Supplementary information

Real-time Effects of Cd (II) on Cellular Membrane Permeability

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Instrumentation:

Scanning electrochemical microscope (CHI920D), 10 μm platinum ultramicroelectrode with disk-shaped (RG=3), platinum wire counter electrode, Ag/AgCl reference electrode were purchased from CH Instruments, Inc. US.. Inverted microscope (Olympus CKX41, Japan), Humidified incubator (Sanyo, Japan, DHP-9053), Centrifuge (Eppendorf, Centrifuge 5415D), enzyme labeling instrument (Molecular Devices, SpectraMaxM4), fluorescence microscope (Olympus, BX53, Japan).

Simulation calculation of theoretical approximation curve

The theoretical approximation curve was calculated according to the parameters in Table S1 and formulas (1) and (3). In the formula, I_T is the probe tip current, $I_{T,\infty}$ is the probe tip current when the probe is infinitely far from the substrate, a is the tip radius of the UME probe, d is the probe-substrate distance, d_0 is the distance from the first scanning point to the substrate surface, and d_{exp} is the distance from the probe to the starting point approaching the starting point. The experimental data are drawn as the function curves of I_T/I_∞=0.75 and fitted with the theoretical approximation curve.[1]

Here is Table.S1

$$I_T \rightarrow I = I_T / I_{T,\infty} \qquad (1)$$

$$d_{exp} \rightarrow L = d/a = (d_o - d_{exp})/a \qquad (2)$$

$$I = 1/[A + B/L + C\exp(D/L)] \qquad (3)$$

MTT cell viability study of MCF-7 cells incubated with $CdCl_2$

Before SECM detection, we used MTT cell proliferation assay to determine the overall activity of MCF-7 cells treated with Cd (II). Cell survival or viability was determined by comparing the treated cells to untreated cells (eq 1):

$$Cell\ viability(\%) = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}}$$

Here is Fig.S1

As shown in Fig.S1a, cells exposed to concentrations below 40 μM showed viability with minimal deviation from the control group, approximately 100%. Exposure concentrations exceeding 40 μM, the survival rate decreased gradually. The decrease of cell viability continued with the increase of exposure concentration. After exposure to 150 μM cadmium chloride for 2 hours, the activity of MCF-7 cells decreased to 69% compared with the control group. This confirms the increase in Cd(II)-induced cellular death in MCF-7 cells with higher Cd(III) concentrations.[3]

Next, we determined the dependence of MCF-7 cell activity on the incubation time of cadmium chloride. In Fig.S1b, the cell viability remains constant at approximately 100% throughout the incubation time of 40 μM cadmium chloride was less than 2 h. Exposure time exceeding 2 h resulted in gradual decreased viability. The decrease in cell viability was found to continue with increasing exposure time. After treated with 40 μM cadmium chloride for 5 h, the activity of MCF-7 cells decreased to 77% of that of the control group. With higher treatment and dosage times, Cd^{2+} is likely to induce cellular death (apoptosis or necrosis) in the MCF-7 cell line similar to other cell lines.[4]

Cell in situ imaging

Here is Fig.S2

In situ imaging of MCF-7 cells treated with different concentrations of cadmium chloride was carried out by using SECM constant height mode. As shown in Fig.S2a, MCF-7 cells incubated without cadmium chloride are oval-shaped and the cell surface imaging is uniform. As the probe passes directly above the cell, the current changes significantly and the cell is firm. MCF-7 cells showed significant contraction after incubated with 40 μM cadmium chloride for 2 h (Fig.S2b). Combined with the cell activity test, there was no significant change in cell activity compared to the control group. The contraction of MCF-7 cells at low concentrations of cadmium chloride may be a manifestation of cells' defense against cadmium toxicity. We observed cell expansion as cadmium incubation concentrations continued to increase to 60 μM (Fig.S2c). The experimental results of cell activity showed that the cell activity began to decrease compared to the control group at this concentration. We speculate that the increase in cell volume observed in the experiment may be that the defense ability of MCF-7 cells is not strong enough to counteract the toxic effects of metal cadmium, and the cell membrane begins to be damaged. Cell membrane damage causes changes in osmotic pressure inside and outside the cell, causing more water molecules to enter the cell and cause the cell to expand. In Fig.S2d, we observed that at cadmium chloride concentrations of 150 μM, the cell morphology did not change much compared with that without cadmium chloride. However, when the probe scanned directly above the

cell, the change of current was significantly lower than that of the latter. At this concentration, cell activity decreased to 31% compared with the control group. At this time, the cell suffered from the severe toxic effect of cadmium, cell membrane permeability increased, and the obstruction effect of cell membrane on ferrocene methanol decreased.

Cytoskeleton fluorescence staining

Here is Fig.S3

The cytoskeleton maintains the stability of cell morphology by finely connecting to the cell membrane and the internal organelles.[5] To detect the effect of the sample on the polymerization of actin in MCF-7 cells, we stained the MCF-7 cells treated with FITC-ghost pen cyclic peptide which only binds to the polymerized microfilament protein, and the structural changes of the treated MCF-7 cells were observed under the confocal microscope. As shown in Fig.S3a, in normal cultured MCF-7 cells, green fluorescence shows a fibrous structure with a clear boundary, indicating that F-actin polymerization is normal. When the samples with a concentration of 40 μM were cultured for 2 h, the reticular structure of green fluorescence began to disperse, indicating that actin polymerization was blocked. By adding a sample culture of 2 h with a concentration of 40 μM, the mesh structure of the green fluorescence began to disperse, indicating that that actin polymerization was blocked. With the increase of sample concentration, the intracellular distribution of green fluorescence became more and more uniform, and the brightness decreased obviously, indicating that with the increase of sample

concentration, the inhibition of actin polymerization in MCF-7 cells became more and more serious. Changes in the cytoskeleton lead to changes in cell morphology and may indirectly affect the function of other cells.[6]

Compared to the normal cultured control group, the mesh structure of green fluorescence in MCF-7 cells began to disperse by adding a sample culture of 2 h at a concentration of 40 μM, but the mesh structure can still be seen, indicating that actin polymerization was blocked at this time, but part of the actin polymer could be maintained. With the extension of the treatment time to 5 h, the fluorescence of the cells was uniformly distributed and the brightness decreased, indicating that the cells could not maintain the reticular actin polymer at this time. The results showed that the blocking effect of the sample on the polymerization of actin in MCF-7 cells was time-dependent. As the processing time is extended to 5 h, the fluorescence within the MCF-7 cells is evenly distributed and the brightness decreases, indicating that the cells could not maintain the reticular actin polymer at this time. It is shown that the sample is time dependent on the blocking of the polymerization of actin in MCF-7 cells.

References

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Table.S1 Parameter Values for Equation (3) (Negative Feedback) at different RG Values[2]

RG	A	B	C	D
10.2	0.40472	1.60185	0.58819	-2.37294
8.13	0.42676	1.46081	0.56874	-2.28548
5.09	0.48678	1.17706	0.51241	-2.07873
3.04	0.60478	0.86083	0.39569	-1.89455
2.03	0.76179	0.60983	0.23866	-2.03267

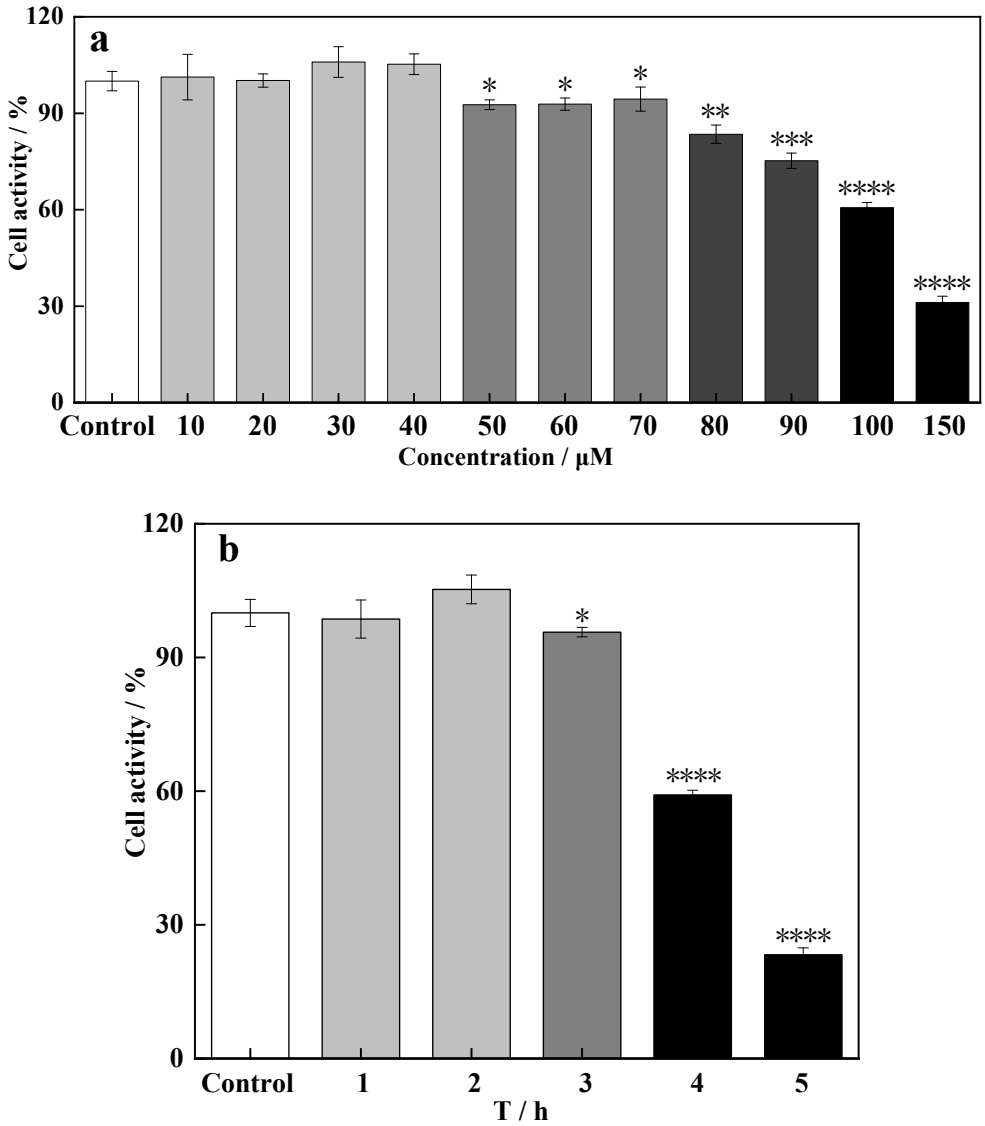

Fig. S1. (a) Different concentrations of cadmium chloride incubate 2 h MCF-7 cells with changes in vitality;(b) Cadmium chloride incubates MCF-7 cells with changes in cell vitality

at different times

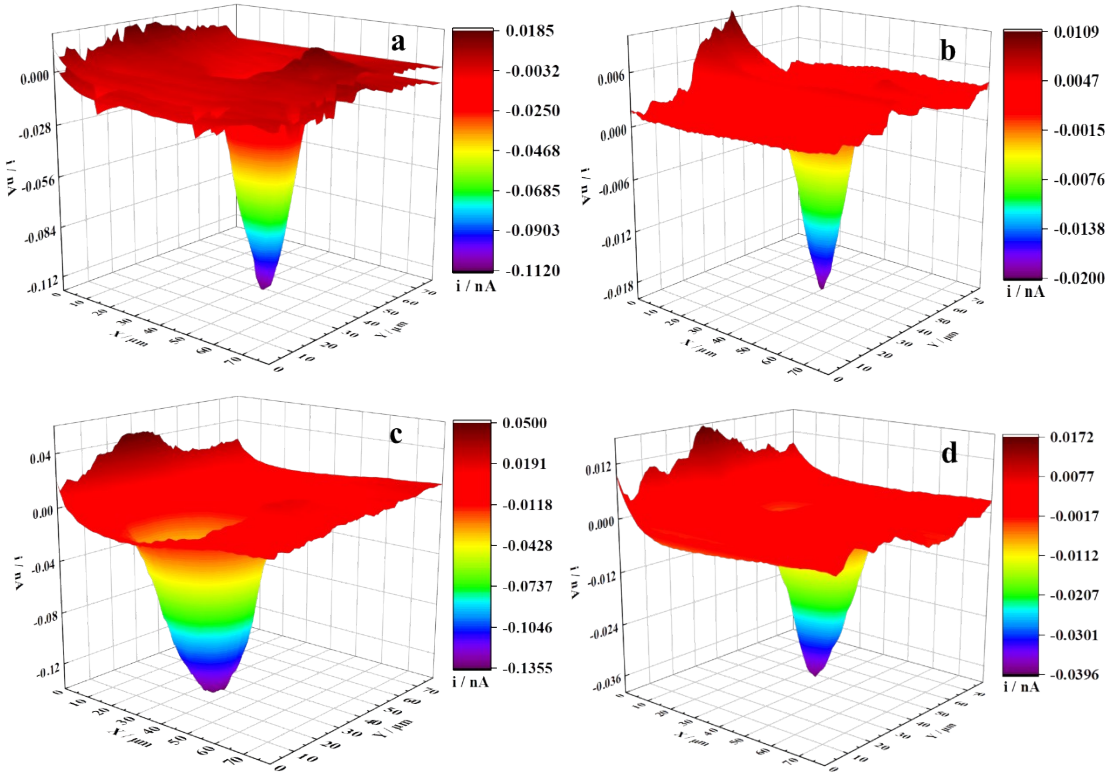

Fig. S2. In situ imaging of MCF-7 cells treated with different concentrations of cadmium chloride，(a) Control; (b)40 μM; (c)60 μM；(d)150 μM

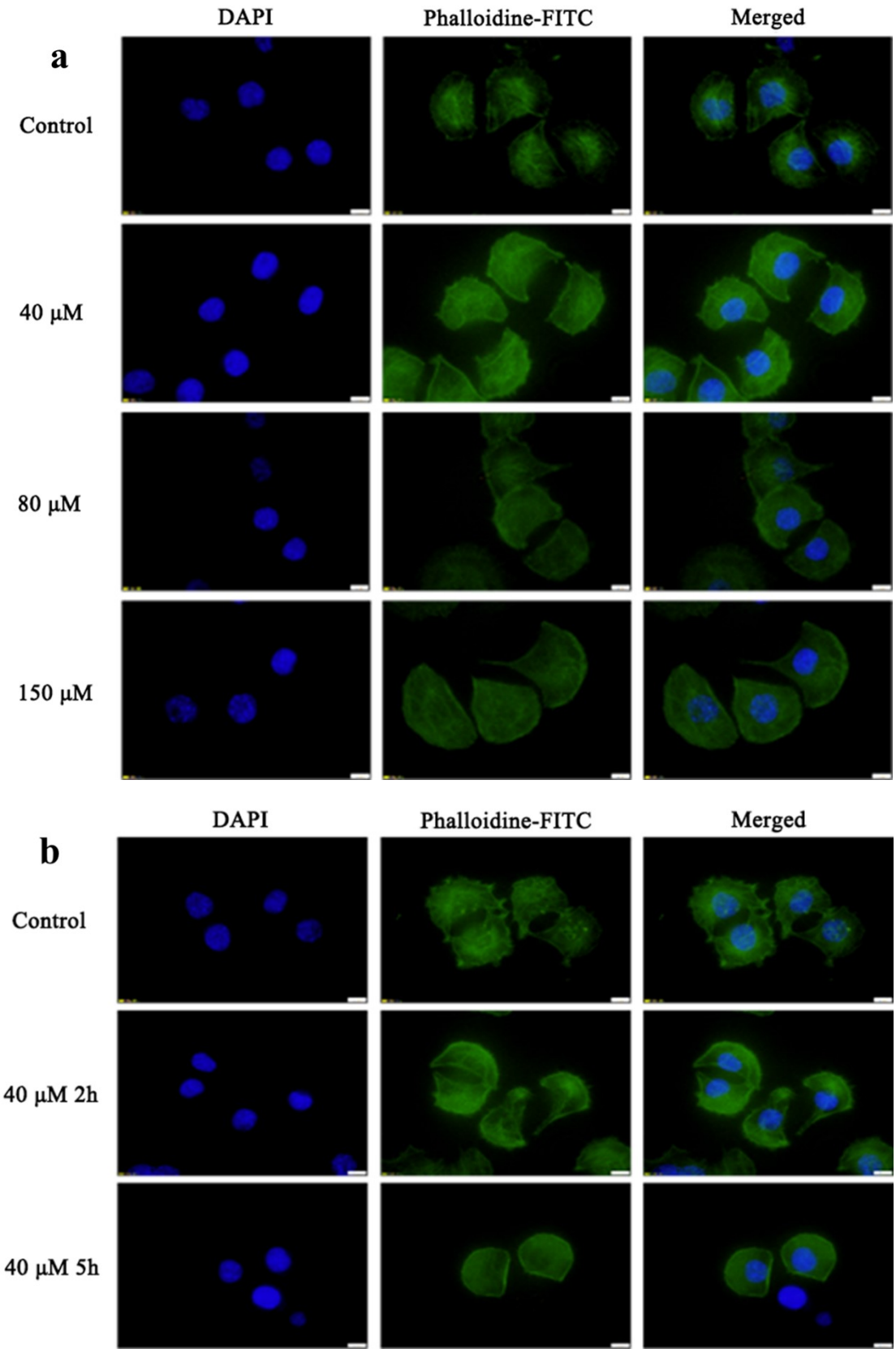

Fig. S3. (a) MCF-7 cell fluorescence imaging effect after 2 h incubation of $CdCl_2$ at different concentrations. (b) Comparison of MCF-7 cell fluorescence imaging effects at different times incubated at 40 μM $CdCl_2$