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# Noninvasive Diabetes Monitoring through Continuous Analysis of Sweat Using Flow-Through Glucose Biosensor

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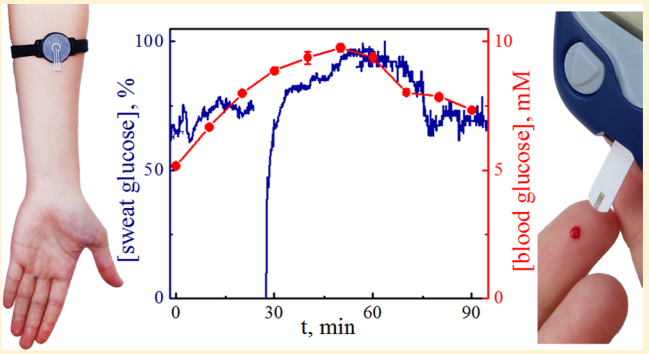
Supporting Information

**ABSTRACT:** We propose monitoring of diabetes through continuous analysis of undiluted sweat immediately after its excretion using a flow-through glucose biosensor. The used biosensors are based on Prussian Blue and glucose oxidase immobilized in perfluorosulfonated ionomer or gel of alkoxysilane; the resulting sensitivity with the latter reaches in batch mode 0.23 A $M^{-1}$ $cm^{-2}$, and the calibration range is from 1 $\mu$M to 1 mM (flow-through mode). On the basis of the glucose tolerance test known to be a clinically relevant procedure to mimic hyperglycemia, a positive correlation between the rates of glucose concentration increase in blood and in noninvasively collected sweat has been observed ($r$ = 0.75). The observed correlation between sweat and blood considering low-molecular weight metabolites is even better than that observed previously between capillary and vein blood, confirming diagnostic value of sweat for diabetes monitoring. The dynamics of sweat glucose concentration, recorded by means of the proposed biosensor, is in a good accordance with the dynamics of blood glucose content without any time delay, thus offering a prospect for noninvasive monitoring of diabetes.






According to the World Health Organization, 422 million people (≈5% of the world's population) suffer from diabetes. Predicted to become the seventh leading cause of death, diabetes is dangerous because of its complications: cardiovascular diseases, blindness, risk of amputation, kidney failure, etc. Glucose concentration in blood is the key parameter for diabetic patients: maintaining it at an appropriate level allows these complications to be postponed.

Noninvasive methods, which exclude not only injury to blood vessels, but also damage to the skin surface, are preferred for diagnostics: such methods are painless and avoid potential infection and trauma to patients. However, despite continuing efforts, the problem of noninvasive evaluation of blood glucose concentration has not yet been solved.

Physical methods for evaluation of blood glucose concentration were not successful, particularly, near-IR spectroscopy could not provide the required sensitivity.[1−4] Hence, for noninvasive diagnostics the analysis of an excreted liquid has to be considered.

Several attempts were made to evaluate blood glucose by measuring the excreted liquid on the other skin surface including application of negative pressure to skin[5] and glucose iontophoresis.[6,7] The latter was realized in commercialized devices (for instance, "Glucowatch", Cygnus Corp.) which, however, have disappeared from the market shortly after being issued. The "tattoo-based noninvasive glucose monitor"[8] is in fact a replica of the mentioned iontophoresis based "Glucowatch". Moreover, for sensor constructions tightly applied to the skin,[8,9] the continuous monitoring is hardly expectable, because in the absence of the solution outlet, the sensor surface would be continuously exposed to the sweat portion excreted initially. Obviously, continuous monitoring is only possible using a flow-through biosensor.

Noninvasively collected sweat is already used in clinical practice; its conductivity allows monitoring cystic fibrosis.[10,11] Chemical analysis of sweat is highly attractive; however, conventional electrochemical clinical analyzers are not applicable for this aim because sweat components inactivate platinum used as the transducer in the corresponding biosensors.

Poisoning the transducer by sweat components has not been observed for ferric hexacyanoferrate (Prussian Blue),[12] apparently the most advantageous electrocatalyst for hydrogen peroxide reduction (see ref 13 for review). In neutral media favorable for biosensor operation, the advantages of Prussian Blue (PB) modified electrodes over platinum, the material most widely used for detection of hydrogen peroxide, are (i) 3 orders of magnitude higher activity in $H_2O_2$ reduction and oxidation in terms of 1000 times higher electrochemical rate constants,[14] which obviously provide similarly improved

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sensitivity, and (ii) 3 orders of magnitude higher selectivity allowing low-potential $H_2O_2$ detection in the presence of oxygen.[15] The recently reported operation of Prussian Blue based biosensors through power generation[16] not only simplifies the detection scheme substituting conventional potentiostat by an ammeter but also 5−10 times improves the signal-to-noise ratio.

Human sweat has already attracted a particular interest for noninvasive diagnostics of hypoxia. The attempts to monitor sweat lactate were reported with the use of biosensors[17,18] as well as the nonenzymatic sensor.[19]

Obviously, sweat also has been considered for noninvasive monitoring of diabetes. In recent times, various devices combining sweat analysis, including glucose detection, with signal transduction and transmission appeared.[20,21] However, variation of sweat glucose concentration encountering almost 3 orders of magnitude[22] is much higher compared to blood glucose, making it impossible to define sweat glucose threshold levels differentiating normal patient conditions from hypo- or hyperglycemia. The attempts to find relations between sweat and blood glucose levels on the basis of reliable sweat sampling through a clinically relevant procedure (pilocarpine stimulation[23]) were made in refs 9 and 24. However, the data which would allow one to predict blood glucose levels on the basis of sweat glucose concentrations are still absent.

Here we report on the flow-through glucose biosensor for continuous analysis of undiluted sweat immediately after its excretion. Concerning the diagnostic value of sweat, a positive correlation between the rates of blood and sweat glucose increase has been observed, which is a sufficient requirement for noninvasive monitoring of diabetes. The reported biosensor offers a prospect for real-time monitoring of blood glucose concentration thus representing a prototype of a noninvasive diabetes monitor.

## EXPERIMENTAL SECTION

**Materials.** Experiments were carried out with Millipore Milli-Q water. All inorganic salts, perfluorosulfonated ionomer MF4 SK (Nafion analogue), organic solvents, and hydrogen peroxide (30% solution) were obtained at the highest purity from Reachim (Moscow, Russia) and used as received. Pilocarpine (1% solution) was purchased from Ferein, Russia. D-Glucose was purchased from ICN Biomedicals. Glucose oxidase (EC 1.1.3.4) from *Aspergillus niger* (type VII, lyophilized powder, activity 200 IU) was purchased from Sigma-Aldrich, Germany. Alkoxysilanes were purchased from Sigma-Aldrich, Germany, and distilled prior to use. Planar sensors were made on the basis of three electrode screen printed structures with a 2 mm in diameter carbon working electrode, produced by Ltd. Rusens (Russia).

**Instrumentation.** Voltammetry and amperometry were carried out using PalmSens 3 potentiostat (PalmSens BV, The Netherlands). As an ammeter in power generation mode the Digital multimeter Tektronix DMM4020 (Tektronix Inc.) was used. The reference instrument for blood glucose detection was EcoBasic (Care Diagnostica, Germany). The flow-injection setup consisted of a syringe pump Perfusor Compact S (Braun, Germany), homemade flow-through wall-jet cell with 0.5 mm nozzle, and a homemade injector. The flow rate used was of 0.8 mL $min^{-1}$. For electrophoresis, we used Potok-1, Russia. Sweat sampling was made with a sweat collector (Macroduct).

**Methods.** Glucose biosensors were made by dipping the glucose oxidase containing mixture (2 $\mu$L) in 85% isopropanol onto the surface of Prussian Blue/(stabilized PB) modified planar working electrode.[25] After evaporation of the solvent at room temperature, the membranes were kept in the refrigerator (+4 °C) for 24 h. For enzyme immobilization in perfluorosulfonated ionomer (Nafion analogue), the casting mixture was prepared suspending the aqueous enzyme (10 mg/mL) with an isopropanol solution of the ionomer to a final concentration of 0.3%.[25]

Interfacial synthesis of Prussian Blue[26] was made by dipping a droplet of 2−5 mM $K_3[Fe(CN)_6]$ and 2−5 mM $FeCl_3$ in 0.1 M HCl and 0.1 M KCl and initiating by addition of $H_2O_2$ to a final concentration of 50−200 mM. Stabilized PB films were synthesized via layer-by-layer deposition of the Prussian Blue and the stabilizing Ni hexacyanoferrate layers.[27] The growing solution for the latter was 0.5−2 mM of $NiCl_2$ and 0.1−1 mM $K_3[Fe(CN)_6]$ in 0.5 M KCl containing 0.1 M HCl. After deposition, the modified electrodes were annealed at 100 °C for 1 h.

The group of 19 healthy human volunteers was formed: from 20 to 30 years old, 5 females and 14 males. Informed consent was obtained from all subjects. All work was carried out in accordance with GCP regulations. Glucose tolerance test protocols were approved by the Ethical Committee of Pulmonology Research Institute (Moscow). After an overnight fast, the subject was loaded orally with 75 g of glucose. Collection of sweat samples was carried out according to the accepted worldwide clinically relevant procedure based on stimulation of the sweat gland with 1% pilocarpine through electrophoresis for 10−15 min.[23,28] Capillary blood samples were collected from a finger using sterile single-use safety lancets 17G (Acti-Lance, Poland).

The flow-through biosensor was made by modifying the outer flat surface of a Macroduct-type collector with double side adhesive forming a channel of 1.5 mm width. Another side of the adhesive was linked to the power generating Prussian Blue based glucose biosensor[16] (Figure S1, Supporting Information).

## RESULTS AND DISCUSSION

**Glucose Biosensor Based on Siloxane Gel.** Sweat glucose content is known to be on average more than an order of magnitude lower as compared to blood.[22] Accordingly, for continuous monitoring of glucose in undiluted sweat, it was essential to access analytical properties of glucose biosensors. Despite a success with glucose oxidase immobilization in Nafion,[25] alkoxysilane gels provide higher effectivity of the resulting enzyme containing membranes in terms of the higher ratio of the biosensor sensitivity to the sensitivity of the transducer used.[12,29] Indeed, sensitivity of the lactate biosensor based on lactate oxidase immobilized in siloxane membranes is just 3 times lower than the sensitivity of the $H_2O_2$ transducer,[29] whereas for glucose oxidase immobilized in Nafion membranes this ratio is around 0.1,[25] despite the latter enzyme being much more active.

The immobilization protocol first proposed by us more than 20 years ago[30] involving enzyme exposure to concentrated organic solvents results in enzyme containing membranes with improved activity and stability.[12,25] Among various alkoxysilanes tested, the best performance characteristics including the highest sensitivity were observed for biosensors based on $\gamma$-aminopropyltriethoxysilane (Figure S2, Supporting Informa-



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tion). The achieved sensitivity was even higher compared to the biosensor based on Nafion membrane.

Figure 1 displays the sensitivity of the γ-aminopropyltriethoxysilane based glucose biosensor in batch mode as a function of the casting mixture composition. The 3-D graph displays the strongly pronounced extreme reaching a sensitivity of 0.23 A $M^{-1}$ $cm^{-2}$. The extreme is reached at enzyme and siloxane concentrations of, respectively, 4 mg $mL^{-1}$ and 1.5% in the casting mixture. The ratio of the biosensor-to-transducer sensitivity reaches the extreme value of 0.4−0.5. Accordingly, the advantage of the alkoxysilane as a matrix for glucose oxidase immobilization is 4−5 times improved sensitivity compared to the Nafion based biosensor. The calibration range of the corresponding biosensor is prolonged from 5 μM to 1 mM of glucose. In flow-through mode the lower limit of the calibration range for a similar biosensor is decreased down to 1 μM due to lower noises.

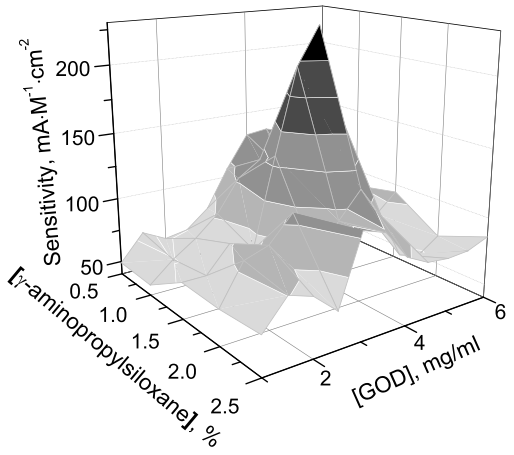


**Figure 1.** Sensitivity of the glucose biosensor as a function of siloxane and glucose oxidase concentrations in the membrane forming mixture.

Prior to use for diagnostics, both Nafion and siloxane based biosensors were validated upon analysis of blood samples. EcoBasic (Care Diagnostica, Germany) was used as a reference. The data obtained using a homemade flow-injection analyzer equipped with both types of glucose biosensors are in a good accordance with the reference method: the value of Pearson correlation coefficient ($r$) exceeds 0.96 (Figure S3, Supporting Information).

**Diagnostic Value of Sweat for Diabetes.** Noninvasive monitoring by means of chemical analysis is often considered unreliable since none of the excreted liquids directly replicate the metabolite content of blood. Indeed, the estimated variation in sweat glucose concentration encounters almost 3 orders of magnitude (from 5 × $10^{-6}$ M to 2 mM),[22] whereas the allowed blood glucose variation is 100 times lower. Our own direct comparison between sweat and blood concerning their glucose content shows rather poor correlation with a Pearson coefficient of 0.44 (Figure S4, Supporting Information).

We note that the majority of existing long-term glucose monitors referred to as "low-invasive" require calibration. Moreover, these monitors are still invasive, because they are based on microsensors positioned at the 5 mm depth under the skin surface and thus analyzing blood. Concerning the mentioned "calibration", it involves a conventional blood probing with subsequent glucose detection in it. Hence, occasional blood probing for calibration does not appear to devalue a diagnostic tool also referred to as "noninvasive". Accordingly, a sufficient requirement for noninvasive diagnostics would be a correlation in variation rates between metabolite concentrations in the excreted liquid and the corresponding values in blood.

The so-called "glucose tolerance" test[31] seems to be the only clinically relevant procedure to mimic hyperglycemia events in healthy subjects. Sweat sample was collected from a healthy subject (after an overnight fast) during 20 min. Simultaneously a blood sample was taken from the subject's finger. These sweat and blood samples are referred to as "before test" ones. After that the volunteer was loaded orally with 75 g of glucose. As known and confirmed previously,[32] glucose concentration in the blood reached its maximum after approximately 60 min. Accordingly, after glucose loading, a blood sample was taken at the 60th minute and a sweat sample was collected from the 50th to the 70th minute. These samples are referred to as "after test" ones.

Figure 2 displays the variation rates (ratios of glucose concentrations in "after test" samples to them in "before test" ones) for blood and sweat. The samples were collected from 19 healthy human volunteers (see Experimental Section); six of them were subjected to this procedure twice: the interval between the procedures was not less than 1 week. The raw data on glucose content in sweat and blood samples are shown in Table S1, Supporting Information. Figure 2 displays the regression line through 25 points with the slope of approximately 1. The Pearson correlation coefficient reaches the value of 0.75. Hence, the variation rates for glucose concentrations in blood and sweat correlate positively.

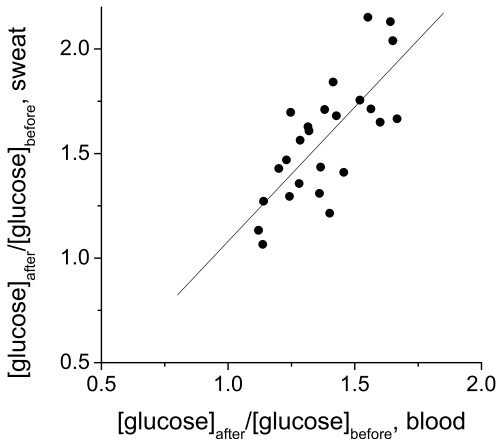


**Figure 2.** Correlation in the rates of glucose concentration increase (ratios of glucose content after glucose loading to the corresponding value before loading) for sweat and for blood: Pearson correlation coefficient $r$ = 0.75.

The important question is whether the ratio of sweat glucose levels to blood glucose levels varies significantly with time for a given subject. Table S1, Supporting Information, shows that this ratio is constant (with the error within 10%) for 2−3 weeks. A significant deviation in the ratio of sweat glucose levels to blood glucose levels has been observed for one subject when the test was repeated after 8 months. However, for the two other subjects with a long time gap between the experiments (3.5 and 8 months), the exact coincidence in


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the ratios of the sweat-to-blood glucose levels has been registered.

One can, however, raise an objection that the observed correlation is not good enough for clinical diagnostics. We note that despite blood being considered as a biological liquid with undoubted diagnostic potential, blood concentrations of low molecular weight metabolites in different vessel systems can vary significantly. It is generally accepted that vein blood glucose is different from capillary blood glucose. Concerning the variation rates, we've recalculated our data on lactate content in capillary and vein blood.[33] Lactate is an intermediate of glucose metabolism, and it serves as a marker for hypoxia being the end product of anaerobic glucose conversion.[34,35] As we've shown recently,[32] glucose metabolizing to lactate occurs even in exhaled breath condensate; hence, in blood and tissues its rate is significant. We conclude that blood samples taken from different vessel systems of the same body also do not replicate each other with high accuracy. The variation rates for capillary blood are on average 3.5 times higher than for vein blood, and the regression line passes far from the origin (Figure S5, Supporting Information). This is much worse than the observed correlation between sweat and blood, displaying the regression line with the slope close to 1.0 (Figure 2). Considering Pearson's correlation coefficient, it is ($r$ = 0.8) at a similar level to the observed above for correlation between sweat and blood glucose.

Hence, considering the content of low-molecular weight metabolites, the observed correlation between sweat and blood (slope close to 1, $r$ = 0.75) is even better as compared to the correlation between capillary and vein blood (similar correlation coefficient, 0.8, but the regression line slope of 3.5). Since blood is undoubtedly considered as biological liquid of primary diagnostic potential, the observed correlation between the variation rates in sweat and blood glucose levels (Figure 2) is sufficient for diagnostics obviously offering the prospect of a noninvasive approach to the monitoring of diabetes.

**Noninvasive Diabetes Monitor.** As mentioned, among the reasons why electrochemical tattoo-like systems were unsuccessful for monitoring of blood glucose was the absence of a flow outlet making it impossible to monitor the content of interstitial liquid. For example, in ref 9 one out of seven experiments displayed even negative correlation: sweat glucose decreases as a result of a blood glucose increase. As an alternative, we've used the flow-through sweat collector integrated with glucose biosensor (Figure S1, Supporting Information). Prior to use, the noninvasive monitor was calibrated with standard glucose solutions directly applied to the skin surface.

Obviously, prior to use in a monitor, the stability of glucose biosensor should be assessed. To study the biosensor operational stability, the flow-through cell mimicking the monitor (Figure S1, Supporting Information) has been constructed. For this aim, the part applied to the skin surface has been substituted by the inlet connected to the pump. As found, at the lowest flow rate possible, the biosensor response remains constant (100%) during 25 h of operation (Figure S6, Supporting Information). Taking into account that sweat gland requires restimulation (see below), this is sufficient for operation of the monitor. Shelf life of the biosensors is characterized by at least a year of storage in packed state without loss of sensitivity.

Additionally, the noninvasive diabetes monitor was validated using the standard analytical method. For this aim, upon monitor operation onto the volunteer's arm, the portions of sweat have been collected from its outlet and analyzed subsequently with the homemade flow-injection analyzer equipped with a glucose biosensor. The latter, as mentioned (Experimental Section), has been validated using EcoBasic (Care Diagnostica, Germany). The readings of the monitor have been found to be in good correlation with the results of the FIA analyzer: the corresponding Pearson correlation coefficient $r$ exceeds 0.99 (Figure S7, Supporting Information).

The proposed noninvasive diabetes monitor operates as follows. After 15 min of sweat gland stimulation (above), the monitor is applied to the same skin spot, and within the first 1−2 min the biosensor displays a response. Constant sweat flow after pilocarpine activation is observed during 20−30 min. Afterward, the same skin spot should be reactivated. Obviously, in clinical practice, subsequent stimulations of the sweat gland has to be automated. Among possible solutions is the delivery of agonists to the sweat glands from the pilocarpine based hydrogel with the aid of an electrical current.[9]

As mentioned, the glucose tolerance test seems to be the only clinically relevant procedure to mimic hyperglycemia events in healthy subjects. Figure 3 displays the real-time

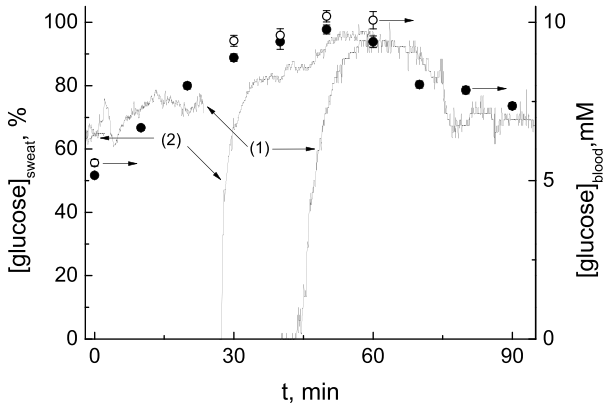


**Figure 3.** Readings of the noninvasive diabetes monitor (relative to the absolute maximum for each experiment) representing sweat glucose level (solid lines) in comparison with blood glucose (scatter, circles) in the course of glucose tolerance test: (●) blood glucose corresponding to curve 1 and (○) blood glucose corresponding to curve 2.

readings of the noninvasive monitor in the course of the two glucose tolerance tests applied to the same human subject (solid lines). As seen from both tests, the subsequent activation of the same skin spot results in similar readings of the monitor, thus allowing in future an automatic stimulation of the sweat gland (above).

Figure 3 presents the two independent glucose tolerance test experiments, separated by a week. During the first experiment, the sweat gland was activated prior to the test. This allows one to observe the monitor readings immediately after the volunteer has been loaded with glucose. The second activation of the same skin spot caused an approximately 25 min break in monitor readings (Figure 3). During the second experiment, the monitor was operated before the test and the second sweat glad activation started at the same time, when the volunteer has been loaded with glucose. This allowed monitoring sweat



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content between 35 and 60 min of the glucose tolerance test, when blood glucose concentration reaches its maximum.

Figure 3 also displays the dynamics of glucose concentration in blood taken from the volunteer's finger during the test (scatter graphs). As seen, the dynamics of sweat glucose is in good accordance with the dynamics of blood glucose. In the chosen time scale, no considerable delay in dynamics of sweat glucose vs blood is registered. The observed similar dynamics in blood and sweat glucose concentrations obviously offers a prospect for monitoring of diabetes through continuous analysis of undiluted sweat. With this example, we thus clearly show that humans (diabetic patients, individuals subjected to glucose tolerance test, etc.) can actually be monitored reliably via the proposed approach.

## CONCLUSIONS

The observed positive correlation between the variation rates in sweat and blood glucose levels undoubtedly offers the prospect of a noninvasive approach to the monitoring of diabetes. Obviously, the presented proof-of-concept data has to be used with caution, and further large-scale validation and clinical trials are required to confirm the diagnostic value of sweat glucose. We note, however, the proposed approach is of great importance, because it would allow diabetic patients to decrease blood probing significantly and monitor blood glucose content through continuous analysis of the excreted sweat. We believe that in addition to the described glucose tolerance test, the monitor will pass validation in clinics for real hyper- and hypoglycemia events.

Continuous monitoring of blood glucose concentration is of high importance because it opens a possibility to cure diabetes or at least to decrease the insulin dependence of the patients. There are also severe cases of diabetes, among which there is a possibility of death of the patient in their sleep caused by hypoglycemia, and the best solution for them is noninvasive diabetes monitoring.

In clinical practice, even current state of art with the proposed noninvasive diabetes monitor would be of interest, for instance, to investigate the kinetics of glucose consumption during glucose tolerance tests. Further efforts in this direction are highly important, because they are targeted to improve the quality of life for hundreds of millions of people.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b05928.

Figure S1, 3-D image and drawings of the flow-through glucose biosensor; Figure S2, sensitivity of glucose biosensors depending on membrane material; Figure S3, validation of glucose biosensors on blood using EcoBasic analyzer; Figure S4, direct correlation between blood and sweat glucose; Figure S5, variation rates of vein and capillary blood lactate; Figure S6, operational stability of glucose biosensor; Figure S7, validation of the diabetes monitor with FIA analyzer; and Table S1, glucose content in blood and sweat samples (PDF)

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

(1) Tura, A.; Maran, A.; Pacini, G. *Diabetes Res. Clin. Pract.* **2007**, *77*, 16−40.
(2) Vashist, S. K. *Anal. Chim. Acta* **2012**, *750*, 16−27.
(3) Goodarzi, M.; Sharma, S.; Ramon, H.; Saeys, W. *TrAC, Trends Anal. Chem.* **2015**, *67*, 147−158.
(4) Yadav, J.; Rani, A.; Singh, V.; Murari, B. M. *Biomed. Signal Proces.* **2015**, *18*, 214−227.
(5) NEC Corp. (Nidec). *Blood glucose concentration sensor-uses a low pressure suction device to extract a decoction through the skin for electrochemical analysis*. Japanese Patents JP2586594-B2 and JP2057238-A, 1997.
(6) Rao, G.; Glikfeld, P.; Guy, R. H. *Pharm. Res.* **1993**, *10*, 1751−1755.
(7) Tierney, M. J. *Electrochemical sensor with dual purpose electrode*. U.S. Patent 5,954,685, September 21, 1999.
(8) Bandodkar, A. J.; Jia, W.; Yardimci, C.; Wang, X.; Ramirez, J.; Wang, J. *Anal. Chem.* **2015**, *87*, 394−398.
(9) Emaminejad, S.; Gao, W.; Wu, E.; Davies, Z. A.; Nyein, H. Y. Y.; Challa, S.; Ryan, S. P.; Fahad, H. M.; Chen, K.; Shahpar, Z.; Talebi, S.; Milla, C.; Javey, A.; Davis, R. W. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 4625−4630.
(10) Barben, J.; Casaulta, C.; Spinas, R.; Schoeni, M. H.; Swiss Working Group for Cystic Fibrosis (SWGCF). *Swiss Med. Wkly.* **2007**, *137*, 192−198.
(11) Naehrlich, L. *Klin. Paediatr.* **2007**, *219*, 70−73.
(12) Yashina, E. I.; Borisova, A. V.; Karyakina, E. E.; Shchegolikhina, O. I.; Vagin, M. Y.; Sakharov, D. A.; Tonevitsky, A. G.; Karyakin, A. A. *Anal. Chem.* **2010**, *82*, 1601−1604.
(13) Karyakin, A. A. *Current Opinion in Electrochemistry* **2017**, *5*, 92−98.
(14) Karyakin, A. A.; Karyakina, E. E.; Gorton, L. *J. Electroanal. Chem.* **1998**, *456*, 97−104.
(15) Karyakin, A. A. *Electroanalysis* **2001**, *13*, 813−819.
(16) Komkova, M. A.; Karyakina, E. E.; Karyakin, A. A. *Anal. Chem.* **2017**, *89*, 6290.
(17) Jia, W.; Bandodkar, A. J.; Valdes-Ramirez, G.; Windmiller, J. R.; Yang, Z.; Ramirez, J.; Chan, G.; Wang, J. *Anal. Chem.* **2013**, *85*, 6553−6560.
(18) Pribil, M. M.; Laptev, G. U.; Karyakina, E. E.; Karyakin, A. A. *Anal. Chem.* **2014**, *86*, 5215−5219.
(19) Zaryanov, N. V.; Nikitina, V. N.; Karpova, E. V.; Karyakina, E. E.; Karyakin, A. A. *Anal. Chem.* **2017**, *89*, 11198−11202.
(20) Gao, W.; Emaminejad, S.; Nyein, H. Y. Y.; Challa, S.; Chen, K. V.; Peck, A.; Fahad, H. M.; Ota, H.; Shiraki, H.; Kiriya, D.; Lien, D. H.; Brooks, G. A.; Davis, R. W.; Javey, A. *Nature* **2016**, *529*, 509.
(21) Lee, H.; Song, C.; Hong, Y. S.; Kim, M. S.; Cho, H. R.; Kang, T.; Shin, K.; Choi, S. H.; Hyeon, T.; Kim, D. H. *Science Advances* **2017**, *3*, e1601314.
(22) Harvey, C. J.; LeBouf, R. F.; Stefaniak, A. B. *Toxicol. In Vitro* **2010**, *24*, 1790−1796.
(23) Burn, J. H. *J. Physiol. (Oxford, U. K.)* **1925**, *60*, 365−378.
(24) Moyer, J.; Wilson, D.; Finkelshtein, I.; Wong, B.; Potts, R. *Diabetes Technol. Ther.* **2012**, *14*, 398−402.



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*Anal. Chem.* 2019, 91, 3778−3783

(25) Karyakin, A. A.; Kotel'nikova, E. A.; Lukachova, L. V.; Karyakina, E. E.; Wang, J. *Anal. Chem.* **2002**, *74*, 1597−1603.
(26) Borisova, A. V.; Karyakina, E. E.; Cosnier, S.; Karyakin, A. A. *Electroanalysis* **2009**, *21*, 409−414.
(27) Karpova, E. V.; Karyakina, E. E.; Karyakin, A. A. *RSC Adv.* **2016**, *6*, 103328−103331.
(28) Gibson, L. E.; Cooke, R. E. *Pediatrics* **1959**, *23*, 545−549.
(29) Pribil, M. M.; Cortes-Salazar, F.; Andreyev, E. A.; Lesch, A.; Karyakina, E. E.; Voronin, O. G.; Girault, H. H.; Karyakin, A. A. *J. Electroanal. Chem.* **2014**, *731*, 112−118.
(30) Karyakin, A. A.; Karyakina, E. E.; Gorton, L.; Bobrova, O. A.; Lukachova, L. V.; Gladilin, A. K.; Levashov, A. V. *Anal. Chem.* **1996**, *68*, 4335−4341.
(31) O'Sullivan, J. B.; Mahan, C. M. *Diabetes* **1964**, *13*, 278−285.
(32) Karyakin, A. A.; Nikulina, S. V.; Vokhmyanina, D. V.; Karyakina, E. E.; Anaev, E. K. H.; Chuchalin, A. G. *Electrochem. Commun.* **2017**, *83*, 81−84.
(33) Sakharov, D. A.; Shkurnikov, M. U.; Vagin, M. Y.; Yashina, E. I.; Karyakin, A. A.; Tonevitsky, A. G. *Bull. Exp. Biol. Med.* **2010**, *150*, 83−85.
(34) Cain, S. M. *Am. J. Physiol.* **1967**, *213*, 57−63.
(35) Lee, D. C.; Sohn, H. A.; Park, Z.-Y.; Oh, S.; Kang, Y. K.; Lee, K.-m.; Kang, M.; Jang, Y. J.; Yang, S.-J.; Hong, Y. K.; Noh, H.; Kim, J.-A.; Kim, D. J.; Bae, K.-H.; Kim, D. M.; Chung, S. J.; Yoo, H. S.; Yu, D.-Y.; Park, K. C.; Yeom, Y. I. *Cell* **2015**, *161*, 595−609.



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