# Supplementary Material

**Intercalating methylene blue to molecular beacon for sensitive detection of salivary TNF-α towards early diagnosis of oral cancer**

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**Optimization of SPCE-GO-Apt (MB) sensing interface**

Fig S1 showed the effect of different conditions including pH, the concentration of $Na^+$, temperature and incubated time, on the sensing performance of the SPCE-GO-Apt (MB). When pH changed from 4 to 7.4, the release of methylene blue (MB) was gradually increased due to the instability of secondary structure of aptamer under the acidic condition, and thus hairpin opened and released the MB. While the DNA was denatured under alkaline conditions[1], and thus less MB loaded (Fig. S1a). An optimal electrochemical signal was obtained when the sodium ion concentration was 100 mM (Fig. S1b). Fig. S1c shows when the temperature was higher than 60 °C, the release of MB has significant difference compared with at room temperature. Thus, the temperature for SPCE-GO-Apt (MB) sensing interface was set at 25 °C for subsequent experiments. Fig. S1d showed the incubation time of the sensing interface with the cytokine TNF-α. When the reaction time was within 30 min, the current signal of MB increased before it reached a plateau, therefore 30 min was selected as the optimal reaction time.

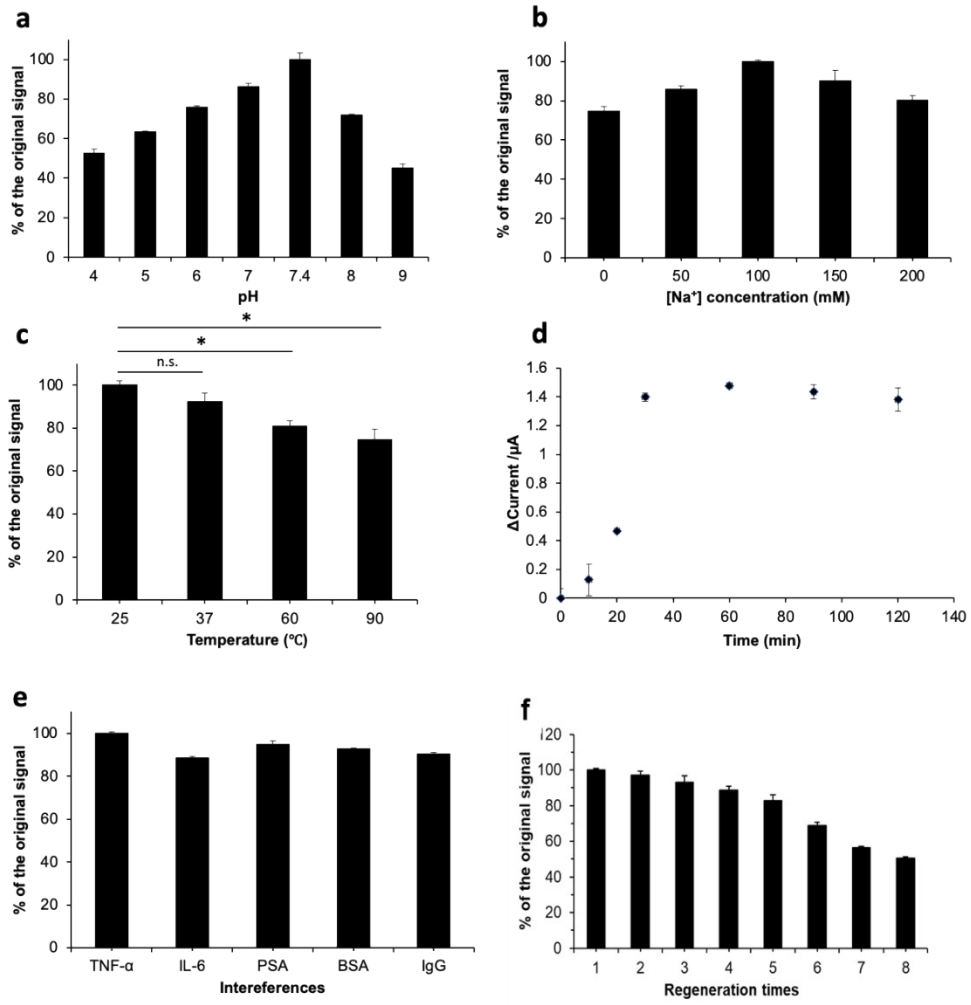


**Fig. S1.** Effect of (a) pH, (b) the concentration of [$Na^+$], (C) temperature and (d) incubation time of TNF-α on the performance of SPCE-GO-Apt (MB) sensing interface for the detection of TNF-α. (e) The current response of SPCE-GO-Apt (MB) sensing interface for the selective study of four non-specific proteins of IL-6, PSA, BSA and mouse IgG in the presence of 50 pg $mL^{-1}$ TNF-γ. (f) The reproducibility of SPCE-GO-Apt (MB) sensing interface for 100 pg $mL^{-1}$ TNF-α. (two-tailed Student's t test; *, $p < 0.05$).

**Table S1** Optimization of sensing interface conditions

| Parameters | Ranges | Optimal condition |
|---|---|---|
| pH value | 4, 5, 6, 7, 7.4, 8, 9 | 7.4 |
| Concentration of Na+ | 0, 50, 100, 150, 200 mM | 100 mM |
| Temperature | 25, 37, 60, 90 °C | 25 °C |
| Incubated time | 0, 10, 20, 30, 60, 90, 120 | 30 min |

**Comparison of developed electrochemical biosensor with other reported assays for the detection of TNF-α**

**Table S2** Side-by-Side Comparison of the Analytical Features of Different electrochemical biosensors developed for TNF-α Detection

| | Recognition element | Sample | Linear range (pg. $mL^{-1}$) | LOD (pg. $mL^{-1}$) | Ref. |
|---|---|---|---|---|---|
| SPCE-GO-Apt-MB | Aptamer | Saliva | 10 -300 | 10 | This study |
| SPCE-GO-Apt (MB) | Aptamer | Saliva | 1 -400 | 1 | This study |
| CMA-gold electrode | Antibody | Saliva | 1 -30 | 1 | 2 |
| SPAuE-Py/Py-COOH/MNPs | Antibody | Saliva | 1 -15 | 0.3 | 3 |
| SPCE-Magnetic beads | Antibody | Serum | 15 -405 | 5.8 | 4 |
| MoS2-based electrochemical biosensor | Antibody | Serum | 1-200 | 0.202 | 5 |
| SPCE@4-ABA | Antibody | Serum | 3250-50,000 | 4100 | 6 |
| SPCE-Magnetic beads | Affibody® | Serum | 76-5,000 | 38 | 7 |
| HOOC-Phe-DWCNTs/SPCEs | Antibody | Serum, Saliva | 1-200 | 0.85 | 8 |
| Ag@Pt-CNTs–CS SPCE | Antibody | serum | 6 - 60 | 1.6 | 9 |
| SPCE-ASPE immunosensor | Antibody | serum | 10 -100 | 5 | 10 |
| MB-tagged aptamer electrochemical sensor | Aptamer | Whole blood | 10,000-100,000 | 10,000 | 11 |
| DSP@AuE | Antibody | Cell | 1-100 | 1 | 12 |

**Abbreviations:** Gold electrode (AuE), carboxymethylaniline (CMA), carbon nanotubes and chitosan (CNTs-CS), graphite screen-printed electrode modified with poly-anthranilic acid (ASPE), screen-printed gold electrode (SPAuE), screen-printed carbon electrode (SPCE),

magnetic microparticles coated with poly(pyrrole-co-pyrrole-2-carboxylic acid) (Py/Py-COOH/MNPs), dithiobis-succinimidyl propionate (DSP)

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