Supporting Information

Construction a desirable hyperbolic microfluidic chip for ultrasensitive determination of PCT based on the chemiluminescence

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Table S1 Comparison of grid independence errors when Re=10

Mesh number	72742	205021	414980	820029	1256127
Velocity error	1.27%	1.11%	0.62%	0.05%	0.03%
Concentration error	205.63%	83.48%	49.72%	0.47%	0.05%
Calculation time (min)	9	37	70	250	820

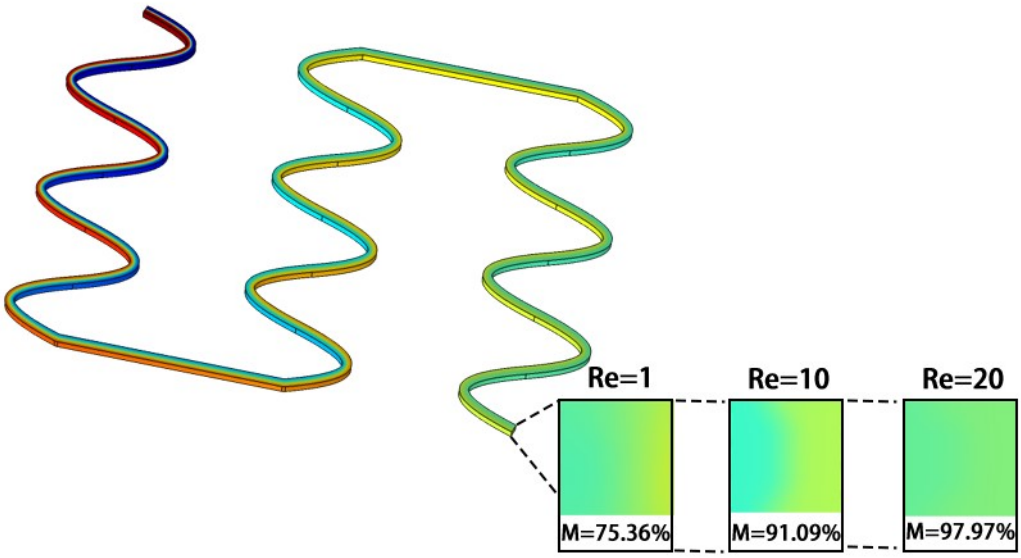

Fig. S1 Concentration distribution of the three-stage hyperbolic micromixer with H_5 curves

As shown in Fig. 3D, the mixing efficiency of the H_5 micromixer was relatively low at Re=1, only 48.32%, which may be due to the mutual inhibition of molecular diffusion and chaotic advection in the channel. In order to obtain an efficient mixing effect under any Re, three sections of hyperbolic curve H_5 were embedded in the detection chip to achieve reagent mixing. Fig. S1 showed the mixing concentration distribution at Re = 1, 10, and 20. The measured mixing efficiencies at the outlet were 75.36%, 91.09%, and 97.97%, respectively. Therefore, The fluid mixing efficiency met the experimental requirements.

The specific design of the hyperbolic microfluidic detection chip

A 4 mm thick microchannel layer at the top, a 0.1 mm thick reaction layer in the middle, and a 3 mm thick substrate layer at the bottom. A rotary valve and an external holder controlled the microchannel access. Using a 1 mm punch, drill the holes in the reservoirs to facilitate reagent dosing and maintain pressure equilibrium inside and outside the chip. This was followed by three double-curved micro-mixing channels and a square assay channel with a micro-mixing channel size of 300 μm wide and 300 μm deep and an assay channel size of 500 μm wide and 500 μm deep, with a total volume of approximately 40 μL. Finally, it led to the square waste liquid pool of the base layer until the negative pressure interface. The substrate layer contains the mounting holes for the rotary valves, the size and location corresponding to the channel layer. The through-hole arrangements on the rotary valve were at 5.5 mm intervals, and each through-hole connected the corresponding reservoir to the branched channel. Turning the rotary valve to different angles controlled the reagent flow in the reservoir. As shown in Fig 5B, the rotary valve was divided into three working states, the first working state was when reservoir one and reservoir two were rotated through, the second working state was when reservoir three was rotated through, and the last working state was when reservoir four was rotated through. To ensure the stability of the valve's operation, we designed external brackets and fixed them on the outer surface of the chip.

The fabrication of DHMC

First, we constructed a 3D model of the chip using SolidWorks, and the chip mold, holder, and rotary valve were printed using an AccuFab-L4K light-curing 3D printer. Then pour the PDMS matrix and curing agent mixture into the mold after evacuating air bubbles in a vacuum drying oven to remove small surface air bubbles and left to stand in a constant temperature drying oven at 65°C for 120 min. After curing and molding, we removed the microchannel and reaction layers from the mold with tweezers. Then punched through holes for the reservoir and negative pressure interface using a 1 mm hole punch. A plasma cleaner performed the hydrophilic surface treatment. With plasma for 60 s at 200 W power bombarded the surface of the microchannel layer, and the reaction also had a 1.5 L min^{-1} oxygen flow rate to break the silicon-oxygen bonds on the surface, as shown in Fig. S2A.

We placed the reaction layer in the middle of the channel and the substrate layers, then aligned the silica film with the square channel in a straight line. Then aligned the edges of the channel and substrated layers and squeezed out the air bubbles in the double PDMS layer to tightly bond them. After finishing, we inserted a rotary valve at the through holes on the side of the double PDMS layer so that the first two through holes on the valve were aligned with reservoirs one and two. Finally, we fixed it firmly to the PDMS chip with a bracket (Fig. S2B).

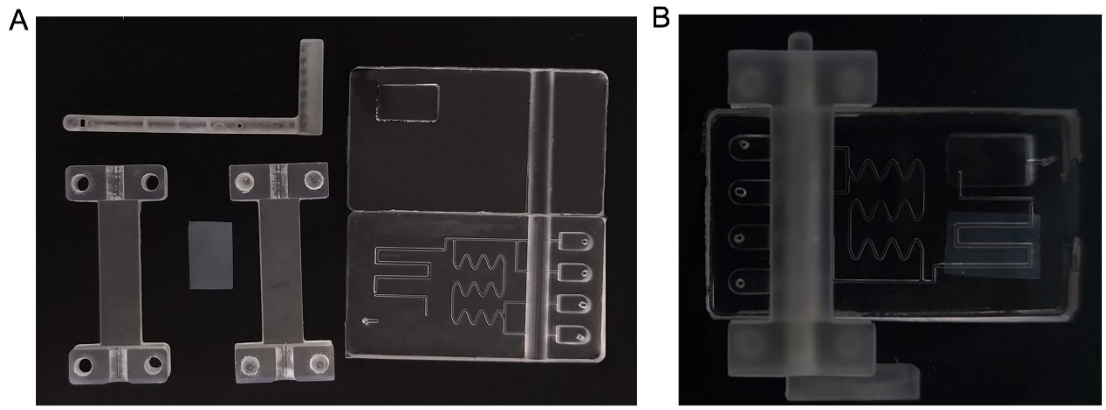

Fig. S2 (A) Physical diagram of each hyperbolic microfluidic chip detection chip component. (B) Physical diagram of the hyperbolic microfluidic detection chip assembly.

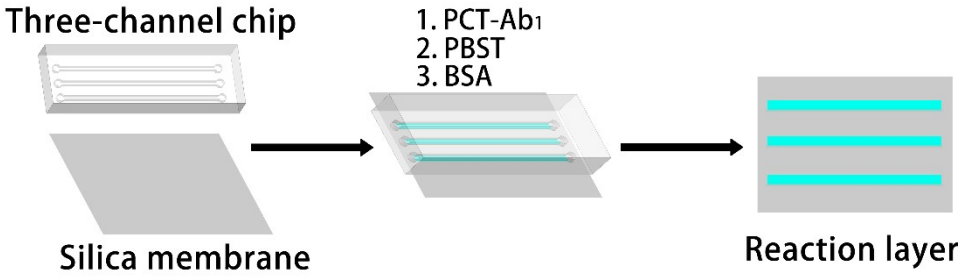

Fig. S3 Schematic diagram of the fabrication of the reaction layer.

Fig. S3 showed that the reaction layer was a silicone membrane coated with capture antibody PCT-Ab1 strips and the fabrication method. Firstly, the three-channel chip was attached flat to the silicone mold, where the size and gap of the channels were the same as the square assay channels; then, the PCT-Ab1 was injected into the three channels with a pipette gun and incubated for 20 min to make the antibody adsorbed on the silicone membrane; secondly, the channels were washed by passing PBST wash solution and incubated again with BSA (5%) for 20 min to occupy the free sites on the membrane and prevent non-specific adsorption. Finally, the BSA solution was aspirated, and the chip was torn open, dried at room temperature, and placed at 4 °C for storage.

The detection principle of the microfluidic chip

After completion of the chip assembly, four reservoirs were added in order from right to left with 20 μL of a certain concentration of PCT sample, 20 μL of PCT-Ab2 -HRP solution, 50 μL of PBST wash solution, and 35 μL of the chemiluminescent substrate. The syringe pump was connected to the negative pressure port via a hose. The reaction process of the assay was divided into the following steps.

(1) Adjustment of the rotary valve so that the reservoir where the test sample and the test antibody solution were located and connected to the main channel, turning on the syringe pump, and the test sample and the test antibody solution were thoroughly mixed and reacted in the microchannel and then flowed into the square test channel (Fig. 5C(b)).

(2) Turning off the syringe pump and incubating for a certain time to allow specific adsorption immunoreactivity of the mixture in the square microchannel (Fig. 5C(c)).

(3) Turning on the syringe pump and drawing all the liquid in the channel to the waste pool.

(4) Adjusting the rotary valve so that the PBST wash entered the main channel and completed about 15 s of continuous washing until all the PBST wash entered the waste pool (Fig. 5C(d)).

(5) Adjusting the rotary valve so that it corresponded to the chemiluminescent substrate reservoir, and the chemiluminescent substrate flowed into the square-shaped microchannel.It reacted with the HRP on the capture antibody and subsequently flowed into the waste pool (Fig. 5C(e)).

(6) After the reaction process, put the chip into the chemiluminescence detector for exposure. The three linear channels of the square microchannel were perpendicular to the three captured antibody bands on the silicone membrane, generating nine detection sites.

In the above experiments, the optimized concentration of PCT-Ab1 on the silica membrane and the concentration of PCT-Ab2-HRP were obtained from the previous study [1]. The optimized concentrations were 60 μg mL^{-1} and 50 μg mL^{-1}, respectively.

Table S2 Comparison of DHMC detection capabilities

Detection method	DHMC	CL[2]	FRET[3]	FUNC[4]	LFD[5]
LOD [PCT (ng mL^{-1})]	0.17	0.17	0.25	0.19	0.54
Linear range (ng mL^{-1})	0.4-12.8	0.31-25	0.1-10	0.5-100	0-50
Standard equation	Y=18959.69X-43922.21 (R^2=0.975)	Y=1594X+1309 (R^2=0.9945)	(R^2=0.9916)	Y=0.1328X+0.0699 (R^2=0.9955)	(R^2=0.9389)
Detection time (min)	15	70	30	> 1 h	> 30
Cost	Low	Low	Medium	Medium	Low

DHMC: Desirable Hyperbolic Microfluidic Chip; **CL:** Chemiluminescence; **FRET:** Fluorescence Resonance Energy Transfer; **FUNC:** Freeze-dried Up-conversion Nanoparticles/Antibody Conjugates; **LFD:** Lateral Flow Device.

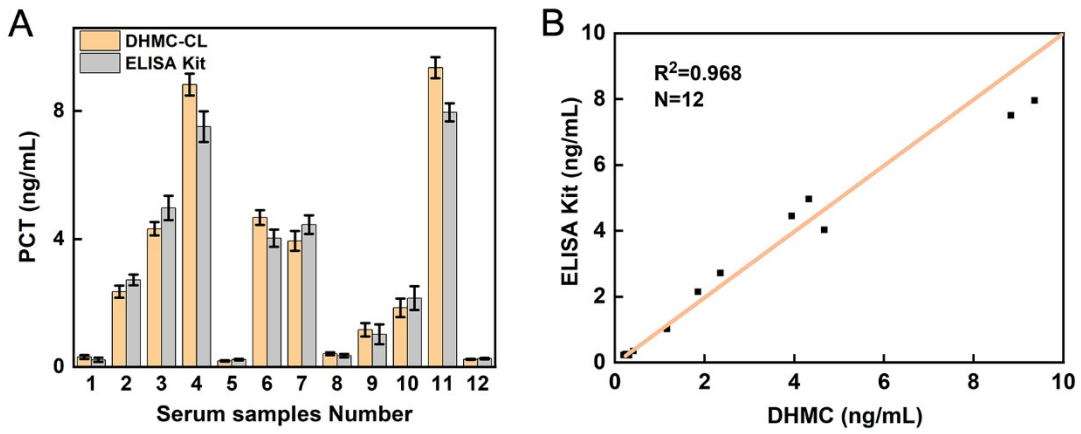

Fig. S4 Comparison of DHMC and ELISA Kits for PCT detection.

As shown in Fig. S4, we compared our DHMC with ELISA Kit in detecting PCT in serum samples. Both the DHMC and ELISA Kit can accurately determine the negative samples (samples No. 1, 5, 8, and 12). For positive samples (samples No. 2-4, 6, 7, and 9-11), the DHMC method can successfully identify all the positive samples (Fig. S4 A). In addition, the quantitative results of this DHMC for detection of PCT in serum samples agreed well with those of ELISA Kit with a correlation coefficient of 0.968 (Fig. S4 B, N=12).

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