ADVANCED MATERIALS

**Supporting Information**

for

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# Porous, Hollow, and Ball-in-ball Metal Oxide Microspheres: Preparation, Endocytosis, and Cytotoxicity

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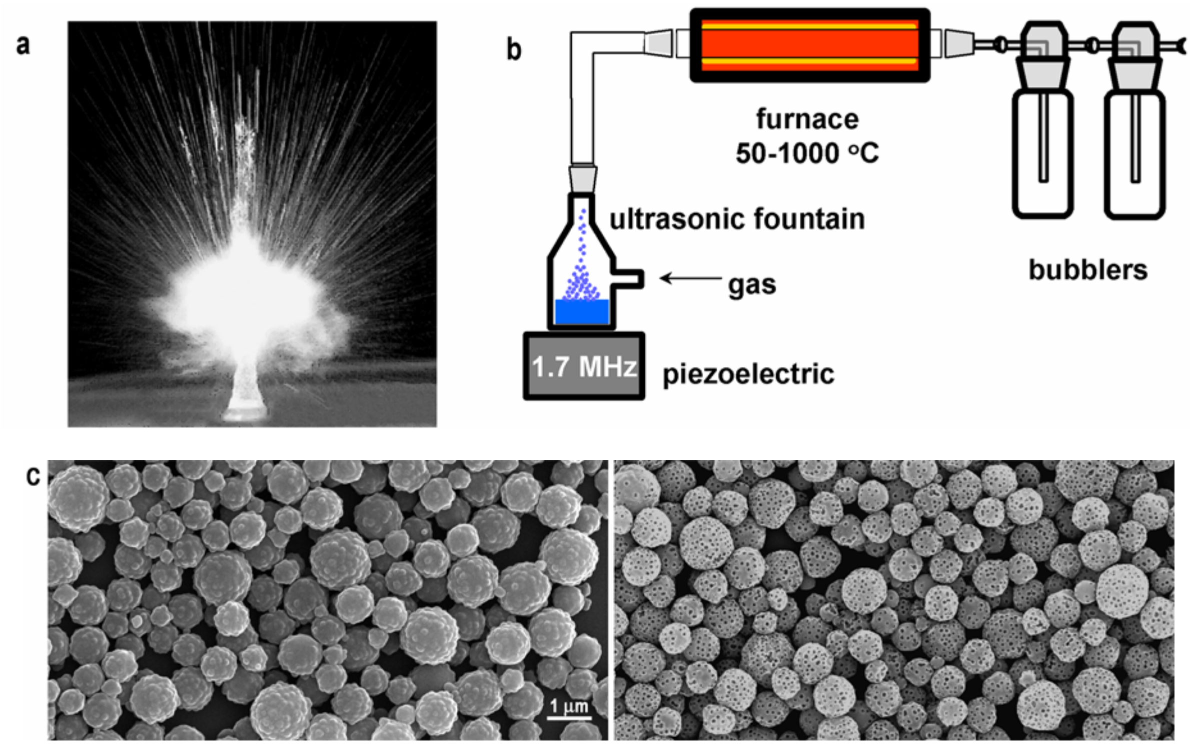


**Figure S1.** Ultrasonic spray pyrolysis (USP). (a) Macrophotograph of an ultrasonic fountain and mist produced at 1.7 MHz (0.033 s exposure, total width ~ 8 cm). W. H. Suh, K. S. Suslick, *J. Am.Chem. Soc.* **2005**, *127*, 12007. (b) Ultrasonic spray pyrolysis (USP) apparatus with single furnace. (c) Low magnification SEM of USP products, **4** (left) and post-etching products, **4-etched** (right).

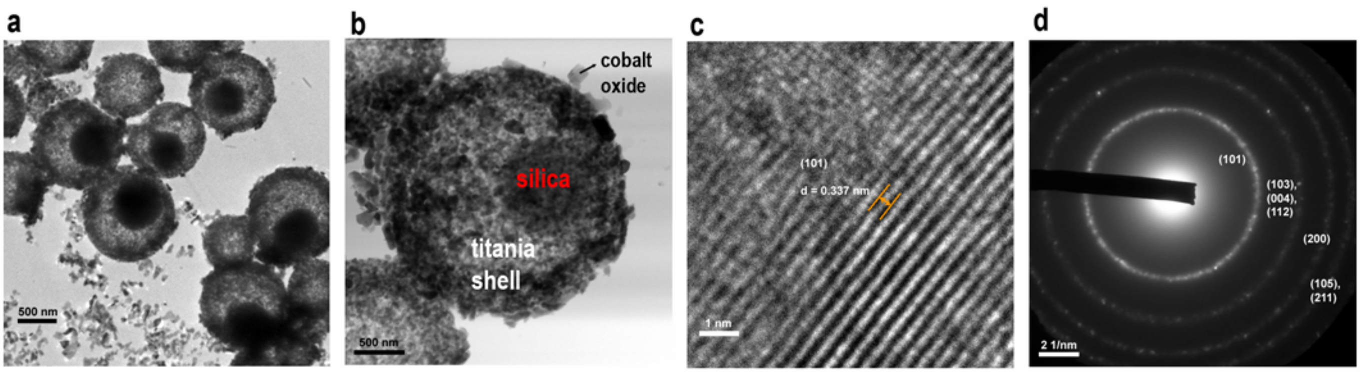


**Figure S2.** TEM and Selected Area Electron Diffraction (SAED) analysis of prepared titania spheres. (a, b) TEM of **2-etched**. (c) Anatase phase USP titania, *d*-spacing = 0.337 nm. (d) SAED of USP titania.

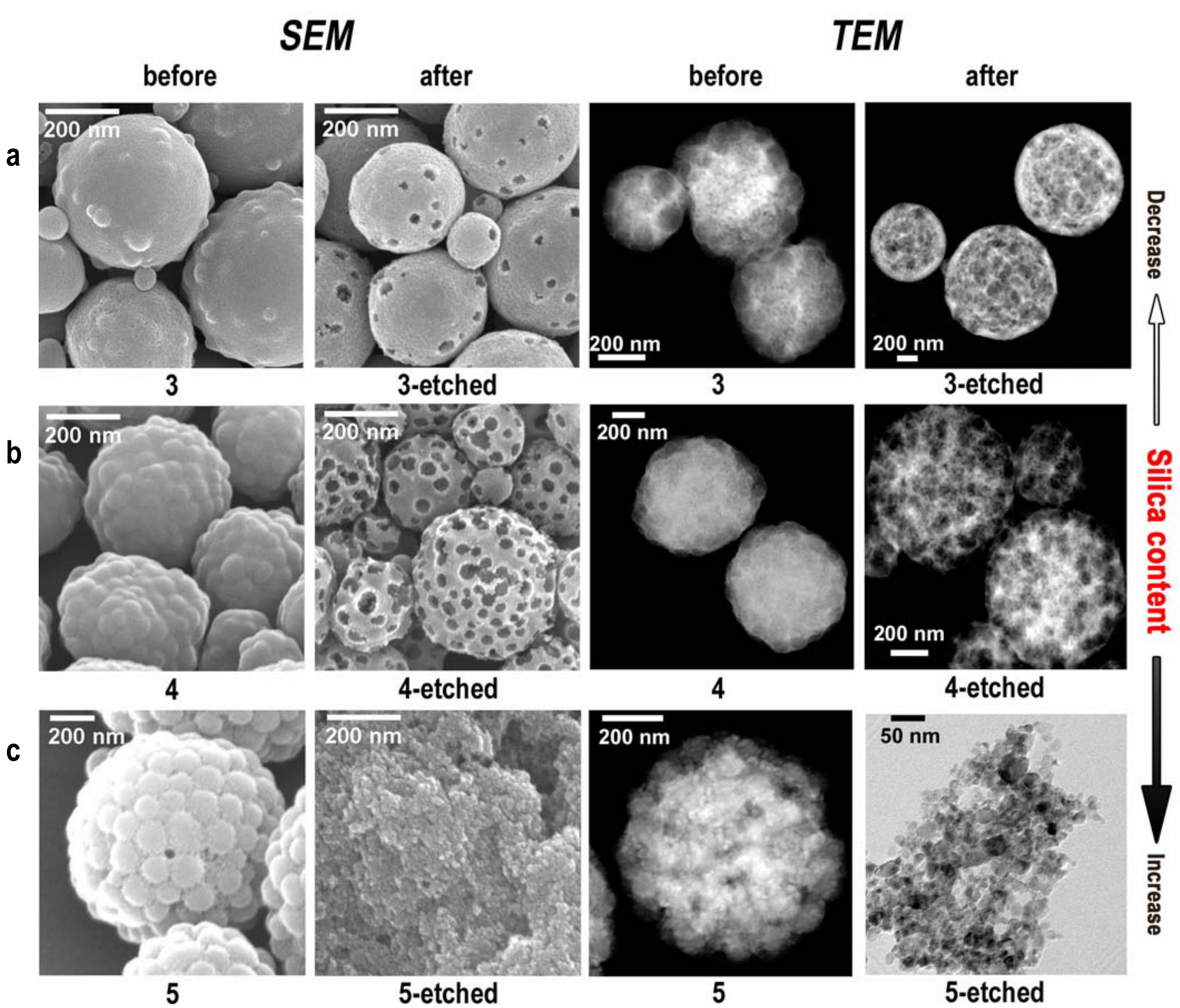


**Figure S3.** Effect of silica/titania ratio on particle morphology. First and third column of images before etching; second and fourth images after etching. The pairs of SEM and (S)TEM images shown are for microspheres **3** (a, Si:Ti = 1:5, 70-100 nm silica), **4** (b, Si:Ti = 1:1, , 70-100 nm silica), and **5** (c, Si:Ti = 8:1, 70-100 nm silica). In a typical preparation, Snowtex ZL (0.02 mol for **4**, 70-100 nm silica), titanium(IV) bis(ammonium lactato) dihydroxide or (in certain cases) titanium(IV) oxysulfate (0.02 mol), and purified water (50 mL, Barnstead Nanopure ion exchanged) were mixed and nebulized. Alternatively, a 1:5 silica/Ti molar ratio for **3** and 8:1 for **5** were used. Furnace temperature was set at 700-900 $^{o}$C with an air flow rate of 1 SLPM. For nebulization, a Sunbeam 1.7 MHz household ultrasonic humidifier (<$30) was used. After 6 hours of collection into water-filled bubblers, the grey colloidal particles were obtained by centrifugation at 8000 x *g*. The products were washed with purified water at least three times and sampled for analysis.

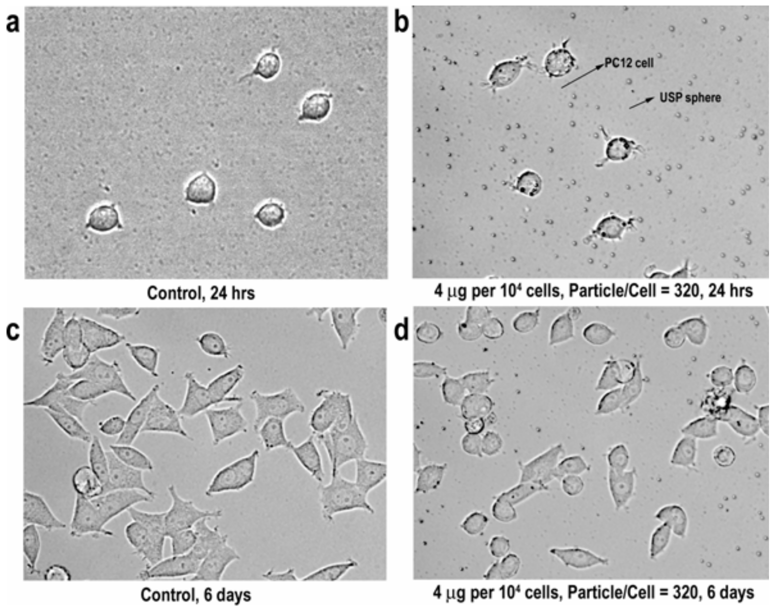


**Figure S4.** Cell culture optical microscope pictures. (a) Control PC12 cells only, 24 hrs. (b) PC12 cell and particle **1** cultured together, 24 hrs. (c) PC 12 cells alone, day 6. (d) PC12 cell and particle **1** cultures together, day 6. **Cell culturing with microspheres.** PC12 cells were incubated with a known number ratio of particles to cells in a 6 well plate at 37 °C under 5% $CO_2$. Cell growth was monitored visually and optical micrographs were taken using a digital camera attached to an Olympus IX50 optical microscope.

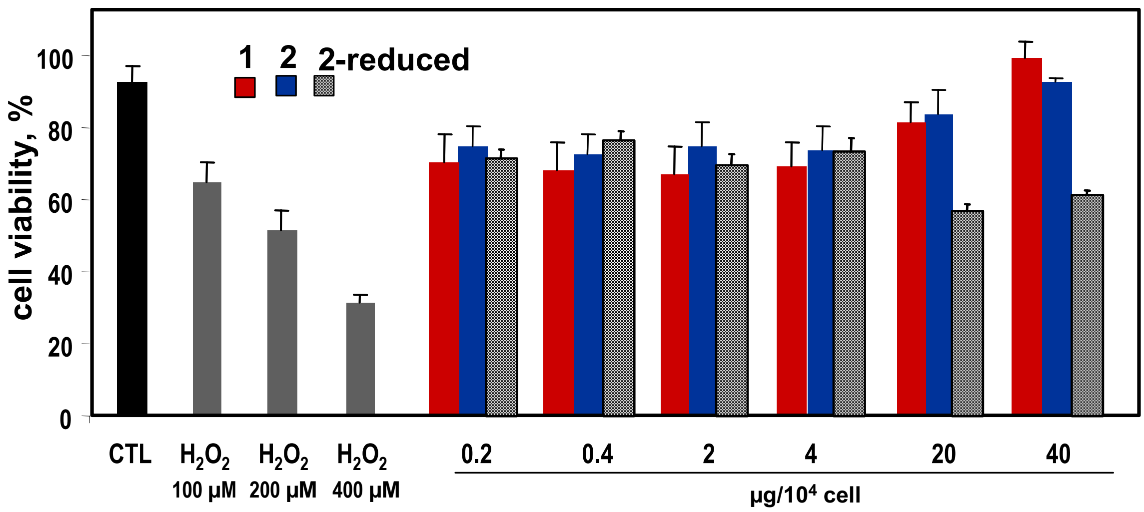


**Figure S5.** WST-1 assay on BV2 macrophage cells. Ctl=control. 0.2 μg of microspheres/$10^4$ cells ~ a number ratio of 16 microspheres/cells. **2-reduced** is microsphere **2** treated with 5% hydrogen (95% nitrogen) at 600 °C for 4 hours, which converts the cobalt oxide into cobalt metal.

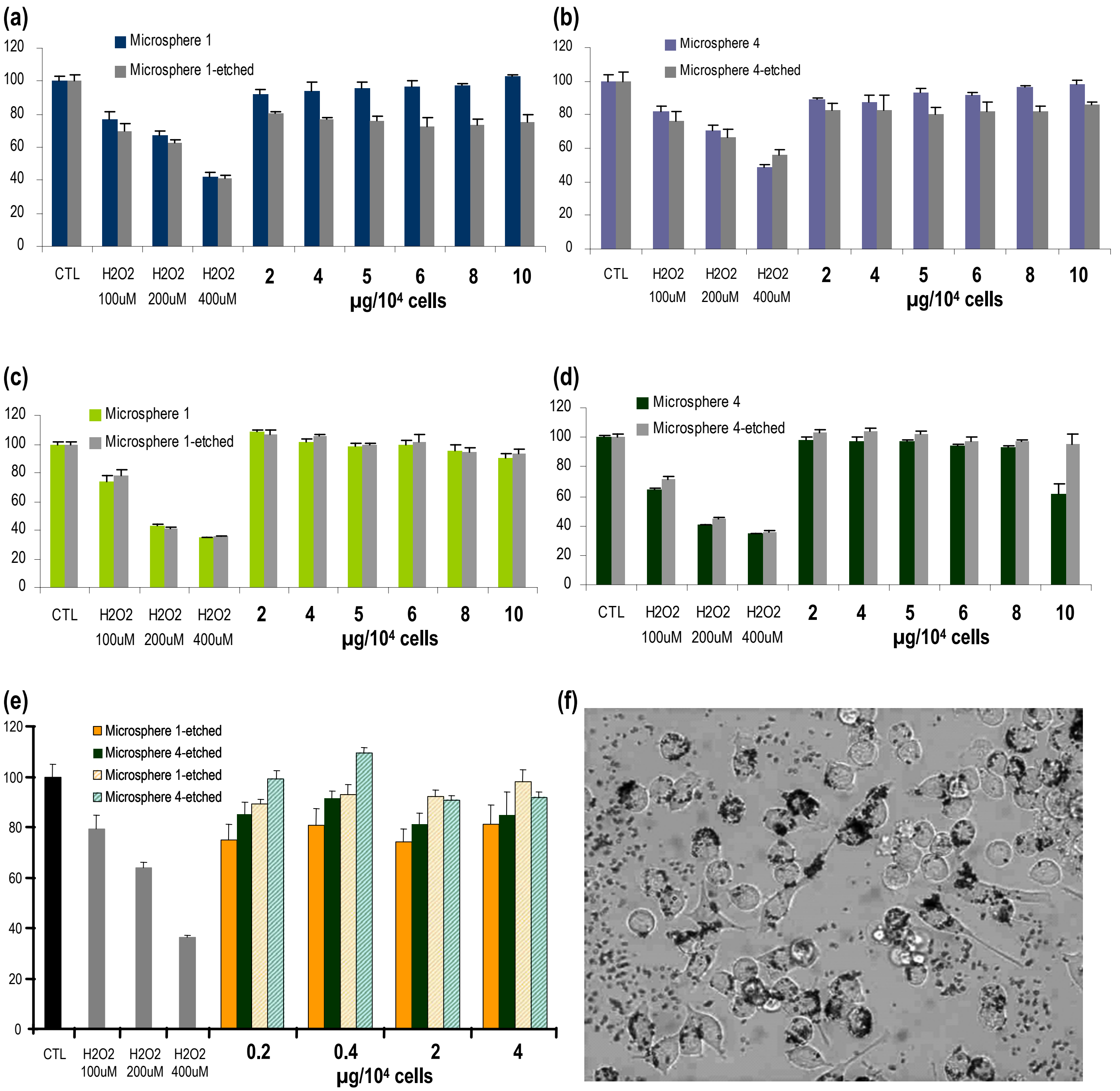


**Figure S6.** WST-1 assay on BV2 and SHSY5Y cells. Ctl=control. 0.2 μg of microspheres/$10^4$ cells ~ a number ratio of 16 microspheres/cells. All tests here were $10^4$ cells in 100 μL). (a) 4 hour BV2 cell assay for microspheres **1** and **1-etched**. (b) 4 hour BV2 cell assay for microspheres **4** and **4-etched**. (c) 4 hour SHSY5Y cell assay for microspheres **1** and **1-etched**. (d) 4 hour SHSY5Y cell assay for microspheres **4** and **4-etched**. (e) 1 day BV2 and SHSY5Y cell assay for **1-etched** and **4-etched**. All the results combined with Figure 4, S4 and S5 are consistent with very low cytotoxicity of the as-prepared titania-based microspheres. (f) Optical micrograph showing 4 μg of microspheres **1-etched** (the black particles) for approximately $10^4$ cells in a 100 μL volume.

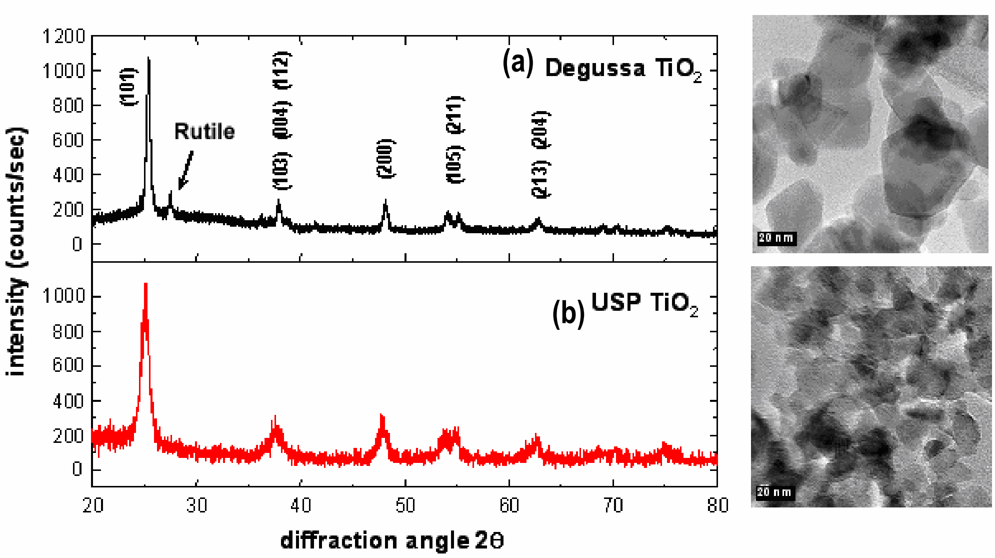


**Figure S7.** XRD distributions of nanocrystalline anatase $TiO_2$ samples and their TEM. (a) Commercially available Degussa $TiO_2$ P25; a small amount of the Rutile phase is also observed. TEM shows particle sizes are between 20-50 nm. According to Degussa average particle size is 21 nm. (b) USP synthesized anastase titania (**1**); no other phase is observed. TEM of **1-etched** shows that particle sizes are sub-20 nm. Similar to **5-etched** in Figure S3c. The broader [101] peak suggests that USP titania is smaller in crystallite size. Calculation of this line broadening using the Scherrer equation[1] revealed that while Degussa P25 is 13 nm, USP titania is 9 nm, which is consistent with the TEM data.

[1] R. Jenkins, R. L. Snyder, *Introduction to X-ray Powder Diffractometry*; John Wiley & Sons, Inc., New York, NY, **1996**, p 90.

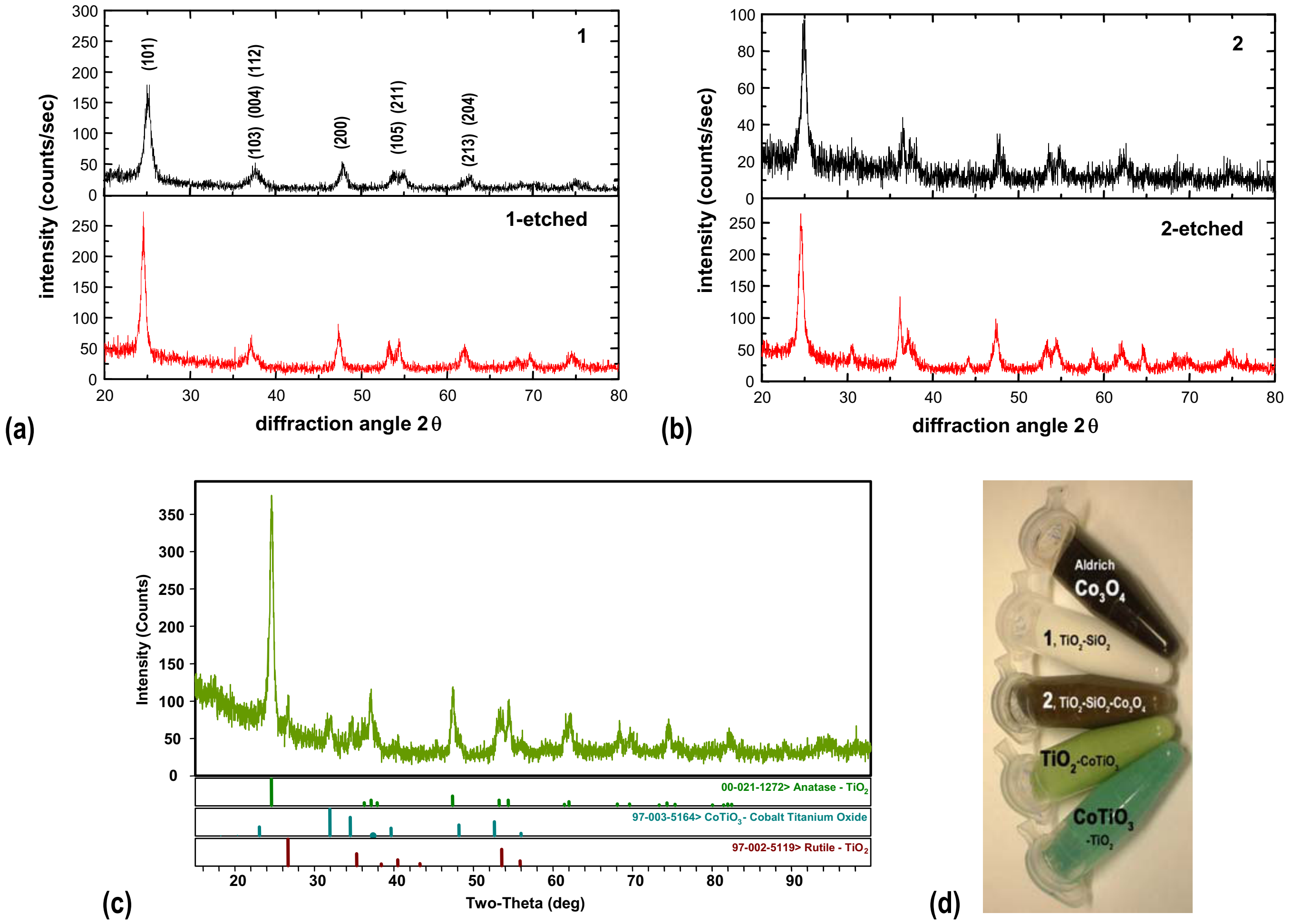


**Figure S8.** XRD patterns of samples in Figure 2. (a) **1** and **1-etched**. (b) **2** and **2-etched**. (c) 900 °C. (d) Colors of the solids.

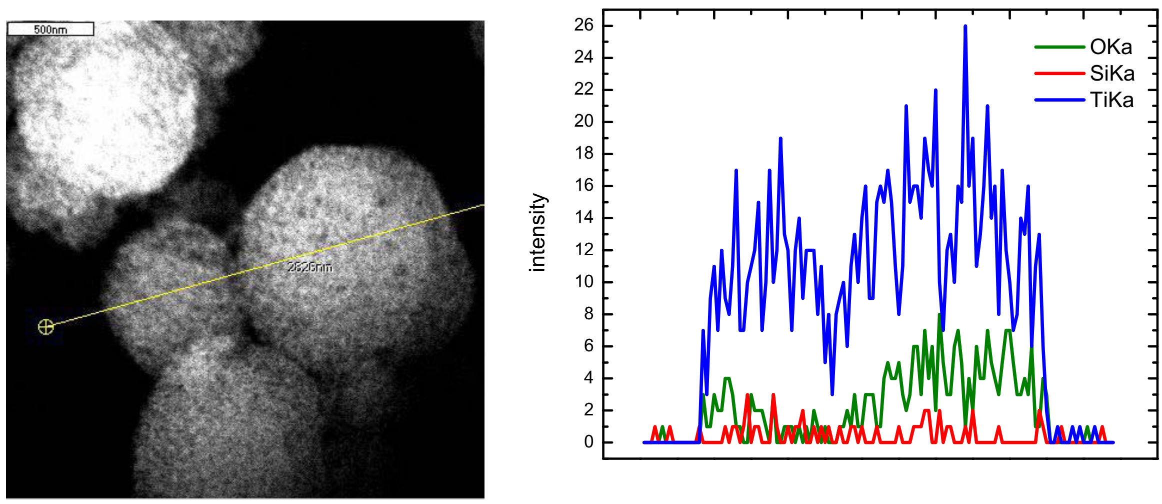


**Figure S9.** STEM line scan analysis of **1-etched**.

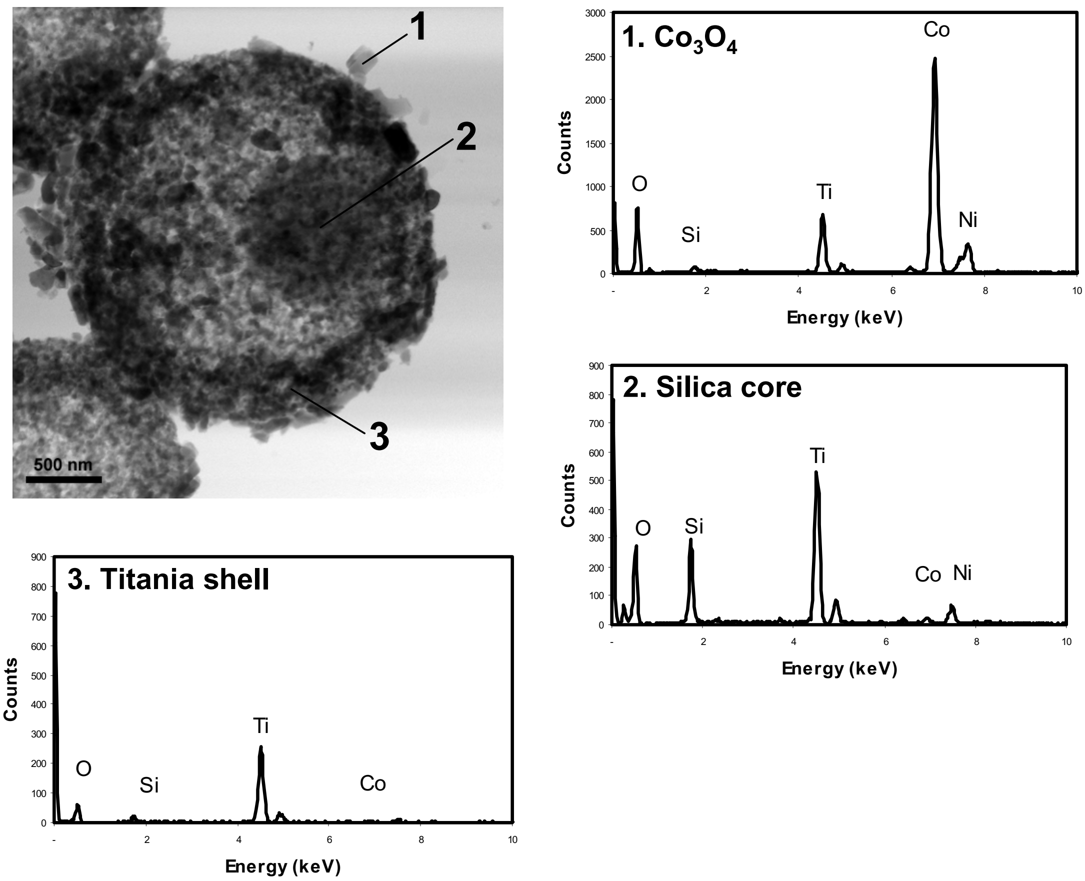


**Figure S10.** STEM spot analysis of **2-etched** from Figures 2f and S2a.

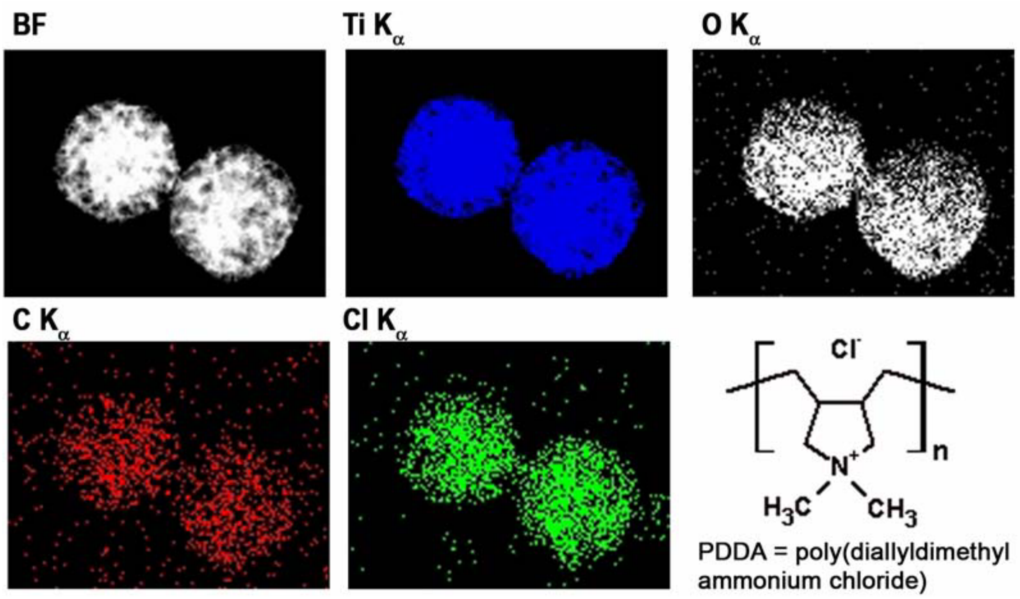


**Figure S11.** STEM elemental map analysis of PDDA filled porous titania **4-etched**. SEM and STEM are shown in Figure 5. BF = bright field, Ti Kα and O Kα comes from titania and C Kα and Cl Kα comes from PDDA. Structure of PDDA is given to show that Cl should be present in the final product.

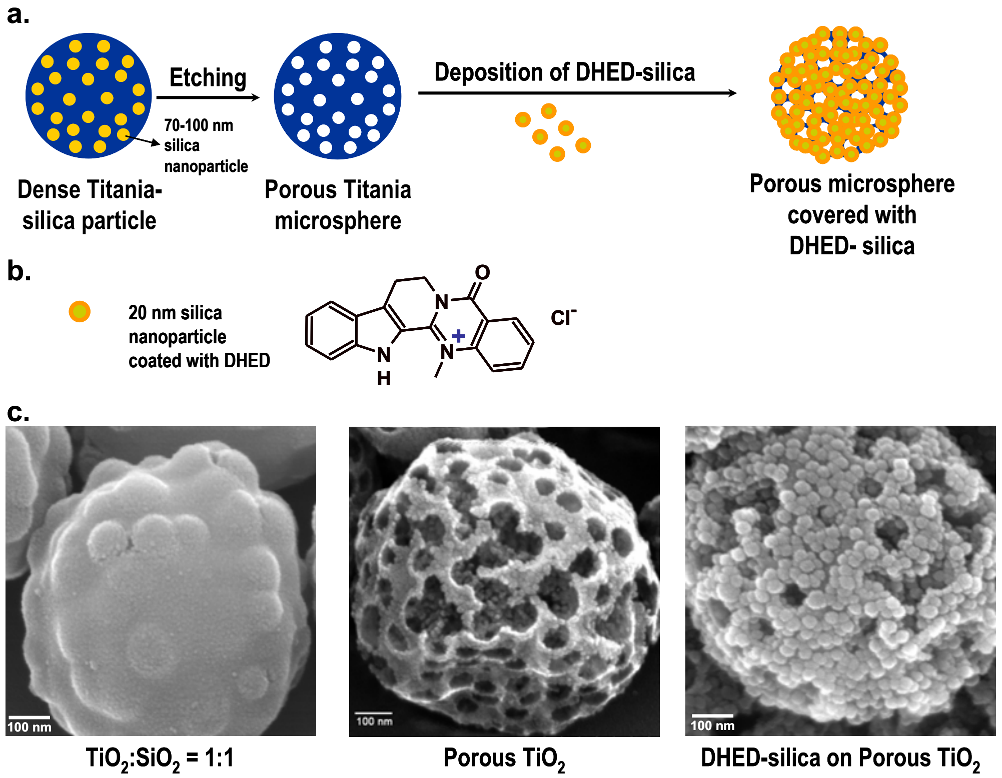


**Figure S12.** Silica deposition on porous titania microspheres. (a) Schematic of the deposition process. (b) The chemical structure of DHED, which was pre-adsorbed on the 20 nm silica nanoparticles. (c) SEM images corresponding to the schematic above; leftmost image is before HF etching, middle image after etching, rightmost image is after coating of the middle material with DHED. Refer to Figure 6 in the text.

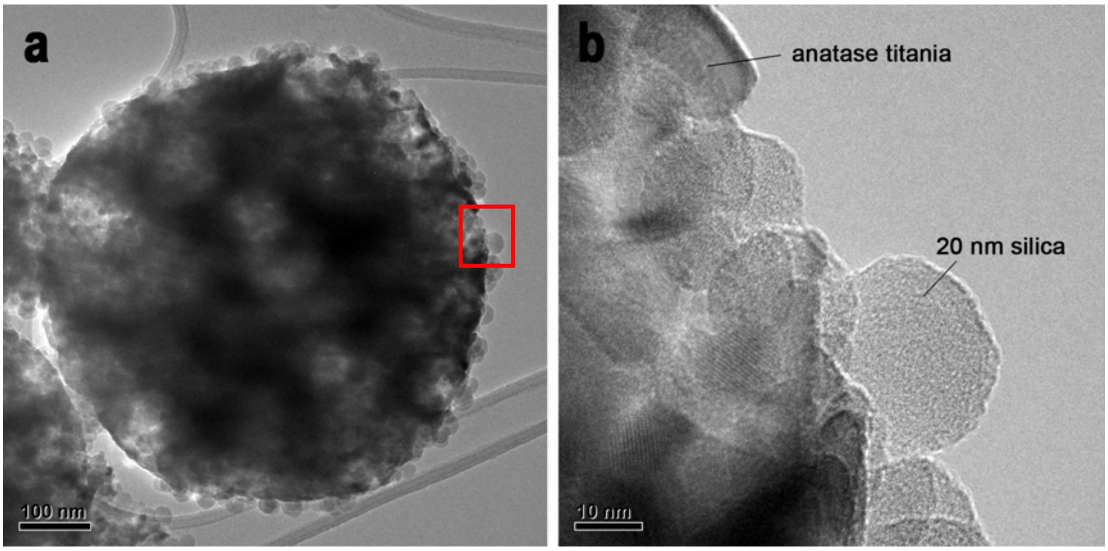


**Figure S13.** TEM images of silica deposited porous titania **4-etched**. (a) TEM on whole particle shows that the internal voids are filled up with 20 nm silica nanoparticles. (b) Close-up of the red box in the left figure. Crystalline titania phases are clearly shown as well as the amorphous and spherical 20 nm silica.

| Product | Before | After (etched) |
|---|---|---|
| 1 | 74 | 94 |
| 2 | 22 | 138 |
| 3 | 27 | 56 |
| 4 | 35 | 86 |
| 5 | 38 | 93 |
| Aldrich $TiO_2$* | 16 | |
| Degussa P25 $TiO_2$** | 38 | |
| Aldrich $Co_3O_4$*** | 32 | |

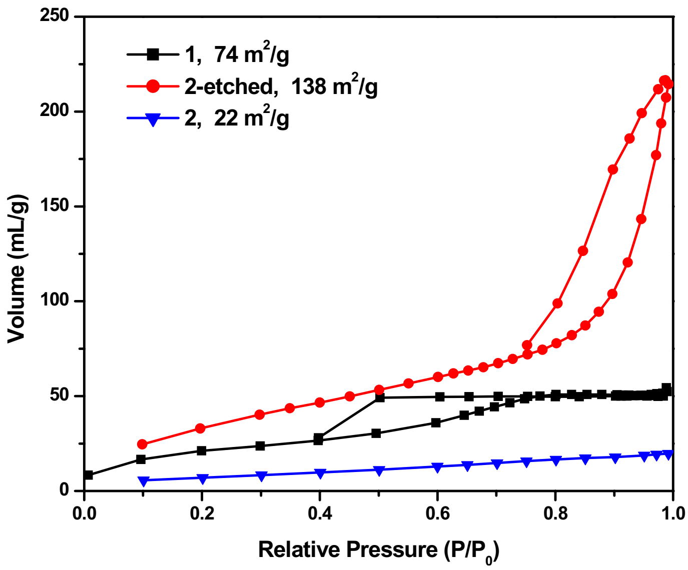


**Table S1.** Surface area ($m^2/g$) measurements for USP microspheres. Before etching (middle column) and after etching (right) values differ in a sense that when silica nanoparticles are sacrificed the surface area increased for all cases. The increased surface area for **2** is much higher than **1** or **4** because there are cobalt oxide nanoparticles supported on to the porous and hollow titania microspheres. In addition, there is a ball of silica inside which also contributes to the total surface area measurement. **5-etched** (basically titania nanoparticles) were measured to have 93 $m^2/g$, which is considerably higher than commercial nanoparticles. Additionally, full isotherms of **1, 2** and **2-etched** is given on the right side. They show that **1** is mesoporous (Type IV), **2** resemble Type II (nonporous) materials where as **2-etched** is type IV, meaning with the hysteresis it is mesoporous or macroporous adsorbents, as expected. Pore volume at relative pressure 0.99 (adsorption) for **2** is 0.03 mL/g (pore size<116 nm) and for **2-etched** it is 0.33 mL/g (pore size<116 nm). 11-fold increase is possibly due to the interior core having more free space. Pore volume of **1** is about 0.084 mL/g (pore size<85 nm).

*20-25 $m^2/g$ according to Sigma-Aldrich, $TiO_2$ nanopowder, 25-70 nm particles, anatase/rutile mixture, product #634662.
**50 ± 15 $m^2/g$ according to Degussa, $TiO_2$ P25, 21 nm (average) particles.
***40-70 $m^2/g$ according to Sigma-Aldrich, $Co_3O_4$ nanopowder, 20-30 nm particles, product #637025.