Supporting information

A portable blood lactate sensor with non-immobilized enzyme for early sepsis diagnosis

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Text S1 Chemicals and materials

Carbon ink and silver/silver chloride (Ag|AgCl) ink were purchased from Gwent group (Torfaen, UK). A commercial screen-printed carbon electrode was purchased from Quasense Co., Ltd (Bangmod, Bangkok, Thailand). Lactate oxidase (L9795-100UN from *Aerococcus viridans*) lyophilized powder containing 2.4 mg solid, 100 $U \cdot mL^{-1}$, Sodium DL-lactate Reagent Plus ≥99%(nt) and titanium diisopropoxidebis (acetylacetonate) or TIAA (75% wt in isopropanol) were purchased from the Sigma Chemical Co. (St.Louis, MO, USA). Conductive graphene dispersion was purchased from Graphene Supermarket (New York, USA). Isopropanol was obtained from Univar Chemical Co. (Illinois, USA). Phosphate buffer saline (PBS) tablets pH 7.4 was purchased from VWR life science AMRESCO Inc. (Denver, CO, USA). 1,3-propanediol (98%wt) was obtained from ACROS organics (Morris plains, NJ, USA). A stock solution of LOx enzyme was prepared in 0.10 M PB (pH 7.4) aliquoted into a tube (10 μL) and stored at−20°C in a normal freezer. All aqueous solutions were prepared in Milli-Q water (resistivity value 18.2 MΩ·cm at 25 °C).

Text S2 Instruments

All amperometric measurements were carried out using an Emstat Pico system developed by PalmSens BV and Analog Devices Inc. (GA Houten, Netherland). A three-electrode cell was used and the working electrode was a TiO_2 sol-G nanocomposite modified SPCE (0.3 cm in diameter). A screen-printing block and machine were fabricated by Chaiyaboon Co., Ltd. (Bangkok, Thailand). A Hitachi/x-4800 transmission electron microscope (TEM) (Japan Electron Optics Laboratory, Japan) was used for TiO_2 sol and TiO_2 sol-G nanocomposite characterization. A standard method for blood lactate measurements was a blood gas analyzer (stat profile prime plus®, NOVA, USA). A customized reader for a portable blood lactate detection kit was developed by Wuxi Admas Technology Co., Ltd (Jiangsu, China).

Text S3 Fabrication of a modified electrode

The SPCE was constructed by using a screen-printing machine to print a carbon ink onto a polyvinyl chloride sheet for a working electrode (WE, area = 0.07 cm^2) and counter electrode (CE). A silver/silver chloride (Ag|AgCl) ink was used as a pseudo-reference electrode (RE) and a conductive pad. The printed electrode was dried in an oven at 55 °C for 15 min. The as-prepared nanocomposite was diluted by adding isopropanol with a 1:5 dilution ratio. Finally, 0.5 μL of the nanocomposite was thoroughly dropped onto the working electrode and allowed to dry at room temperature and

atmospheric pressure, then the modified electrode was kept in a desiccator when not in use. As for comparison, LOx modified TiO_2 sol-G/SPCE was prepared by dropping 1 µL of LOx onto TiO_2 sol-G/SPCE, and incubated at - 4 ºC for overnight.

Text S4 Electrochemical analysis

Amperometry was conducted to optimize the experiment parameters by mixing 1 µL of LOx (50 $U \cdot mL^{-1}$) with 100 µL of 5 mM lactate in 0.1 M PB solution (pH 7.4) for 5 min and measuring on a TiO_2 sol-G/SPCE at an optimized potential of +0.5 V for a fixed time duration following the previous literature [22]. Amperometric lactate detection on the TiO_2 sol-G/SPCE electrode was carried out at the optimized parameters and the anodic current was recorded at a steady state current of 170 s. All measurements were carried out at room temperature, 27 ± 2 °C

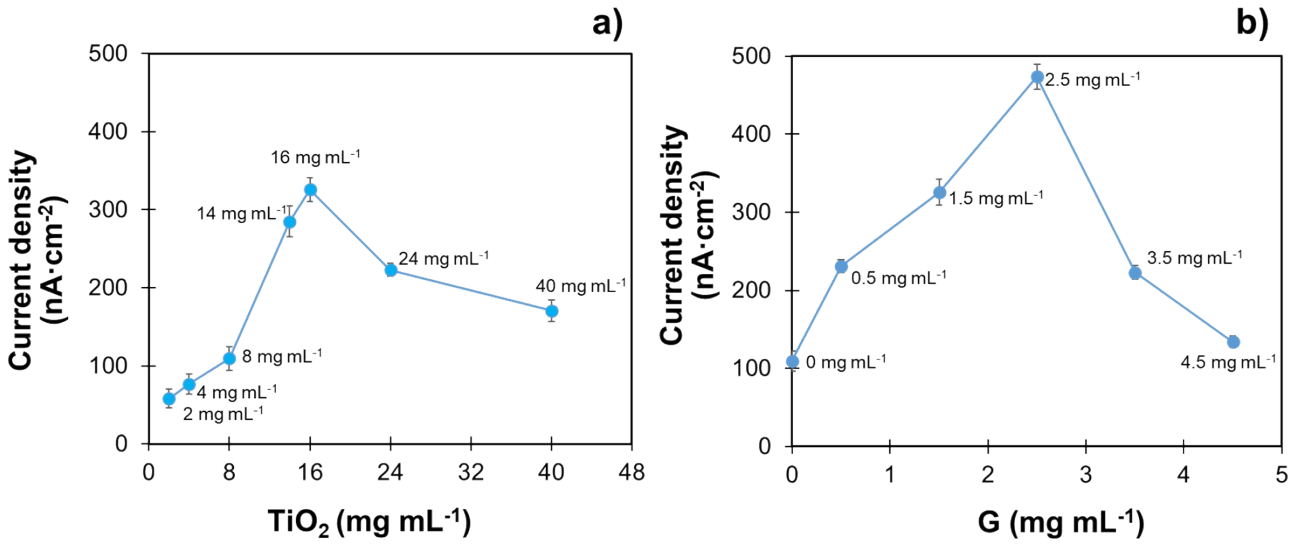
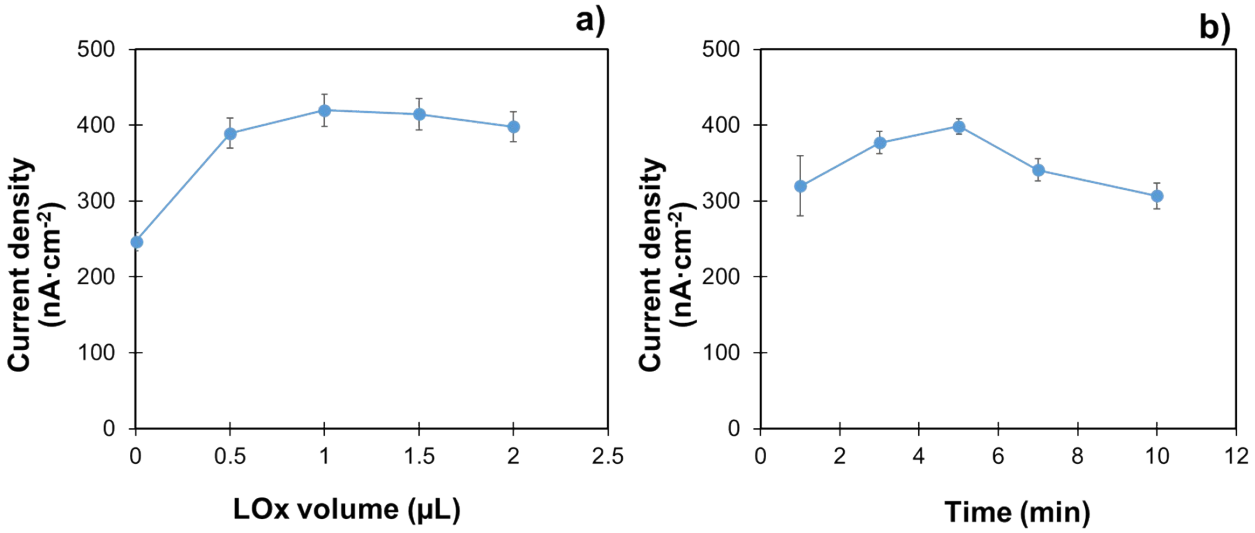


Fig. S1 Effect of TiO_2 sol (a) and G (b) concentration on the current response to 5 mM lactate in 0.1 PB (pH 7.4) at +0.5 V; each data point has been obtained by measurements with 3 individual single-use electrode; error bars indicate the standard deviations.



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77 **Fig. S2** Effect of LOx enzyme volume (a) and incubation time (b) on the current response to 5 mM

78 lactate in 0.1 M PB (pH 7.4) at +0.5 V; each data point has been obtained by measurements with 3

79 individual single-use electrode; error bars indicate the standard deviations.

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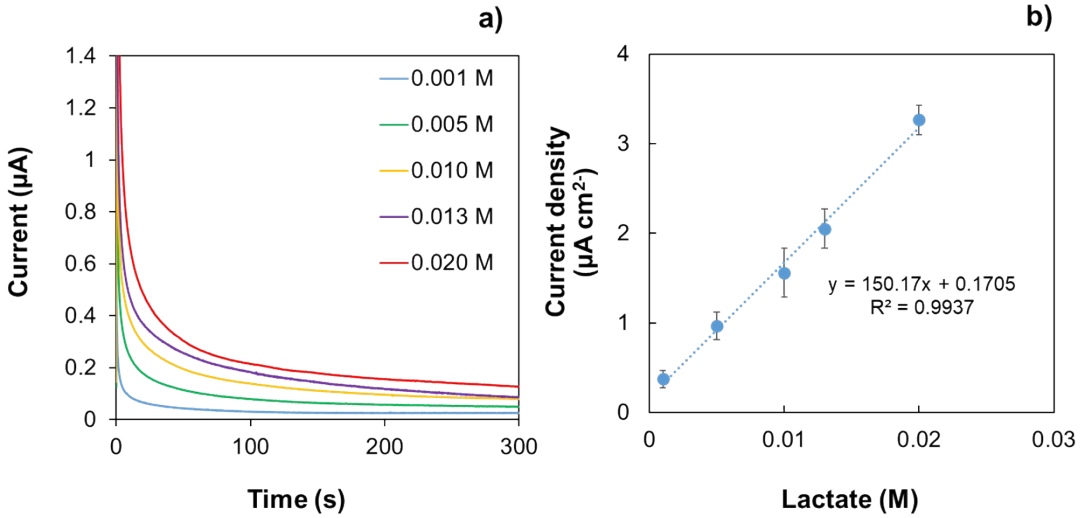

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82 **Fig. S3.** Amperometric responses of TiO_2 sol-G nanocomposite/SPCEs (n=5) for various lactate concentrations

83 (0.001-0.020 M) in 0.1 M PB (pH 7.4) measured at +0.5 V (a). Calibration plot constructed from the signal

84 measurements presented in a (b).

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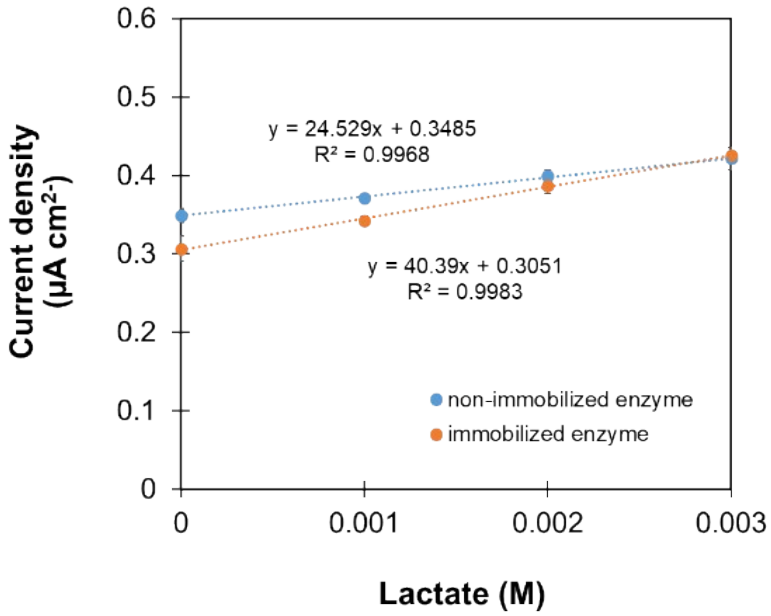

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87 **Fig. S4.** Calibration plot of of TiO_2 sol-G/SPCEs (a) and LOx/TiO_2 sol-G/SPCEs (b)

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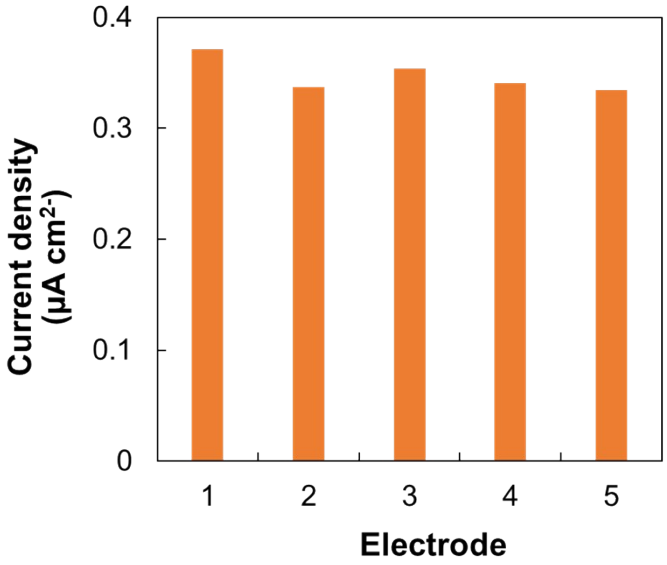

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90 **Fig. S5.** Amperometric current responses of five different LOx/TiO_2 sol-G/SPCEs

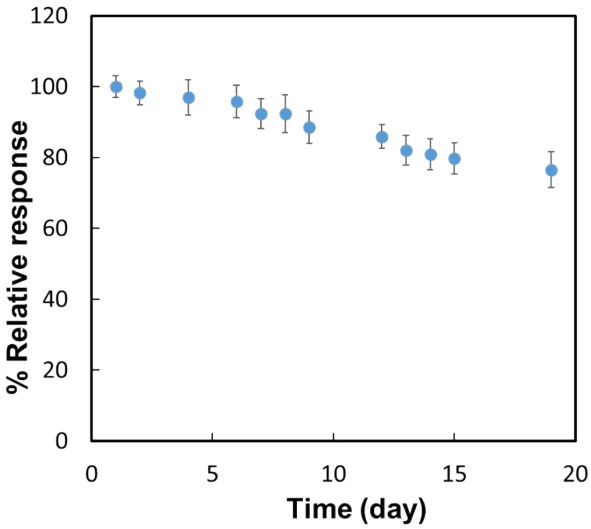

Fig. S6. Long-term stability of LOx/TiO_2 sol-G/SPCEs

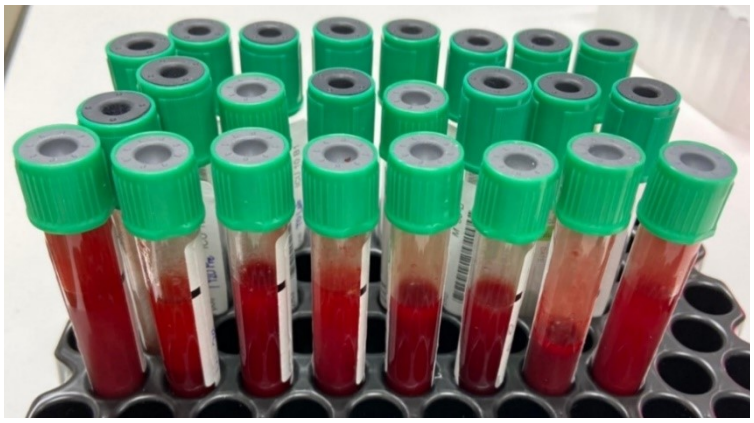

Fig. S7. A photograph of whole blood lactate samples obtained from ICU patients collected in lithium heparin tubes.

Table S1: Characteristics of sepsis patients' subject.

Characteristic	Value, median (interquartile range)
Number of patients	50
Gender	
male	28
female	22
Age (years) mean ± SD	60.2 ± 20.8
range	18 - 98
Arterial lactate level (mmol/L) mean ± SD	6.4 ± 5.7
range	0.8 - 20

Table S2. Determination of blood lactate levels in healthy people validated with a blood gas analyzer (*n*=3).

sample	Lactate concentration (mmol/L)		Relative standard deviation; %RSD	%Accuracy[a]
	Blood gas analyzer	Electrochemical		
1	3.0	2.6 ± 0.1	3.8	86.7
2	5.7	5.6 ± 0.1	1.7	98.2
3	3.8	3.8 ± 0.1	2.6	100.0
4	4.0	4.3 ± 0.2	4.7	107.5
5	2.1	2.2 ± 0.1	4.5	104.8

[a]Accuracy (%) = Mean value of [(mean concentration observed from electrochemical)/concentration observed from blood gas analyzer)] *100.

Two samples from healthy individuals (4.3 ± 0.2 and 5.6 ± 0.1 mM) had intensive exertion before sample collection, which may have arisen from lactate accumulation in the blood.

Table S3. Determination of blood lactate levels in ICU patients validated with a blood gas analyzer (*n*=3).

sample	Lactate concentration (mmol/L)		Relative standard deviation; %RSD	%Accuracy[a]
	Blood gas analyzer	Electrochemical		
1	1.6	1.5 ± 0.2	13.3	93.8
2	2.2	2.5 ± 0.2	8.0	113.6
3	3.0	2.6 ± 0.1	3.8	86.7
4	5.7	5.7 ± 0.1	1.7	100.0
5	17.0	16.8 ± 0.2	1.2	98.8
6	20.0	15.7 ± 0.4	2.5	78.5
7	5.7	4.5 ± 0.3	6.7	78.9
8	1.1	1.0 ± 0.1	10.0	90.9
9	19.5	15.0 ± 0.6	4.0	76.9
10	12.9	11.2 ± 0.2	1.8	86.8
11	11.5	12.9 ± 0.2	1.6	112.2
12	2.3	2.35 ± 0.3	12.8	102.2
13	1.7	1.8 ± 0.2	11.1	105.9
14	13.8	14.8 ± 0.2	1.4	107.2
15	16.1	12.1 ± 1.5	12.4	75.2
16	2.5	2.9 ± 0.4	13.8	116.0
17	2.6	2.5 ± 0.2	8.0	96.2
18	4.2	5.1± 0.2	3.9	121.4
19	2.2	2.3 ± 0.1	4.3	104.5
20	5.2	5.0 ± 0.2	4.0	96.2
21	10.8	11.5 ± 0.8	7.0	106.5
22	8.6	8.3 ± 0.2	2.4	96.5
23	5.9	6.5 ± 0.1	1.5	110.2
24	7.2	8.5 ± 0.1	1.2	118.1
25	6.9	6.1 ± 0.4	6.6	88.4
26	4.9	5.7 ± 0.6	10.5	116.3
27	0.8	0.9 ± 0.1	11.1	112.5
28	1.7	1.9 ± 0.3	15.8	111.8
29	7.9	6.0 ± 0.3	4.3	75.9
30	1.0	1.0 ± 0.1	10.0	100.0
31	19.7	16.1 ± 1.0	6.2	81.7
32	17.7	16.8 ± 1.3	7.7	94.9
33	1.9	1.9 ± 0.1	5.3	100
34	1.4	1.4 ± 0.1	7.1	100

35	3.9	4.0 ± 0.1	2.5	102.6
36	3.8	4.2 ± 0.1	2.4	110.5
37	4.2	4.6 ± 0.3	6.5	109.5
38	7.6	8.9 ± 0.8	9.0	117.1
39	3.8	3.6 ± 0.2	5.6	94.7
40	11.5	12.4 ± 0.4	3.2	107.8
41	5.8	6.4 ± 0.3	4.7	110.3
42	0.9	0.9 ± 0.1	11.1	100
43	1.2	1.3 ± 0.2	15.4	108.3
44	1.0	1.0 ± 0.1	10.0	100.0
45	14.8	14.1 ± 0.5	3.5	95.3
46	0.9	0.9 ± 0.1	11.1	100.0
47	2.8	3.1 ± 0.1	3.2	110.7
48	5.0	4.8 ± 0.6	12.5	96.0
49	2.3	2.3 ± 0.3	13.0	100.0
50	1.5	1.6 ± 0.2	12.5	106.7

[a]Accuracy (%) = Mean value of [(mean concentration observed from electrochemical)/concentration observed from blood gas analyzer)] *100

The blood samples were collected from patients who were admitted to all sections of the intensive care units (ICUs) at King Chulalongkorn Memorial Hospital at the specified time as described in the experimental section. Due to the samples being collected without the duration of treatment, some patients might recover from treatment, resulting in a lower lactate level than the cut-off.