An all-in-one microfluidic slipchip for power-free and rapid biosensing of pathogenic bacteria

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Materials

Carboxylated MNPs (180 nm) from Allrun Nano (Shanghai, China) were used with the anti-*Salmonella* polyclonal antibody (pAb) from Fitzgerald (Birmingham, AL, USA) for magnetic separation of the target *Salmonella* cells. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysulfosuccinimide sodium (NHSS) from Sigma Aldrich (St. Louis, MO, USA) were used to actuate the carboxyl groups on the surface of MNPs. Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), chloroplatinic acid ($H_2PtCl_6 \cdot 6H_2O$) and Sodium tetrachloropalladate(II) (Cl_4Na_2Pd) from Sigma Aldrich, and trisodium citrate and ascorbic acid from Aladdin (Shanghai, China) were used to synthesize the gold@platinum palladium nanocatalysts (Au@PtPd NCs). Anti-*Salmonella* monoclonal antibody (mAb) from Meridian (Memphis, TN, USA) was used to modify the Au@PtPdNCs for labelling the *Salmonella* cells specifically.

Silicone elastomer kit from Dow Corning (Sylgard 184, Auburn, MI, USA) was used for fabricating microfluidic chips. The Objet30 Pro 3D printer from Stratasys (Eden Prairie, MN, USA) was used to fabricate the holder and molds of the microfluidic chips.

Culture of the bacteria

The culture and preparation of the target and non-target bacteria for obtaining a serial dilution of bacterial samples. *Salmonella* typhimurium was used as target bacteria. *Staphylococcus aureus*, *Bacillus cereus*, *E. coli* O157:H7, and *Listeria monocytogenes* were used as the non-target bacteria. All these bacterial strains were stored at -20°C in a glycerol/broth medium (30%, v/v) and re-activated by incubating the bacteria in sterile Luria-Bertani (LB) medium (Aoboxing Biotech, Beijing, China) at 37°C for 16-24 h with shaking at 180 rpm. The bacterial cultures were 10-fold diluted with sterile PBS to obtain the bacteria at the concentrations of 10^1-10^6 CFU/mL, respectively. Bacterial enumeration was conducted using the standard culture plating. In brief, the bacterial samples were serially 10-fold diluted with sterile PBS, and 100 μL of the diluents were surface plated on the LB agar plates. After incubation at 37℃ for 22-24 h, the visible colonies were counted for enumeration of the bacteria.

Preparation of the spiked bacterial samples

To evaluate the performance of this microfluidic biosensor on *Salmonella* detection in the real food samples, milk and chicken were purchased from a local supermarket and has been confirmed the absence of *Salmonella* using selective culture plating. According to China's food safety national standards (GB 4789.4), 25 g chicken or 25 mL milk was first added into 225 mL sterile PBS in a homogeneous bag. The chicken sample was homogenized using a homegenizer (BagMixer, InterScience, Paris, France) for 5 min, followed by standing for 15 min to obtain the supernatant. Then, pure *Salmonella typhimurium* was mixed with diluted the chicken supernatant or diluted milk to prepare the artificially contaminated the spiked milk and chicken samples with the bacterial concentrations from 3.0×10^2 to 3.0×10^4 CFU/mL.

Development of smartphone App

A smartphone (Huawei, Shenzhen, China) with an Android operation system was used in this study. A proprietary App programmed in JAVA was developed and downloaded on the smartphone with two built-in cameras (camera 1's resolution: 20 million pixels; camera 2's resolution: 12 million pixels; sensor: CMOS). First, the image of catalysate was collected using the camera. After the image was uploaded and displayed on the App, its detection area was cropped and processed using the median filter to smooth the selected image. Then, the RGB value of each pixel in the image was obtained and converted to the Hue-Saturation-Lightness (HSL) value through a matrix transformation algorithm (equation 1) and each saturation was extracted. Finally, the average saturation was obtained for each image, and the linear calibration model was built between the saturation of the image and the concentration of the target bacteria.

max=MAX (R, G, B)
min= MIN (R, G, B)

$$H = \begin{cases} 0°, & if\ max = min \\ 60° \times \frac{G-B}{max-min} + 0°, & if\ max = R\ and\ G \geq B \\ 60° \times \frac{G-B}{max-min} + 360°, & if\ max = R\ and\ G < B \\ 60° \times \frac{B-R}{max-min} + 120°, & if\ max = R \\ 60° \times \frac{R-G}{max-min} + 240°, & if\ max = B \end{cases}$$

(equation 1)

$$S = \begin{cases} 0, & if\ L = 0\ or\ max = min \\ \frac{max-min}{max+min}, & if\ 0 < L \leq \frac{L}{2} \\ \frac{max-min}{2-(max+min)}, & if\ L > \frac{1}{2} \end{cases}$$

$$L = \frac{1}{2}(max + min)$$

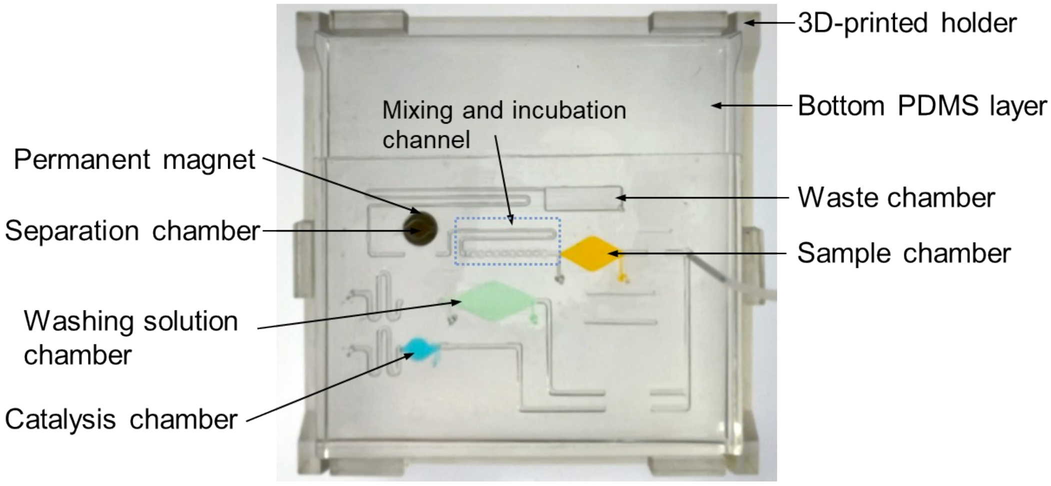

Fig. S1 Photo of the microfluidic slipchip

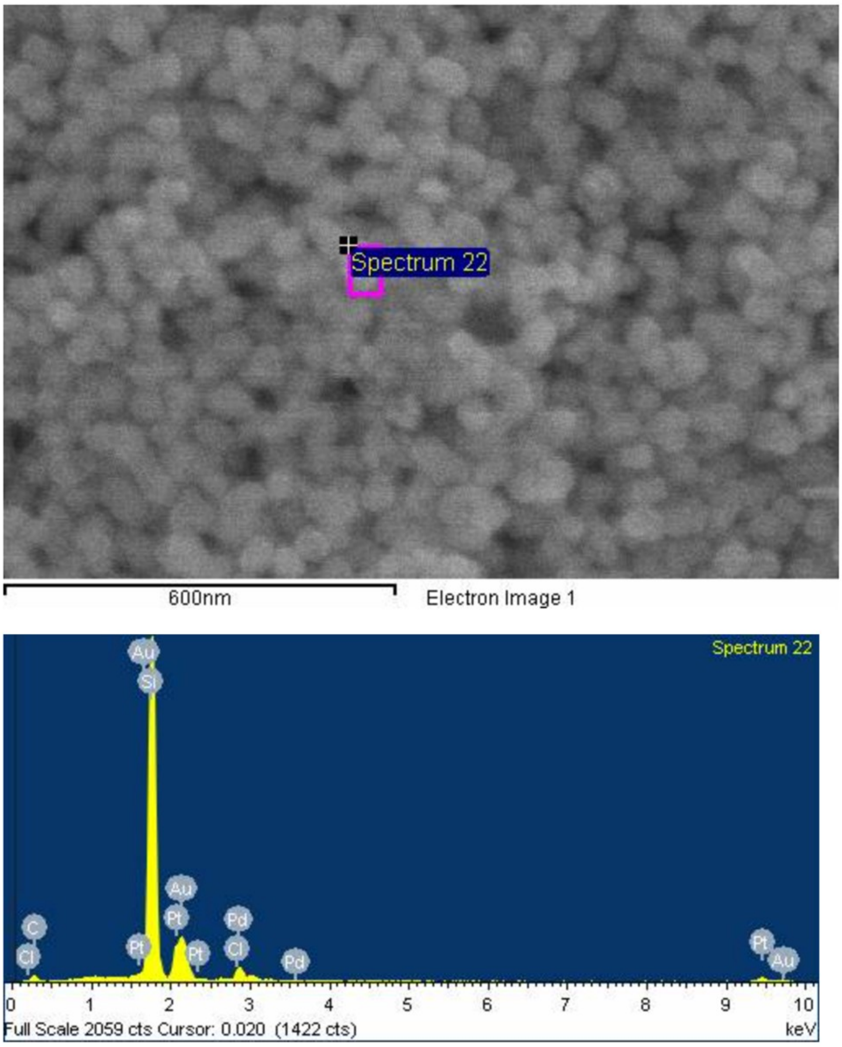

Fig. S2 The EDS elemental spectrum of Au@PtPd nanocatalysts

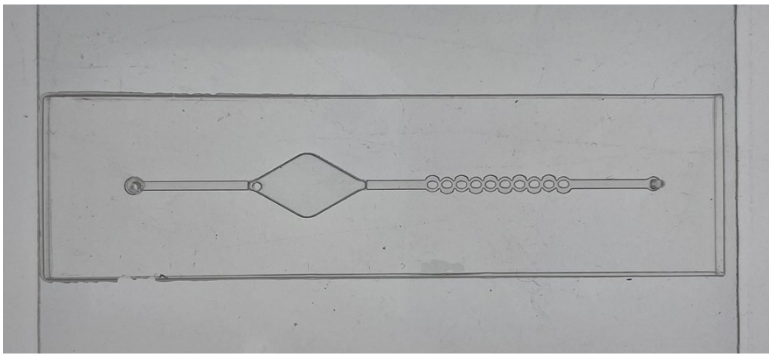

Fig. S3 Photo of microfluidic chip with the ASAR micromixer

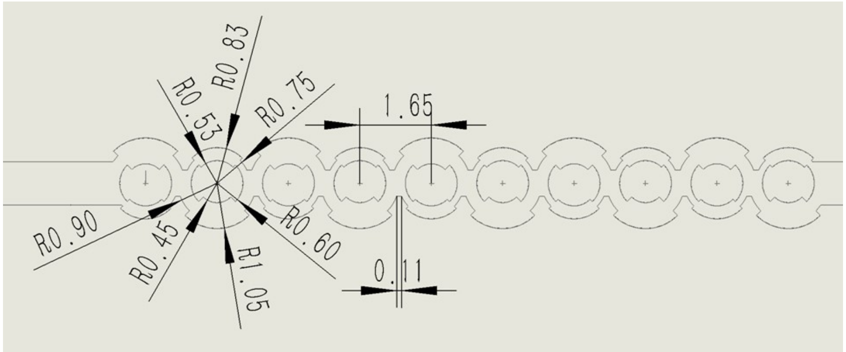

Fig. S4 The specific design parameters of the ASAR micromixer (unit: mm)

Table S1. Cost for all the components of this biosensor.

Item	Amounts	Cost (US $)
Syringe (1 mL)	1	0.02
PDMS	1	1.0
Duct	1	0.08
Silicon oli	1	0.01
3D print holder	1	0.5
Magnet	1	0.05
Magnetic nanobeads	1	0.50
Au@PtPd nanocatalysts	1	0.05
Antibodies	1	6.69
Total		8.9

Table S2. Comparison of this biosensor with other reported microfluidic methods.

Methods	Target	Instrument	LOD (CFU/mL)	Linear Range (CFU/mL)	Total time (h)	References
Electrochemical	*E. coli* O157:H7	Electrochemical workstation	500	2×10^3-2×10^5	1.0	[1]
Electrochemical	*Salmonella*	Impedance analyzer	100	1.0×10^2-1.0×10^5	2.0	[2]
SERS	*E. coli* O157:H7	Raman Spectrometer	30	-	-	[3]
SPR	*Staphylococcus aureus*	Spectrophotometer	10^3	-	1.0	[4]
Fluorescent	*Salmonella*	microplate reader	4.9×10^3	4.9×10^3-4.9×10^7	3.5	[5]
Fluorescent	*Salmonella*	Inverted fluorescence microscope	50	50-10^5	5.0	[6]
Fluorescent (RCA-CRISPR/Cas12a)	*Salmonella*	Smartphone	1.93×10^2	1.93×10^2-1.93×10^8	~3.0	[7]
Colorimetric	*Salmonella*	microplate reader	3×10^2	1.0×10^4-1.0×10^6	1.0	[8]
Colorimetric	*Salmonella*	Smartphone	276	10^3-10^8	~1.0	[9]
Colorimetric	*Salmonella*	smartphone	101	2.0×10^2-2.0×10^5	0.5	This biosensor

SERS: Surface-enhanced Raman spectroscopy. SPR: Surface plasmon resonance. RCA: rolling circle amplification.

App Code:

```
int ddw = iv_image.getDrawable().getBounds().width();
int ddh = iv_image.getDrawable().getBounds().height();
int TotalPxiel = ddw * ddh;
int TotalR = 0, TotalG = 0, TotalB = 0;

for (int i = 0; i <= ddw - 1; i++) {
    for (int j = 0; j <= ddh - 1; j++) {
        TotalR += Color.red(bitmap.getPixel(i, j));
        TotalG += Color.green(bitmap.getPixel(i, j));
        TotalB += Color.blue(bitmap.getPixel(i, j));
    }
}
float averageR = (float)(TotalR/TotalPxiel)/255, averageG = (float)(TotalG/TotalPxiel)/255, averageB =
(float)(TotalB/TotalPxiel)/255;
float max = MAX(averageR, averageG, averageB);
float min = MIN(averageR, averageG, averageB);
float L = (max + min)/2;
float S;
if(L > 0.5) { S = (max - min)/(max + min);}
else { S = (max - min)/(2 - max - min);}
float h;
if(averageR == max) { h = (averageG - averageB)/(max - min);}
else if(averageG == max) { h = 2 + (averageB - averageR)/(max - min);}
else { h = 4 + (averageR - averageG)/(max - min);}
float H;
if(h >= 0) { H = h * 60;}
else H = 360 + h * 60;

for (int count = 1; count <= 3; count++) for (int i = 1; i < bitmap.getWidth()-1; i++) {
    for (int j = 1; j < bitmap.getHeight() - 1; j++) {
    int Array[] = {bitmap.getPixel(i - 1, j - 1), bitmap.getPixel(i - 1, j), bitmap.getPixel(i, j - 1),
    bitmap.getPixel(i, j), bitmap.getPixel(i - 1, j + 1), bitmap.getPixel(i + 1, j - 1),
    bitmap.getPixel(i + 1, j), bitmap.getPixel(i, j + 1), bitmap.getPixel(i + 1, j + 1),};

        int result = ChoiceSort(Array, 9);
        count++;
        bitmap.setPixel(i, j, result);
        }
    }
}
int ChoiceSort(int arr[], int n){
    for(int i = 0;i < n; i++){
        int m = i;
        for(int j = i + 1;j < n;j++){
            if(arr[j] < arr[m]){
                m = j;
            }
        }
        if(i != m){
            int t = arr[i];
            arr[i] = arr[m];
            arr[m] = t;
        }
    }
            return arr[4];
}
```

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