Electronic Supplementary Material (ESI) for RSC Advances.

An Electrochemiluminescence lab-on-paper device for Sensitive Detection of Two Antigens at the MCF-7 Cell Surface Based on Porous Bimetallic AuPd Nanoparticles

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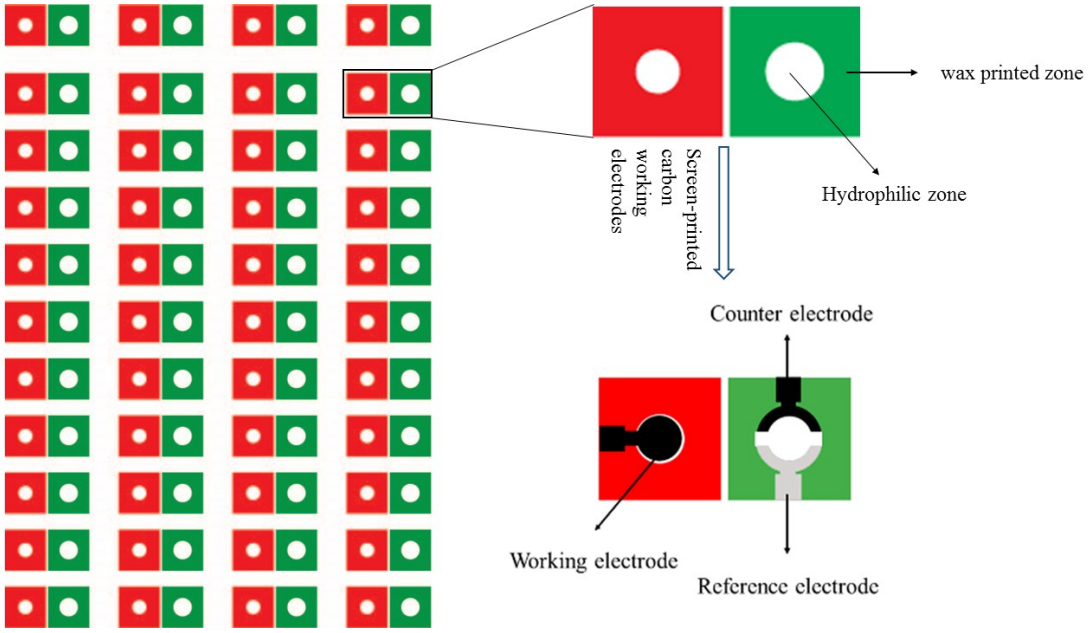

Fig.S1. Paper sheets were firstly patterned in bulk using a wax printer. The schematic representation and shape of this μ-PAD with the screen-printed reference and counter electrode, and the working electrode on the other side.

Fabrication process of the wax patterns

(1) Printing the wax patterns onto the surface of paper sheet (A4) with wax printer (FUJIXEROX Phaser 8560DN, Japan) set to the default parameters for photo-quality printing (the most inkjet quantity) in a high-resolution printing mode (Fig.S2); (2) Baking the wax-printed papers in an oven at 130 °C for 150 s to let the printed wax melt and penetrate through the paper to form the hydrophobic and insulating patterns (Fig.S3). These two steps can be finished within 2 min.

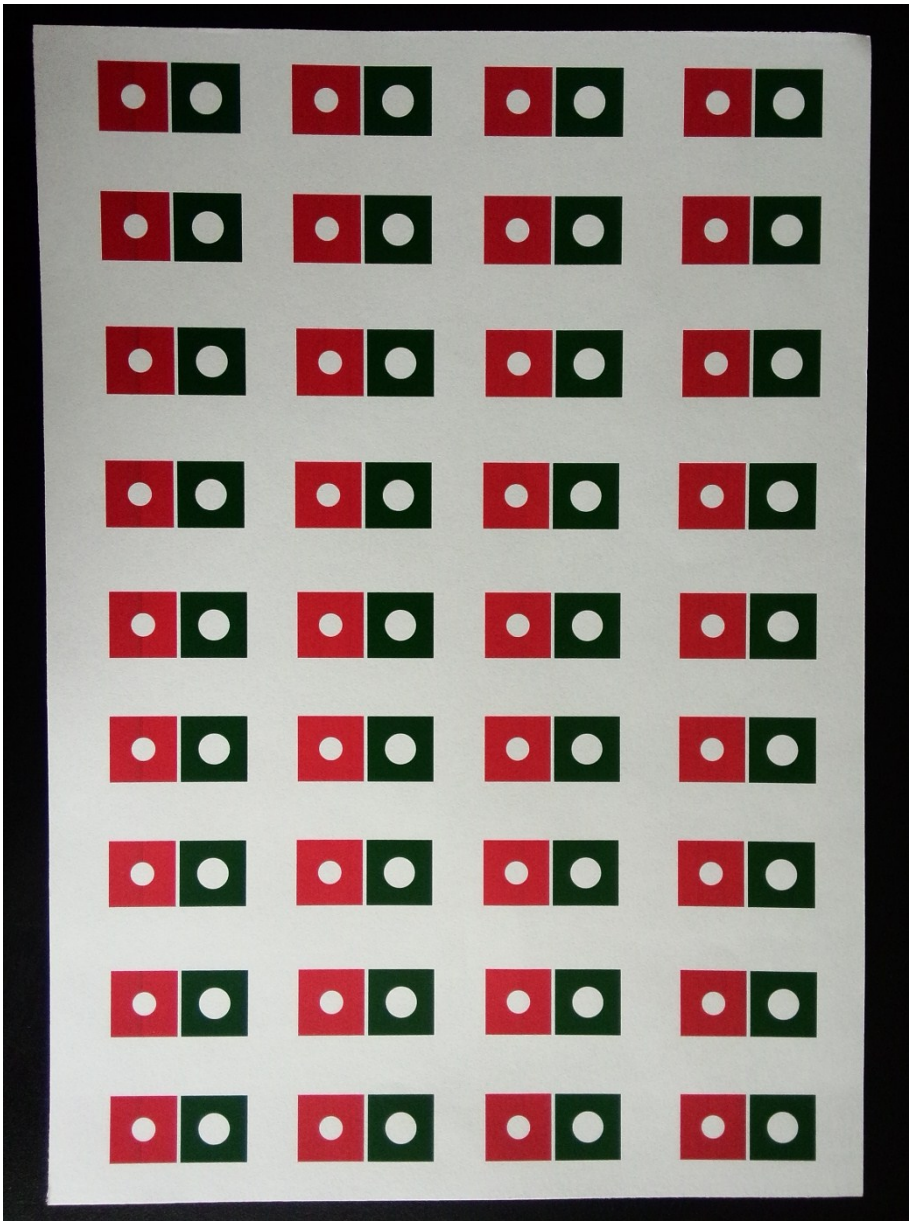

Fig.S2. Wax-patterns of this paper device on a paper sheet (A4) before baking

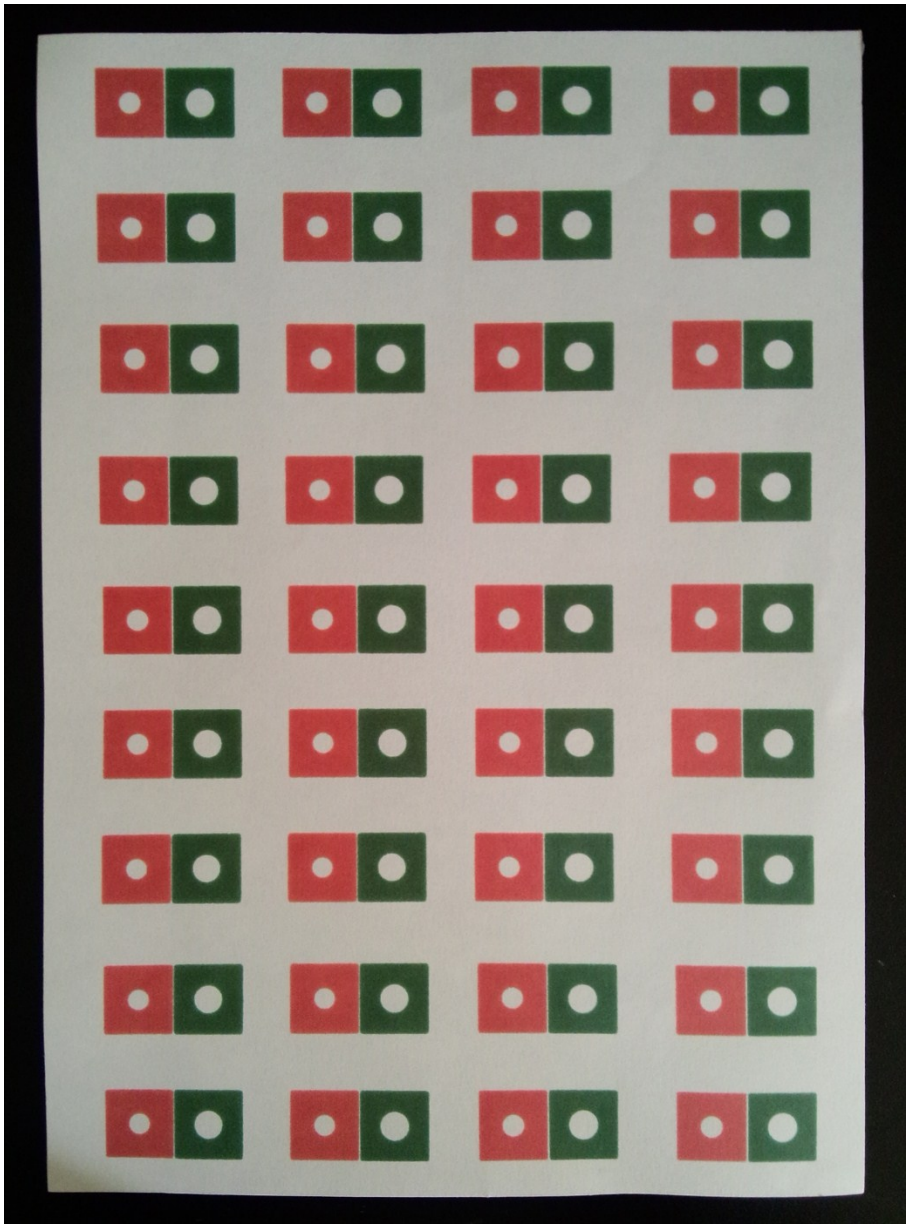

Fig.S3. Wax-patterns of this paper device on a paper sheet (A4) after baking.

Fig.S4. After screen-printing of electrode, including working electrode, one counter electrode, and one Ag/AgCl reference electrode.

Preparation of AFP antibodies Associated Luminol-AuPd Complex

Luminol with amino functional groups was easier to facilitate biomolecules binding via EDC and NHS. Briefly, the luminol was mixed with 200 mM EDC and 50 mM NHS in 1.0 mL of pH 7.4 PBS buffer and activated for 40 min in the dark. The mixture was centrifuged at 13 000 rpm for 15 min. Subsequently, the resulting soft sediment was dispersed in 1.0 mL of pH 7.4 PBS. The Luminol-Ab_2 was obtained by adding 20 μL of Luminol (20 mg·mL^{-1}) mentioned above to 50 μL of Ab_2 (20 μg·mL^{-1}) [1]. After incubation overnight at room temperature in the dark, the mixture was washed with PBS (pH 7.4). The purified Luminol-labeled Ab_2 was further diluted with PBS (pH 7.4) to 1.0 mL and kept at 4 °C for later use. AuPd/Luminol-labeled Ab_2 was obtained by adding Luminol-Ab_2 into the obtained AuPd solution (200 μL, 2.0 mg·mL^{-1}), followed by gently shaking at 4 °C for 1 h. The reaction mixture was then centrifuged at 10 000 rpm for 5 min, and the supernatant was discarded. The AuPd/Luminol-labeled Ab_2 tracer was repeatedly washed 3-4 times with PBS buffer

and was blocked by BSA (0.1% (w/v)) after incubation for 1 h. Finally, the nanocomposites was collected by centrifuging and redispersed in 200 μL PBS as the assay solution. The AuPd/Luminol-labeled Ab_2 bioprobe integrated both the specific recognition of corresponding AFP antigen on the captured MCF-7 cell surface by antibody and amplification of ECL signal based on the AuPd/Luminol with the H_2O_2 as the coreactant.

Choice of Materials

The working electrode were scanned from -1.8 V to -0.5 V with scan rate of 100 $mV \cdot s^{-1}$. To investigate the amplification technique of the Au-PWE and AuPd nanostructures for ECL analysis, control experiments without the involvement of Au-PWE which replaced by normal paper working electrode or AuPd nanoparticle were carried out respectively and the results are shown in Fig. S5. We compared the ECL intensity of pure CdTe QDs labeled Ab_2 (curve a) and CdTe@AuPd composites labeled Ab_2 (curve b). The quantity of the CdTe QDs and CdTe@AuPd composites was equal in both labels and Au nanocages composites were used to capture Ab_1 with the same quantity. As can be observed that CdTe@AuPd composites labeled Ab_2 reveals excellent ECL performance compared with pure CdTe QDs. From the Fig.S4, we can see that the CdTe@AuPd composites can be used as excellent ECL labels. It is observed from Fig.S5 (curve d) that a greatly enhanced ECL performance is obtained by applying Au-PWE to immobilizing Ab_1 instead of normal AuNPs functionalized paper working electrode (curve c). In a conclusion, the ECL immunosensors showed a greatly amplified ECL signal based on CdTe@AuPd composites and Au-PWE and

hence enhances the detection sensitivity.

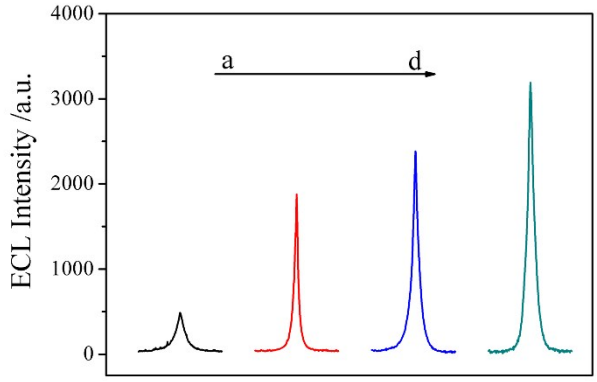

Fig. S5. ECL curves (a) neither CdTe@AuPd composites nor Au-PWE, (b) Au-PWE, but only $CdTe/Ab_2$ is used as the ECL tag, (c) with AuPd but not Au-PWE, (d) both CdTe@AuPd composites and Au-PWE

Optimization of the cell assay conditions

The pH of the detection solution is another important factor in the ECL response, and the effects of the solution pH on ECL responses to CEA were investigated (Fig. S6A). The response increased as the pH value increased from 6.2 to 8.6 and then decreased at pH values higher than 7.4. Thus, PBS at pH 7.4 was selected for preparing the solutions used for detection. The incubation time is another critical parameter that affects the analytical performance and the time efficiency of the cell assay. At room temperature, the ECL response increased with increasing incubation times in the sandwich-type cell assays and then stabilized, which indicates the achievement of saturated binding in the immunoreaction (Fig. S6B). The optimal incubation time of CEA immuno-complexes was 50 min, and accordingly, an

incubation time of 50 min was selected for the subsequent experiments.

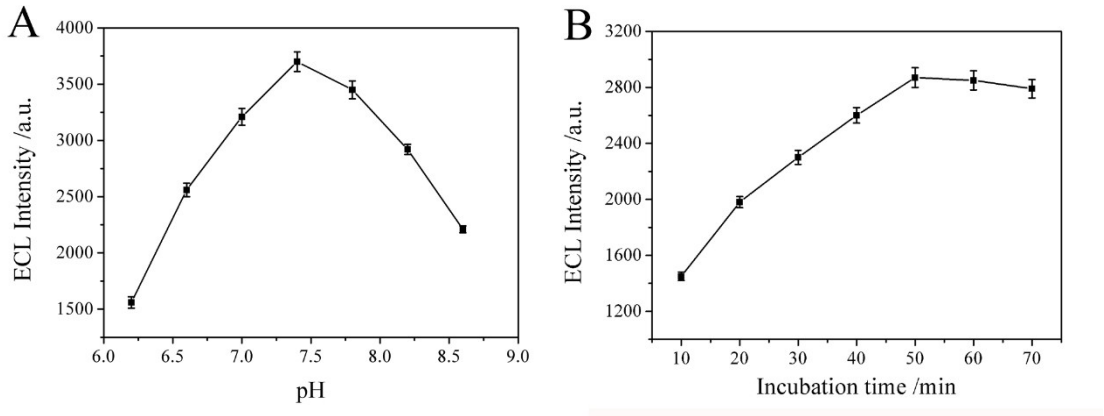

Fig. S6. (A)The effect of pH on ECL response; (B) The effect of incubation time on ECL response

3.8. Cell viability

To inspect whether the prepared materials affect the cell viability, a standard staining method was adapted by the use of the calcein-AM (a living cell dyes). Low-toxic calcein-AM, which could be incorporated into living cells through the membrane, was chosen to carry out staining experiment for the determination of cell viability. Under the fluorescent microscope, green fluorescence could be observed in cytoplasm of MCF-7 cancer cells [2, 3]. To perform these tests, MCF-7 cancer cells were incubated with CdTe QDs/AuPd-Ab_2 for 8 h. Media in the dishes were discarded and the cells were gently rinsed twice with PBS. 3 mL of the calcein-AM solution was added into the dishes and incubated for 30 min at 37 °C. It should be noted that, CdTe QDs/AuPd-Ab_2 have no apparent effect on cancer cells (Fig.S7A). After captured by the indium tin oxide electrode, MCF-7 cancer cells showed a good living morphology as shown in Fig. S7B. These results demonstrated that the materials as-prepared used in this work have no apparent harm to MCF-7 cancer cells.

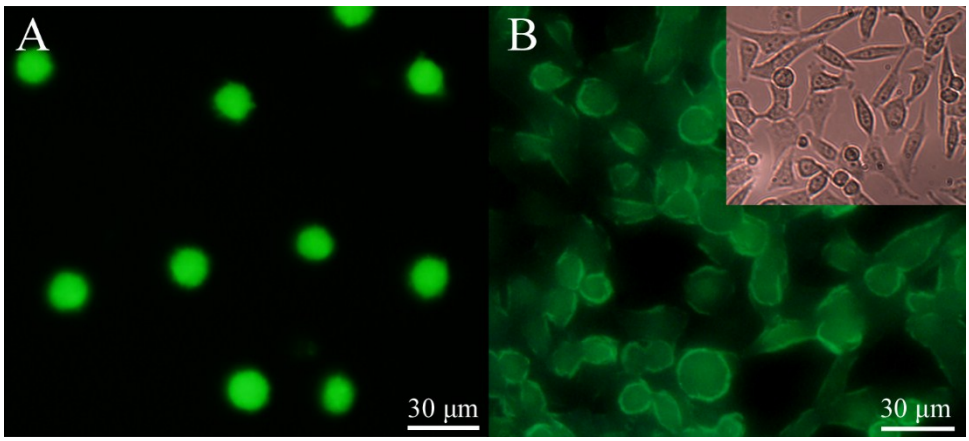

Fig.S7. (A) optic microscopy image of MCF-7 cell (B) optic microscopy image of MCF-7 cell after the incubation with CdTe@AuPd composites-labled Ab_2 in the culture dish for 8 h

References

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