## Spatiotemporal 3D cells impedance monitoring for metal nanoparticle

## risk assessment by plug-in vertical electrode array

Yimin Shi<sup>a,b,c</sup>, Hui Liu<sup>a,b,c</sup>, Mingda Zhao<sup>a,b</sup>, Sheng Sun<sup>a,b</sup>, Meiyan Qin<sup>a,b</sup>, Yang Zhao<sup>a</sup>, Mingxiao Li<sup>a</sup>, Lina Zhang<sup>d\*</sup>, Lingqian Zhang<sup>a\*</sup> and Chengjun Huang<sup>a,b,c\*</sup> <sup>a</sup>Institute of Microelectronics of the Chinese Academy of Sciences, Beijing 100029, China <sup>b</sup>University of Chinese Academy of Sciences, Beijing 100049, China <sup>c</sup>School of Future Technology, University of Chinese Academy of Sciences, Beijing, 100049, China <sup>d</sup>Cancer Research Center, Beijing Chest Hospital, Capital Medical University, Beijing Tuberculosis and Thoracic Tumor Research Institute, 101149, Beijing, China E-mail addresses: <u>lina\_zhang912@ccmu.edu.cn</u>, <u>zhanglingqian@ime.ac.cn</u> and <u>huangchengjun@ime.ac.cn</u>

# Supplementary information

#### **Fabrication of MEA**

Metal electrodes were patterned by wet etching. Using DZS500 (Shenyang Keyi, China), 10 nm chromium was evaporated on the surface of glass as an adhesive layer, followed by evaporation of 200 nm gold layer. S1813 (AZ, USA) photoresist was spin-coated at 3000 rpm with soft bake and post-exposure bake at 110 °C for 300 s each. Photolithography was performed by MA6 DUV Mask Aligner (SUSS, Germany) and development for 60 s using a bath of RZX3038 developer (Ruihong Electronic Chemical, China).

The gold corrosion solution was prepared using potassium iodide (KI): iodine  $\binom{l_2}{}$ : water (DI)

= 4 g: 1 g: 40 g, and the chromium corrosion solution was prepared using ceric ammonium nitrate: acetic acid: water (DI) = 20 g: 10 g: 70 g. The chip was immersed in the gold corrosion solution for 45 s and chromium corrosion solution for 30 s. After removal of residual photoresist by acetone and cleaning, the electrode patterns have been successfully fabricated.

#### Impedance spectroscopy measurements

An electrochemical workstation (PGSTAT302N, Autolab) was used to record EIS data. In long-term testing, a 10  $\mu$ A sine excitation signal was applied for measurement in a point measurement system in the frequency range of 10 Hz to 1M Hz every 30 minutes (**Fig. S2**). The entire operation was carried out in a sterile environment. It took about 1 minute to measure a complete impedance spectrum at an electrode position and repeated the measurement three times at each point.

#### Simplify Fick's Second Law

For the diffusion process of ZnO nanoparticles in Matrigel, we use Fick's second law, which formula is:

 $\frac{\mathrm{dC}}{\mathrm{dt}} = \frac{\mathrm{d}}{\mathrm{dx}} \left( D \frac{\mathrm{dC}}{\mathrm{dx}} \right) \#(1)$ 

Where C represents concentration, x represents position, t represents time, and D represents diffusion coefficient.

Establish the unsteady diffusion of the semi-finite system, with the assumption that the concentration of ZnO nanoparticles remains constant at the top (x = 0) position. We assume that the initial concentration remains constant during the diffusion process. The initial and boundary conditions are represented as:

 $C_{(x=0,t)} = C_0 \#(2)$   $C_{(x=\infty,t=0)} = 0 \#(3)$  $C_{(x=\infty,t=\infty)} = C_0 \#(4)$ 

where  $C_0$  represents the ZnO nanoparticles concentration to be solved at x = 0,  $C_{(x,t)}$  represents the concentration at position x at time t.

After simplifying with the above conditions and Gaussian error function, we obtain:

$$C_{(x,t)} = C_0 \left(1 - \operatorname{erf}\left(\frac{x}{2\sqrt{Dt}}\right)\right) = C_0 \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right) \#(5)$$
where  $\operatorname{erf}\left(\frac{x}{2\sqrt{Dt}}\right)$  is the Gaussian error function and  $\operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}}\right)$  is the complementary Gaussian

where  $(2\sqrt{D0})$  is the Gaussian error function and  $(2\sqrt{D0})$  is the complementary Gaussian error function, which can be queried the Gaussian error function value table.

### The relationship between diffusion coefficient (D) and molar mass (M)

To simplify the model, ZnO nanoparticles were simplified as geometric spheres with a radius of 80 nm. The average pore size of Matrigel is about 142 nm, which is lager than ZnO nanoparticles <sup>1</sup>. The Stokes-Einstein equation can relate the diffusion coefficient to the radius of the sphere <sup>2</sup>:

$$D = \frac{kT}{6\pi\eta r} \#(6)$$

Where k represents Boltzmann constant, T represents absolute temperature,  $\eta$  represents solvent viscosity and r represents hydrodynamic radius. The hydrodynamic radius can be estimated as:

$$r = \sqrt[3]{\frac{3M}{4\pi\rho N_A}} \#(7)$$

All other parameters being equal, the diffusion coefficient (D) is proportional to the -1/3 power of the molar mass (M).

### **Supplementary Fig. 1**



We have established three electrical models for analyzing electrode sensitivity. The first model is the structure of our sensor, with electrode arrays on both sides. The second model is a structure with an electrode array on one side and a large-area single electrode on the other side, adopted by Yong Qiu et al <sup>3</sup>. The third model has a large-area single electrode structure on both sides <sup>4</sup>. When the electrode spacing is the same, our design sensitivity is highest in these three models.

### **Supplementary Fig. 2**



This is the change in EIS impedance spectrum during long-term monitoring. We selected impedance at a specific frequency for analysis. We obtained EIS impedance spectra at different time points through continuous impedance scanning and ultimately generated a graph showing the relationship between impedance changes and time.



Significance analysis of the effect of impedance sensors on ZnO nanoparticles in 2D and 3D cell models. The data analyzed here adopts the impedance data after 24 hours of nanoparticle addition in Figures 3b&c. All statistical analysis was performed with GraphPad Prism 6.0 (GraphPad software, USA). All the data are presented as the means  $\pm$  standard deviations for at least three repeats. Comparisons between two groups were analyzed by Student's t test (two tailed) with two-sample unequal variances, and p < 0.05, 0.01, 0.001 and 0.0001 were considered significant (\*), very significant (\*\*\*), highly significant (\*\*\*), and extremely high significant (\*\*\*\*), respectively.



### **Supplementary Fig. 4**

To verify the accuracy of our established nanoparticle diffusion model, we monitored the diffusion of RhB particles in Matrigel. Since RhB possesses fluorescent properties and the excited fluorescence intensity is proportional to its concentration, we can utilize this characteristic for quantitative analysis.

We first prepared a 200  $\mu$ M RhB solution in deionized water and then dispensed it onto the upper surfaces of three PMMA cell chambers that had been pre-coated with Matrigel. After allowing the RhB to diffuse for 6 h, 12 h, and 18 h respectively, we rapidly froze the samples to halt the diffusion process. The samples were then sectioned vertically, and fluorescence excitation images were captured at depths of 2 mm, 4 mm, 6 mm, and 8 mm (**Fig. S4a-c**). RhB emits red light when excited by green light, and care was taken to ensure that all images were captured under the same conditions. The captured images were then processed in ImageJ to select the red channel and measure the gray values. The concentration at each location was obtained using the following formula:

$$\frac{C_X}{C_0} = \frac{g_X}{g_0} \#(8)$$

Where  $C_X$  represents the RhB concentration at position X, while  $C_0$  denotes the initial concentration, which is 200  $\mu$ M.  $g_X$  stands for the gray value of the image captured at position X, and  $g_0$  represents the fluorescence intensity of the 200  $\mu$ M RhB solution. By using this formula, we can calculate the RhB concentration at different positions (**Fig. S4e**).

We also used this model to calculate the theoretical concentration of RhB at different times and locations (**Fig.S4d**). The comparison between the two proves the accuracy of the diffusion model we established for solving nanoparticles.

### **Supplementary Fig. 5**



We can obtain the distribution of ZnO concentration at different times through Fick's second law. This graph shows diffusion starting at an initial concentration of 200  $\mu$ M, with concentrations at different locations at 6 h, 12 h, and 18 h.

### Supplementary Fig. 6



Evaluation of  $^{IC_{50}}$  of  $^{SiO_2}$  nanoparticles in 3D A549 cells. The results obtained by evaluating the  $^{IC_{50}}$  of  $^{SiO_2}$  using impedance all exceeded the maximum concentration, and the main reason for this is that the toxicity of 200  $\mu$ M  $^{SiO_2}$  is too weak.

# References

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