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Reductase-like Activity of Silicon Nanowire Arrays

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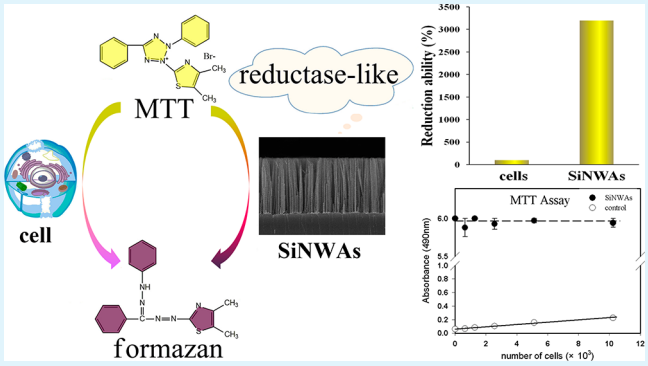
Supporting Information

ABSTRACT: The MTT (3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide) reduction method is widely used for measuring cell viability and proliferation. However, when MTT was used to study cells on silicon nanowire arrays (SiNWAs), the measured viability was much higher than normal values, resulting in a misleading estimate of cell viability. Our results demonstrated that the apparent high viability of cells is due to the fact that the SiNWAs itself was capable of reducing MTT in the absence of cells. In the presence of coenzyme, its reducing capacity was enhanced, thus showing the reductase-like function of SiNWAs. Furthermore, the chemical composition and nanostructure of Si surface had a strong influence on MTT reduction with the HF-treated SiNWAs (H-SiNWAs) showing significant reducing capacity. For example, the reduction capacity of H-SiNWAs samples was significantly higher than that of HF-treated planar silicon, whereas Piranha-treated SiNWAs and planar silicon did not reduce MTT. H-SiNWAs were also used for the reduction of azo dyes and showed a decolorization rate of more than 65% and as high as 90%. These findings suggest the potential use of SiNWAs as enzyme-mimics in biotechnology and environmental chemistry.



KEYWORDS: *silicon nanowire arrays, MTT, cell viability, reductant*

1. INTRODUCTION

Intracellular reduction of the water-soluble dye MTT (3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide) is mainly attributed to the mitochondrial enzyme succinate dehydrogenase and electron carriers and produces water-insoluble blue-purple formazan. MTT reduction may also be catalyzed by other non-mitochondrial enzymes. Therefore, intracellular reduction of MTT is the result of several reductases in cells and is closely related to intracellular metabolism.

The MTT assay has been widely used for evaluation of in vitro cytotoxicity and cell interactions with biomaterials. However, drugs and materials may react with MTT leading to erroneous results in these assays. For example, Chakrabarti et al. found that ascorbic acid could reduce MTT to formazan, and retinol acted as a reductase in catalyzing this reaction.[1] In recent years, there have been many reports concerning the toxicity testing of biomaterials using the MTT assay, but the method remains controversial, especially in the case of nanomaterials.[2−4] For example, the assay gave inaccurate data for cell toxicity because of superoxide formation induced by nanoscale TiO_2.[4]

Silicon-based nanostructured materials are biocompatible and have a wide range of applications in biomedical research.[5−8] Bayliss et al. showed that porous Si offers significant advantages over macroscopic Si in terms of cell viability.[9] However, Low et al. found that the apparent high viability of cells on porous Si was in part due to the fact that the porous Si itself was capable of reducing MTT in the absence of cells in a culture system.[10] The culture medium composition may also influence MTT reduction, and further investigation of MTT reduction in a non-cell-culture system is required. Laaksonen et al. showed that MTT reacted with porous silicon microparticles; the response due to the Si particles in cell tests was 3-fold higher than in the same conditions in the absence of cells. They suggested that the combined effect of cells and particles may give increased reduction of MTT.[11] As a kind of one-dimensional silicon nanomaterial, silicon nanowire arrays (SiNWAs) are widely used as biomaterials[5,12] and therefore it is necessary to study the toxicity of SiNWAs.

However, when MTT was used to study cells on SiNWAs, we found the measured viability was much higher than normal values, independent of the number of cells, resulting in a misleading estimate of cell viability. Here, we demonstrate that the apparent high viability of cells due to the fact that the SiNWAs itself is capable of reducing MTT in the absence of cells. Furthermore, the influence of the chemical composition and nanostructure of Si surface on MTT reduction is

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investigated. In addition we demonstrate that H-SiNWAs can degrade organic azo dyes. We expect that the reductase-like property of H-SiNWAs will be applicable as a promoter/catalyst in biochemical reactions more generally.

2. EXPERIMENTAL SECTION

2.1. . Materials and reagents. Silicon wafers (n-doped, (100)-oriented, 0.56 mm thick, 100 mm diameter) were purchased from Guangzhou Semiconductor Materials (Guangzhou, China). The as-received silicon wafers were cut into 0.5 cm×0.5 cm square chips. 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT, Amresco, 98%), Cell Counting Kit-8 (WST-8, Beyotime), RPMI medium 1640 (Hyclone), NADH (Solarbio), NADPH (Solarbio) and Remazol dyes (brilliant red F3B, brilliant orange 3R, golden yellow RNL 150% and brilliant violet 5R) (DyStar) were used as received. Deionized water purified by a Millipore water purification system to give a minimum resistivity of 18.2 MΩ cm was used in all experiments. Nitrogen gas was of high purity grade. All other solvents were purified according to standard methods before use.

2.2. Preparation of SiNWAs. The SiNWAs investigated in this study were prepared by chemical etching of silicon wafer in $AgNO_3$/HF aqueous solution as described.[13] Briefly, silicon wafers were cleaned in a freshly prepared Piranha solution (H_2SO_4:H_2O_2 = 7:3 (v/v)) at 90 °C for 0.5 h and then rinsed with distilled water and dried in a stream of argon. The cleaned silicon wafers were immersed in an etching solution containing HF (5 M) and $AgNO_3$ (0.015 M) at 50 °C for 30 min. The resulting materials were immersed in 20% nitric acid for 1 min and then rinsed extensively with deionized water. The surface morphology of as-prepared silicon nanowire arrays (SiNWAs) was observed using a field-emission scanning electron microscope (FESEM, S-4800, Japan). Most of the nanowires are perpendicular to the silicon wafer substrate and have uniform lengths of about 24 μm and diameters in the range 50−100 nm.

HF treatment: silicon wafers and SiNWAs were immersed in HF solution (5% in deionized water, v/v) for 5 min. After treatment, the materials were rinsed with deionized water and then dried in a nitrogen stream.

Piranha treatment: silicon and SiNWAs were treated in "Piranha" solution at 90 °C for 2 h. After treatment, the materials were rinsed with deionized water and then dried in a stream of nitrogen.

2.3. Cell Culture. L929 cells, derived from mouse connective tissue fibroblast, were cultured in RPMI medium 1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were cultured in 25 cm^2 culture flasks in a humidified incubator at 37 °C with 5% CO_2. After the L929 cells grew to the expected concentration, they were harvested by trypsinization; trypan blue staining tests showed that cell viability was above 95% after trypsinization. L929 cells were seeded at different density (0, 640, 1280, 2560, 5120, and 10240 cells/well) into the wells of 96-well plates for MTT and WST-8 tests. The cells were incubated for 24 h with 5% CO_2 at 37 °C before assays.

2.4. MTT and WST-8 Assays. Cell viability was assessed using MTT and WST-8 tests. First, the medium was replaced by 200 μL fresh medium and one piece of SiNWAs (0.5 cm×0.5 cm) was added, with control wells containing only cell culture medium. Then 20 μL of 5 mg/mL MTT or WST-8 was added and the wells were incubated for another 4 h. For the MTT assay, the medium was aspirated, the MTT-formazan generated by live cells was dissolved in 220 μL of DMSO; while for the WST-8 assay, the formazan dissolved directly in medium. Finally, 200 μL of the solution from each well was transferred into the adjacent empty wells, and absorbance values at 490 nm for MTT and 450 nm for WST-8 were measured using a microplate reader (Thermo Fisher Scientific Inc). Data are presented as average ± SE (n = 3).

2.5. Extracellular MTT Reduction in Aqueous Solution. 20 μL (5 mg/mL) of MTT was added to 200 μL aqueous solution, and the final concentration of NAD(P)H was 1.36 mM. After mixing, the SiNWAs sample was added immediately, and the control was absent of SiNWAs. The reactions were carried at 37 °C for 1 h. The solution was aspirated, and 220 μL of DMSO was added to dissolve the formazan adsorbed on the SiNWAs surface. Finally, 200 μL of the solution from each well was transferred into the adjacent empty wells and absorbance was measured as previously described.

2.6. Influence of Surface Properties of Si on MTT Reduction. 20 μL (5 mg/mL) of an aqueous solution of MTT was added to 200 μL deionized water. After mixing, a single piece of HF- or Piranha-treated Si or SiNWAs (H-Si, H-SiNWAs, P-Si, and P-SiNWAs) was added; reactions were carried at 37 °C for 3 h and absorbances were measured as described above.

2.7. Reductive Degradation of Remazol Dyes by H-SiNWAs. The Remazol dyes chosen for the present study were brilliant red F3B, brilliant orange 3R, golden yellow RNL 150% and brilliant violet 5R, azo, or diazo reactive dyes. The dyes were dissolved and diluted in distilled water to give absorbance values between 0.6 and 0.8. Then five pieces of H-SiNWAs were added to 150 μL of the dye solution. After incubation at 37 °C for 3 h, the product solution was centrifuged at 12 000 rpm for 1 min. Ultraviolet/visible absorption spectra were measured using a spectrophotometer.

3. RESULTS AND DISCUSSION

MTT and WST-8 Assays. It is of great importance to be able to evaluate accurately the toxicity of SiNWAs when they interact with organisms. There are many reports on the in vitro interactions of SiNWAs with cells, assessed using, for example,

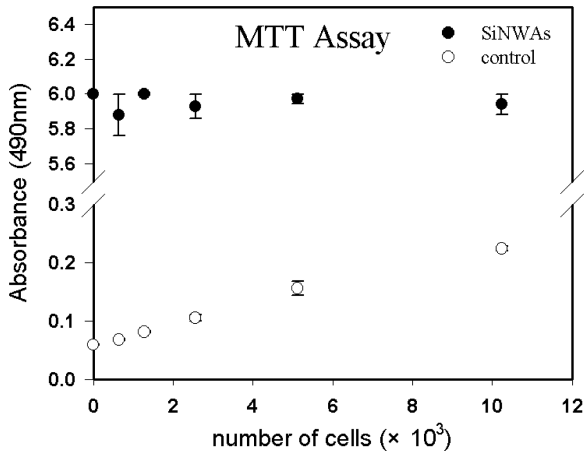

Figure 1. Density-dependent L929 cell viability determined by MTT assay in the presence (dots) or absence (circles) of SiNWAs. Absorbance at 490 nm was measured and data are presented as mean ± standard error, n = 3.

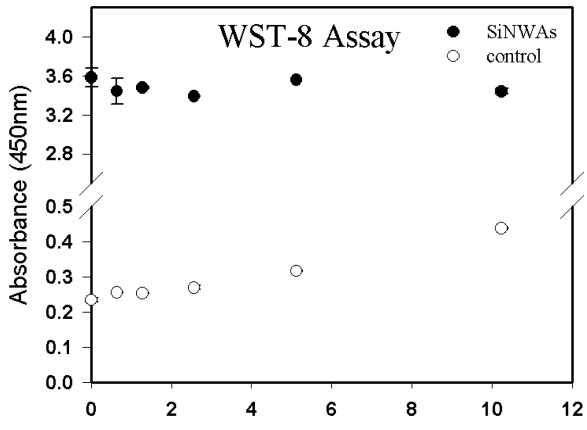

Figure 2. Density-dependent L929 cell viability determined by WST-8 assay in the presence (dots) or absence (circles) of SiNWAs. Absorbance at 450 nm was measured and data are presented as mean ± standard error, n = 3.

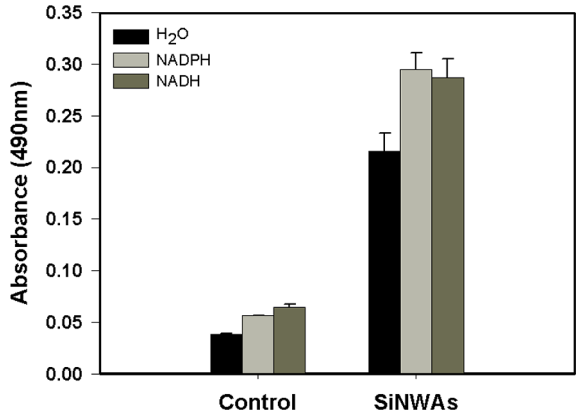

Figure 3. Extracellular reduction of MTT in water solution. The experiments were performed using H_2O (black bars), NADH solution (dark gray bars), and NADPH solution (light gray bars) to react with MTT in the presence or absence (control) of SiNWAs.

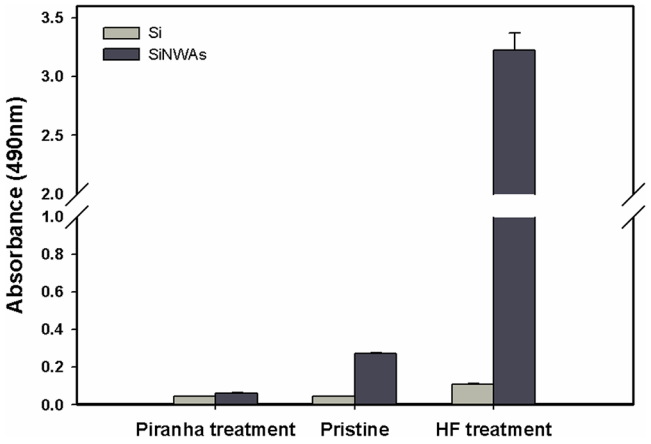

Figure 4. Comparative reduction of MTT by Si surfaces. Experiments were performed using HF or Piranha treated Si or SiNWAs. Pristine surfaces were used as control. The data shown are mean ± standard error, $n = 3$.

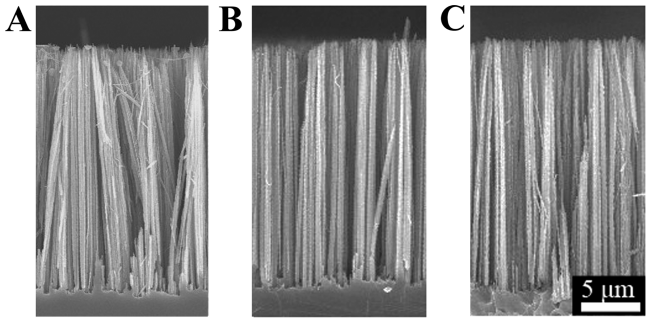

Figure 5. Morphologies of SiNWAs. Typical SEM images of sample surfaces: (A) cross-sectional view of the as-prepared SiNWAs with nanowires' length of 24 μm. Cross-sectional view of the H-SiNWAs (B) before and (C) after MTT reduction.

the Alamar Blue assay.[14] However, there are no reports using the common cytotoxicity testing methods MTT and WST-8 for assessing the toxicity of SiNWAs. In our experiment with these methods the estimates of the viability of L929 cells in the presence of SiNWAs are consistently too high and it appears that SiNWAs may affect the accuracy of these tests. In this study, different densities of cells were grown on culture plates and MTT and WST-8 assays were used to test cell activity in the presence of SiNWAs. Specifically, cells were cultured for 24 h on normal culture plates, then MTT or WST-8 was added without SiNWAs. As shown in Figures 1 and 2, the absorbance (background not subtracted) increased linearly with increasing cell number (the linear correlation coefficient $r > 0.99$). In the presence of SiNWAs, the absorbance values were much higher, and there was no correlation with cell number, indicating that the MTT and WST-8 assays did not accurately reflect cell activity in these conditions. More importantly, the readings were the same whether or not cells were present indicating that the MTT reaction did not reflect cell activity but rather interactions of the materials with the assay components.

Low et al. reported that porous Si surface could reduce MTT in the absence of cells. Thus, cytotoxicity tests based on oxidation-reduction, such as Alamar Blue and MTT, did not give "normal" results when used to test the activity of Si surface.[10] In addition, Laaksonen et al. using the MTT assay to test the activity of Caco-2 cells in the presence of porous Si particles, found activity greater than 300% while in the absence of cells activity was about 70%, i.e., a greater than 3-fold difference.[11] They hypothesized that intermediate products might be reactive in the cells and might also form formazan, causing the observed enhancement of the redox reaction between MTT and porous Si particles. However, our results showed that in the culture media, the reduction ability of SiNWAs is much stronger than that of attached cells. Thus, the MTT and WST-8 assays do not accurately reflect the activity of cells adherent to the material and thus this method cannot be used to assess the biocompatibility of SiNWAs.

In contrast to the weak effect of other Si micro nanomaterials in these assays, the reduction effect of SiNWAs prepared by chemical etching is more obvious. For the WST-8 test the effect gave values more than 15-fold greater than normal; for the MTT test increases the values were more than 30-fold greater, again indicating that these tests are not valid for estimation of the effects of SiNWAs on cells because the material itself has a clear reduction effect. These results suggest that the specific morphology of SiNWAs results in extremely high activity in the reduction of MTT.

From these results, we conclude as follows: (1) The reduction of MTT by SiNWAs is greater than that of other nanomaterials tested; (2) The reduction of MTT in the culture media was due to only the SiNWAs and was not influenced by the cells.

Extracellular MTT Reduction in Aqueous Solution. The intracellular reduction of MTT is a complicated process involving the synergistic effects of a variety of enzymes and electron donors. This process is affected by many factors, one of which is the concentration of intracellular NAD(P)H.[15] The principle based on NADH reduction of tetrazolium salts to generate the colored formazan product and quantification of NADH using the color signal has been widely used in clinical and cell proliferation/toxicity testing.[16] The complexity of the culture medium makes it difficult to identify the components that promote or inhibit MTT reduction, especially when nanomaterials are present. In this work, we used pure water solution as the medium, and coenzymes (NADH and NADPH) as reducing agents to investigate whether SiNWAs affect MTT reduction. The results, as shown in Figure 3, indicate that the reducing ability of NADH was stronger than that of NADPH, in agreement with findings on the intracellular reactions.[17] In the absence of any other agent, the reducing power of SiNWAs

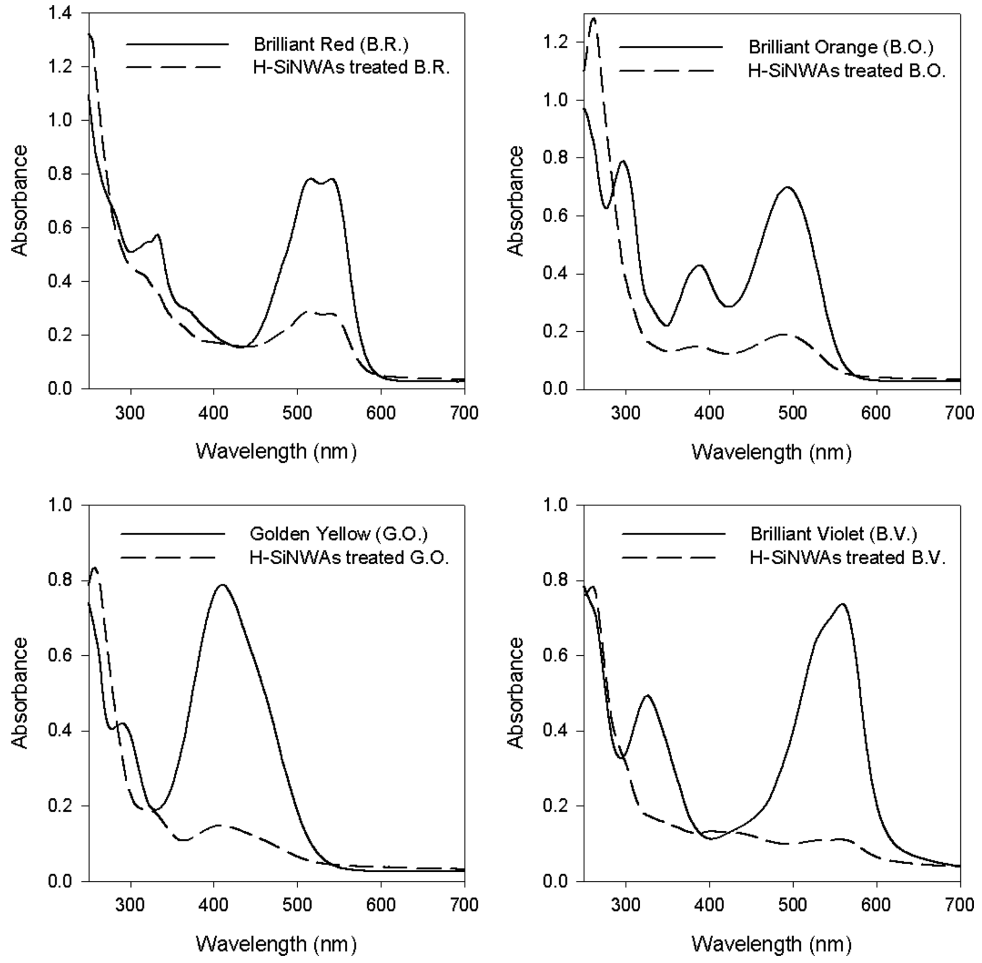

Figure 6. UV−vis spectra of four Remazol dyes. Solid lines, Remazol dyes in distilled water; dashed lines, dye solutions treated with HF-treated SiNWAs (H-SiNWAs) for 3 h at 37 °C.

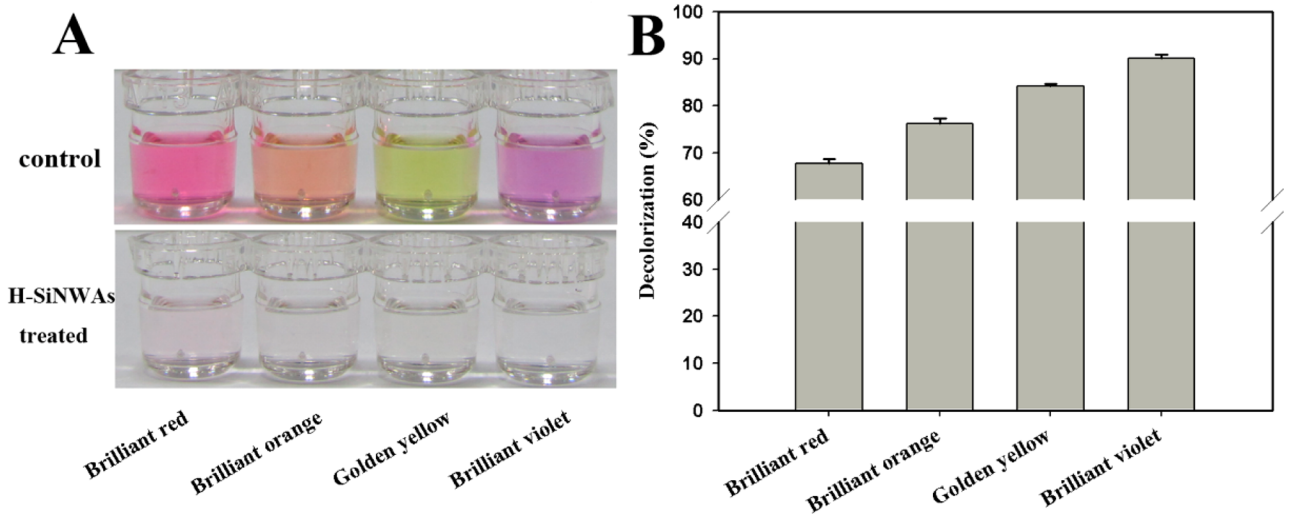

Figure 7. (A) Decoloration in the presence of HF-treated SiNWAs (H-SiNWAs); (B) percentage dye decoloration calculated from the absorbance spectra.

(0.25 cm^2) was 5.7-fold greater than that of NADH (1.36 mM), and 8.7-fold greater than that of that of NADPH (1.36 mM). MTT was reduced even more in the presence of both SiNWAs and NAD(P)H, illustrating that along with the coenzyme, SiNWAs had properties similar to reductases.

In addition, the reduction of MTT by SiNWAs in pure culture medium was about 30-fold greater than in water solution. We speculate that MTT reduction may be influenced by components in the culture medium. Chakrabarti et al. have pointed out that ascorbic acid and retinol affect MTT

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reduction,[1] but unlike M199, RPMI 1640 culture medium does not contain these components. Further work is required to identify possible reducing agents in RPMI 1640.

Influence of the Surface Properties of Si on MTT Reduction. The chemical and physical properties of Si surface have an important influence on various chemical reactions. A one-dimensional nanostructure forms on Si surface upon chemical etching, thereby increasing its surface-to-volume ratio. HF-treated or metal modified silicon nanowires had excellent photocatalytic activity.[18−20] And thermally oxidized or carbonized porous silicon particles can also reduce MTT. The conversion of MTT to formazan was 14% for HF-treated porous silicon, 5−7% for the thermally oxidized and carbonized surfaces.[11] So the differences were attributed to the incomplete loss of activity of the porous microparticles, and clearly better passivation methods are required to permit definitive conclusions.

To further investigate the influence of the chemical composition and nanostructure of Si surface on MTT reduction, we used Si materials (SiNWAs and Si wafer) prepared by two different surface processing methods, viz., hydrogenation and hydroxylation (see XPS result in Figure S3 in the Supporting Information). The data in Figure 4 indicate that surface processed with HF reduced MTT to a greater extent than unmodified surface or surface treated with Piranha solution, suggesting strongly that the chemical composition of the surface was of vital importance. Treatment with HF has been shown to introduce Si−H groups into the surface of SiNWAs (see Raman spectrum in Figure S4 in the Supporting Information). Surface with Si−H had greater reduction capacity than unprocessed surface,[20] and H-SiNWAs had 45-fold greater capacity than H−Si implying that both SiNWAs and Si surfaces could reduce MTT. In addition, the introduction of nanostructure also introduced catalytic activity thus reducing the activation energy and making the reaction faster. When processed with "Piranha" solution, Si−O bonds were introduced[21,22] reducing activity to the extent that MTT reduction barely occurred. The reduction capacity of the unmodified surface was intermediate between those of the HF and Piranha treated surfaces. Unlike the SiO_2 layer coated Si wafer surface, it is known that some reactive hydride species are still left on the surface after fabrication that are available for various reactions.[5] However, the reduction capacity of pristine SiNWAs was 12.5-fold lower than that of H-SiNWAs because of the incomplete hydrogenation. In summary, H-SiNWAs showed the greatest reduction capacity of the materials examined and displayed reductase-like function in the reduction process. Furthermore, the ability of SiNWAs in MTT reduction was found to increase with increasing nanowire length (see Figure S2 in the Supporting Information). The surface morphology of SiNWAs before and after MTT reduction were investigated by SEM, and no obvious change was observed (Figure 5).

Reductive Degradation of Remazol Dyes by H-SiNWAs. Because H-SiNWAs can reduce MTT and WST-8, it seemed possible that they might also reduce other substances, we investigated the use of H-SiNWAs to reduce various Remazol azo dyes including Red F3B, Brilliant Orange 3R, Golden RNL 150%, and Brilliant Purple 5R (for structural formulae, see Figure S5 in the Supporting Information). After treatment with H-SiNWAs, the color of the dyes in aqueous solution was significantly reduced, presumably by reduction of azo bonds. At the wavelength of maximum absorption (red, 540 nm;[23] orange, 492 nm;[24] golden yellow, 410 nm;[25] purple, 560 nm.[26]) the absorbance values were clearly reduced (Figure 6) and the decolorization rate was greater than 65% after 3 h, in some cases as high as 90% (Figure 7), indicating that the H-SiNWAs degraded the azo dyes efficiently.

Chen et al. reported that HF-treated SiNWs prepared by the oxide-assisted-growth (OAG) method could degrade methyl red under ultrasonic oscillations. The reaction did not take place in the absence of ultrasound, suggesting that the oscillations activated the chemical bonds in the contacting surface, broke Si−H bonds and generated $*Si^+$ and $*H^-$ radicals; the $*H^-$ then formed *H radicals in water, and the unstable *H reacted with N═N bonds to form amino groups; $*Si^+$ formed SiO_x in water by oxidation and was eventually hydroxylated on the surface of the nanowires.[27] In our experiments, the reaction of H-SiNWAs with Remazol was conducted without ultrasound, so that the HF-treated SiNWAs could break N═N bonds directly to decolorize the dye. Thus, we conclude that the Si−H formed on SiNWAs after HF treatment was more active and had better reduction capacity than that on silicon nanowires prepared by the OAG method.

4. CONCLUSION

SiNWAs showed surface-related reducing properties in MTT and WST-8 tests as a result of a spontaneous oxidation-reduction reaction, wherein the tetrazolium salt was reduced. Thus the biocompatibility of SiNWAs could not be determined accurately using the tetrazolium salt reduction method. It was found that SiNWAs could reduce MTT directly in water. In the presence of coenzyme its reducing capacity was enhanced, thus showing the reductase-like function of SiNWAs. Furthermore, the chemical composition and nanostructure of Si surface had a strong influence on MTT reduction with the H-SiNWAs showing significant reducing capacity. In addition we demonstrated that H-SiNWAs can degrade organic azo dyes via its ability to break N═N bonds. We expect that the reductase-like property of H-SiNWAs will be applicable as a promoter/catalyst in biochemical reactions more generally.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Hela cell experimental result, MTT reduction on SiNWAs with different lengths of nanowires, XPS result, Raman spectrum, and structural formulae of Remazol dyes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Chakrabarti, R.; Kundu, S.; Kumar, S. *J. Cell. Biochem.* **2000**, *80*, 133.
(2) Ciofani, G.; Danti, S.; D'Alessandro, D.; Moscato, S.; Menciassi, A. *Biochem. Biophys. Res. Commun.* **2010**, *394*, 405.
(3) Belyanskaya, L.; Manser, P.; Spohn, P.; Bruinink, A.; Wick, P. *Carbon* **2007**, *45*, 2643.
(4) Wang, S.; Yu, H.; Wickliffe, J. K. *Toxicol. In Vitro* **2011**, *25*, 2147.
(5) Brammer, K. S.; Choi, C.; Oh, S.; Cobb, C. J.; Connelly, L. S.; Loya, M.; Kong, S. D.; Jin, S. *Nano Lett.* **2009**, *9*, 3570.
(6) Low, S. P.; Voelcker, N. H.; Canham, L. T.; Williams, K. A. *Biomaterials* **2009**, *30*, 2873.
(7) Bimbo, L. M.; Sarparanta, M.; Santos, H. A.; Airaksinen, A. J.; Mäkilä, E.; Laaksonen, T.; Peltonen, L.; Lehto, V. P.; Hirvonen, J.; Salonen, J. *ACS Nano* **2010**, *4*, 3023.
(8) Cheang, T.; Tang, B.; Xu, A.; Chang, G.; Hu, Z.; He, W.; Xing, Z.; Xu, J.; Wang, M.; Wang, S. *Int. J. Nanomed.* **2012**, *7*, 1061.
(9) Bayliss, S. C.; Buckberry, L. D.; Harris, P. J.; Tobin, M. J. *J. Porous Mater.* **2000**, *7*, 191.
(10) Low, S. P.; Williams, K. A.; Canham, L. T.; Voelcker, N. H. *Biomaterials* **2006**, *27*, 4538.
(11) Laaksonen, T.; Santos, H.; Vihola, H.; Salonen, J.; Riikonen, J.; Heikkilä, T.; Peltonen, L.; Kumar, N.; Murzin, D. Y.; Lehto, V.-P.; Hirvonen, J. *Chem. Res. Toxicol.* **2007**, *20*, 1913.
(12) Shalek, A. K.; Robinson, J. T.; Karp, E. S.; Lee, J. S.; Ahn, D. R.; Yoon, M. H.; Sutton, A.; Jorgolli, M.; Gertner, R. S.; Gujral, T. S.; MacBeath, G.; Yang, E. G.; Park, H. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 1870.
(13) Chen, L.; Liu, M.; Bai, H.; Chen, P.; Xia, F.; Han, D.; Jiang, L. *J. Am. Chem. Soc.* **2009**, *131*, 10467.
(14) Qi, S.; Yi, C.; Ji, S.; Fong, C. C.; Yang, M. *ACS Appl. Mater. Interfaces* **2008**, *1*, 30.
(15) Vistica, D. T.; Skehan, P.; Scudiero, D.; Monks, A.; Pittman, A.; Boyd, M. R. *Cancer Res.* **1991**, *51*, 2515.
(16) Carloni, P.; Greci, L.; Maurelli, E.; Stipa, P.; Wozniak, M.; Marrosu, G.; Petrucci, R.; Trazza, A. *Res. Chem. Intermed.* **1993**, *19*, 643.
(17) Berridge, M. V.; Herst, P. M.; Tan, A. S. *Biotechnol. Ann. Rev.* **2005**, *11*, 127.
(18) Shao, M. W.; Cheng, L.; Zhang, X. H.; Ma, D. D. D.; Lee, S. T. *J. Am. Chem. Soc.* **2009**, *131*, 17738.
(19) Tsang, C. H. A.; Liu, Y.; Kang, Z. H.; Ma, D. D. D.; Wong, N. B.; Lee, S. T. *Chem. Commun.* **2009**, 5829.
(20) Sun, X. H.; Peng, H. Y.; Tang, Y. H.; Shi, W. S.; Wong, N. B.; Lee, C. S.; Lee, S. T.; Sham, T. K. *J. Appl. Phys.* **2001**, *89*, 6396.
(21) Giesbers, M.; Kleijn, J. M.; Cohen Stuart, M. A. *J. Colloid Interface Sci.* **2002**, *252*, 138.
(22) Zhuravlev, L. T. *Langmuir* **1987**, *3*, 316.
(23) Laszlo, J. A. *Environ. Sci. Technol.* **1997**, *31*, 3647.
(24) Zanoni, M. V. B.; Sene, J. J.; Anderson, M. A. *J. Photochem. Photobiol. A: Chem.* **2003**, *157*, 55.
(25) Kirby, N.; Marchant, R.; McMullan, G. *FEMS Microbiol. Lett.* **2000**, *188*, 93.
(26) Lourenço, N. D.; Novais, J. M.; Pinheiro, H. M. *J. Biotechnol.* **2001**, *89*, 163.
(27) Chen, Z. H.; Tang, Y. B.; Liu, Y.; Kang, Z. H.; Zhang, X. J.; Fan, X.; Lee, C. S.; Bello, I.; Zhang, W. J.; Lee, S. T. *J. Appl. Phys.* **2009**, *105*, 10467.