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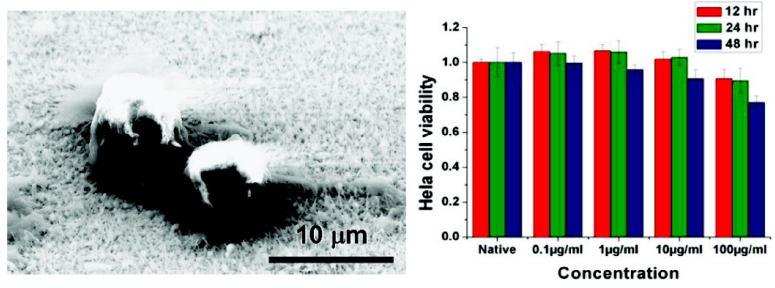

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Cellular Level Biocompatibility and Biosafety of ZnO Nanowires

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We report the first cellular level study on the biocompatibility and biosafety of ZnO nanowires (NWs). Hela cell line showed a complete biocompatibility to ZnO nanostructures from low to high NW concentrations beyond a couple of production periods. The L929 cell line showed a good reproduction behavior at lower NW concentration, but when the concentration was close to 100 μg/ml, the viability dropped to ~50%. Our study shows the biocompetability and biosafety of ZnO NWs when they are applied in biological applications at normal concentration range.

1. Introduction

Nanostructures of zinc oxide (ZnO) have attracted much interest because of their unique piezoelectric, semiconducting, and catalytic properties[1,2] and a wide range of applications in optoelectronics, sensors, transducers, energy conversion and medical sciences.[3−10] Recently, utilizing the coupled piezoelectric and semiconducting properties, ZnO nanowire (NW) arrays and nanobelts have been applied for converting mechanical energy into electricity and building piezotronic devices.[3,5,11] One of the objectives for harvesting energy from the environment, such as body movement, muscle stretching, vibration (such as acoustic/ultrasonic wave) and blood flow energy, is to build self-powered nanosensors for implantable biomedical detections.[12] For these in vivo applications, it is essential to examine the biocompatibility and biosafety of ZnO nanostructures. But to our knowledge, limited literature exists about the biocompatibility of ZnO NWs and nanobelts.[13] Some literature indicates that it is necessary to characterize the physicochemical properties of ZnO nanoparticles (NPs) and their behavior in biological medium, which provide significative clues about the biocompatibility and biosafety of ZnO as NPs.[14−20] In this paper, we report the first study on the cellular level biocompatibility of ZnO nanowires. Our data show that ZnO NWs are completely biocompatible and biosafe. ZnO NWs are reliable and trustworthy in further biomedicine and engineering application.

2. Experimental Details

2.1. Synthesize ZnO Nanowires and Specimen Preparation. The ZnO NWs to be used for this study were grown by a vapor−liquid−solid (VLS) process in a horizontal tube furnace, as reported previously.[21,22] Gold nanoparticles were used as the catalyst and the NWs were supported by a polycrystalline alumina substrate (Figure 1a). For the biocompatible study, 5 mg of ZnO NWs were used as the test sample, which were removed from the polycrystalline alumina substrate, then put into a polypropylene centrifuge tube, which was filled with 5 mL sterile phosphate buffer solution (PBS, pH 7.2). The average diameter of the NWs is 1 μm and the average length is 200 μm. The NWs were dispersed by ultrasonication for 10 min. Then samples with concentrations of 1000, 100, 10, and 1 μg /ml, respectively, were prepared.

2.2. Cell Culture and Counting. For our study, two cell lines from different origins of tissues were utilized.[23] One was Hela cell line (American type Culture Collection, ATCC, CCL-2, *Homo sapiens*), a kind of epithelial cell. The other one was L-929 cell line (ATCC, CCL-1, *Mus musculus*), from subcutaneous connective tissue. Two cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM, ATCC) supplemented with 10% fetal bovine serum (FBS, ATCC) and 1% penicillin/streptomycin (ATCC). Cell suspension was added to 25 cm^2 vials and put into an incubator (37 °C, 5% CO_2).

After incubation, the cells were trypsinized with 0.05% trypsin solution and rushed down from the bottom of 25 cm^2 vials when they were in a semiconfluent state and still in log phase of growth. Ten microliter cell suspension was mixed with 10 μL Trypan Blue. The 10 μL mixture was added into the hemocytometer and the live cell amount under an optical microscope was counted to calculate the total number of harvest cells. The density of the cell was counted and cellular viability was greater than 90%. The cell population was 5000 per well (90 μL). These cells were planted in 96-well plates and incubated in DMEM with 10% FBS, 37 °C, 5% CO_2 for 12 h. Four concentrations of ZnO NWs in PBS (10 μL) were added into these 96-well plates, so the concentrations in cell culture medium were 0.1, 1, 10, and 100 μg/ml, respectively. Some wells were preserved without adding NWs for comparison purpose. After dispersing NWs with micropipettes, the 96-well plates were incubated at 37 °C in 5% CO_2 for 12, 24, and 48 h, respectively.

2.3. Physical Interaction Between the Nanowires and the Cells. The physical interaction of NWs and Hela cells was investigated by inverted microscopy and scanning electron

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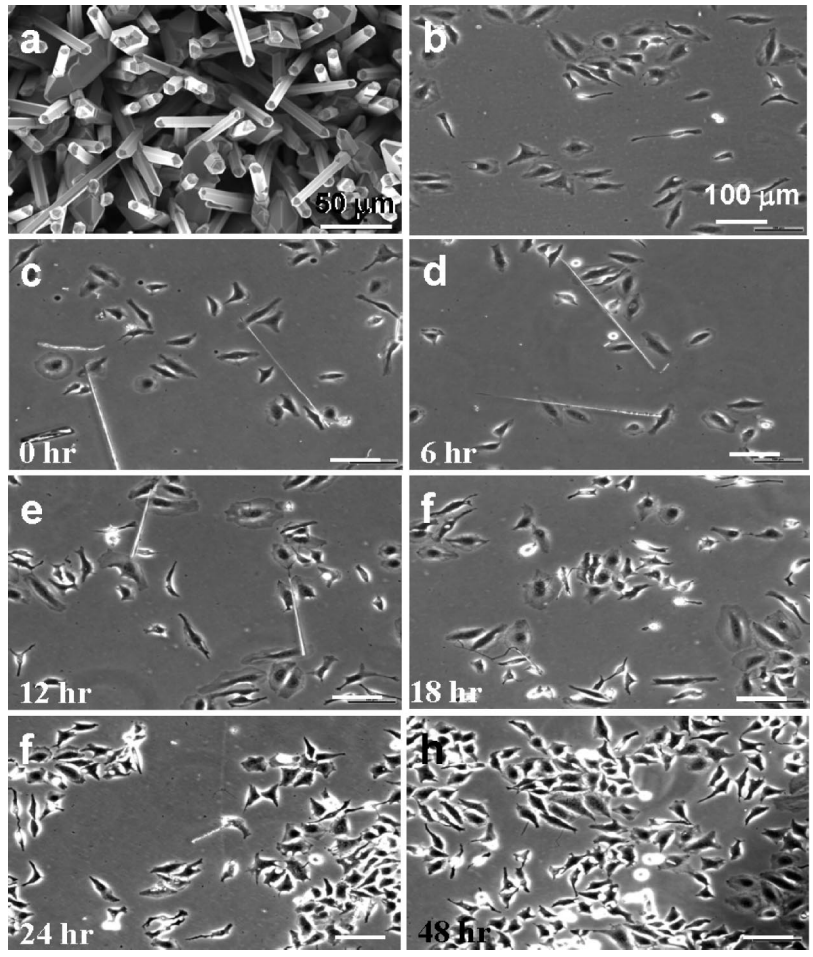

Figure 1. Effect of ZnO NWs on the growth and reproduction of cells as a function of time. (a) As-grown ZnO nanowires on an alumina substrate. (b) Hela cells have been cultured for 4 h. (c−h) Hela cells cultured with ZnO nanowires in solution after growing for 0, 6, 12, 18, 24, and 48 h, respectively. The cells grew and reproduced with the presence of ZnO nanowires. Panels b−h were recorded at the same magnification so that the number of cells in each image represents its concentration. The scale bar is 100 μm.

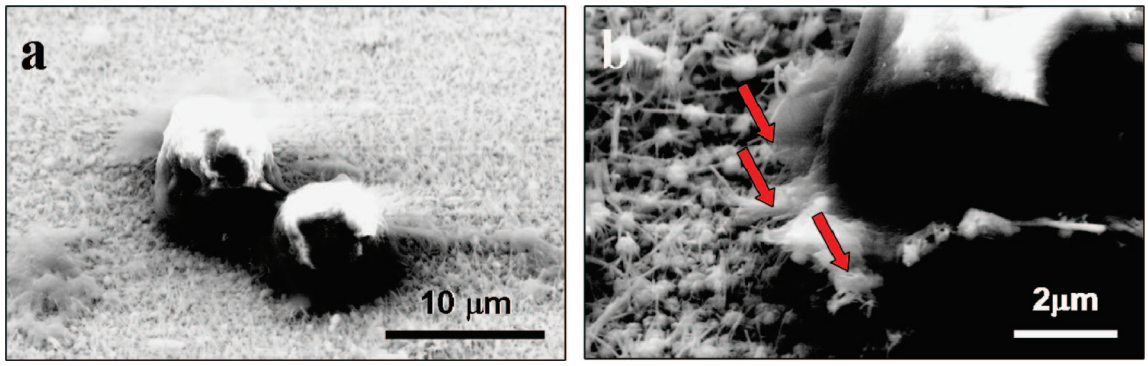

Figure 2. SEM images of Hela cells on ZnO NWs arrays. (a) Two Hela cells are growing on the surface of ZnO NWs arrays. (b) Cells are upheld by the NWs. Some ZnO NWs are phagocytosed into the Hela cell (pointed out by the red arrow). The diameter and the length of the nanowires are ~100 nm and ~1.5 μm, respectively.

microscopy (SEM). ZnO NWs array was in high density and the average diameter and the length of the nanowires were ~100 nm and ~1.5 μm, respectively. After seeding Hela cells on the ZnO NWs arrays, the substrate was incubated in 37 °C, 5% CO_2 for 24 h with DMEM cell culture medium. As the cells in the culture medium subsided on the NWs substrate, the cells were upheld by ZnO NWs (Figure 2a). To maintain the morphology of the cells for SEM, immobilized process will be actualized to the cells adhered on the substrate. The samples were prepared via a critical point drying technique after the treatment with glutaraldehyde for fixation. SEM images of the samples are shown in Figure 2a,b. Figure 2a shows two Hela cells began to extend and grow on the surface of ZnO NWs. Hela cells phagocytosed some of the broken ZnO NWs into the cell membrane (Figure 2b). No external force or inducement was necessary for the phagocytosis, owing to the self-activity of the cell. The results indicate that ZnO nanowires have fine biocompatibility and can be readily phagocytosed by the cells.

2.4. In vitro Cellular Viability Test (MTT). The response of the cells to the addition of NWs was examined by measuring the activity of the mitochondrial enzyme succinate dehydrogenase (SDH).[24] Specimens were not removed from each well,

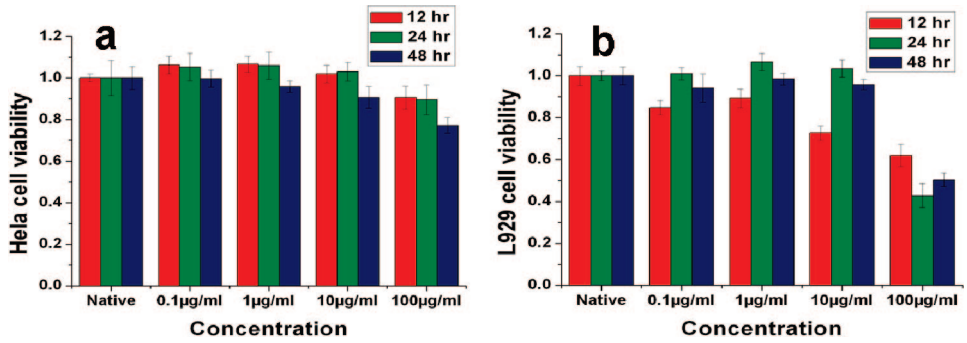

Figure 3. Cell viability tested by MTT method as a function of ZnO NW concentration and time. (a) Cell viability of Hela cell line in MTT test, cultured with different concentration of ZnO NWs for 12 h, 24 h, 48 h. (b) Viability of L929 cell line in MTT test, cultured with different concentrations of ZnO NWs for 12 h, 24 h, 48 h.

TABLE 1: Paired-Sample T-test, SPSS

Hela/concentration (μ g/ml)	0.1	1	10	100
12 h Sig.(2 tailed)	0.087	0.103	0.541	0.059
24 h Sig.(2 tailed)	0.471	0.346	0.524	0.060
48 h Sig.(2 tailed)	0.124	0.736	0.131	0.000
L929/concentration (μ g/ml)	0.1	1	10	100
12 h Sig.(2 tailed)	0.039	0.155	0.007	0.001
24 h Sig.(2 tailed)	0.725	0.059	0.287	0.000
48 h Sig.(2 tailed)	0.182	0.545	0.142	0.000

and an MTT (3-[4, 5-dimethyl-thiazol-2-yl-]-2, 5-diphenyl tetrazolium bromide)-succinate solution (20 μL, 0.5 mg/mL MTT) was added and the plates were incubated again at 37 °C for 4 h. Then all solution in the 96-well plates was removed. The MTT-formazan formed by SDH activity in the cells was preserved at the bottom of the well and was dissolved by dimethylsulfoxide (DMSO). The plate was shaken softly for 5 min to thoroughly dissolve the formazan. Then the plate was put in a microplate reader (EL808 IU-PC, BioTek Instruments, Inc.) for recording the photon-absorbance of each well at 630 nm wavelength.

2.5. Cellular Morphologic Images. On the other hand, we took some phase contrast images of living cells interaction with ZnO NWs at every 6 h (Figure 1b−h). The Hela cells were still growing and reproducing even though the NWs were directly in contact with them (Figure 1c−h). Also, we can see the degradation of ZnO NWs in cell culture medium with the time changing (Figure 1c−h).

3. Results and Discussion

We found that ZnO NWs are biocompatible and biosafe to the two cell lines (Figure 3). The viabilities of Hela cells cultured with NWs for 12 and 24 h showed no difference. The 48 h cultured cells showed only a slight reduction in viability at a high concentration of 100 μg/ml (Figure 3a). More than 95% of the Hela cells were alive after the test, and there was no significant difference in viability among the plates of three time groups (SPSS, Paired-sample *t* test) (Table 1). In the 48 h MTT experiment with the highest NW concentration of 100 μg /ml, the viability of the Hela cells was a little lower than that of the sample without NWs, but the viability was still more than 75% (Figure 3a).

Table 1 shows the statistical analysis of Paired-sample *t* tests using SPSS. If the values of paired-sample *t* tests are larger than 0.05, it means that there is no significant difference between the compared groups. Most of the values shown are larger than 0.05, indicating adding ZnO NWs did not affect the viability of the cells.

For 96-well plates of planting L929 cells, the viabilities showed some variations (Figure 3b). For the 12 h plate, the viability of L929 cells was lower than those of the other two 24 and 48 h time-sequence plates (Table 1), indicating that the L929 cell was in a frail period and more sensitive to ZnO NWs when it was first cultured for less than 12 h. After the culturing time exceeded 12 h, the viability of the cells remained strong and was better than 95% even at relatively high NWs concentration. However, the viability of the cells dropped significantly when the NW concentration reached 100 μg/ml. Therefore, the NWs are considered to be completely biocompatible and biosafe at NWs concentration lower than 100 μg/ml.

4. Conclusion

In conclusion, we have reported the first cellular level study on the biocompatibility and biosafety of ZnO NWs. Two different cell lines from different origins of tissues were utilized in this study. Hela cell line showed a complete biocompatible to ZnO nanostructures from low to high NW concentrations beyond a couple of production periods. The L929 cell line showed good reproduction behavior at lower NW concentration, but when the concentration was close to 100 μg/ml, the viability dropped to ~50%. In general, our study shows the biocompatibility and biosafety of ZnO NWs when they are applied in biological applications at normal concentration range. This is an important conclusion for their applications in in vivo biomedical science and engineering.

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