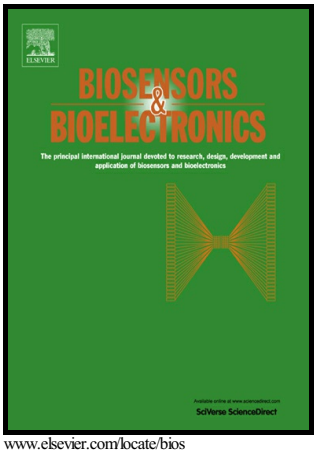


www.elsevier.com/locate/bios

In-vitro model for assessing glucose diffusion through skin

Sana Ullah, Fadi Hammade, Urte Bubniene, Johan Engblom, Arunas Ramanvicius, Almira Ramanaviciene, Tautgirdas Ruzgas

PII: S0956-5663(18)30212-4
DOI: https://doi.org/10.1016/j.bios.2018.03.039
Reference: BIOS10365

To appear in: *Biosensors and Bioelectronic*

Received date: 13 November 2017
Revised date: 24 February 2018
Accepted date: 17 March 2018

Cite this article as: Sana Ullah, Fadi Hammade, Urte Bubniene, Johan Engblom, Arunas Ramanvicius, Almira Ramanaviciene and Tautgirdas Ruzgas, *In-vitro* model for assessing glucose diffusion through skin, *Biosensors and Bioelectronic,* https://doi.org/10.1016/j.bios.2018.03.039

In-vitro model for assessing glucose diffusion through skin

Sana Ullah[1], Fadi Hammade[1,2], Urte Bubniene[3], Johan Engblom[1], Arunas Ramanvicius[3], Almira Ramanaviciene[4], Tautgirdas Ruzgas[1*]

[1]Department of Biomedical Science, Faculty of Health and Society, Malmö University, SE-205 06, Malmö, Sweden. Biofilms - Research Center for Biointerfaces, Malmö University, SE-205 06, Malmö, Sweden

[2]Sterisol AB, 592 23 Vadstena, Sweden

[3]Department of Physical Chemistry and Geoscience, Vilnius University, LT-03225 Vilnius, Lithuania

[4]NanoTechnas – Center of Nanotechnology and Materials Science, Faculty of Chemistry and Geosciences, Vilnius University, LT-03225 Vilnius, Lithuania

*Corresponding author: tautgirdas.ruzgas@mau.se

Abstract

Pig ear skin membrane-covered glucose biosensor based on oxygen electrode has been assessed as a tool to evaluate glucose penetration through skin *in-vitro*. For this, glucose oxidase (GOx) was immobilised on oxygen electrode and covered with the skin membrane. Exposing this electrode to the solution of glucose resulted in glucose penetration though skin membrane, its oxidation catalysed by GOx, consumption of O_2 and decrease of the current of the oxygen electrode. By processing the biosensor responses to glucose, we found that glucose penetration through 250 µm thick skin membrane is slow; 90% of steady-state current response was reached in 32(±22) min. Apparent diffusion coefficient for glucose in skin was found to be equal to 0.15(±0.07)*10^{-6} cm^2s^{-1}. This value is 45 times lower than glucose diffusion coefficient in water. Tape-stripping of stratum corneum (SC) allows considerably faster glucose penetration. The electrodes covered with tape-stripped skin reached 90% of steady-state current response in 5.0(±2.7) min. The theoretical estimate of glucose flux through SC was considered exploiting four-pathway theory of transdermal penetration. Theoretical flux values were more that three orders lower than measured experimentally. This high discrepancy might indicate that glucose penetration through healthy human skin could be even slower, allowing much lower flux, than it was found in our study for skin membranes from pig ears.

Keywords: glucose biosensor; attachable; skin; topical; epidermal

1. Introduction

The number of people affected by diabetes is constantly growing. Among adults, it has increased from 4.7% in 1980 to 8.5% in 2014 (WHO). Frequent monitoring of blood glucose, followed by an adequate changed life style or/and appropriate use of medication, are the most vital measures that supports management of diabetes (Rise et al. 2013). This is a reason behind the global growth of the glucose biosensor market, which according to Grand View Research Inc. estimate will reach USD 31 billion by year 2022. The majority of blood glucose measurements are done by sampling of blood from a punctured finger, which is regarded as non-convenient and painful (Heinemann 2008; Yoo and Lee 2010). This stimulates tremendous research efforts to develop non-invasive or minimally invasive glucose monitoring approaches as well as implantable glucose biosensors (Kim et al. 2018; Lee et al. 2017; Lheureux and Preiser 2014; Pickup et al. 2011). Development of new medical devices usually rely on high number of clinical trials, which could be minimised if an appropriate *in-vitro* system could be introduced. In this work, we propose and examine a simple tool to study glucose diffusion through skin *in-vitro*. The system consists of an electrochemical glucose biosensor based on an oxygen electrode covered with skin membrane from pig ear. Other biosensor designs relying on

direct electrochemical registration of H_2O_2 (O'Neill et al. 2008) or mediated electron transfer (Bandodkar et al. 2015) could be also suitable. The choice of the oxygen electrode based setup, in this study, was motivated by its robustness, simplicity of the preparation and high stability.

In general, skin membrane-covered electrode setup is a rather universal tool and has previously been used to study the kinetics of transdermal penetration of hydrogen peroxide, ascorbic acid, and quercetin (Gari et al. 2015; Rembiesa et al. 2015). Recently we also exploited this tool to study function of native enzymes in skin, specifically, skin catalase (Nocchi et al. 2017). In this work, we exploit the electrode design to experimentally assess passive transdermal glucose penetration. For the interpretation of the results we applied the four-pathway theory of transdermal penetration comprehensively summarised by Mitragotri in 2003 (Mitragotri 2003).

2. Material and methods

2.1. Material

Glucose, tablets of phosphate buffer saline (PBS), glucose oxidase from *Aspergillus niger* (lyophilized, powder, ~200 U/mg) were purchased from Sigma Aldrich. Oxygen electrode (Fig. 1) was purchased from UAB "OPTRONIKA", Vilnius, Lithuania. All solutions have been prepared by using deionised water (Milli-Q system, Merck Millipore, Billerica, USA) with resistivity of 18.2 Ω cm.

2.2. Preparation of skin membranes

Fresh pig ears were obtained from a local abattoir and stored at -80°C if not immediately used. Video about skin membrane preparation is provided in supplementary material. In brief, pig ears were rinsed with cold water and cut into 2 cm broad strips with a scalpel. The strips were shaved with trimmer machine for shaving and the 250 µm thick skin membranes were sliced with a dermatome. The resulting skin stripes were punched to make circular membranes (16 mm diameter, see Fig. 1). If not immediately used the membranes were kept in the freezer (-18°C). Before mounting on the electrode the membrane was thawed and kept at least 1 h on a filter paper soaked in PBS.

2.3. Preparation of skin membrane-covered glucose biosensor based on the Clark oxygen electrode

The tip of the oxygen electrode body (Fig. 1A, item 1), containing glass melted 250 µm diameter Pt wire was polished with alumina suspension (1 µm alumina, Buehler, Lake Bluff, IL) and rinsed with deionised water. The cup of the electrode (Fig. 1A, item 4) was covered with 5 µm thick Teflon (Fig. 1A, item 2) and 250 µm thick skin (Fig. 1A, item 3) membranes using rubber ring (Fig. 1A, item 5). The cup then was filled with saturated KCl solution and screwed onto the electrode body giving a completely assembled skin membrane-covered oxygen electrode (Fig. 1B). For making skin membrane-covered glucose biosensor based on oxygen electrode, a drop of 10 µL solution comprised of 10 mg/mL GOx in water was placed on Teflon membrane and allowed to dry under blowing air. The modified Teflon membrane was then covered with skin membrane and fixed on the electrode cup by using rubber ring. The cup was filled with saturated KCl solution and screwed onto the electrode body giving a completely assembled skin membrane-covered glucose biosensor based on oxygen electrode (Fig. 1B). It was important that this type of biosensor construction showed high stability of baseline current and low noise (see Fig. S1, supplementary material).

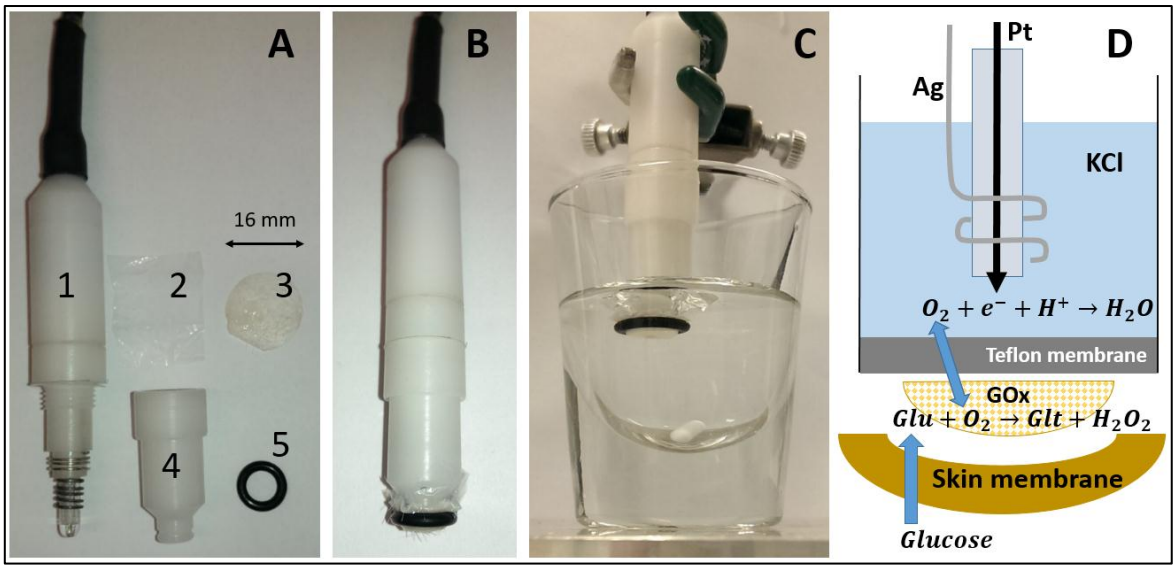


Fig. 1. Components and assembly of skin membrane-covered electrode. (**A**) Oxygen electrode body (1) comprised of silver wire serving as Ag/AgCl electrode and Pt wire melted into the glass tip of the electrode. Other parts are: (2) 5 µm thick Teflon membrane, (3) 250 µm thick, 16 mm diameter skin membrane, (4) electrode cup and (5) rubber o-ring. (**B**) A completely assembled skim membrane-covered glucose biosensor based on oxygen electrode. (**C**) Skin membrane-covered glucose biosensor in PBS solution. (**D**) Schematic representation of glucose biosensor based on oxygen electrode including essential redox reactions: GOx – glucose oxidase, Glu – glucose, Glt – gluconolactone.

2.4. Amperometric monitoring of transdermal glucose penetration in-vitro

Skin membrane-covered glucose biosensor based on oxygen electrode (Fig. 1B) was dipped into a glass filled with 10 mL PBS solution, pH 7.4 (Fig. 1C). Amperometric measurements were performed using a potentiostat AMEL (Milano, Italy) by sampling the values of the electrode current each second. The electrode was connected to potentiostat in two-electrode configuration with minus 0.7 V (*vs* Ag/AgCl/KClsat) applied on the Pt cathode of the oxygen electrode. After 15-30 min stabilisation of the baseline current, a defined amount of 1 M glucose solution was pipetted into the glass giving concentration-dependent decrease of the reduction current of the biosensor electrode (examples of current traces are presented in Fig. S2 and S3, supplementary material). The solution was mixed with magnetic stirrer. All measurements were conducted at room temperature, 22°C.

2.5. Calculation of lag-time value of the biosensor current response to glucose

For assessing apparent coefficient of diffusion for glucose in skin membranes, lag-times of the membrane-covered biosensor responses to glucose were determined as described previously (Friend 1992; Selzer et al. 2013). For this, the value of baseline current (Fig. 2A, marked with boxes) was subtracted from the current response to glucose. This gave delta current values, which were nonzero (higher than zero) only for the current measurements obtained after the addition of glucose. Then the time zero was assigned to the moment of glucose addition into the solution and the delta current values were integrated over the time. The integral *vs* time curve became linearly dependent on time (Fig. 3) when approaching a steady-state of the current response. This linear part of the integral *vs* time dependence was fitted to a linear equation and extrapolated towards time-axis (Fig. 3, dotted line). The intercept was taken for a lag-time value. All results are presented as a mean ± SD (standard deviation).

3. Results and discussion

To access the penetration of glucose through skin *in-vitro*, a 250 µm thick skin membrane from pig ear was mounted on top of glucose biosensor based on oxygen electrode. A photo of the electrode is presented in Fig. 1B. An example of the current trace recorded with the electrode in PBS solution before and after the addition of glucose is shown in Fig. 2 (curve 1, the biosensor covered with intact skin membrane). The baseline current (Fig. 2, marked with box) is due to O_2 that diffuses from PBS solution throughout the skin and Teflon membranes and gets electroreduced at the Pt cathode of the oxygen electrode. Deoxygenation of the solution by bubbling with Argon results in close to zero electrode current (see Fig. S4, supplementary material). After the addition of 3 mM glucose into the solution the electrode current decreases (Fig. 2, curve 1). This is due to the consumption of O_2 in glucose oxidation reaction (Fig. 1D). This current response indicates that glucose penetrates through 250 µm thick, intact skin membrane. Control experiments have been done to confirm that in the absence of O_2 or without an immobilised layer of GOx the electrode does not respond to glucose (see Fig. S4 and S5, respectively, supplementary material).

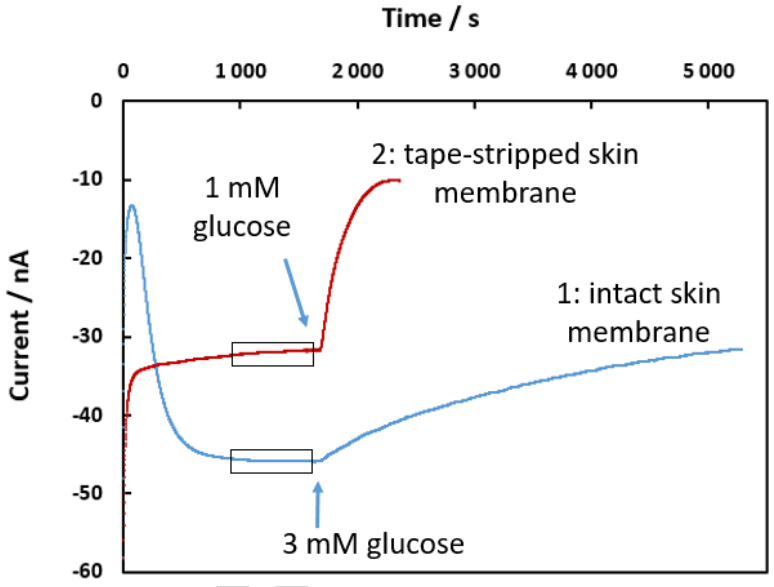
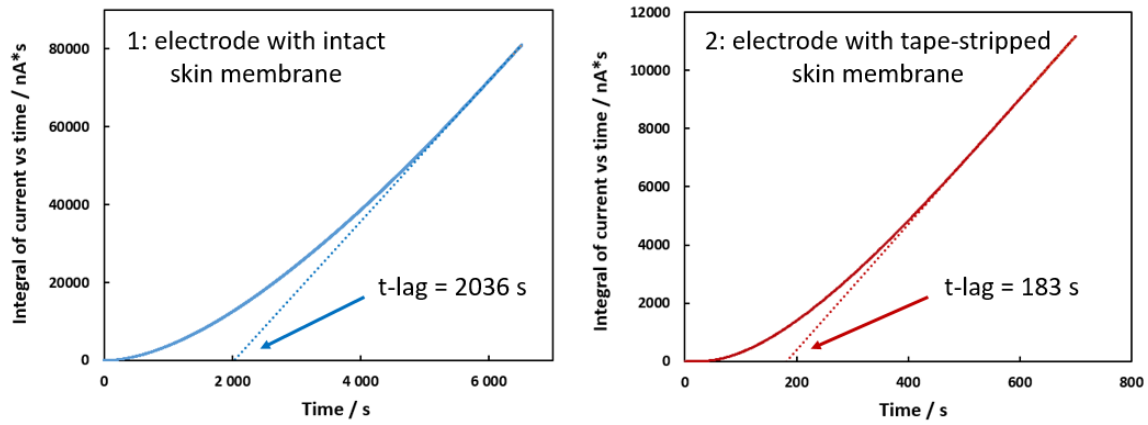


Fig. 2. Amperometric response of skin membrane-covered glucose biosensor to glucose. The electrode is immersed in stirred PBS solution, pH 7.4. The electrodes were covered by (1) intact and (2) tape-stripped (removed stratum corneum) skin membrane, respectively. Addition of glucose into PBS solution are indicated by arrows. Baseline current is marked by a box.

It is well accepted that the toughest penetration barrier is the most distal skin layer comprised of dead corneocytes surrounded by skin lipids (Prausnitz et al. 2012). This, approximately 10 µm thick, layer is named stratum corneum (SC). Thus, it is of importance to investigate the response of the electrode covered with skin membrane lacking SC. Measurements with SC-free skin membrane resembles situation of implantable biosensor or the case when SC is damaged by electroporation, microneedles, ultrasound, electroosmosis, etc. SC was removed by well-known 20-times tape-stripping procedure of skin membrane (Nocchi et al. 2017; Tsai et al. 2003). The response to glucose obtained with the electrode covered with SC-free skin membrane is shown in Fig. 2 (curve 2). It can be seen that the response is much quicker though only about 10 µm thick layer is removed from the total 250 µm thick skin membrane.

To assess apparent diffusion constants characterising permeation of glucose through intact and tape-stripped skin, current response measurements (similar as in Fig. 2) were carried out for several skin membranes and used to determine lag-time values. Fig. 3 illustrates the procedure for the data



presented in Fig. 2.

Fig. 3. Illustration of the procedure for determining lag-time of the current response of skin membrane-covered biosensor to glucose. Integral of the current response (baseline current subtracted current) over time for data presented in Fig. 2. Time zero in this plot corresponds to the moment of glucose addition into the solution (marked with arrows in Fig. 2). The linear fit, its extrapolation to time-axis, and determination of the intercept marked with arrow illustrate the procedure of the lag-time determination; the electrode covered with (1) intact and (2) tape-stripped skin membrane, respectively.

The values of lag-time were used to calculate apparent diffusion coefficients characterising passive glucose penetration (or flux) in skin (Eq. 1) (Friend 1992; Selzer et al. 2013).

$$D_{app} = \frac{d^2}{6t_{lag}} \qquad (1)$$

D_{app} is apparent diffusion coefficient, d is thickness of the membrane equal to 250 µm for intact skin and 240 µm for skin membranes with removed SC (SC is assumed to be equal to 10 µm, (Mitragotri 2003; Prausnitz et al. 2012)), t_{lag} is a lag-time of the current response.

The average lag-times for transdermal glucose penetration were found to be equal to 860 ± 520 (n = 8) and 150 ± 69 (n = 4) s for the electrodes covered with intact and tape-stripped skin membranes, respectively. Corresponding times for reaching 90% current response were 1900 ± 1300 and 300 ± 160 s, respectively. The values of the apparent diffusion coefficient for the intact skin and tape-stripped skin membranes were found to be equal to $0.15(\pm0.07)*10^{-6}$ and $0.84(\pm0.59)*10^{-6}$ cm^2s^{-1}, respectively. Graphical presentation and the table with the values can be found in supplementary material, Fig. S6. The D_{app} for case of intact skin, $0.15(\pm0.07)*10^{-6}$ cm^2s^{-1}, is close to previously reported glucose diffusivity, $0.075(\pm0.05)*10^{-6}$ cm^2s^{-1}, in human epidermis (Khalil et al. 2006). The diffusion coefficient found for case of tape-stripped skin approaches the diffusivity of glucose in dermis, $2.64(\pm0.42)*10^{-6}$ cm^2s^{-1}. Slightly lower value obtained in our experiments is reasonable since tape-stripping removes only SC and leaves viable epidermis, which is still a considerable biological barrier (Khalil et al. 2006).

Comparing the values of D_{app} to the known diffusion coefficient for glucose in water, $6.7*10^{-6}$ cm^2s^{-1}, it can be concluded that diffusion of glucose is about eight times slower in tape-stripped skin which comprises dermis and viable epidermis. Adding 10 µm thick SC layer makes D_{app} about 45 time lower than the value of the diffusion coefficient for glucose in water. The experiments conducted with skin membrane-covered glucose biosensor presented in this work confirm that thin SC layer as well as viable epidermis constitute a considerable diffusional barrier for passive glucose penetration (Khalil et al. 2006).

The experiments with the removed SC model an implantable sensor situation where glucose from blood capillaries might need to, passively, diffuse for few 10^{th} or 100^{th} of µm in order to reach the implanted biosensor. Our experiments indicate that in this case sensor response might be delayed for few minutes, e.g., on average 300 s were needed to reach 90% of the steady-state current response for electrodes covered with tape-stripped skin membranes.

In addition to lag-time determination, the steady-state current for 1 mM glucose was measured for eight, intact skin membrane-covered electrodes and was found to be equal to 3.1(±2.1) nA. The appreciation of this experimentally derived current value can be theoretically rationalized by exploiting the four-pathway penetration theory (Mitragotri 2003). We hope that the following theoretical considerations will enable better understanding how glucose penetrates the skin tissue and will provide the basis for developing and optimising advanced methods of topical glucose sampling. Since the main barrier to the penetration has been found to be SC we consider the penetration of glucose through SC more thoroughly.

The penetration of hydrophilic solutes though SC has been well described. The best flux estimation through SC is obtained by exploiting the four-pathways penetration theory (Chen et al. 2013; Mitragotri 2003). The theory accounts for structural features of SC and states that depending on the molecular weight (MW) and the lipophilicity (log K_{ow}) of the compound its transdermal penetration proceed through four routes with possibly one being a dominant pathway. Basically, low MW lipophilic compounds penetrate mainly through lipid layers of SC by a mechanism of free volume diffusion. High MW (> 500 Da) lipophilic molecules will also penetrate through lipid structures of SC, however, their penetration is much slower since they diffuse together with a relatively slow lateral translocation (mobility) of skin lipids. Water and hydrophilic molecules such as H_2O_2, glucose, etc. will mainly penetrate through 2-3 nm diameter pores (pore pathway). Due to the fact that the pore network in SC is very scarce, the transdermal flux of hydrophilic molecules usually is low (Prausnitz et al. 2012). High MW hydrophilic compounds penetrate through hair follicles, sweat ducts, imperfections, etc., commonly named as shunts. Their penetration is very slow due to low abundance of the mentioned structural organizations. According to the four-pathway penetration theory, glucose should mainly penetrate SC though pores. The theoretical flux of solute or permeant (in our case - glucose) can be expressed by the following equation:

$$flux\left[\frac{mol}{cm^2 s}\right] = (K_p^{fv} + K_p^{lateral} + K_p^{pore} + K_p^{shunt}) \cdot C\left[\frac{mol}{cm^3}\right] \qquad (2)$$

K_p^{fv}, $K_p^{lateral}$, K_p^{pore}, and K_p^{shunt} in $\left[\frac{cm}{s}\right]$, are permeability constants accounting for free-volume, lateral, pore and shunt mode of solute diffusion, respectively. C is the concentration of permeant at the surface of SC.

Assuming that the experimentally used skin membranes contain SC with average structural features of human skin, the K_p constants can be represented by specific expressions (Mitragotri 2003). The specific expressions for Eq. 2 are provided in supplementary material. The calculated values for

respective permeability constants and total flux of glucose penetration through SC are presented by Eq. 3, giving appreciation of the contribution of each individual pathway to the flux.

$$flux\left[\frac{mol}{cm^2 s}\right] = (0.26 \cdot 10^{-9} + 7.8 \cdot 10^{-12} + 8.2 \cdot 10^{-9} + 2 \cdot 10^{-9}) \cdot C\left[\frac{mol}{cm^3}\right] \quad (3)$$

From Eq. 3 it follows that pore pathway dominates (the highest K_p, compare Eq. 2 and 3) contributing with 75 % of glucose flux. The total theoretically estimated flux at 1 mM glucose concentration is equal to $0.011\left[\frac{pmol}{cm^2 s}\right]$:

$$flux\left[\frac{mol}{cm^2 s}\right] = 1.1 \cdot 10^{-8} \cdot 1 \cdot 10^{-6}\left[\frac{mol}{cm^3}\right] = 1.1 \cdot 10^{-14} = 0.011\left[\frac{pmol}{cm^2 s}\right] \quad (4)$$

The theoretical value of flux is practically the same to 0.01 $\left[\frac{pmol}{cm^2 s}\right]$, which was determined experimentally by Pellett *et al* for human skin membranes (Pellett et al. 1999). By using this flux estimate the expected current of 250 µm diameter oxygen electrode would be equal to $1\ pA$. The calculations are presented in supplementary material.

The experimentally measured current is however about three orders of magnitude higher, i.e., 3.1(±2.1) nA. There might be several reasons for this, theory *vs* experiment, discrepancy. First, in calculations we considered planar diffusion to 250 µm diameter microelectrode. Contribution from radial diffusion could probably be substantial and results to experimentally higher current by up to two orders of magnitude. Secondly, the assumptions accounting for an average SC structure are taken from the literature and are for human SC (Mitragotri 2003). Generally, the skin from pig ear is considered to be a good model of human skin, especially, for lipophilic molecules. However, the permeability of hydrophilic compounds through skin from pig ear might be an order higher (Barbero and Frasch 2009; Dick and Scott 1992). Thus, the reasoning of high discrepancy between the theory and experiments is possible, unfortunately, it diminishes the applied value of the theory. The theory-experiment mismatch, especially, for highly hydrophilic solutes has been recently criticized (Chen et al. 2013). The prediction might give errors of theoretically estimated flux reaching 2 to 6 order differences from the experimentally measured values. E.g., more than two orders higher, experimentally found flux for glucose (3.8 $\left[\frac{pmol}{cm^2 s}\right]$ compare to 0.011 $\left[\frac{pmol}{cm^2 s}\right]$, Eq. 4) has been reported (Youenang Piemi et al. 1998). Despite the theory-experiment mismatch being high, the theoretical exercise (Eqs. 2, 3 and 4) provides an important estimate on relative contribution of different pathways to passive glucose penetration through skin.

The experimental results and theoretical consideration presented above indicate advantages and disadvantages of skin membrane-covered biosensor setup for characterization of glucose flux trough skin. This characterization is, obviously, important in the development of attachable glucose biosensors. The tool, however, might become important for studies of glucose containing cosmetic or health care formulations (see Fig. S7, supplementary material).

4. Conclusions

In this work we demonstrate that skin membrane-covered glucose biosensor based on oxygen electrode allows characterization of glucose penetration through skin *in-vitro*. The results show that intact skin imposes considerable lag-time for transdermal penetration of glucose. Experimentally, we determined that it took about 30 min to reach 90% of steady-state response for glucose at electrodes covered with intact 250 µm thick skin membranes. By tape-stripping the layer of SC we demonstrate that this time has been reduced to about 5 minutes. Theoretical glucose diffusion analysis using the

four-pathway penetration theory confirms that glucose penetration through skin is dominated by pore pathway. High discrepancy between the theoretical and experimental values of transdermal glucose flux might indicate that glucose penetration through healthy human skin might be even slower, and allowing smaller flux, than it was found for skin membranes from pig ear used in this study.

Acknowledgements

Financial support from The Knowledge Foundation (FH, JE, TR) and The Gustaf Th. Ohlsson Foundation (JE, TR) are greatly acknowledged. AR and AR are acknowledging financial support from Research Council of Lithuania for project No SEN-21/2015.

REFERENCES

Bandodkar, A.J., Jia, W., Yardimci, C., Wang, X., Ramirez, J., Wang, J., 2015. Tattoo-based noninvasive glucose monitoring: A proof-of-concept study. Anal. Chem. (Washington, DC, U. S.) 87(1), 394-398.

Barbero, A.M., Frasch, H.F., 2009. Pig and guinea pig skin as surrogates for human in vitro penetration studies: A quantitative review. Toxicol. in Vitro 23(1), 1-13.

Chen, L., Han, L., Lian, G., 2013. Recent advances in predicting skin permeability of hydrophilic solutes. Adv. Drug Delivery Rev. 65(2), 295-305.

Dick, I.P., Scott, R.C., 1992. Pig ear skin as an in vitro model for human skin permeability. J. Pharm. Pharmacol. 44(8), 640-645.

Friend, D.R., 1992. In vitro skin permeation techniques. J. Controlled Release 18, 235-248.

Gari, H., Rembiesa, J., Masilionis, I., Vreva, N., Svensson, B., Sund, T., Hansson, H., Morén, A.K., Sjöö, M., Wahlgren, M., Engblom, J., Ruzgas, T., 2015. Amperometric in vitro monitoring of penetration through skin membrane. Electroanalysis 27, 111 - 117.

Heinemann, L., 2008. Finger pricking and pain: a never ending story. J Diabetes Sci Technol 2(5), 919-921.

Khalil, E., Kretsos, K., Kasting, G.B., 2006. Glucose partition coefficient and diffusivity in the lower skin layers. Pharm. Res. 23(6), 1227-1234.

Kim, J., Campbell, A.S., Wang, J., 2018. Wearable non-invasive epidermal glucose sensors: A review. Talanta 177, 163-170.

Lee, H., Song, C., Hong, Y.S., Kim, M.S., Kang, T., Shin, K., Hyeon, T., Kim, D.-H., Cho, H.R., Choi, S.H., 2017. Wearable/disposable sweat-based glucose monitoring device with multistage transdermal drug delivery module. Sci Adv 3(3), e1601314.

Lheureux, O., Preiser, J.-C., 2014. Year in review 2013: Critical Care--metabolism. Critical care (London, England) 18(5), 571.

Mitragotri, S., 2003. Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. J. Controlled Release 86, 69-92.

Nocchi, S., Bjoerklund, S., Svensson, B., Engblom, J., Ruzgas, T., 2017. Electrochemical monitoring of native catalase activity in skin using skin covered oxygen electrode. Biosens. Bioelectron. 93, 9-13.

O'Neill, R.D., Rocchitta, G., McMahon, C.P., Serra, P.A., Lowry, J.P., 2008. Designing sensitive and selective polymer/enzyme composite biosensors for brain monitoring in vivo. TrAC, Trends Anal. Chem. 27(1), 78-88.

Pellett, M.A., Hadgraft, J., Roberts, M.S., 1999. The back diffusion of glucose across human skin in vitro. International Journal of Pharmaceutics 193(1), 27-35.

Pickup, J.C., Freeman, S.C., Sutton, A.J., 2011. Glycaemic control in type 1 diabetes during real time continuous glucose monitoring compared with self monitoring of blood glucose: meta-analysis of randomised controlled trials using individual patient data. BMJ (Clinical research ed.) 343, d3805.

Prausnitz, M.R., Elias, P.M., Franz, T.J., Schmuth, M., Tsai, J.C., Menon, G.K., Holleran, W.M., Feingold, K.R., 2012. Skin Barrier and Transdermal Drug Delivery. In: Bolognia, J., Jorizzo, J.L., Schaffer, J.V. (Eds.), Dermatology, pp. 2065-2073. Saunders (W.B.) Co. Ltd., London, 2012.

Rembiesa, J., Gari, H., Engblom, J., Ruzgas, T., 2015. Amperometric monitoring of quercetin permeation through skin membranes. Int. J. Pharmaceutics 496, 636-643.

Rise, M.B., Pellerud, A., Rygg, L.O., Steinsbekk, A., 2013. Making and maintaining lifestyle changes after participating in group based type 2 diabetes self-management educations: a qualitative study. PLoS One 8(5), e64009.

Selzer, D., Abdel-Mottaleb, M.M.A., Hahn, T., Schaefer, U.F., Neumann, D., 2013. Finite and infinite dosing: Difficulties in measurements, evaluations and predictions. Adv. Drug Delivery Rev. 65, 278-294.

Tsai, J.C., Shen, L.C., Sheu, H.M., Lu, C.C., 2003. Tape stripping and sodium dodecyl sulfate treatment increase the molecular weight cutoff of polyethylene glycol penetration across murine skin. Arch. Dermatol. Res. 295(4), 169-174.

Yoo, E.-H., Lee, S.-Y., 2010. Glucose biosensors: an overview of use in clinical practice. Sensors (Basel) 10(5), 4558-4576.

Youenang Piemi, M.P., de Luca, M., Grossiord, J.-L., Seiller, M., Marty, J.-P., 1998. Transdermal delivery of glucose through hairless rat skin in vitro: effect of multiple and simple emulsions. International Journal of Pharmaceutics 171(2), 207-215.

Highlights

- Skin membrane-covered glucose biosensor can be used to study glucose penetration through skin
- 250 µm thick skin from pig ear delays the biosensor response for 30 min
- The response time decreases to 5 min after removing stratum corneum from the skin membrane
- The four-pathway penetration theory points to holes in skin as the main transdermal glucose diffusion pathway