Electronic Supplementary Material (ESI) for Analytical Methods.

Visual detection of glucose based on the QDs aerogel in microfluidic chips

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Keywords: microfluidic chip, QDs aerogel, glucose, visual detection.

1. Materials and methods

1.1 Materials

All chemicals were analytical grade. $CdCl_2 \cdot 2.5H_2O$, $NaBH_4$, L-Glutathione(L-GSH) and Na_2TeO_3 were purchased from Alfa Aesar (Shanghai, China). NaOH and isopropanol (C_3H_8O) were purchased from KESHI (Chengdu, China). Glucose Oxidase (GOx) was purchased from Sigma-Aldrich (St. Louis, USA). Ultrapure water (≧18 $M\Omega \cdot cm^2$) was prepared by a Millipore water purification system. Urine samples were collected from healthy volunteers. Serum samples were provided by Zhongda Hospital (Nanjing, China).

1.2 Appratus

Fluorescence spectra was recorded by Lengguang Tech F96 pro fluorescence spectrophotometer (Lengguang Tech, Shanghai, China). The morphology of the synthesized CdTe-GOx aerogel was analyzed by a FEI Sirion field emission scanning electron microscope (FEI, Hillsboro, America). Fluorescence images were taken with a Canon 7D® digital camera (Canon, Tokyo, Japan), using a Qinke ZF-7A UV lamp (Qinke, Shanghai, China) as a light source.

1.3 Fabrication of microfluidic chips

We used CAD software to draw the pattern of the microfluidic chip channel shape and made into a photolithographic mask. A layer of positive photoresist was dumped on silicon wafer and solidified in high temperature (the thickness of the photoresist is 50μm), then it was exposed on the lithography machine and developed through the developer solution. The remaining photoresist and silicon were used to form a male mold. The surface of the mold was cleaned by ethanol and drying. PDMS matrix was mixed with the curing agent at mass ratio of 10:1 and placed in a vacuum oven to remove air bubbles from the solution. The mixed solution without bubbles was then placed on the hot plate curing at 80°C for 1h, and was taken out and cooled to room temperature. The PDMS was separate from the mold and produced a PDMS microfluidic chip structure with a flat surface. After the PDMS and glass substrate are treated with plasma cleaning agents and bonded to each other, it was placed in an oven at 120℃ for 2h. Finally, we got the microfluidic chip we need.

The reactor design consists of a 12 mm long, 0.5 mm wide and 0.05 mm deep channels with 2 in/outlets to the chip periphery. The reactor design is placed five times on the reactor chip to enable five parallel measurements. The deep packed bed reactor chips have the outer dimensions of a microscope slide, 76 mm´26 mm´1 mm.

1.4 Preparation of CdTe QDs solution

103 mg of $CdCl_2 \cdot 2.5H_2O$ and 184 mg of GSH were first mixed in 55 mL of ultrapure water, stirred and adjusted to a pH of 10.5. Then, 22 mg of Na_2TeO_3 and 3.8 mg of $NaBH_4$ were added to the mixed solution and again was stirred for until the color of the solution change from colorless to pale green. After stirring, the solution was put into three necked flasks, and heated by oil bath at 110℃ for 2.5h. The fabricated CdTe QDs were characterized using a fluorescence microscope. An emission peak at 605 nm is observed.

1.5 Preparation of CdTe microfluidic glucose sensor

First, 1 mL QDs were purified by precipitation with isopropanol and dispersed in 0.545 mL PBS with 100 U GOx. Then the mixed solution was injected into the flow channel of the microfluidic chip by micro-injector, the microfluidic chip was put into refrigerator at -20℃ for 48h. Finally, the chip was freeze-dried in a freeze dryer, and the microfluidic glucose sensor is obtained.

1.6 Characterization

Different concentrations of glucose were dissolved in PBS buffer. Then, 0.8 μL solutions of each concentration were injected into the flow channel of the microfluidic chip by a microinjector. After 5 minutes, the microfluidic chip was put in the same place of ultraviolet analyzer and taken photos under a 254 nm UV lamp. Subsequently, the gray value of the flow channel was obtained by detecting the photos. I is the gray

value of the flow channel with different concentrations of glucose. I_0 is the gray value of the flow channel with PBS. C is the concentration value of glucose. Calculate the value of I/I_0 and $\log_{10}$ (C) and draw the curve of I/I_0 with $\log_{10}$(C).

1.7 Determination Glucose in serum and urine

Fluorescent detection of glucose in urine: Different concentrations of glucose were dissolved in urine samples, which were collected from volunteers. Then, 0.8 μL solution was injected into the flow channel of the microfluidic chip by microinjector. After 5 min, the microfluidic chip was put in the same place of ultraviolet analyzer and taken photos under a 254 nm UV lamp. Subsequently, the gray value of the flow channel was obtained by detecting the photos. I, I_0 and C is same as defined in section 1.8 Calculate the value of I/I_0 and $\log_{10}$ (C) and draw the curve of I/I_0 with $\log_{10}$(C).

Fluorescent detection of glucose in serum: Different samples of serum supported by Zhongda Hospital was injected into the flow channel of the microfluidic chip by microinjector until there was 0.8 μL solution in per channel and then treated as above procedure. The gray value of the flow channel was obtained by detecting the photos.

1.8 Ethics statement

This study was performed with the approval of the IEC for Clinical Research of Zhongda Hospital, Affiliated to Southeast University. ALL human urine and human serum were treated as the guidelines of the IEC for Clinical Research of Zhongda Hospital and informed consent was obtained.

Figure S1. The photographic images of the microfluidic chip with QDs-GOx based aerogel under indoor light (a) and 254 nm UV light (b).

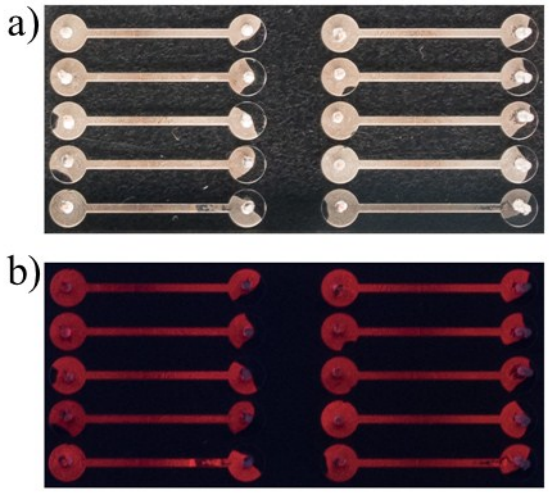


Figure S2. The photograph of the glucose microfluidic sensor with H_2O (left) and the response to H_2O_2 (right).

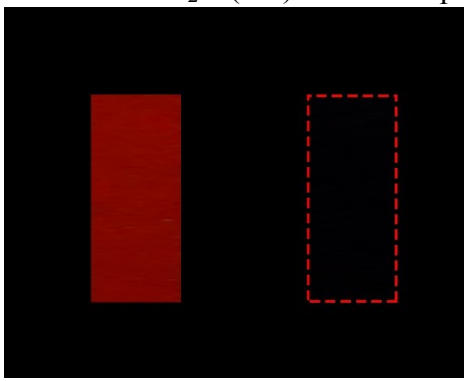


Table S1. The urine glucose value obtained by commercial instrument(Uritest-500B Automated Urine Chemistry Analyzer, URIT Corporation, China) and by glucose microfludic sensor

sample	detected by our method(mM)	detected by hospital(mM)	Photo
1	3.63	-	
2	3.74	-	
3	6.46	-	
4	11.58	+	
5	13.73	+++	
6	15.69	+++	

Table S2. The blood glucose value obtained by commercial instrument (Synchron LX-20 automatic biochemical analyzer, Beckman Coulter Inc., CA, USA) and by glucose microfludic sensor

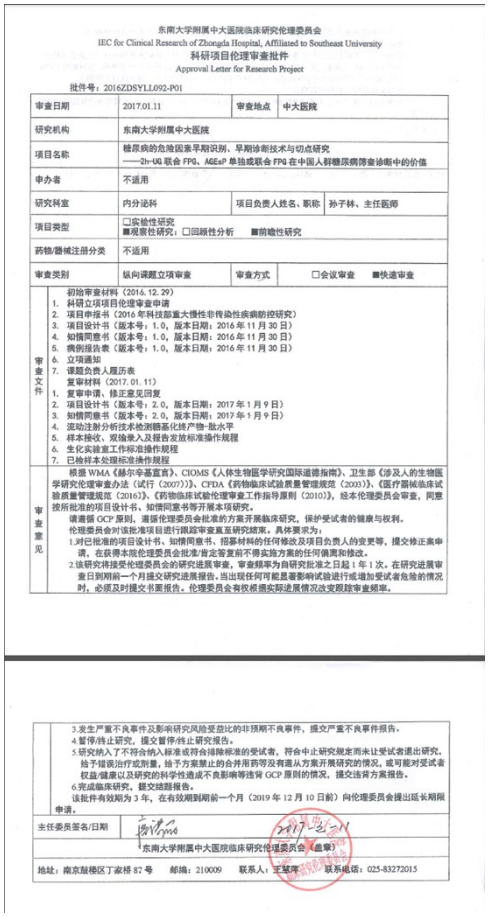
sample	blood glucose(mM)	found(mM)	Recovery
a	2.72	2.80	99.11%
b	5.65	5.64	96.21%
c	8.49	9.17	104.18%
d	10.98	12.16	106.81%
e	20.49	20.13	94.74%

Table S3. The comparison among our sensor and recently reported methods for detection of glucose

analytical system	**range**	**LOD**	**reaction place**	**reaction volume**	**reactiom Time**	**visual detection**	**measurement equipment**	**Reference**
GOx/HRP-luminol	0.1-100mM	0.0948mM	cloth	10μL	5.5min	Yes	camera	1
GQD-chitosan	1.2-120μM	0.3pM	solution	not given	not given	No	electrochemistry workstation	2
GOx-Mn3(PO4)2	0.1-20mM	0.01mM	paper	8μL	10min	Yes	camera	3
GOx&CCP/QDs	0.1-5mM	0.05mM	solution	not given	3h	Yes	fluorescence spectrometer	4
fiber-based scheme	0-270mg dL^{-1} 300-3000mg dL^{-1}	3mg dL-1	fiber	not given	not given	No	Mach-Zehndr interferometer	5
GOx-CdTe	0-12mM	0.135mM	microfludic chip	0.8μL	5.5min	Yes	camera	This work

Table S4. The comparison among our sensor and commercial glucose meters for detection of glucose.

Brand	**ACCU-CHEK®**	**Onetouch**	**Our sensor**
Model	**Active**	**UltraEazy**	
Instrument price	¥131	¥247	Unwanted
Instrument weight	46g	40g	Unwanted
Test type	blood	blood	blood/urine
Test range	0.6-33.3mM	1.1-33.3mM	0.14-12mM
Accuracy	≤15%	≤15%	≤8%
Test time	5s	5s	5.5min
Test cost	¥3.85/time	¥4.56/time	¥0.46/time



东南大学附属中大医院临床研究伦理委员会
IEC for Clinical Research of Zhongda Hospital, Affiliated to Southeast University
科研项目伦理审查批件
Approval Letter for Research Project

批件号：2016ZDSYLL092-P01

审查日期	2017.01.11	审查地点	中大医院
研究机构	东南大学附属中大医院		
项目名称	糖尿病的危险因素早期识别、早期诊断技术与切点研究 ——2h-UG 联合 FPG、AGEsP 单独或联合 FPG 在中国人群糖尿病筛查诊断中的价值		
申办者	不适用		
研究科室	内分泌科	项目负责人姓名、职称	孙子林、主任医师
项目类型	□实验性研究 ■观察性研究：□回顾性分析　■前瞻性研究		
药物/器械注册分类	不适用		
审查类别	纵向课题立项审查	审查方式	□会议审查　■快速审查

审查文件

初始审查材料（2016.12.29）
1. 科研立项项目伦理审查申请
2. 项目申报书（2016 年科技部重大慢性非传染性疾病防控研究）
3. 项目设计书（版本号：1.0，版本日期：2016 年 11 月 30 日）
4. 知情同意书（版本号：1.0，版本日期：2016 年 11 月 30 日）
5. 病例报告表（版本号：1.0，版本日期：2016 年 11 月 30 日）
6. 立项通知
7. 课题负责人履历表

复审材料（2017.01.11）
1. 复审申请、修正意见回复
2. 项目设计书（版本号：2.0，版本日期：2017 年 1 月 9 日）
3. 知情同意书（版本号：2.0，版本日期：2017 年 1 月 9 日）
4. 流动注射分析技术检测糖基化终产物-肽水平
5. 样本接收、双输录入及报告发放标准操作规程
6. 生化实验室工作标准操作规程
7. 已检样本处理标准操作规程

审查意见

根据 WMA《赫尔辛基宣言》、CIOMS《人体生物医学研究国际道德指南》、卫生部《涉及人的生物医学研究伦理审查办法（试行（2007））》、CFDA《药物临床试验质量管理规范（2003）》、《医疗器械临床试验质量管理规范（2016）》、《药物临床试验伦理审查工作指导原则（2010）》，经本伦理委员会审查，同意按所批准的项目设计书、知情同意书等开展本项研究。

请遵循 GCP 原则，遵循伦理委员会批准的方案开展临床研究，保护受试者的健康与权利。

伦理委员会对该批准项目进行跟踪审查直至研究结束。具体要求为：

1.对已批准的项目设计书、知情同意书、招募材料的任何修改及项目负责人的变更等，提交修正案申请，在获得本院伦理委员会批准/肯定答复前不得实施方案的任何偏离和修改。

2.该研究将接受伦理委员会的研究进展审查，审查频率为自研究批准之日起 1 年 1 次。在研究进展审查日到期前一个月提交研究进展报告。当出现任何可能显著影响试验进行或增加受试者危险的情况时，必须及时提交书面报告。伦理委员会有权根据实际进展情况改变跟踪审查频率。

3.发生严重不良事件及影响研究风险受益比的非预期不良事件，提交严重不良事件报告。

4.暂停/终止研究，提交暂停/终止研究报告。

5.研究纳入了不符合纳入标准或符合违除标准的受试者，符合中止研究规定而未让受试者退出研究。给予错误治疗或剂量，给予方案禁止的合并用药等没有遵从方案开展研究的情况，或可能对受试者权益/健康以及研究的科学性造成不良影响等违背 GCP 原则的情况，提交违背方案报告。

6.完成临床研究，提交结题报告。

该批件有效期为 3 年，在有效期到期前一个月（2019 年 12 月 10 日前）向伦理委员会提出延长期限申请。

主任委员签名/日期　2017-2-11

东南大学附属中大医院临床研究伦理委员会（盖章）

地址：南京鼓楼区丁家桥 87 号　邮编：210009　联系人：王慧萍　联系电话：025-83272015

Fig. S3 The approval of ethics committee to this experiments

Notes and references

1 H. Li, C. Liu, D. Wang and C. Zhang, *Biosens. Bioelectron.*, 2016, **91**, 268-275.

2 K. Tian, F. Nie, K. Luo, X. Zheng and J. Zheng, *Journal of Electroanalytical Chemistry*, 2017.

3 W. Y. Li, S. Y. Lu, S. J. Bao, Z. Z. Shi, Z. Lu, C. M. Li and L. Yu, *Biosens. Bioelectron.*, 2017, **99**, 603-611.

4 M. Yu, K. Zhao, X. Zhu, S. Tang, Z. Nie, Y. Huang, P. Zhao and S. Yao, *Biosens. Bioelectron.*,2017, **95**, 41-47.

5 N. Xie, H. Zhang, B. Liu, J. Wu, B. Song and T. Han, *Journal of Optics*, 2017.