Supplementary Materials

FROM FUNCTIONAL STRUCTURE TO PACKAGING: FULLY-PRINTING FABRICATION OF MICROFLUIDIC CHIP WITHIN BIOSENSORS

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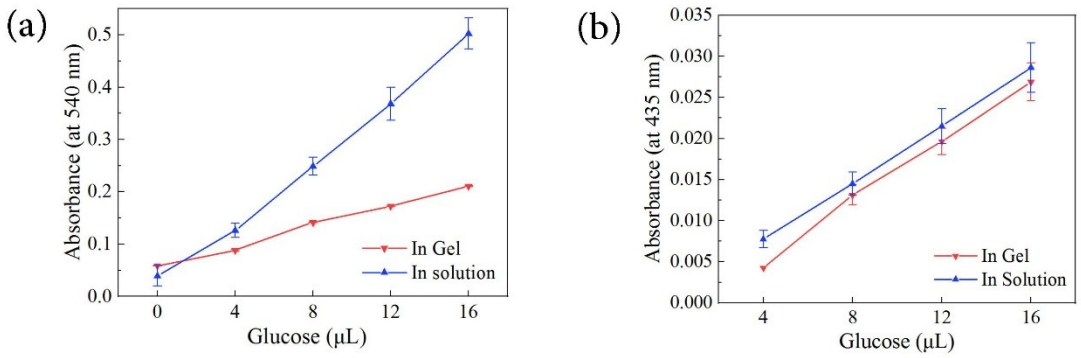

Figure S1. The result of enzyme activity testing via spectrophotometry. (a) The absorbance of the completely reacted mixture solution with the stop buffer at 540 nm; (b) The average value of the variation per minute of the absorbance in the first five minutes. The concentration of the glucose is 0.55 M.

Principle

Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase.[1] Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase (POD) to form a colored product. The intensity of the brown color measured at 435 nm is proportional to the original glucose concentration, according to the following formula, [2]

$$U/_{mg} = \frac{\Delta A}{t} \cdot \frac{1}{\varepsilon_b} \cdot V_S \cdot \frac{1}{V_E} \cdot \frac{1}{C_E} \cdot D \quad (1)$$

Among the formula, U is the activity of the enzyme, ΔA is the variation of the absorbance at 435 nm, ε_b if the absorbance of o-dianisidine with concentration of 1 mM, V_S is the volume of the sample, V_E is the volume of the glucose oxidase, C_E is the concentration of the glucose oxidase and D is the dilution ratio of the enzyme. Under the same experimental conditions, we could make a conclusion that

$$\frac{U_{enzyme\ in\ solution}}{U_{enzyme\ in\ gel}} = \frac{\Delta A_{enzyme\ in\ solution}}{\Delta A_{enzyme\ in\ gel}} \quad (2)$$

Furthermore, Oxidized o-dianisidine reacting with sulfuric acid can form a more stable cored product. The intensity of the pink color measured at 540 nm is also proportional to the original glucose concentration. The standard curve, plotting absorbance at 540 nm as y axis vs concentration of glucose as x axis, is supposed to be linear if the activity of glucose oxidase is normal.[3]

Experimental section

Glucose oxidase (GOx, Type X-S, lyophilized powder, 100 units/mg) was purchased from Sigma-Aldrich. Peroxidase (S10064-10), o-Dianisidine (R-076N) and β-D-glucose (S47608-25) were purchased from Accustandard (Beijing, China). Sulfuric Acid (98%) was purchased from Beijing Chemicals (Beijing, China). 0.1 M standard phosphate buffer (PBS, pH = 7.4) was prepared in the same way as the body text.

5 mg of glucose oxidase and 1 mg peroxidase were dissolved with 39.2 ml of deionized water. 5 mg of o-Dianisidine was reconstituted with 1 mL deionized water and 0.8 mL of the o-Dianisidine was mixed with 39.2 mL of Glucose oxidase/Peroxidase solution to obtain a standard glucose oxidase reagent (marked as “enzyme in solution”).

The gel was prepared in the same way as the body text. 5 mg of o-Dianisidine was reconstituted with 50 mL deionized water. 15 μL of the reagent mixture was polymerized into gel and then the

gel was soaked in 100 μL of o-Dianisidine solution (marked as “enzyme in gel”). In these case 100 μL of these two glucose oxidase solution contained the same quantity of glucose oxidase.
The following solutions were pipetted into the ELISA plate.

Plate well	*Water*	*Glucose Standard*
Blank test	100 μL	0 μL
Test 1	98 μL	2 μL
Test 2	96 μL	4 μL
Test 3	94 μL	6 μL
Test 4	92 μL	8 μL

For the static test, the reaction was started by adding 100 μL of “enzyme in solution” in one group and 100 μL of “enzyme in gel” in another group at zero time. Then each well of the ELISA plate reacted exactly 30 minutes at 37-degree C. The reaction was stopped by adding 100 μL of 12 N H_2SO_4. The absorbance of each well against the blank test was measured at 540 nm by a microplate reader. As Figure S1(a) shows, the linearity between the absorbance and the volume of glucose proves the nice activity of the enzyme in the gel.
For the dynamic test, the reaction was started by adding 100 μL of “enzyme in solution” in one group and 100 μL of “enzyme in gel” in another group at zero time. Then each well of the ELISA plate reacted at 25-degree C and the absorbance at 435 nm was measured at 60-second intervals in the first 5 minutes. Figure S1(b) demonstrates the average change rate of the absorbance. According to formula (2), the inactivation rate of glucose oxidase in gel was less than 4%.

References:

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