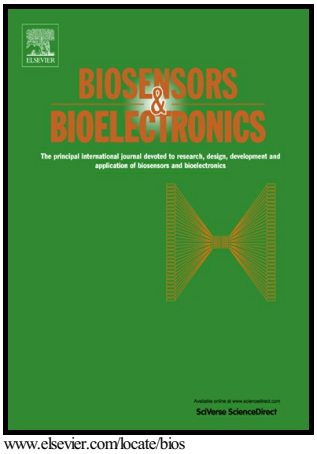

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PII: S0956-5663(16)31293-3
DOI: http://dx.doi.org/10.1016/j.bios.2016.12.044
Reference: BIOS9437

To appear in: *Biosensors and Bioelectronic*

Received date: 25 October 2016
Revised date: 15 December 2016
Accepted date: 17 December 2016

Cite this article as: Longyi Chen, Wai Hei Tse, Yi Chen, Matthew W. McDonald, James Melling and Jin Zhang, Nanostructured Biosensor fo Detecting Glucose in Tear by Applying Fluorescence Resonance Energy Transfe Quenching Mechanism, *Biosensors and Bioelectronic* http://dx.doi.org/10.1016/j.bios.2016.12.044

Nanostructured Biosensor for Detecting Glucose in Tear by Applying Fluorescence Resonance Energy Transfer Quenching Mechanism

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ABSTRACT

In this paper, a nanostructured biosensor is developed to detect glucose in tear by using fluorescence resonance energy transfer (FRET) quenching mechanism. The designed FRET pair， including the donor, CdSe/ZnS quantum dots (QDs), and the acceptor, dextran-binding malachite green (MG-dextran), was conjugated to concanavalin A (Con A), an enzyme with specific affinity to glucose. In the presence of glucose, the quenched emission of QDs through the FRET mechanism is restored by displacing the dextran from Con A. To have a dual-modulation sensor for convenient and accurate detection, the nanostructured FRET sensors were assembled onto a patterned ZnO nanorod array deposited on the synthetic silicone hydrogel. Consequently, the concentration of glucose detected by the patterned sensor can be converted to fluorescence spectra with high signal-to-noise ratio and calibrated image pixel value. The photoluminescence intensity of the patterned FRET sensor increases linearly with increasing concentration of glucose from 0.03 mmol/L to 3 mmol/L, which covers the range of tear glucose levels for both diabetics and healthy subjects. Meanwhile, the calibrated values of pixel intensities of the fluorescence images captured by a handhold fluorescence microscope increases with increasing glucose. Four male Sprague-Dawley rats with different blood glucose concentrations were utilized to demonstrate the quick response of the patterned FRET sensor to 2 μL of tear samples.

Keywords: Fluorescence resonance energy transfer (FRET); quantum dots; silicone hydrogel; tear glucose; non-invasive biosensor

[1] L. Y. Chen and W.H. Tse contributed equally

1. Introduction

Tear fluid cleans and lubricates the eye while nourishing. Over 20 components have been found in tears, including salt water, proteins, glucose, and some small metallic ions, etc. (Ohashi et al., 2006). Diagnosis of biomolecules in tear fluid pertaining to ocular diseases such as ocular rosacea, have been performed primarily by clinicians to examine the high molecular-mass glycoproteins in tears (An et al., 2005). The detection of ocular glucose dates back to 1930 (Ridley et al., 1930). Following that, Michail and his collaborators first demonstrated the level of glucose in tears is often elevated in diabetic patients (Michail et al., 1937). Sen and Sarin studied over 200 cases, their statistic results indicated the blood glucose is about 2 times higher in diabetic patients than that in non-diabetics, whereas tear glucose levels are ~5 times higher in diabetics than that in the general population (Sen et al., 1980). In past decades, different research groups found a definite relationship between tear glucose and blood glucose, concluding hyperglycemia could be detected by measuring tear glucose levels (Lewis et al., 1958; Gasset et al., 1968; Motoji et al., 1971). Recent studies indicate tear glucose mean values were 0.35 ± 0.04 mmol/L and 0.16 ± 0.03 mmol/L, for patients with diabetes and healthy subjects, respectively (Lane et al., 2006). It is also noted time lag in measuring tear glucose is common to other glucose meters as it takes 5-15 minutes to allow the change of glucose in blood to eventually reflect in tear/interstitial fluids (Boyne et al., 2003); while it can be solved by a series of calibrations (Lodwig et al., 2003; Kvist et al., 2007).

However, it is very difficult to acquire enough tear samples in a short period. Furthermore, the concentration of glucose in tears is much lower than that in blood. Very few methods so far can measure such low concentration of glucose in a rapid fashion

(O'Donnell et al., 2001; March et al.; 2004). Compared to many other techniques, fluorescence resonance energy transfer (FRET), an inexpensive and very sensitive method, has been used in molecule imaging and glucose test (Tyagi et al. 2000; Forster et al. 1959; Moschou et al., 2004). It is a distance-dependent energy transfer from a fluorophore donor (D) to a fluorophore acceptor (A) in a nonradioactive process. When the distance of the two fluorophores is very close (< 10 nm), the excited D will transfer some of its energy to excite A that then emits light at a longer wavelength (Moschou et al., 2004). Using FRET technique for detecting the competitve reactions of glucose and other polysaccharide, such as dextran, to concanavalin A (Con A), a protein, may result in accurate and convenient measures (Ballerstadt et al., 2006; Russell et al. 1999; Pickup et al., 2005). However, fluorophores/organic dyes used in FRET pair are subject to low fluorescent intensity, and poor signal responses influenced by the decay of chromophores and external environment (Resch-Genger et al., 2008). Fluorescence nanostructures have been recognized as excellent materials used in FRET sensor due to their photo-stability and narrow emission peaks (Jamieson et al., 2007). We developed a nanostructured sensor by using fluorescence resonance energy transfer (FRET) technique which is able to be incorporated into hydrogel-based contact lenses for measuring low concentration of glucose directly (Zhang et al., 2011, 2013). While, most reported FRET sensors have an inversely proportional relationship between the ratio of the fluorescence intensity of the donor to that of the acceptor (I_D/I_A) and the amount of the targeted molecules because the rate constant of the energy transfer, k_{FRET}, decreases while the distance between the FRET pair increases. Consequently, most reported FRET sensors have significant

measurement errors when detecting the increase of concentration of the targeted molecules because of the low fluorescence signals-to-noise ratio .

Quite recently, fluorescent quenching-based assay in which the donor fluorescence can be quenched by the acceptor without analytes has shown significant advantages in biosensing, as it allows the restored fluorescence signal proportional response to the concentration of target analytes and lower background fluorescence (Tyagi et al., 2000; Chen et al., 2016). Consequently, a new nanostructured sensor by applying FRET quenching mechanism is developed here to detect small concentration of tear glucose from very small volume of tear samples in a fast and accurate fashion.

Figure 1a displays a FRET pair-labeled Concanavalin A (Con A), an enzyme with specific affinity to glucose (Ballerstadt et al., 2006; Russell et al. 1999; Pickup et al., 2005). The donor in this designed FRET transducer is made of quantum dots with an emission (λ_{em}) in the visible range; the acceptor is dextran-bound malachite green (MG) which has an absorption at the same wavelength of the emission of the quantum dots. The emitted fluorescence of the quantum dots can be quenched by malachite green through the FRET mechanism. In the presence of glucose, the binding between Con A and dextran is out competed by the affinity of Con A for glucose. As a result, dextran-bound MG will not quench the fluorescence of quantum dots, and therefore fluorescence intensity is restored. The change in fluorescence intensity is correlated to the amount of glucose reacting with Con A.

In addition, we would like to further develop a dual-method for detecting glucose through both of fluorescence spectra and fluorescence images. To quickly and accurately detect the amount of glucose in a very small volume of tear sample by measuring the

changes of fluorescence intensity of the sensor, the numerous nanostructured FRET sensors are immobilized on a single ZnO nanorod (NR) which have a high surface area to volume ratio as shown in Figure 1b. This design allows numerous FRET biosensors located at a nanoscale, and therefore, is able to amplify the resolution of the sensor (Chen et al., 2015). Moreover, to quickly and accurately detect the amount of glucose in a very small volume of tear sample by fluorescence images, the designed pattern made of ZnO NR array is utilized here which can be recognized easily through fluorescence image process. The pixel intensity values in a taken fluorescence image corresponding to the concentration of glucose can be calculated through an imaging process. Consequently, the patterned nanostructured FRET sensor on silicone hydrogel can realize the dual modulation for monitoring glucose level in tear samples through both of fluorescence spectrum and calibrated image pixel value.

2. Material and Methods

2.1 Fabrication patterned QDs decorated-ZnO NRs Array on silicone hydrogel

Cadmium selenium/zinc sulfide (CdSe/ZnS) core-shell quantum dots (QDs) were chosen as the donor of the FRET pair. The core-shell QDs were further functionalized with amine groups (Lee et al., 2010). Briefly, CdSe/ZnS (20 mg) dissolved in 3 mL of chloroform was mixed with a solution of cysteamine (Cys) hydrochloride (100 mg) dissolved in 5 mL of water. The solution was sonicated until the chloroform layer became clear. The remaining cysteamine was reacted with the addition in excess of 2-mercaptoethanol (20 mmol/L). CdSe/ZnS/Cys QDs were purified by threefold centrifugation (15 minutes at 10000 rpm) and rinsed with ethanol. Purified particles were re-dispersed in water.

Silicone hydrogel with 150 μm in thickness was produced using a photochemical process (Kim et al., 2008). The patterned ZnO nanorod (NR) array was fabricated on the silicone hydrogel by a lift-off process (see the supplementary materials, Figure S1). The surface modification of the patterned ZnO NR array with amino groups is described as follows; the ZnO NR array on silicone hydrogel was immersed into 2 mL DMSO and 270 μL 3-aminopropyltriethoxysilane (APS) suspension. After having reacted at 120 °C for 2 h, the ZnO hydrogel was taken out and washed with ethanol to remove unreacted compounds (Kim et al., 2005; Costenaro et al., 2011). Please note that a small corner of the ZnO NR array on silicone hydrogel was not modified by 3-APS for further conjugation of FRET pair which will be used as a control area for imaging analysis.

Prior to coating QDs-based FRET sensor onto the surface of lithographically patterned ZnO NRs, glutaraldehyde first reacted with amino group modified ZnO NRs. Following that, ZnO NRs array on silicone hydrogel merged in a 20 mL CdSe solution for 3 days at 4°C, then wash with DI water to remove excess glutaraldehyde and CdSe/ZnS QDs. The obtained film was dried in the dark under vacuum to preserve sensing capabilities.

2.2 Synthesis of Malachite Green Dextran

Malachite green (MG) isothiocyanate and 70,000 MW amino-dextran purchased from Life Technologies (Burlington, Ontario, Canada) were mixed in a sodium bicarbonate buffer with 0.05 M at pH 9.6 (McCartney et al., 2001). Successful conjugation of the isothiocyanate and dextran to form MG-dextran was verified by thin layer chromatography as shown in Figure S2. Concentration of MG-dextran ($\varepsilon=10^5$ M^{-1} cm^{-1} for malachite green at 621 nm) was determined by UV/Vis (the supplementary materials).

2.3 Synthesis and Optimization of FRET Sensor

The detailed process of assembling FRET sensor on silicone hydrogels is shown in the S3 section of supplementary materials. Briefly, the dropwise application of Con A bonded with MG-dextran to the surface of QDs-decorated ZnO NRs with the aid of glutaraldehyde. The weight ratio of CdSe/ZnS/Cys QDs to Con A bonded with MG-dextran was 7:1. Non-conjugated Con A was rinsed off with DI water.

2.4 Sensor Measurement

10 µL of glucose in different concentrations at pH=7.0, 0.03 mmol/L, 0.05 mmol/L, 0.2 mmol/L, 0.75 mmol/L, 1 mmol/L, 2 mmol/L, 3 mmol/L, were dropped on the patterned FRET sensors on silicone hydrogels with 30 seconds of interacting time. The fluorescence response of the patterned FRET sensors on silicone hydrogels to different concentrations of aqueous glucose were recorded by the fluorospectrometer at excitation wavelength (λ_{em}) = 490 nm. At least five independent measures were conducted to measure the response of the sensor according to each glucose level. The standard deviation for each measured points was also calculated.

2.5 Fluorescence Sensor Signal Converting to Image Pixel Intensity

A USB powered portable digital microscope, MiScope, from Zarbeco (40X, pixels 640 × 480), was used to measure and digitally capture the fluorescence intensity of the 0.5 mg of glucose sensor conjugated onto ZnO NRs. 10 µL of various concentrations of glucose was added; 0.03, 0.05, 0.2, 0.75, 1, 2, and 3 mmol/L. Glucose was allowed to react with the sensor for 5 minutes prior to fluorescent measurement. The donor molecule was excited with the UV light built into the MiScope.

The captured images were converted to RBG colours using Matlab as our previous reported results (Zhang et al., 2013). Both green and red channels were applied to record

the pixel intensity pixel values. The values of pixel intensity of the patterned sensors responding to glucose amounts were calculated in comparison with that of the control areas where no FRET pair is conjugated. The recorded fluorescence images taken by the handhold fluorescence microscope can be converted to the value of pixel intensity through Matlab's imaging process. In this imaging conversion process, the image matrix of the control area were used to compare with that of sensing area of the FRET sensor to obtain the value of pixels intensity corresponding to the concentration of glucose.

The captured FRET sensing image with pixels $\sum XY$ is calibrated in comparison with the image of the control area (lowest fluorescence signal from substrate) with pixels $\sum X'Y'$. The calibrated pixel intensity (I_p) generated from FRET sensors can be expressed as follow;

$$I_p = \sum I(X_i Y_i) - \sum I\ (X_i' Y_i'), \quad (1)$$

Where, i is the number of pixels in the chosen areas.

2.6 Animal tear test

Following the positive results of cytotoxicity (see S4), we conducted the animal test to evaluate the response of designed sensor to tear glucose. Four male Sprague-Dawley rats (Charles River Laboratories, St. Constant, QC, Canada) were housed in a 12-hour light/dark cycle room with humidity (50%) and temperature (21.5 °C) kept constant. Rats were given water and chow *ad libitum* and made diabetic with streptozotocin (STZ; Sigma-Aldrich, Oakville, ON, Canada). Intraperitoneal injections of STZ (20mg/kg) dissolved in a citrate buffer (0.1M, pH 4.5) were given over five consecutive days. Following the confirmation of diabetes (two blood glucose readings greater than 18 mmol/L) subcutaneous insulin pellets were implanted in the abdominal region of rats.

Rats were anesthetized with isoflurane for ease of application of the sensors to their eyes. Tear fluid was collected from the ocular surface with a 1 µL glass capillary tube (P1424 SIGMA). 2 µL of rat tear sample were collected from each male Sprague-Dawley rats. All samples were diluted by PBS with pH= 7.0 to 7 µL. Diluted tear samples （2 µL） were then dropped on the FRET sensors (with five independent measures). The standard deviation for each measured points was also calculated. Fluorospectrometry was used to measure the fluorescence intensity of quantum dots (donor) after tear samples interact with FRET sensor for 30 second (s). A blood sample was taken from the saphenous vein concurrently with the application of the sensor for blood glucose concentration (Freestyle Lite Blood Glucose Monitoring System, Abbott Diabetes Care Inc., Mississauga, Ontario).

Ethics approval was obtained through the University of Western Ontario Research Ethics Board, in accordance with Canadian Council on Animal Care guidelines.

3. Results and Discussion

3.1 Characterization of nanostructured FRET sensor deposited on silicone hydrogel

The ZnO NR array pattern was fabricated on the silicone hydrogel using it as a substrate by a lift-off process shown in the Supplementary materials (Figure S1). SEM was used to study the patterned ZnO NR array deposited on the synthetic silicone hydrogel as shown in Figure 2a and Figure 2b. The designed pattern is made for easy identification of the captured fluorescence images to calibrate the pixel intensity depending on the concentration of glucose.

The patterned ZnO NR arrays grown on the synthetic silicone hydrogel were modified with amino groups ($-NH_2$) to immobilize CdSe/ZnS QDs onto the ZnO NRs.

Glutaraldehyde first reacted with $–NH_2$ functionalized ZnO NRs following the linkage formed by the reaction of glutaraldehyde with the amino group modified QDs. In Figure 2c, the hexagonal rods with a smooth surface were grown on the silicone hydrogel. The average dimensions of the ZnO NRs are estimated at 120 ± 5 nm in diameter and 2.00 ± 0.05 μm in length. The rough surface of the NRs can be observed when decorated with QDs, as shown in Figure 2d. Furthermore, the TEM micrograph (Figure 2e) clearly shows the QDs are decorated on the ZnO NRs. The HRTEM micrograph (Figure 2f) further indicates the core-shell CdSe/ZnS QDs with 5 ± 2 nm in diameter are decorated on the highly crystalline ZnO NRs with a lattice fringe of 0.255 nm, which corresponds to the (0002) lattice planes (Chen, et al., 2011). Figure 3 shows the FTIR spectra of the ZnO NRs with surface modification, amino modified QDs, and the hybrid ZnO NRs coated with QDs (ZnO NR-QDs). All samples demonstrate the typical –CH stretch, and $–CH_2$ stretch in the FTIR spectra. The –C=O stretch at 1750 cm^{-1} is observed when glutaraldehyde was modified onto ZnO NRs, while this stretch does not show up to sample of ZnO NR-QDs. The –C=N stretch of the imine group, and –C=N–R located around 1620 cm^{-1} appears in the spectrum of ZnO NRs-QDs. Thus, two carbon–nitrogen double bonds (C=N), i.e. Schiff bases, were formed to allow QDs to coat on the ZnO NRs through the linkage of glutaraldehyde.

3.2 Fluorescence Intensity of the FRET sensor as a function of glucose level in aqueous

The optimization of the weight ratio of the donor (QDs) to enzyme (Con A) bonded with the acceptor (MG-dextran) was determined at 7:1 as shown in the supplementary materials. The photoluminescence (PL) properties of the nanostructured FRET

transducer-coating ZnO NR array (i.e. the patterned FRET sensor), the nanostructured donor of the sensor (i.e. CdSe/ZnS QDs) were studied by a fluorospectrometer in the range of 550 nm to 700 nm. In Figure 4a, the photoluminescence (PL) spectrum of ZnO in the visible range is observed with centering at 628 nm. The maximum intensity of PL speactrum of CdSe/ZnS QDs is observed at 648 nm. The PL spectrum of ZnO NR-QDs is dominated by the decorated QDs because of the large amount of QDs on a single ZnO NR, centered at 648 nm. When the acceptor of FRET sensor, MG-dextran, bound to QDs through Con A, the PL peak of ZnO NRs can be observed with centering at 652 nm; whereas the PL peak at 652 nm attributed to the donor (QDs) of the patterned FRET sensor is significantly suppressed. The slight redshift of the emission of QDs may be caused by the surface conjugation of Con A and the acceptor of MG-dextran. MG quenches the fluorescence signal of QDs through the FRET mechanism because of its broad absorbance around 650 nm.

10 µL of aqueous glucose of various concentrations were dropped onto the patterned FRET sensors on silicone hydrogels with 30 seconds of interaction time. The fluorescence measurements were determined by fluorospectrometry. As shown in the illustration of Figure 1, the fluorescence emission of the donor (CdSe/ZnS QDs) of the designed FRET sensor was quenched by the acceptor MG. In the presence of glucose, the quenched fluorescence of QDs is restored after the addition of glucose and the fluorescence emission of the patterned FRET sensors centered at 652 nm increases with increasing aqueous glucose. The FRET emission (λ_{em} = 652 nm) as a function of the concentration of aqueous glucose were measured. The relative fluorescence intensity (I_{re}) is calculated by using the equation below;

$$I_{re} = I'/I_o \ , \quad (2)$$

where I' is the restored intensity according to a glucose level, I_o the intensity of the sensor without glucose, or 0 glucose concentration. Figure 4b shows that two linear regions; (1) from 0.03 to 3 mmol/L; and (2) from 0 to 0.03 mmol/L. It is noted that the concentration of glucose in the range of 0.03 to 3 mmol/L can cover the tear glucose lever of both the diabetes and healthy subject. The linear relationship between relative fluorescence intensity and glucose concentration in the range of 0.03 to 3 mmol/L can be expressed as follows;

$$Y=0.239X+1.132 \quad (3)$$

3.3. Pixel Intensity Value of Fluorescence image of the FRET sensor as a function of glucose level in aqueous

The fluorescence images of the patterned FRET sensors on silicone hydrogel interacting 10 μL of various concentrations of aqueous glucose were monitored by a handheld fluorescence microscope. Figure 5a shows two fluorescence images of the patterned FRET sensor interacting with two samples of aqueous glucose; 0.04 mmol/L (sample A) and 0.4 mmol/L (sample B). The recorded images by the handheld microscope were converted to the readable signal through Matlab's imaging process. The pixel intensity was calibrated using MatLab to plot the intensity as a function of aqueous glucose concentration. The calibrated pixel intensities of the patterned FRET sensor is clearly increasing as glucose increases from 0.03 mmol/L to 0.6 mmol/L as shown in Figure 5b. The difference in pixel intensity value does not change significantly when the concentration of glucose is beyond 0.6 mmol/L. It is expected that a more advanced fluorescence microscope will be able to overcome the limit.

3.4 Response of the designed FRET sensor to 2 μL rat tears

The concentrations of blood glucose of the rats used in the current study were maintained in a range similar to patients with 'poorly' managed Type 1 diabetes (Nathan et al., 2014). Specifically, rats were representative of low to moderate hyperglycemic patients with Type 1 diabetes mellitus under conventional insulin therapy (Melling et al., 2013). Typically blood glucose concentrations in patient populations with type 1 diabetes fluctuate considerably; therefore, four rats with a range of blood glucose concentrations were utilized in the experiment to demonstrate.

The rats' tear samples were diluted 3.5 times. The patterned FRET sensor deposited on silicone hydrogels were used to measures the 2 μL diluted rats' tear samples. The PL spectra of the sensor corresponding to glucose in tears are shown in Figure S5 after interacting with rat tears. The detected glucose level in rats' tear samples were calculated by using Eq. 3. Meanwhile, the blood glucose levels to the four rats were measured by Freestyle Lite Blood Glucose Monitoring System, Abbott Diabetes Care Inc. Table 1 shows the glucose level in rats' tear samples measured by the nanostructured FRET sensor as compared to the blood glucose level of the four rats. The small value of the calculated standard deviation of the measurement indicates the repeatability of the measure.

In the supplementary materials, Figure S6 indicates that other saccharides molecules and some small biomolecules existing in tears do not interfere with the measure of glucose. Thus, our design allows Con A specifically interact with saccharide with smaller molecular weight, i.e. glucose.

4. Conclusion

In summary, the patterned nanostructured FRET sensor deposited on the synthesized silicone hydrogel were designed for monitoring glucose in tears. The emission (λ_{em}) = 650 nm of the CdSe/ZnS QDs can be quenched by the acceptor of dextran-bound malachite green (MG) which has an absorption at the same wavelength of the emission of the QDs. The dual modulation of detection is realized by depositing CdSe/ZnO QDs-based FRET sensors on the ZnO nanorod array. When the interaction time was maintained at 30 seconds, a linear relationship of the photoluminescence of the patterned nanostructured FRET sensor to the concentration of glucose from 0.03 mmol/L to 3 mmol/L is observed. It noted that the tear glucose of diabetics is > 0.35 ± 0.04 mmol/L, and the average value of tear glucose for healthy subjects is around 0.16 ± 0.03 mmol/L. Meanwhile, the calibrated values of pixel intensities are increasing with increasing glucose from 0.03 mmol/L to 0.6 mmol/L. Four rats with a range of blood glucose concentrations were utilized in the experiment. The results clearly show that the patterned nanostructured FRET sensor is capable of quickly monitoring tear glucose in an extremely small drop (2 μL) of tear sample. Consequently, our designed nanostructured FRET glucose sensor is suitable for monitoring tear glucose in a non-invasive and quick manner.

Acknowledgement

Authors are thankful for the financial support from the Grand Challenges Canada- Rising Star and Canada Innovation Fund-Leaders Opportunity Fund. Tse W. H. is thankful for the Graduate Scholarship offered by the Natural Sciences and Engineering Research Council of Canada (NSERC).

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Figure 1. (a) Illustration of the designed FRET transducer made of Con A-conjugating quantum dots (donor) and MG (acceptor) for detecting glucose. Competitive affinity for glucose displaces MG to restore the quenched fluorescence. (b) Immobilization of the nanostructured FRET transducers on ZnO nanorod array deposited on silicone hydrogel.

Figure 2. Characterization of nanomaterials by electron microscopes. (a) SEM micrographs of patterned ZnO NRs deposited on a silicone hydrogel. The small inset is the photomask. (b) The magnified SEM micrograph of ZnO nanorod array deposited on a silicone hydrogel. (c) SEM micrograph of ZnO nanorods. (d) SEM micrograph of QDs coated ZnO nanorods. (e) TEM micrograph of QDs coated ZnO nanorods. (f) HRTEM of CdSe/ZnS QDs.

Figure 3. FTIR spectra of the cysteamine (Cys) modified QDs and ZnO nanorod (NR), and the hybrid ZnO NR coated with QDs (ZnO NR-QDs).

Figure 4. (a) Photoluminescence of ZnO NR arrays on silicone hydrogel with/without QDs, and QDs-based fret sensors, and the UV-vis absorption of MG. (b). A linear relationship between fluorescence intensity of the designed sensor and the concentration of glucose.

Figure 5. Fluorescence images of the patterned FRET sensor on silicone hydrogel and the relative pixel intensities of the sensors responding to the concentrations of glucose. (a) Fluorescence images of the patterned FRET sensor to aqueous glucose with 0.04 mmol/L and 0.4 mmol/L, respectively. (b) The relative pixel intensities of the sensors vs. the concentration of aqueous glucose.

Figure 1

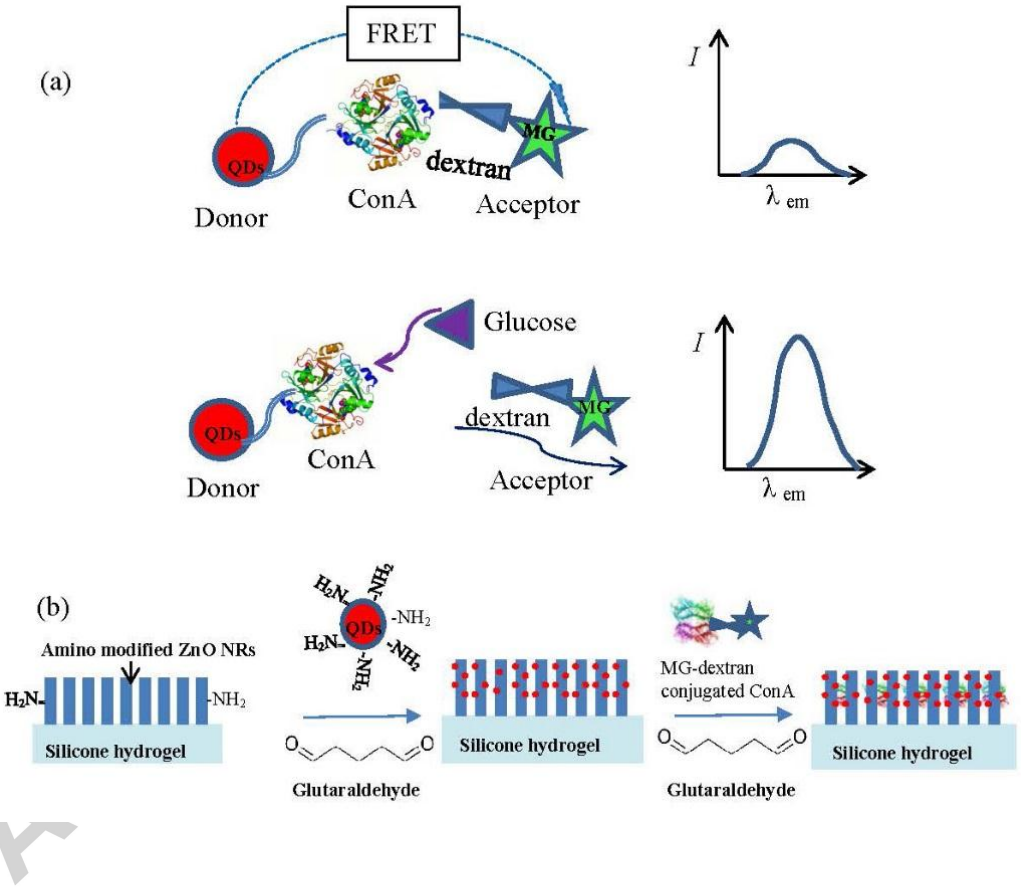
(a)
FRET
QDs
Donor
ConA
dextran
MG
Acceptor
I
λ em
Glucose
(b)
Amino modified ZnO NRs
H2N-
-NH2
Silicone hydrogel
Glutaraldehyde
MG-dextran
conjugated ConA

Figure 2

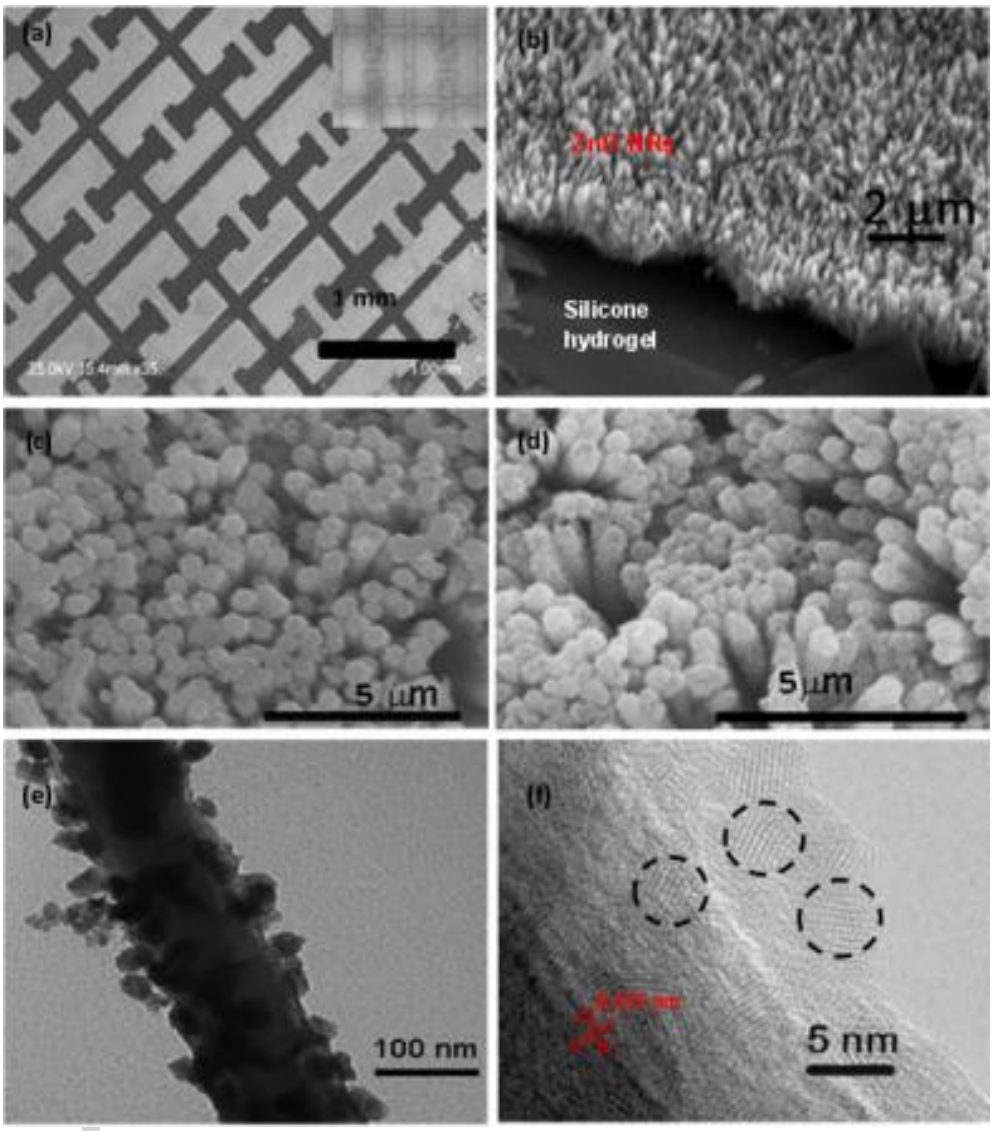

Figure 3

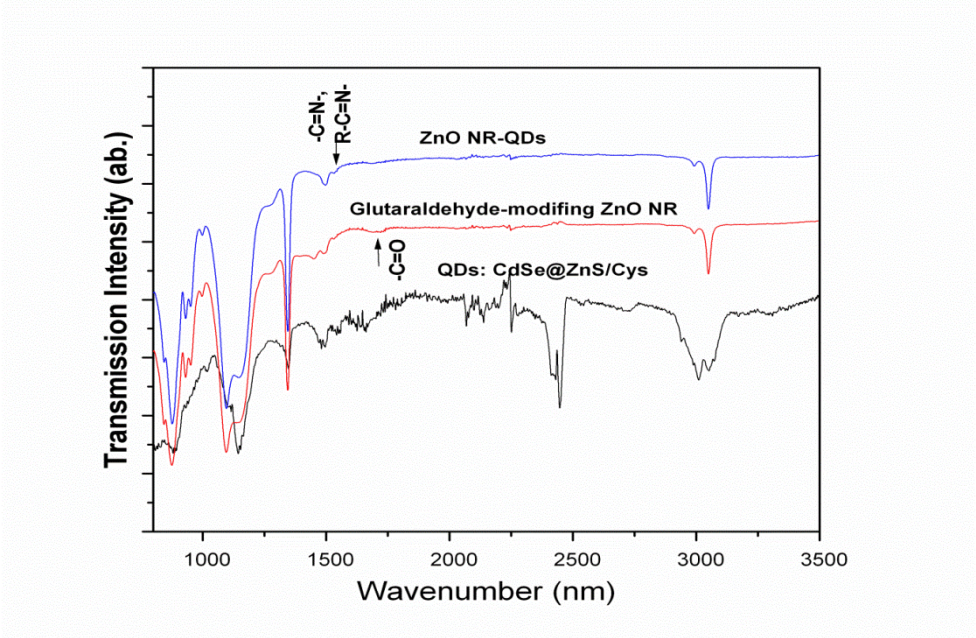

Figure 4

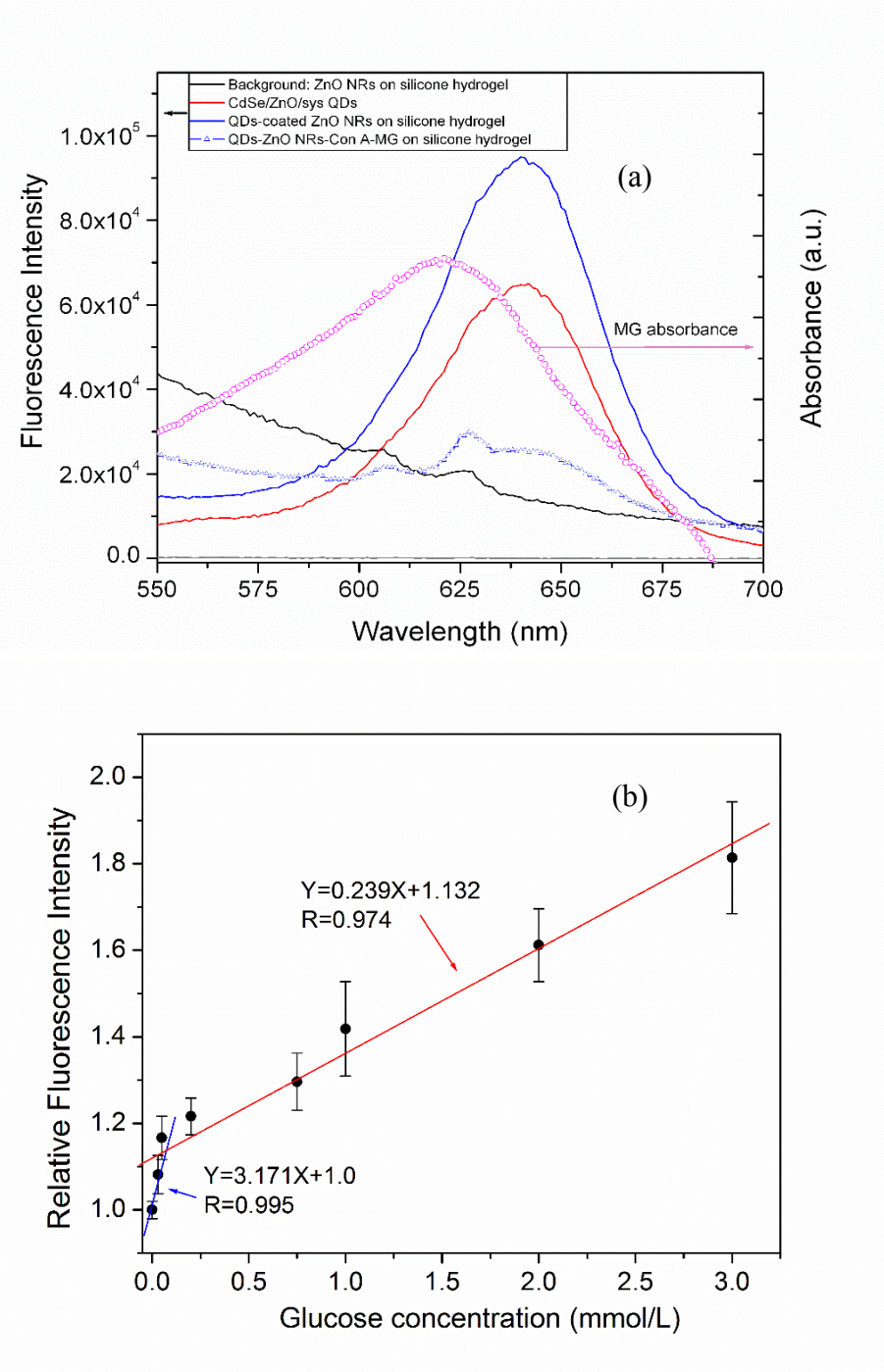
Background: ZnO NRs on silicone hydrogel
CdSe/ZnO/sys QDs
QDs-coated ZnO NRs on silicone hydrogel
QDs-ZnO NRs-Con A-MG on silicone hydrogel
(a)
MG absorbance
Fluorescence Intensity
Absorbance (a.u.)
Wavelength (nm)
1.0x10^5
8.0x10^4
6.0x10^4
4.0x10^4
2.0x10^4
0.0
550
575
600
625
650
675
700
(b)
Y=0.239X+1.132
R=0.974
Y=3.171X+1.0
R=0.995
Relative Fluorescence Intensity
Glucose concentration (mmol/L)
2.0
1.8
1.6
1.4
1.2
1.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Figure 5

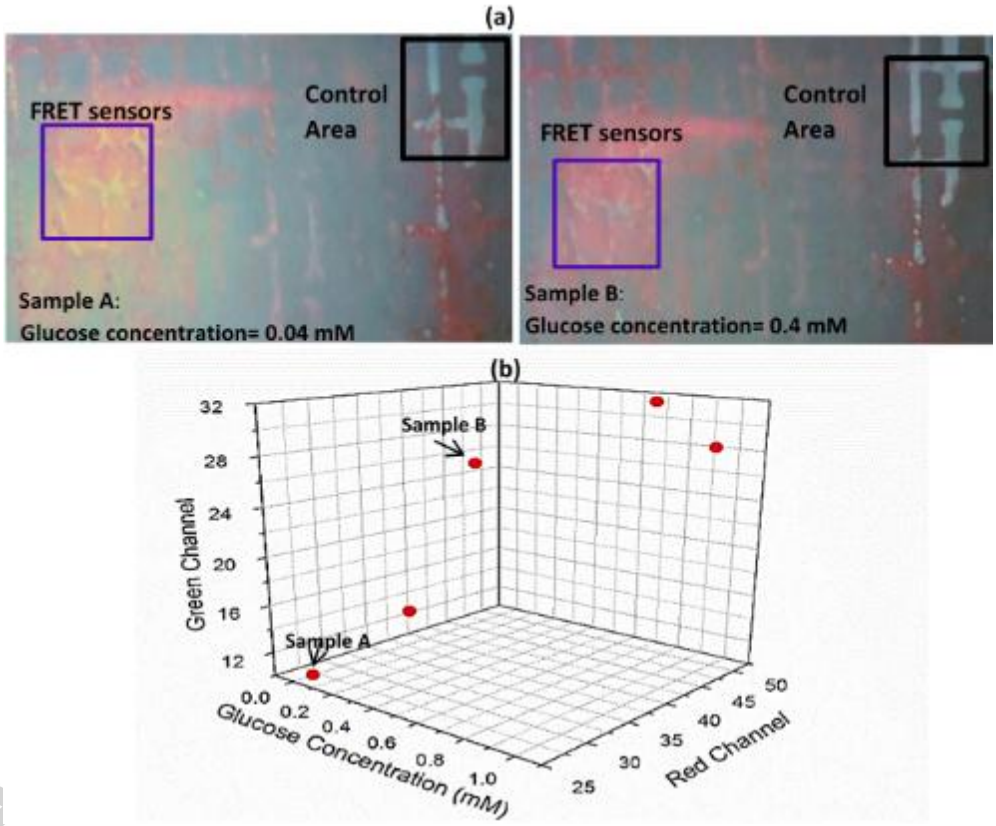
(a)
FRET sensors
Control Area
Sample A:
Glucose concentration= 0.04 mM
FRET sensors
Control Area
Sample B:
Glucose concentration= 0.4 mM
(b)
Sample B
Sample A
Green Channel
32
28
24
20
16
12
0.0
0.2
0.4
0.6
0.8
1.0
Glucose Concentration (mM)
25
30
35
40
45
50
Red Channel

Table 1. Glucose level in rats' tear samples measured by the nanostructured FRET sensor as compared to the blood glucose level of the four rats.

Blood glucose (mmol/L)	Tear glucose (mmol/L)
5.3	0.14±0.03
9.7	0.42±0.04
12.3	0.95±0.05
18.2	1.28±0.05

Highlights

- The nanostructured biosensor is designed for dual detection of tear glucose
- Fluorescence intensity increases linearly with glucose level from 0.03 to 3 mM
- Image pixel intensity value of the sensor is corresponding to the glucose level
- Animal test indicates the sensor can measure 2μL tear glucose in 30 second