$.

References

1. Henzie, J.; Lee, M. H.; Odom, T. W. *Nature Nanotech.* **2007**, *2*, 549-554