One-pot construction of mediatorless bi-enzymatic glucose biosensor based on organic-inorganic hybrid

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Supplementary data
Figure S1 Electrochemical impedance spectra of (a) bare ITO, (b) ITO/PTMSPA, (c) ITO/PTMSPA/HRP and (d) ITO/PTMSPA/HRP-GOx modified electrodes in the solution containing K₃Fe(CN)₆ and K₄Fe(CN)₆ (1 mM each) and 0.1M KCl.
Figure S2 (A) Plot of anodic and cathodic peak currents vs. $\nu$ and (B) Plot of anodic and cathodic peak potential vs. $\ln \nu$ obtained from the cyclic voltammograms recorded at PTMSPA/HRP modified electrode in PBS at various scan rates ($\nu$).
Figure S3 Cathodic peak currents (faradic current) observed at -150 mV on different electrodes (platinum disc electrode (Pt), indium-doped tin oxide (ITO), PTMSPA and PTMSPA/HRP electrodes) in PBS containing 0.5 mM H₂O₂; scan rate of 50 mV s⁻¹.
Figure S4 (A) Influence of pH, (B) effect of film thickness on loading of HRP (Electrolyte: 50 mM TMSPA and 10 mg mL⁻¹ of HRP in 50 mM β-NSA), (C) influence of HRP loading and (D) influence of temperature on current response (faradic current) for 0.5 mM H₂O₂ in PBS at PTMSPA/HRP modified electrode; potential: -150 mV vs. Ag/AgCl.
Fabrication of silica based modified electrodes

The protocol for the fabrication of three different bi-enzymatic electrodes (ITO/TMSPA/HRP-GOx, ITO/silica/HRP-GOx and ITO/silica-graphite/HRP-GOx) is outlined.

1. Fabrication of ITO/TMSPA/HRP-GOx modified electrode

N[3-(trimethoxysilyl)propyl]aniline (TMSPA) (50 mM) in 1M β-NSA (1M) was added to the solution containing fixed ratio of HRP and GOx. This solution was dispersed onto the ITO electrode and allowed to dry for 30 min.

2. Fabrication of ITO/TMSPA/HRP-GOx and ITO/silica-graphite/HRP-GOx modified electrodes

About 1.0 mL of tetramethoxysilane (TMOS from Aldrich), prepared in double distilled water was added to 0.05 M HCl (10 µL) and stirred for 30 min at 4°C. Subsequently, fixed ratio of HRP and GOx in 0.01 M PBS (pH 8) was prepared and added drop wise to TMOS solution. Stirring was continued for 4 h. This solution was dispersed onto the ITO electrode and allowed to dry at 4°C for 30 min. Note that alcohol is not used for the preparation of sol-gel silica in order to prevent the denaturation of the enzymes. In the case of ITO/silica-graphite/HRP-GOx modified electrode, 2.0 mg of graphite powder (< 45 microns from Aldrich) was added to the TMOS solution and the same protocol (mentioned above) was followed for the electrode fabrication.
Figure S5 Cyclic voltammograms recorded at (a) ITO/TMSPA/HRP-GOx, (b) ITO/silica/HRP-GOx, (c) ITO/silica-graphite/HRP-GOx and (d) ITO/PTMSPA/HRP-GOx modified electrodes in PBS (pH 7) coating 7.5 mM glucose; scan rate: 50 mV s⁻¹.
Figure S6

Current response DA, UA, AA (of 5 mM each) and glucose (1.5 mM) measured individually at PTMSPA/HRP-GOx modified electrode, when it was poised at different applied potentials in PBS.
Figure S7 Effect of storage time on the response of glucose (5 mM) at PTMSPA/HRP-GOx modified electrode (The electrode was stored in PBS at 4°C when not in use).