Au nanoparticles decorated ZnO nanorods as fluorescent non-enzymatic glucose probe

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Abstract

ZnO nanorods (NRs) synthesized by a hydrothermal method and decorated with Au nanoparticles (NPs) were used for fluorescent non-enzymatic glucose detection. The detection is based on the photoluminescence (PL) quenching of ZnO NRs/Au NPs (at 382 nm under 325 nm excitation) exposed to glucose. The sensor exhibits a high sensitivity of $(22 \pm 2) \% \text{ mM}^{-1}$ (defined as percentage change of the PL peak intensity per mM) and a limit of detection (LOD) as low as 0.01 mM, along with an excellent selectivity and a short response time (less than 5 s). In comparison with a fluorescent non-enzymatic ZnO nanostructure-based glucose sensor, the addition of Au NPs significantly enhances the sensitivity. This is attributed to the surface plasmon resonance, which not only increases the photoluminescence intensity but also the photo-oxidation property of the ZnO NRs. Thus, ZnO NRs/Au NPs can act as an efficient photocatalyst for glucose detection. Most importantly, the applicability for glucose detection in human blood serum demonstrates the accuracy of the probe. The outstanding performance of the material and its cost effectiveness allow for application in single-use, non-invasive glucose devices.

Keywords: ZnO nanorods, Au nanoparticles, glucose sensor, non-enzymatic sensor, fluorescent sensor.

Introduction

Fast and precise glucose modulation is crucial to control diabetes. The most common method for glucose monitoring is the invasive finger prick test, which requires blood extraction from patients. Recently, other approaches for glucose monitoring that use easily accessible fluids such as urine, sweat, saliva, and tear fluid for glucose measurement have attracted a lot of attention. These new approaches provide a great potential for the development of noninvasive diagnosis methods and devices, which are more convenient for patients. However, the glucose concentrations in those physiological fluids are rather low. For example, the glucose concentration in sweat ranges from 0.2 mM to 0.6 mM, in saliva ranges from 0.55 mM to 1.77 mM, and in tears it is more than 0.92 mM [1, 2]. To use those fluids, the next generation of glucose sensors should thus have a higher sensitivity and higher accuracy.

ZnO can be considered as one of the most promising semiconductor materials since it has been used in a wide range of optical and electrical applications. For glucose sensing, ZnO nanostructures integrated in various kinds of sensors have demonstrated outstanding properties such as high sensitivity, high selectivity, low limit of detection (LOD), and good biocompatibility. Due to its intense photoluminescent emission at room temperature [3–6] with a fluorescence quantum yield of approximately 0.45 [7, 8], its high isoelectric point (IEP) of about 9.5 enabling the adsorption of low IEP materials like proteins or glucose [9], and especially its photo-oxidation property [10, 11], ZnO nanostructures have been successfully implemented in fluorescent glucose probes [12–16]. Kim et al., Sodzel et al., and Sarangi et al. are pioneers who developed glucose sensors, which can measure glucose concentration through the PL quenching of ZnO nanocrystals [12–14]. They observed a linear PL quenching with increasing glucose concentration. However, the sensitivities of 0.7 % mM⁻¹, 0.5% mM⁻¹, and of 1.4% mM⁻¹, respectively, are rather low. The LOD of these sensors are 0.33 mM, 10 mM and 0.5 mM, respectively. Besides ZnO, other nanomaterials were also successfully implemented in fluorescent glucose sensor such as graphene quantum dots in nanofibrous membrane [17], platinum – porphyrin doped polymeric hybrid NPs [18], and lanthanide ions doped in vanadium oxide NPs [19]. The sensitivities of these sensors are 4.4% mM⁻¹, 1.9% mM⁻¹, and 0.7 % mM⁻¹, respectively. In view of their low sensitivity, those sensors are not suitable for glucose sensing in saliva, tear, or sweat. We recently developed and investigated non-enzymatic glucose probes based on ZnO nanorods (NRs) and ZnO nanotubes (NTs) [15, 16]. Our sensors exhibited an improved sensitivity of 2.3% mM⁻¹ for the NR based probe and of 3.5% mM⁻¹ for the NT based probe. Lower LODs of 1.0 mM and of 0.07 mM were obtained for the NR and NT based probes, respectively. Furthermore, a high selectivity toward other interfering species and a good reproducibility were also obtained with these probes. Despite this progress, further enhancement in sensitivity is still a demand for future human health care.

In order to improve the sensitivity of ZnO nanostructure based materials for glucose detection, one of the ideas is to use metal nanoparticle (NP) decorated ZnO nanostructures [20–22]. For example, Lin et al. stated that by Au NPs decoration of the surface of ZnO nanowires, implemented in their electrochemical sensor, the response current doubles compared to that of a sensor without Au NPs when measuring the same glucose concentrations [20]. Similarly, K. Tian et al. showed that the combination of ZnO and Au NPs enables to electrochemically detect glucose concentrations in a wide range of 50 mg dL⁻¹ to 400 mg dL⁻¹ with a high sensitivity of 3371.9 $\mu\text{A cm}^{-2} \text{mM}^{-1}$ [21]. The increased sensitivity is related to the effect of the metal NPs on the optical properties of the ZnO nanostructures. In optical sensors, metal NPs were also successfully implemented in surface enhanced Raman scattering (SERS) sensors based ZnO nanostructures for sensitivity enhancement [23, 24]. The sensitivity enhancement was attributed to resonant coupling of spontaneous emissions in ZnO with the surface plasmon resonance (SPR) of metal NPs. The SPR also leads to a significant PL enhancement in the UV. It was shown that the PL intensity of ZnO NRs at 382 nm can be increased up to 25 times by decoration with Cr NPs [25]. Similarly, a significant PL enhancement was observed followed decoration with Pt, Au and Ag NPs [26–29].

Despite of the extensive investigation of SPR properties of metal NPs in SERS based sensors, there have been very few studies dealing with metal nanoparticle decorated ZnO nanostructures for fluorescent sensing. To the best of our knowledge, there is, despite its highly promising applicability, no publication about fluorescent glucose biosensors based on metal NP decorated ZnO nanostructures. Similar to electrochemical sensors, metal NP decoration could significantly improve the sensitivity.

In this work, we present a remarkably high sensitivity fluorescent non-enzymatic glucose probe based on ZnO NRs decorated with Au NPs: ZnO NRs/Au NPs. Its demonstrated sensitivity is high enough for glucose sensing in physiological fluids such as tears, sweat, and saliva. The probe exhibits high selectivity when it is treated with interference species that typically appear in blood such as ascorbic acid (AA), uric acid (UA), bovine serum albumin (BSA), L-cystine, fructose, maltose, and sucrose. The probe's response time was also measured, and it was also treated with human blood serum to verify its accuracy.

Experimental

Materials and reagents

Chemical reagents zinc (II) nitrate hydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), hexamethylenetetramine ($\text{C}_6\text{H}_{12}\text{N}_4$), D-glucose, hydrogen peroxide (H_2O_2), potassium iodide (KI), acetic acid (CH_3COOH), ascorbic acid (AA), uric acid (UA), bovine serum albumin (BSA), L-cystine, fructose, maltose, and sucrose were purchased from Aldrich-Sigma without further purification. Copper (Cu) substrate with 99% purity was purchased at a local market.

Synthesis of ZnO NRs and ZnO NRs decorated Au NPs

Synthesis of ZnO NRs is described in detail elsewhere [15]. In short, the ZnO NRs are grown on Cu substrates, cleaned in ultrasonic bath in acetone, ethanol, and deionized (DI) water, and immersed in an equivalent saturated nutrition solution of 100 mM $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and of 100 mM $\text{C}_6\text{H}_{12}\text{N}_4$ at 90 °C for 3 hours. The as-prepared ZnO NRs were thereafter covered with Au, sputtered for 30 s with a 30 mA current, corresponding to a calculated layer thickness of 10 nm.

Sample treatment with glucose, H_2O_2 , interfering species, and human blood sera

Sample treatment procedure with glucose, H_2O_2 , interfering species, and human blood sera is described elsewhere [16]. In short, different concentrations of glucose (0.01 mM - 12 mM) and H_2O_2 (0.05 mM - 6 mM) were prepared by dissolving in phosphate buffer saline (PBS) solution, and in DI water, respectively. Other interfering solutions such as 0.1 mM AA, 0.1 mM UA, 0.6 mM BSA, 0.1 mM L-cystine, 1 mM fructose, 1 mM maltose, and 1 mM sucrose were prepared. The concentrations of AA, UA, BSA and L-cystine were chosen based on their normal present concentrations in human blood serum. Human blood sera, provided by our local hospital, had glucose concentrations of 4.35 and 6.29 mM. Lower glucose concentrations ranging from 0.1 mM to 2 mM of human serum were obtained by diluting the original human blood serum in PBS solution.

For all the measurements, 15 μL solution was dropped-cast directly onto the $3 \times 3 \text{ mm}^2$ area of ZnO NR sample. The immobilization, storage, and PL measurement time were the same for all samples. The PL measurements were performed at room temperature. Each measurement was repeated for three times; the average values are taken into account and error bars represent the standard deviation of the mean.

Characterization of the sample

Prior to glucose immobilization, the sample morphology was studied by scanning electron microscopy (SEM, Nova NanoSEM 450) and energy dispersive X-ray spectroscopy (EDS, Ametek EDAX). The crystallinity was investigated by a X-ray diffractometer (Siemens D5005 Brucker). PL spectra were measured by a spectrometer (Princeton, SP -2500i, resolution of 0.1 nm) upon excitation of the samples with a 325 nm He-Cd laser (Kimmon Koha). A longpass filter with cut-off wavelength of 370 nm (SCF-50S-37L, SigmaKoki) was placed in front of the spectrometer to filter out the majority of the excitation wavelength from the laser and the possible UV autofluorescence of the samples.

Results and Discussion

Surface morphologies and optical properties of ZnO NRs/Au NPs

Figure 1 shows the side view and the top view of the ZnO NRs/Au NPs samples, as studied by SEM. The ZnO NRs reveal hexagonal structures with diameters ranging from 150 nm to 250 nm. The zoom in Fig. 1b, clearly shows the NPs decorating the ZnO nanostructures. The Au NPs were well dispersed on the nanostructure of the

ZnO NRs. The Au NPs diameters range from 5 nm to 10 nm. Furthermore, the EDS elemental mapping of the ZnO NRs/Au NPs in Fig 1(c) further demonstrates the uniform distribution of Au over the entire ZnO rod. The EDS spectrum of ZnO NRs/ Au NPs provides a Au weight percentage of approximately 2% (see Fig. S1 in the supporting information).

The XRD patterns of the ZnO NRs are available as supporting information (Fig. S2). The diffraction peaks of ZnO NRs match well with JCPDS card No. 36-1451 of wurtzite ZnO. After decorating Au NPs, a weak intensity peak at 38.27° appears which can be assigned to the diffraction peak of face center cubic (fcc) Au, according to JCPDS Card No. 04-9748. Other peaks corresponding to other planes of fcc Au such as (200) and (220) are not observed.

Figure 2 shows the PL spectra of the ZnO NRs and ZnO NRs/Au NPs samples. The PL spectrum of ZnO NRs demonstrates two typical emission bands in which the UV emission band (382 nm) is due to the free exciton recombination and the broad green emission band (575 nm) is due to the intrinsic defects like interstitial zinc and oxygen vacancies [30]. The PL spectrum of ZnO NRs/Au NPs exhibits a remarkable intensity enhancement of the UV emission band by a factor of 4.5. This can be attributed to the SPR (schematically illustrated in the inset). Since the green emission energy of ZnO NRs is close to the surface plasmon absorbance energy of Au NPs, thus, the green emission energy can be absorbed by the Au NPs. Once the surface plasmon of Au NPs are excited, electrons of the Au NPs are pumped into higher energy states. These electrons then can easily transfer from Au NPs to the conduction band of ZnO NRs, which leads to the accumulation of electrons in the conduction band. The electrons can recombine with holes in the valence band of ZnO, thus, leading to significant enhancement of the UV emission and suppressions of the green emission [31].

Fig. 1 (a) Side view and top view and (b) the zoom in SEM images of the ZnO NRs/Au NPs; (c) SEM image (left) and corresponding EDS mappings for Zn (middle) and Au (right) of a single ZnO NR decorated with Au NPs.

Fig. 2 Photoluminescent spectrum of ZnO NRs and ZnO NRs/Au NPs. The inset explains how the surface plasmon resonance of the Au NPs contributes to the PL enhancement in the UV range, concomitant with the suppression of the emission in the visible range of the as-grown ZnO NRs.

Glucose and H_2O_2 sensing of the ZnO NRs/Au NPs based fluorescent sensor

The PL responses of ZnO NRs/Au NPs with respect to different glucose concentrations in the range of 0.01 mM – 12 mM (corresponding to 0.2 – 216 mg/dL) are shown in Fig. 3a. Since the broad green emission is suppressed, we only focus on the PL change in the UV emission band at 382 nm. All the PL intensity peaks are normalized to the peak intensity of the as-grown ZnO NRs to eliminate intensity variation among different samples. As seen in the figure, the peak intensity decreases strongly with the increase of the glucose concentrations without any shift in the peak position. The calibration plot of the probe reveals a linear quenching in the range of 0.01 – 2 mM (Fig. 3b). From the slope of the calibration plot we can derive the sensitivity, which is defined as the percentage change per mM. The probe exhibits a remarkable high sensitivity of $(22 \pm 2) \% \text{ mM}^{-1}$ with a correlation coefficient of 0.99 in the range of 0.01 – 2 mM. The error bar was added in the figure with the error less than 2%. The LOD of the probe, which is calculated as $\text{LOD} = 3\sigma/S$ with σ is the standard deviation of blank samples and S is the slope of the calibration plot, respectively [32], is 0.01 mM. Above 2 mM, the PL intensity decreases only slightly with further increase of the glucose concentration, what can be interpreted as saturation.

Fig. 3 (a) Normalized PL spectra of the ZnO NRs/Au NPs at different glucose concentrations varying from 0.01 mM to 12 mM. Spectra for some concentrations are not shown on the figure for a clearer view; (b) Calibration plot of the sensor demonstrating the change in PL peak intensity at 382 nm as a function of glucose concentration.

For glucose concentrations below 2 mM, the sensor exhibits a sensitivity is $(22 \pm 2) \% \text{ mM}^{-1}$.

The PL quenching mechanism of ZnO nanostructures is explained by the oxidation of glucose into gluconic acid and H_2O_2 due to the photo-oxidation properties of ZnO under the illumination of UV light [14–16, 33]. ZnO nanostructures acts as photocatalyst, while H_2O_2 functions as a quencher since it decomposes easily into H_2O and O_2 after acceptance of an electron from the conduction band of ZnO. This eliminates radiative

recombination of electrons and holes, thus quenching the PL intensity. To prove the presence of peroxide as a product of glucose's photo-oxidation a mixture of 1 ml glucose 1mM with 1 ml CH₃COOH and 0.1 g KI was prepared. A ZnO NRs/Au NPs sample was immersed in this solution. The dashed circle in Fig. 4a. indicates the area where the ZnO NRs are grown on the Cu substrate. Before UV laser illumination, the as-prepared solution is transparent without any peak in absorbance spectrum. After illumination, the solution turns brown-yellow (Fig. 4b) and the absorbance spectrum of the solution presents two distinct peaks at 286 nm and 354 nm. The brown yellow color of the mixed solution and the two peaks in the absorbance spectrum can be explained by the presence of I⁻ and IO₃⁻ in the solution. This is evidence that H₂O₂ was generated as a result of photo-oxydation of glucose under UV illumination. The presence of peroxide turns the mixture brown-yellow due to iodine liberation from KI. The liberated I₂ interacts with H₂O to generate I⁻ + IO₃⁻, which causes the two absorbance peaks at 286 nm and 354 nm [34–36]. The chemical reactions are written as follows:

Fig. 4 (a) Image of ZnO NRs/Au NPs immersed in a mixed solution of KI, CH₃COOH and 1 mM glucose, the dashed circle indicates the position of the ZnO NRs; (b) Absorbance spectra of the mixture before and after UV illumination. The insets demonstrate the images of the mixed solution before and after UV illumination.

To further confirm the PL quenching is caused by the generation of H₂O₂, the material's behavior in an H₂O₂ environment was also investigated for H₂O₂ concentrations in the range of 0.05 mM to 6 mM. The PL behavior at 382 nm shown in Fig. 5 demonstrates a quenching in the range of 0.05 – 2 mM with an even higher sensitivity of (29 ± 2) % mM⁻¹. The similar PL quenching behavior following exposure of the ZnO NRs/Au NPs to different concentrations of glucose and H₂O₂ suggests that the quenching mechanism of glucose sensing is indeed caused by the generation of H₂O₂. Above 2 mM, a slow decrease in PL intensity is again observed. This confirms the saturation state of the sensor.

The higher sensitivity when the sensor was treated with H₂O₂ can be explained as follows. In the case of H₂O₂ sensing, the PL quenching depends only on the interaction of H₂O₂ with ZnO NRs/Au NPs and the sensitivity is directly related to the H₂O₂ concentrations. In the case of glucose sensing, only the part of glucose that is photo-oxidized into gluconic acid and H₂O₂ will actively interact with ZnO NRs/Au NPs and thus contributes to the PL quenching. In addition, a high IEP material, such as ZnO NRs, can strongly attach molecules of lower IEP, such as glucose or gluconic acid, the end-product of the photo-oxidation process. During the photo-oxidation process, these materials could partly cover the active areas of ZnO NRs/Au NPs, preventing direct contact with H₂O₂ and eliminating the PL quenching effect.

Fig. 5 (a) Normalized photoluminescence (PL) spectra of the ZnO NRs/Au NPs at different H_2O_2 concentrations varying from 0.05 mM – 6 mM. Some spectra were erased from the figure for a clearer view; (b) Calibration plot of the probe demonstrating the change in PL peak intensity at 382 nm as a function of glucose concentration varying from 0.1 mM to 6 mM. For H_2O_2 concentrations below 2 mM, the probe exhibits a sensitivity of $(29 \pm 2)\% \text{ mM}^{-1}$.

In comparison with previous works on fluorescent H_2O_2 /glucose probes based ZnO nanomaterials [12–16], the probe based on ZnO nanorods decorated Au NPs exhibits a significantly higher sensitivity of $29\% \text{ mM}^{-1}$ for H_2O_2 sensing and of $22\% \text{ mM}^{-1}$ for glucose sensing in the concentration range below 2 mM, with a lower LOD of 0.01 mM for glucose sensing (see **Table 1**). The high sensitivity in the range below 2 mM demonstrates that the probe enables to monitor glucose in sweat, tears, and saliva. This can solve the current drawback of low sensitivity of reported fluorescent glucose sensors based on ZnO nanostructures. Furthermore, the probe is non-enzymatic, its behavior solely depends on the intrinsic properties of the as-synthesized ZnO NRs/Au NPs. This can enhance the stability of the probe compared with that of the enzymatic glucose probe. Additionally, the cost-effectiveness of the synthesizing hydrothermal method for ZnO NRs and of the Cu substrate, are other advantages allows for the production of a single use probe. Single use significantly reduces the risk of infection.

The sensitivity enhancement is attributed to the enhancement of photocatalytic efficiency of ZnO NRs/Au NPs due to SPR. The possible reason for the improved photocatalytic activity can be explained as follows. Under UV illumination, electron – hole pairs are generated in ZnO. The photo-electrons in the conduction band react with O_2 to produce a superoxide radical anion $\text{O}_2^{\cdot-}$, while the photogenerated holes in the valence band react with surface hydroxyl ion OH^- or H_2O molecules to produce a $\text{OH}^{\cdot}$ radical. These $\text{O}_2^{\cdot-}$ and $\text{OH}^{\cdot}$ radicals are active oxidants, which can oxidize adsorbed molecules such as rhodamine 6G [37, 38], rhodamine B [26, 39], methylene blue [40], benzene [41]. In studying the photocatalytic oxidation of glucose in water, Cheng et al. identified the presence of $\text{OH}^{\cdot}$ and $\text{O}_2^{\cdot-}$ active oxidants during the photo-oxidation of glucose by ZnO powder under UV illumination. The ESR signals of these active oxidants proved that they are the main sources of the glucose photooxidation process [42]. It was found that the nanocomposites of ZnO and metal nanoparticles such as Ag, Au, Pt exhibit better photocatalytic degradation of rhodamine B, rhodamine 6G, and methylene blue than the individual ZnO nanostructures [31, 43]. In Fig. 6a we propose a plausible mechanism of photocatalytic efficiency enhancement of the ZnO/Au NPs. If ZnO NRs without Au NPs are excited by UV light, only a few electrons reach the conduction band and holes in the valence band can take part in the oxidation process to generate active oxidants $\text{O}_2^{\cdot-}$ and $\text{OH}^{\cdot}$. These active oxidants are the main sources to oxidize glucose into gluconic acid and H_2O_2 . In the ZnO NRs/Au NPs system, the collective photoexcitation of the SPR in the Au NPs enhances the concentration of hot electrons that can be injected in the ZnO conduction band. This produces superoxide radical anions $\text{O}_2^{\cdot-}$ at the surface, which are involved in the photooxidation of glucose. The enhanced electron concentration increases the photocatalytic efficiency and thus results in a higher sensitivity.

Fig. 6 (a) The plausible schematic diagram of the glucose photo-oxidation on (a) ZnO NRs and (b) Zn NRs/Au NPs. The excitation of the SPR in Au NPs is followed by hot electron injection in the conduction band of the ZnO NRs, which enhances the photo-oxidation efficiency; (b) Comparison of the HOMO and the LUMO of glucose, fructose, lactose, maltose, and sucrose with the top of the valence band (VB) and the bottom of the conduction band (CB) of ZnO [44, 45].

Table 1. Comparison with ZnO nanostructure based fluorescent H₂O₂ and glucose sensors in literature

Nanostructures used	Sensor type	Synthesizing method	H ₂ O ₂ Sensing range (mM)	H ₂ O ₂ Sensitivity (%·mM ⁻¹)	Glucose Sensing range (mM)	Glucose Sensitivity (%·mM ⁻¹)	Glucose LOD (mM)	Response time (s)	Ref.
ZnO NPs	Enzymatic	Commercial ZnO nanoparticles immobilized GO _x	0.05 - 100	2.5	10 - 300	0.5	10	N/A	[13]
ZnO NPs	Enzymatic	Synthesized ZnO nanoparticles immobilized mercaptoundecanoic acid (MUA) and GO _x	0 - 14	N/A	1.6 - 33.3	0.7	0.33	5	[12]
ZnO NRs grown on ITO substrate	Non-enzymatic	Synthesized ZnO NRs by hydrothermal method	1 - 30	1.4	0.5 - 40	1.4	0.5	N/A	[14]
ZnO NR grown on Cu substrate	Non-enzymatic	One step, seedless hydrothermal method	N/A	N/A	1 - 100	2.3	1	N/A	[15]
ZnO NT grown on PCB substrate	Non-enzymatic	One step, seedless hydrothermal method	N/A	N/A	0.1 - 15	3.5	0.07	N/A	[16]
ZnO NRs / Au NPs grown on Cu substrate	Non-enzymatic	One step, seedless hydrothermal method	0.05-2	29	0.01 - 2	22	0.01	5	This work

Selectivity and response time of the sensor

Selectivity is a vital characteristic of a sensor. In order to evaluate the sensor's performance in the presence of common interfering species, solutions were prepared with different interfering species with their typical concentrations in the human serum, namely 0.1 mM AA, 0.1 mM UA, 0.6 mM BSA, 0.1 mM L-cystine, and different sugars such as sucrose, fructose, lactose, and maltose [46]. The influences of these interfering species toward the PL emission at 382 nm were taken into account. As shown in Figure S3 in the supporting information, a small tolerance of less than 3% was observed in the case of AA, UA measurements. These minor changes can be ascribed to the repulsion of negatively charged ZnO (the isoelectric point both are about 9.5) and the negatively charged AA and UA. This observation is in agreement with our previous work on ZnO NTs based fluorescent glucose sensor [16], and with Zhou et al.'s work on electrochemical glucose sensor [47]. They found that the presence UA and AA resulted in a minor change in current response of their electrochemical glucose sensor based

on hierarchical nanocomposites of CuO and ZnO. Furthermore, BSA, an autofluorescent molecule, which normally shows a noticeable PL emission in the range of below 360 nm [48], contributed to a small PL change of less than 3% at 382 nm. This is consistent with previous works on ZnO NRs and NTs based fluorescent glucose sensors [14, 16]. We therefore conclude that the influence of AA, UA and BSA on the sensing behavior is minor.

The selectivity of the probe towards other sugars such as sucrose, fructose, lactose, and maltose was also investigated. Similar to our previous work on ZnO NTs based fluorescent glucose detection, we also observed that the presence of fructose, maltose, and sucrose led to a small tolerance of less than 3% at 382 nm PL emission of ZnO NRs/Au NPs based probe. We indeed currently are not sure about all details of the mechanism (the photo-oxidation glucose is complicated and involves several reaction steps), we propose a mechanism which is consistent with the observed selectivity as the energy levels of different sugars are different. Figure 6b presents the energy band diagram of ZnO, glucose, fructose, lactose, maltose, and sucrose in water [44, 45]. The energy band gap of ZnO is of 3.4 eV. According to ab initio studies, the energy band gap of glucose in water is in the range of 3.3 eV to 4.9 eV [44, 49, 50]. The exact value depends on the number of binding units between glucose and water molecules, or between glucose and enzyme glucose oxidase. As seen in the figure, among the sugars, glucose dissolved in water has the smallest energy band gap, and its HOMO and LUMO band levels best aligned with those of ZnO. Therefore, upon UV excitation, electrons from the CB of ZnO NRs/Au NPs can transfer to glucose's LUMO band, while holes on the VB can transfer to the HOMO band of the glucose. These electrons and holes can take part in the oxidation process to generate active oxidants $O_2^{\cdot -}$ and $OH^{\cdot}$ which are the main sources to oxidize glucose. Due to the difference in energy band gap, and in HOMO, LUMO energy levels, the transfer of electrons to other sugars is less preferred, which is consistent with a lower sensitivity of the probe toward other sugars.

Fig. 7 PL peak intensity of ZnO NRs/Au NPs treated with 1 mM glucose with respect to time. The plot shows the response time of the sensor.

The response time, which is defined by the amount of time it takes to response to a change in glucose concentration, is also an important characteristic of the sensor. The PL intensity as a function of time, measured every 5 s for a fixed glucose concentration of 1 mM, is plotted in Fig. 7. The fraction of change within the first 5 s is defined as $\frac{I_0 - I(t=5s)}{I_0 - I_{final}}$ in which I_0 and $I(t=5s)$ are the intensity at 0 s and 5 s, respectively and I_{final} after extended exposure to the glucose mixture. The fraction of change within the first 5 s amounts 76 %, which indicates the non-enzymatic sensor has a very fast response to glucose molecules. The glucose response time is comparable to other fluorescent and electrochemical glucose sensors based on ZnO nanostructures [12, 47].

Glucose sensing in real human blood sera

The potential application of ZnO NRs/Au NPs was confirmed by measuring real blood samples. Two human blood sera with different glucose concentrations of 4.35 mM and 6.29 mM were supplied by a local hospital. Lower glucose concentration from 0.01 mM to 2 mM were obtained by diluting the human blood sera with proper amounts of PBS solution. The concentration of glucose and other substances in the human blood sera were pre-measured by clinical chemistry analyzer AU 5800 (Beckman Coulter) and available as supplementary information

(Table S1). The autofluorescence of the biological substances appearing in human blood serum (see Fig. S4 in the supplementary information) is negligible in comparison with the high PL emission from ZnO NRs/Au NPs (Fig. 2). The PL response of our probe treated with human blood sera is shown in Fig. 8a. For higher glucose concentrations a PL quenching was observed. Based on the calibration plot (Fig. 3), the glucose concentration values of the human blood sera could be determined. The correlation of glucose concentrations provided by the local hospital and by our sensor is shown in Fig. 8b. In fact, slightly higher glucose concentrations were obtained from our fluorescent glucose detector. Small tolerances of less than 5% were obtained with the concentration of above 4 mM. Note that, with this concentration range the probe was treated with human blood sera without dilution. The tolerances can be attributed to the possible influence of different molecules such as cells, proteins fragments, ions....[47]. Additionally, interfering species such as AA, UA, BSA, L-cysteine, maltose, sucrose, and fructose... can contribute a small amount on the tolerance of the PL intensity. Interestingly, our sensor demonstrates nearly identical concentrations with clinical values at concentrations below 2 mM. As mentioned before, concentrations below 2 mM were obtained by diluting human blood serum with PBS solution. The dilution process reduces the concentrations of the interfering species in the human blood serum and thus their influence on the quenching behavior. This again confirms the high accuracy of the probe. We believe that the good responsibility and high accuracy of our sensor to real human blood serum demonstrates that our probe is a useful for glucose concentration determination.

Fig. 8 (a) PL spectra of ZnO NRs/Au NPs treated with human blood serums; (b) Correlation of the glucose concentrations as measured by PL quenching of the ZnO NRs/Au NPs based sensor and by the standard clinical method.

Note that, the glucose concentrations of diabetes patient in sweat are of 0.2 mM – 0.6 mM, tears of > 0.92 mM, saliva of 0.55 mM– 1.77 mM, urine of > 5.55 mM, ocular fluid of 0.5 mM – 5 mM, interstitial fluid of 1.99 mM – 22.2 mM, and in blood of > 5.6 mM [2]. The remarkable sensitivity of our sensor in the range below 2 mM, and the cost-effectiveness of the synthesizing method and of the Cu substrate demonstrates a promising potential as an effective noninvasive, single used probe based device for glucose sensing in sweat, tears, and saliva.

Besides several outstanding properties, the probe also has some drawbacks. Its function is based on the detection of PL emission at 382 nm in the UV range, a wavelength range that is more challenging for practical applications than the VIS range with strict requirements for excitation light sources, detectors and other optical elements (lenses, long-pass filters...). Furthermore, in the practical applications, a cost-effective UV light source having a low excitation intensity might be implemented. Thus, the influence of autofluorescence from biomatter cannot be neglected. Since it might result in a strong background and lower the signal to noise ratio which is another issue that needs to be addressed in a sensor product.

Conclusion

In this study ZnO NRs/Au NPs were successfully synthesized and implemented for non-enzymatic fluorescent glucose sensing. It was shown that due to the adhesion of Au NPs, the sensitivity was significantly increased in compared with that of the sensor based ZnO NRs. The sensor exhibits a working range of 0.01M – 12 mM with a remarkable sensitivity of $(22 \pm 2) \% \text{ mM}^{-1}$ for glucose sensing, and of $(29 \pm 2) \% \text{ mM}^{-1}$ for H_2O_2 sensing in the range of less than 2 mM. This ultra-high sensitivity enables its application in noninvasive, highly sensitive glucose devices, which can monitor glucose levels in low glucose concentration fluids such as tears, sweat, and saliva. Additionally, good selectivity, high response time are other advantages of our sensor. The significant enhancement in sensitivity is due to SPR effect, while the good selectivity toward other interfering species is probably based on energy level matching of ZnO NRs/Au NPs and glucose. Most importantly, when treated with human blood serum, the probe exhibits a high accuracy, which is compatible with that of clinical devices. This ensures the potential application of ZnO NRs/Au NPs for a single use, high sensitivity, high selectivity, non-invasive glucose sensing in clinical measurements.

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