**Supplementary Information**

**Non-invasive oral cancer detection from saliva using ZnO-rGO nanocomposite based bioelectrode**

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**Antibody immobilization**

Figure S1(a) shows the FTIR spectra of ZnO-rGO thin films before and after Anti-IL8 immobilization. The attachment of antibodies to the nanocomposite surface through amide bonding was confirmed by FTIR spectrum of Anti-IL8/ZnO-rGO/ITO which gave a prominent peak of amide C=O at 1624 cm-1. Emergence of new peaks at 3319 cm-1, 2983 cm-1 and 1267 cm-1 belonging to N-H, C-H and C-N stretches further confirmed the presence of antibodies on the surface of ZnO-rGO/ITO electrodes.

To find out the optimum concentration of antibody that would lead to most effective analyte binding was tested by immobilizing a range of different concentrations of antibodies on GO surface and recording its DPV response for 700 pg/mL of IL8. The obtained results, shown in figure S1(b), revealed maximum deflection in current due to formation of less conducting antigen-antibody layer, when 1 µg/mL concentration of Anti-IL8 was used, indicating it to be the ideal concentration for the developed system. The lower deflection in oxidation current in case of antibody concentrations lower than 1 µg/mL was due to the presence of insufficient amount of antibodies on the film that could not effectively bind with available antigen molecules. While, in case of higher antibody concentrations, over-saturation of active sites on the film hampered their proper binding with antigen.



**Figure S1:** (a) FTIR spectra of ZnO-rGO thin film before and after Anti-IL8 immobilization. (b) Graph showing current responses obtained for 700 pg/mL IL8 using immunoelectrodes with different concentrations of Anti-IL8.

**IL8 detection without using antibodies (Control study)**

A control study for detection of IL8 using bare ZnO-rGO/ITO electrodes (i.e. without the addition of antibodies) was performed using DPV method. The obtained current values from the DPV curves for IL8 concentrations ranging from 100 fg mL-1 to 5 ng mL-1 (shown in figure S2), did not show significant variation in current with respect to bare ZnO-rGO/ITO electrodes. Besides, a calibration curve could not be formed since the obtained current values were quite irregular without any specific pattern of increment or decrement. Another disadvantage posed by the antibodies-free detection process was that specificity of the biosensor was lost, as some deflection in current could be obtained on exposure to other biomarkers as well.



**Figure S2:** Bar graph showing the oxidation current values obtained for different concentrations of IL8 on exposure to antibodies-free ZnO-rGO/ITO electrodes.

**Repeatability Measurements**

The Anti-IL8/ZnO-rGO/ITO electrodes were used to take 15 sets of DPV measurements for four different concentrations of IL8 i.e. 400 pg mL-1, 700 pg mL-1, 1 ng mL-1 and 2 ng mL-1 as shown in figure S3. The uncertainty and standard deviation values were calculated for the analytical responses in each case and summarized in the table T1. The results presented high repeatability in the measurements with overall RSD below 3.2%.

**Table T1:** Table showing the calculated uncertainty and RSD values in the response current values for 15 sets of measurements.

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No.** | **IL8 concentration** | **Current (µA)** | **% RSD** |
| 1. | 400 pg mL-1 | 53.16 ± 0.66 | 1.24 |
| 2. | 700 pg mL-1 | 58.25 ± 1.71 | 2.94 |
| 3. | 1 ng mL-1 | 47.62 ± 1.10 | 2.32 |
| 4. | 2 ng mL-1 | 37.13 ± 1.17 | 3.14 |



**Figure S3:** DPV curves showing analytical responses of the Anti-IL8/ZnO-rGO/ITO electrodes for 15 sets of measurements obtained towards (a) 400 pg mL-1 (b) 700 pg mL-1 (c) 1 ng mL-1 (d) 2 ng mL-1 concentrations of IL8.