Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Short-Term and Long-Term Toxicological Effects of Vanadium Dioxide Nanoparticles on A549 Cells

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1. Quantification of cells after live/dead cell staining

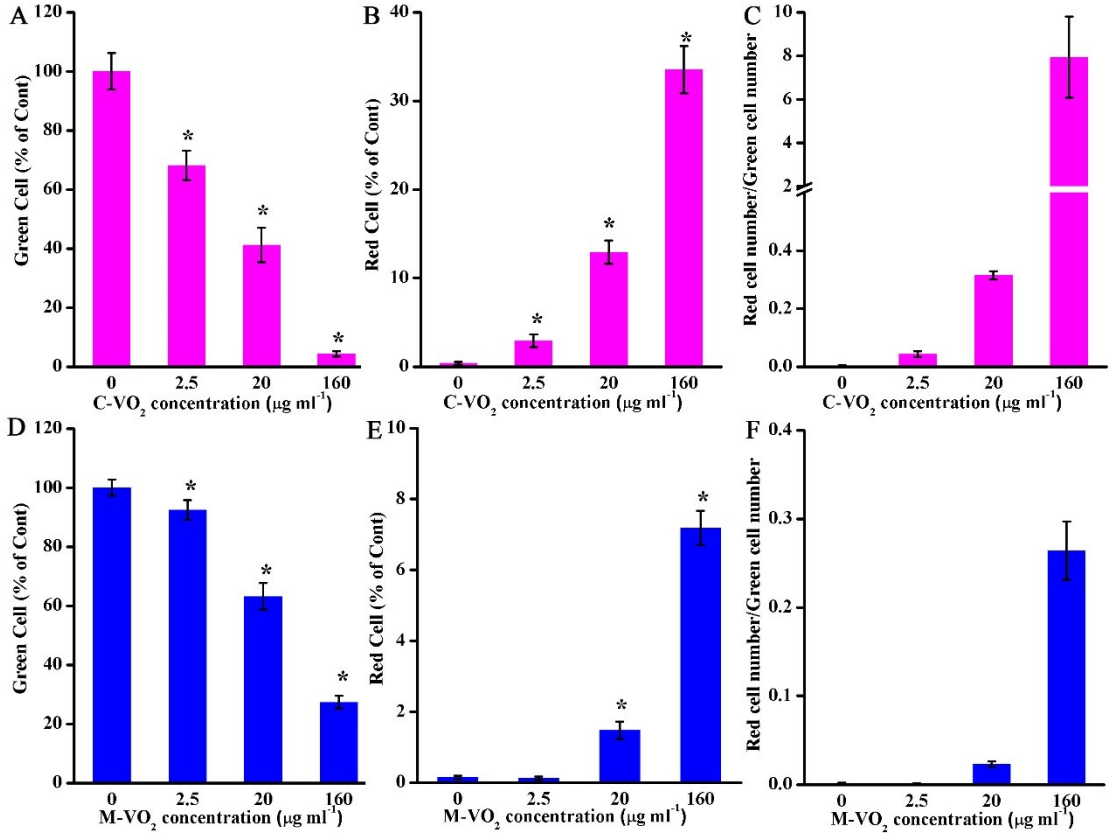

Fig. S1 Quantification of cells after live/dead cell staining. The cells were exposed to C-VO_2 (A-C) and M-VO_2 (D-F). (A and D) The ratios of live cells (green) to total number of control cells. (B and E) The ratios of dead cells (red) to total number of control cells. (C and F) The ratios of live cells (green)/dead cells (red). All data are represented as the mean ± SD (n= 3). *$p < 0.05$ comparing with the 0 μg ml^{-1} control.

2. Viability of A549 exposed to dissolved fraction of VO_2

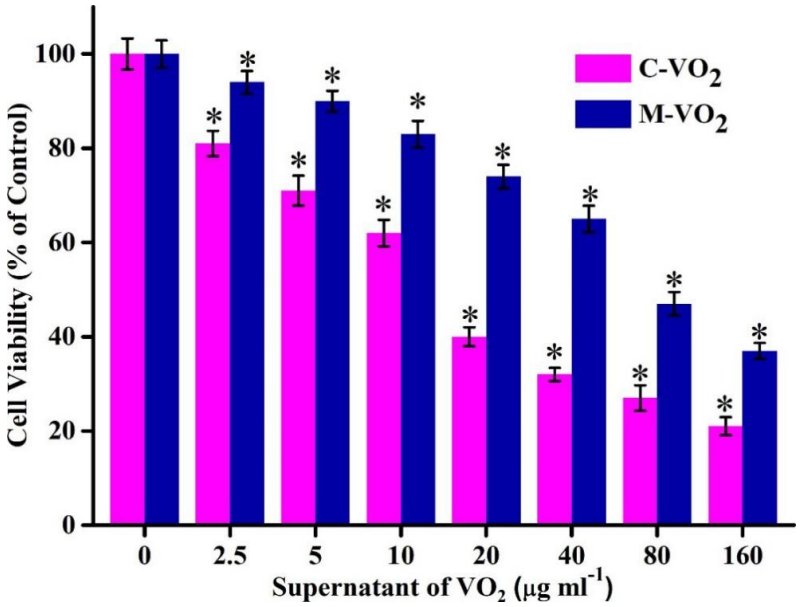

Fig. S2 Viability of A549 cells after exposure to supernatants of VO_2 cultured in DMEM for 24 h. All data are represented as the mean ± SD (n= 6). *$p < 0.05$ comparing with the 0 μg ml^{-1} control.

3. Dissolution of C-VO_2 and M-VO_2

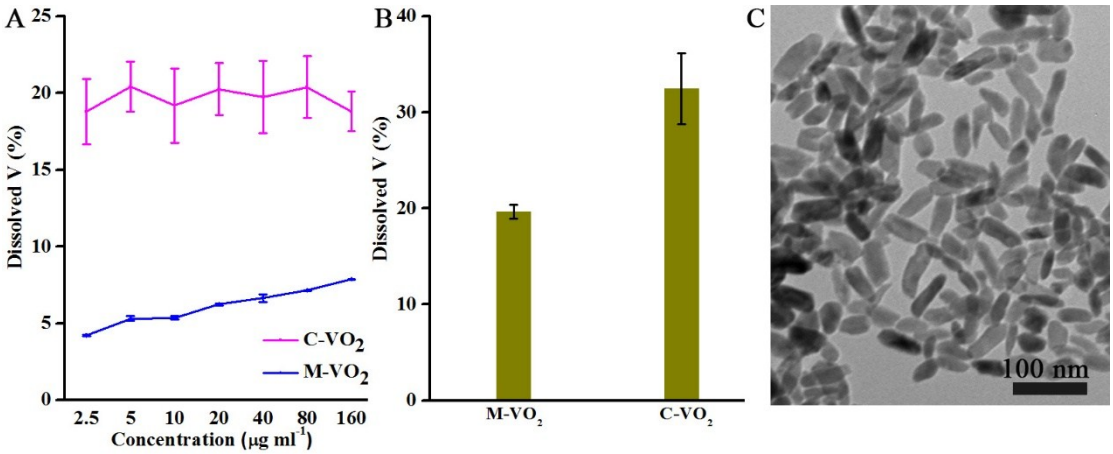

Fig. S3 Dissolution of VO_2 under the cell culture condition. (A) The 24-h dissolution of VO_2 at different concentrations. (B) The 48-h dissolution of VO_2 at a concentration of 0.2 μg ml^{-1}. (C) The TEM image of C-VO_2 after 48-h culture. All data are represented as the mean ± SD (n=3).

4. Viability of A549 exposed to V(IV) and V(V) ions at equivalent concentrations with corresponding dissolved fractions of C-VO_2

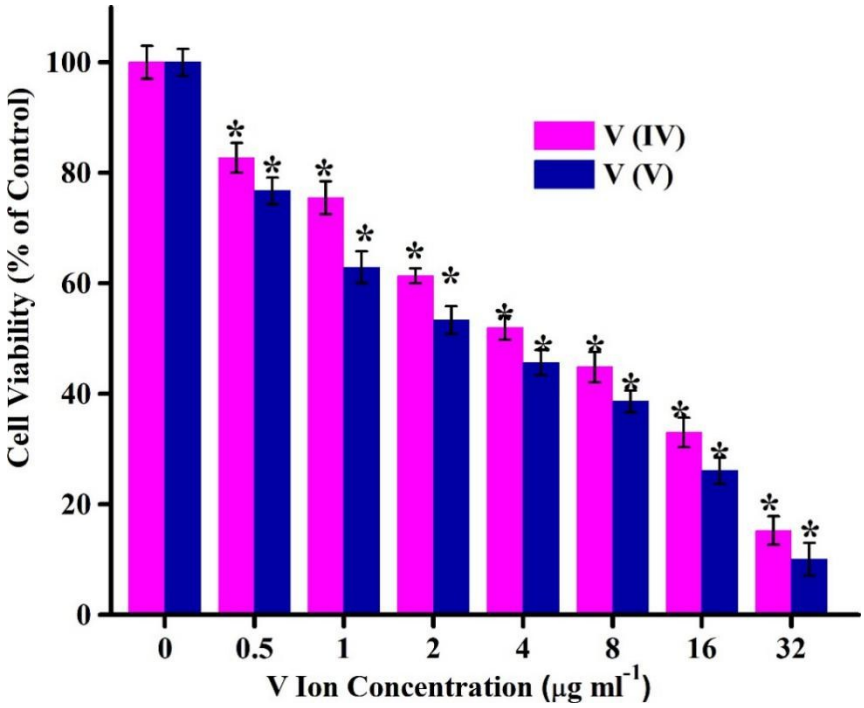

Fig. S4 Viability of A549 cells after exposure to V ions at equivalent concentrations with corresponding dissolved fractions of C-VO_2 for 24 h. All data are represented as the mean ± SD (n=6). *p < 0.05 comparing with the 0 μg ml^{-1} control.

5. Apoptosis and cell cycle arrest induced by V (IV)

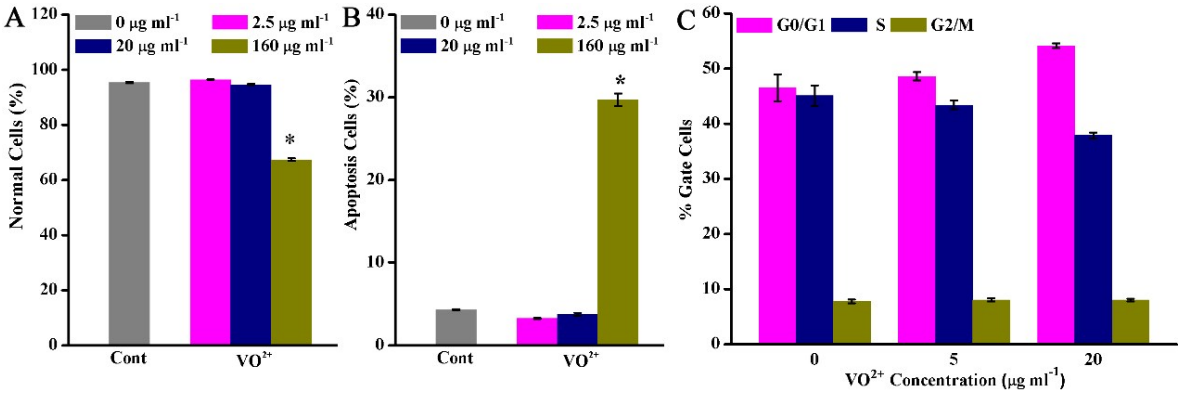

Fig. S5 Apoptosis and cell cycle analyses after exposure to V(IV) for 24 h. (A-B) Summary of FACS scatter analysis of normal (A) and apoptosis (B) cells by staining with the Annexin V-FITC and PI. (C) Cell cycle distribution of V(IV). *P < 0.05 compared with the corresponding control.

6. Viability of A549 cell after exposure to C-VO_2 at low concentrations

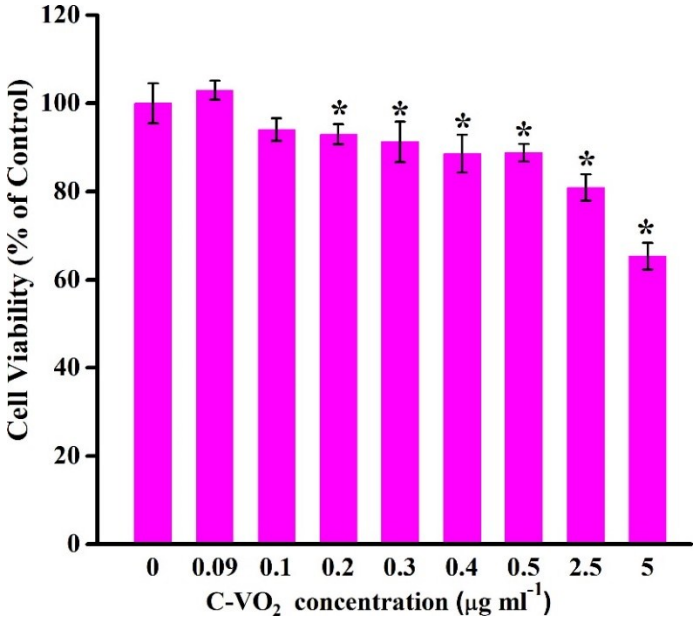

Fig. S6 Viability of A549 cells after exposure to C-VO_2 for 24 h. All data are represented as the mean ± SD (n=6). *$p < 0.05$ comparing with the control.

7. Live/dead staining of cells during the long-term exposure to VO_2

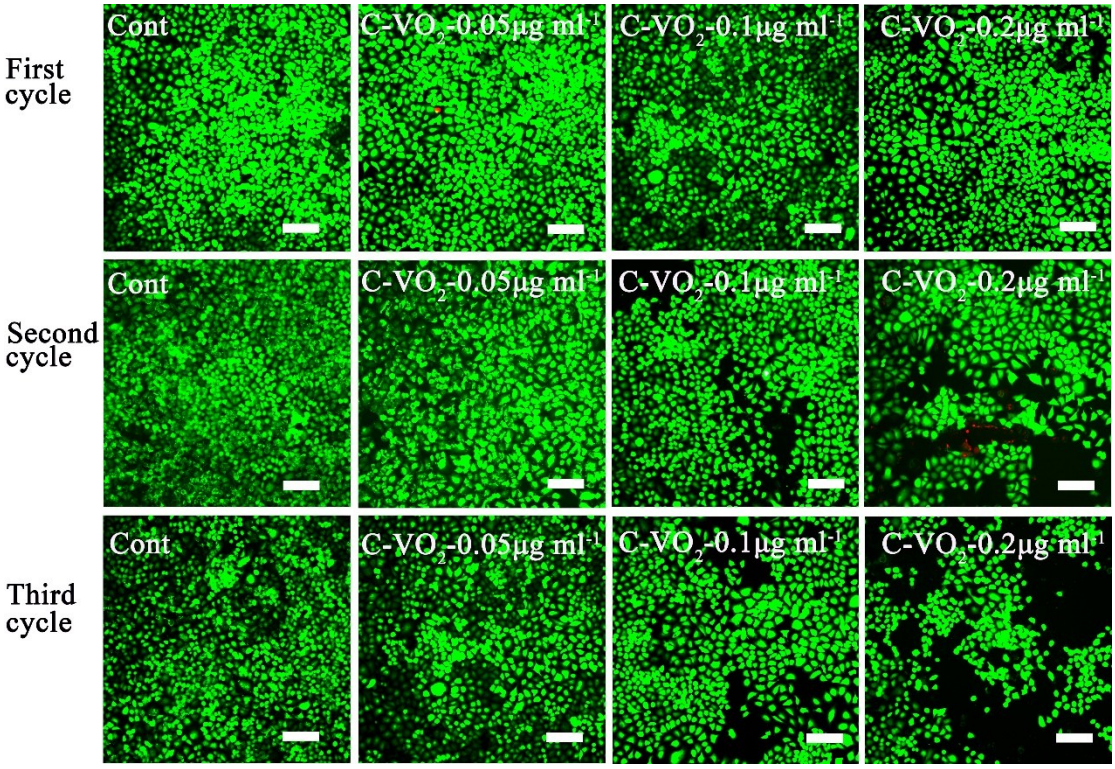

Fig. S7 Representative live/dead staining images of A549 cells after the first to the third cycles of C-VO_2 exposure. The corresponding controls, including positive controls, experienced exactly the same plating process as the cells treated with C-VO_2. Scale bar: 100 μm.

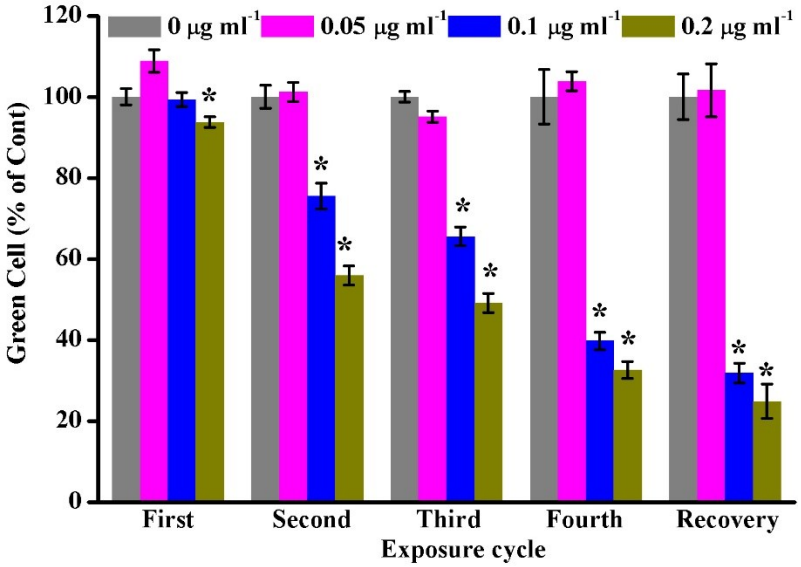

Fig. S8 Quantification of cells after live/dead cell staining. The cells were exposed to C-VO_2 for four cycles and recovery cycle. The results are expressed as percentages of live cells (green) to total number of control cells. All data are represented as the mean ± SD (n= 3). *p < 0.05 comparing with the 0 μg ml^{-1} control.

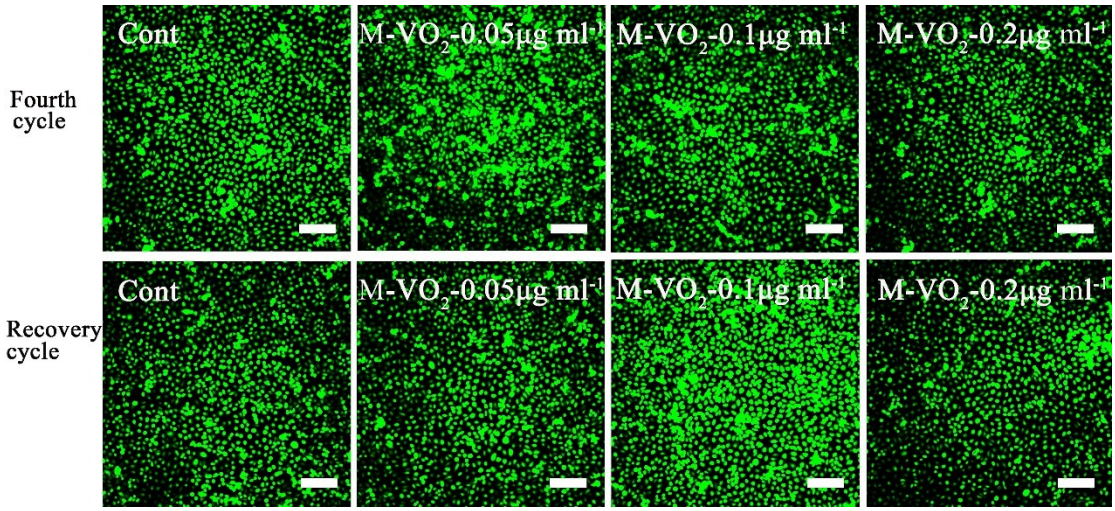

Fig. S9 The representative live/dead staining images of A549 cells after the fourth and recovery cycles of M-VO_2 exposure. Scale bar: 100 μm.

8. Cellular uptake of VO_2

We adopted flow cytometric light scatter analysis to investigate the uptake of C-VO_2 by A549 cells. After uptake of particles, the granularity of cells increased and could be detected by flow cytometric analysis using the side scatter parameter. A549 cells were plated in 6-well plates (1.8×10^5 cells per well) and cultured overnight. Then, culture medium was replaced with fresh culture medium containing 20 μg ml^{-1} VO_2 particles. After incubated for 0, 2, 4, 6 h, the cells were carefully washed with D-Hanks solution

for three times, detached with trypsin and then suspended in 200 μl culture medium for the side scatter measurement on the flow cytometry (BD Biosciences, FranklinLakes, NJ).

We measured the uptake of C-VO_2 by A549 cells using the flow cytometric light scatter analysis by recording the scattering of the laser light (Environ. Sci. Technol., 2007, 41, 3018-3024; Proc. Natl. Acad. Sci., USA, 2018, 115(1), E34-E43; Nanotoxicology, 2015, 9, 568-578; Environ. Sci. Technol., 2015, 49, 5003-5012). As the increase of culture time, side scatter (SSC) values of cells increased only slightly, but the increase of mean SSC values of cells was not significant (Fig. S10), indicating that few VO_2 particles entered cells.

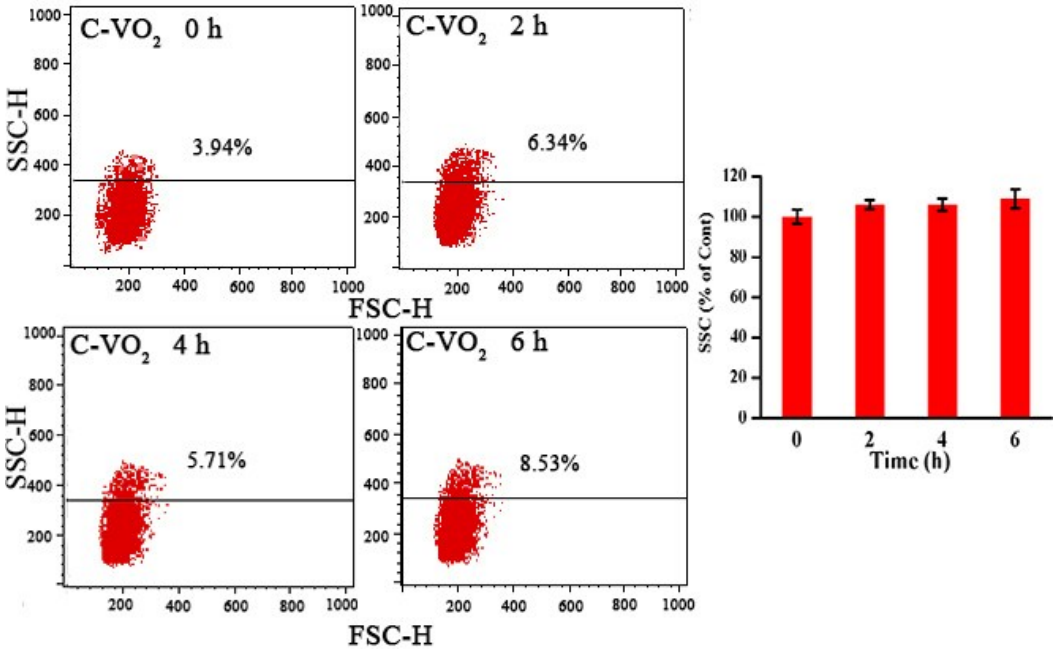

Fig. S10 Scatter diagrams (left) and relative mean SSC values (right) of A549 cells after exposure to 20 μg ml^{-1} of C-VO_2 for 0, 2, 4 and 6 h.

In addition, ICP-MS was used to measure the total amount of V in A549 cells after exposure to C-VO_2. The cell culture and C-VO_2 treatment were same as the above description. The cells were collected and counted. Then they were digested for the V content measurements on ICP-MS as described in Section 2.1. After 6 h incubation with C-VO_2 at a concentration of 20 μg ml^{-1}, the V content in cells, including both particles and ions, was around 25 ng/million cells after cells were exposed to C-VO_2 for 6 h.

This is about 2-4 orders of magnitude lower than the typical uptake of some other metal oxide nanoparticles by cells (e.g. uptake values of several μg/million cells reported in Environ. Sci.: Nano, 2016, 3, 365-374 and Environ. Health Perspect., 2007, 115, 403-409).

Therefore, we concluded that C-VO_2 were difficult to be internalized by A549 cells.