Electronic Supplementary Material (ESI) for Lab on a Chip.

All-in-one platform for salivary cotinine detection integrated with microfluidic channel and electrochemical biosensor

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Supporting Information

Optimization of Detection Conditions

The analytical performance of the immunosensor depends on the immunoassay procedure and other factors including the thionine (Thi) concentration, H_2O_2 concentration, pH value, and target incubation time. Therefore, to enhance the immunosensor efficiency, the mentioned parameters were optimized. The effects of the Thi and H_2O_2 concentrations on the current responses were studied in detail. As shown in Fig. S3A as the concentration of Thi was increased, the currents density increased and reached plateau regions at a concentration of 0.3 mM. Thus, the optimal concentration of Thi was chosen as 0.3 mM. Moreover, the effect of the H_2O_2 concentration on the enzymatic reaction was examined (Fig. S3B). Increments of H_2O_2 concentration up to 2 mM increased the Thi reduction current density. At higher H_2O_2 concentrations, not only the current responses did not increase but also slightly decreased owing to the denaturation of HRP.[1] Therefore, the optimal H_2O_2 concentration was chosen as 2 mM. The pH value of the dilution buffer significantly affects on both electrochemical characteristics of redox probe and the activity of HRP. The immunosensor efficiency was investigated over a pH range of 5.0 to 8.0. Fig. S1C illustrates the effect of the pH on the redox current responses of Thi at the surface of the modified electrode in PBS, and the optimized current response obtained at pH 5.5. The decrease in current responses in high-pH solutions is mostly related to active site changes in the HRP, which results from the movement or deformation of amino acid residues in HRP molecules at the higher pHs.[1] These changes might be ascribed to a hydrogen bond loss between the oxygen ligand and amino acid residue in the HRP molecules at lower pHs. Moreover, the immunoreaction conditions significantly affect the current responses of the immunosensor. These conditions include the C-HRP and cotinine immobilization time. The current density of the immunosensor increased with an increase in the C-HRP immobilization time and became stable at 0.5 hour (Fig. S4A). Since the longer immobilization time did not increase the response, 0.5 hour was selected as the optimized immobilization time for the C-HRP/anti-cotinine reaction. Furthermore, the incubation time for replacing

C-HRP with cotinine was optimized (Fig. S4B). The optimum replacement time was determined to be 0.5 hours. Longer times did not significantly improve the signal.

Table S1. Questionnaire of exposure level to secondhand smoke.

Volunteer No.	Age	SEX	Unrecognized	Recognized	
			Rarely	Sometimes	All of the time
1	20	Female	✓		
2	24	Female	✓		
3	20	Female	✓		
4	22	Female		✓	
5	21	Female			✓
6	20	Female		✓	
7	22	Female		✓	
8	20	Female			✓

Table S2. Comparison between the present immunosensor and other reported methods for cotinine detection.

Technique	LDR ($pg \cdot ml^{-1}$)	LOD ($pg \cdot ml^{-1}$)	Sample	Analysis time (min)	Reference
DPV	**1×10^{-1} to 1×10^{4}**	**6×10^{-2}**	**Saliva**	**31**	**This Work**
CV	1.8×10^{3} to 1.8×10^{5}	5.8×10^{1}	Saliva	12	[2]
SWV	1×10^{3} to 1×10^{5}	1.0×10^{3}	Serum	14	[3]
Amperometry	8.8×10^{7} to 1.8×10^{10}	1.07×10^{4}	Saliva	6	[4]
Impedance spectroscopy	1.6×10^{5} to 1.6×10^{6}	1.6×10^{5}	-	22	[5]
CLI	1×10^{4} to 1×10^{6}	5.0×10^{3}	Serum	6	[6]
HPLC	Up to 4×10^{6}	1.2×10^{4}	Urine	20	[7]
SERS	-	8.8×10^{3}	Saliva	34	[8]

LDR: Linear dynamic range, LOD: Limit of detection, DPV: Differential pulse voltammetry, CV: Cyclic voltammetry, SWV: Square wave voltammetry, CLI: Chemiluminescence immunoassay, HPLC: High-performance liquid chromatography, SERS: Surface-enhanced Raman spectroscopy.

Table S3. Quantitative analysis of spiked cotinine levels in the artificial saliva.

Spiked cotinine[a] ($pg \cdot ml^{-1}$)	Cotinine found by Immunosensor ($pg \cdot ml^{-1}$)	Recovery (%) [c]	Cotinine found by LC-MS/MS ($pg \cdot ml^{-1}$)	Recovery (%) [d]
1	9.1×10^{-1}	91.3	n.m.[b]	-
1×10^{1}	9.5±0.1	95	n.m.[b]	-
1×10^{2}	$9.4\pm0.1\times10^{1}$	92.4	$1.2\pm0.1\times10^{2}$	121
1×10^{3}	$1.1\pm0.1\times10^{3}$	107.1	8.3×10^{2}	83.3
1×10^{4}	9.7×10^{3}	97.2	1.0×10^{4}	100.1

[a]Concentration of cotinine added to the artificial saliva samples. [b]n.m.=not measured. [c]Calculated as $(C_{Immunosensor}/C_{Spiked})\times100$, [d]Calculated as $(C_{LC\text{-}MS/MS}/C_{Spiked})\times100$, where C_{Spiked}, $C_{Immunosensor}$ and $C_{LC\text{-}MS/MS}$ are the concentrations listed in column 1, 2 and 4 respectively.

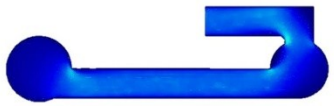
Flow rate ($ml \cdot min^{-1}$)	Linear velocity ($m \cdot s^{-1}$)	Channel width (μm)			
		500	1,000	1,500	2,000
0.1	7.9×10^{-5} 0				
1	7.8×10^{-4} 0				
9	7.0×10^{-3} 0				
15	1.4×10^{-2} 0				

Fig. S1: Velocity profiles at various channel widths (500, 1,000, 1,500, and 2,000 μm) and flow rates (0.1, 1, 9, 15 $ml \cdot min^{-1}$) using COMSOL Multiphysics.

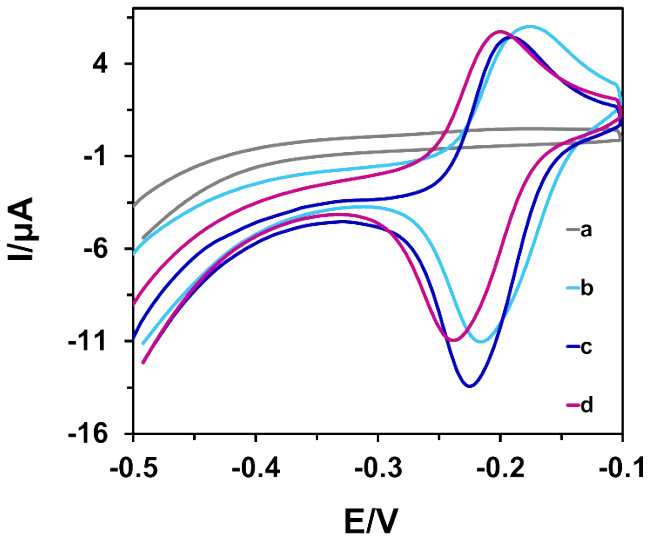


Fig. S2: Cyclic voltammograms of C-HRP/BSA/anti-cotinine/MPA/SPGE in (a) dilution buffer, (b) dilution buffer containing 0.3 μM Thi, (c) probe buffer and cotinine/BSA/anti-cotinine/MPA/SPGE in (d) probe buffer.

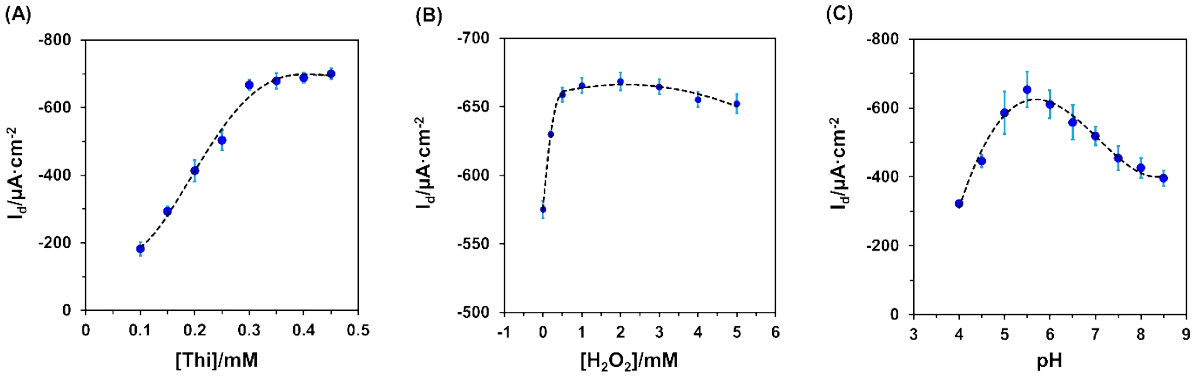


Fig. S3: Effects of (A) Thionine concentration in dilution buffer containing 2.0 mM H_2O_2 (B) H_2O_2 concentration in dilution buffer containing 0.3mM Thionine and (C) pH on the current density of the C-HRP/BSA/anti-cotinine modified SPGE.

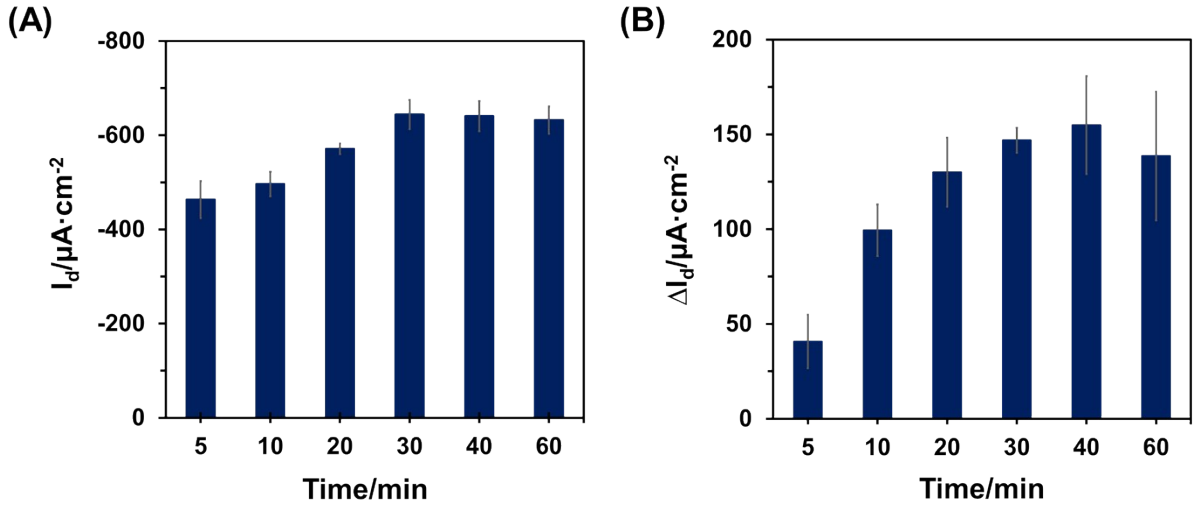


Fig. S4: Immunoreaction conditions (A) Effects of C-HRP/anti-cotinine interaction time (B) Cotinine/C-HRP replacement time on the current density of immunosensor. Cotinine concentration is 100 pg·ml^{-1}.

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