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Transdermal Glucose Monitoring Using Glucose Binding Protein-Based Fiber Optic Biosensor Coupled with Microneedles

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Abstract: In this study, microneedles were used to facilitate a faster diffusion of transdermal glucose. Microneedles were used in transdermal drug delivery studies. A franz cell with pigskin as a membrane was employed as the main setup for *in vitro* passive diffusion studies. Real time monitoring of diffusion of transdermal glucose was monitored using a fiber optic biosensor with glucose binding protein immobilized on Nickel-Nitriloacetic acid agarose beads as sensing membrane. Results showed faster diffusion rates with the use of microneedles compared with uncompromised skin and the transdermal glucose concentrations were also increased. This showed that microneedles could be promising tool for noninvasive glucose sensing.

Keywords: Microneedles, Transdermal glucose, Fiber optic biosensor.

1. Introduction

Current FDA-approved practice involves collecting a small blood that is then analyzed at the point of care (POC) or a slightly larger amount of serum sent to the lab for analysis. Alternatively, a probe is inserted transcutaneously for continuous monitoring. These can be painful and can add to the trauma of children in the pediatric intensive care unit (PICU), where the monitoring of glucose is an essential part of proper care. To eliminate pain and discomfort associated with blood draws or inserting an indwelling needle will be a welcome development for these young patients, their parents and their caregivers. Currently, glucose measurements are taken every hour upon admission and may continue for many hours after.

In previous work, we have shown that we can collect and measure transdermal glucose (TG) by passive diffusion through to a small amount of buffer on the skin surface [1]. The results of the oral glucose challenge test on healthy adults and diabetic patients

showed a good correlation between blood glucose (BG) and TG. This is done without any drastic pre-treatment on the skin beyond washing and drying. The low levels of TG were detected by a highly selective and sensitive biosensor glucose binding protein (GBP). Because the concentrations of TG are in the micromolar levels, the GBP provides the optimum sensitivity. At these concentrations, conventional glucose oxidase sensors are not practical to use.

The long-term objective of this study is a painless, bloodless and safe minimally invasive glucose monitoring system for the pediatric intensive care unit. Microneedles are known to be used in transdermal drug delivery studies [2–5]. Some groups have fabricated microneedle arrays for continuous glucose monitoring [6, 7] and insulin therapy [8]. In this study, microneedles (MN) were used to create microchannels of twenty to several hundred microns deep through the stratum corneum [9]. Pain was minimum and no blood was collected. A fiber optic sensor with highly sensitive fluorescently labeled glucose binding protein was used to measure the rate of diffusion of glucose. In previous work, we have shown that we can detect transdermal glucose (TG) by passive diffusion through skin to a small amount of buffer on the skin surface. This was possible because of the extremely sensitive glucose biosensor with a *K*d ~ μM. Other researchers working on the GBP attempted to alter the sensitivity of GBP to match the mM blood and interstitial fluid concentrations [10, 11]. We kept the GBP structure close to the wild type to preserve its sensitivity and selectivity while altering it enough to attach an environmentally sensitive fluorescent probe and a His-tag for immobilization [12].

The innovation in this study is the combination of microneedles (MNs) and the optical GBP biosensor. By applying MNs briefly onto the skin to create shallow microchannels on the stratum corneum, the rate of glucose diffusion and the rate of sampling can be enhanced relative to simple passive diffusion. The process is similar to the use of a lancet and a glucometer, which is very familiar to health care professionals and ambulatory patients. However, the technology behind it is very different and no bleeding is required. In addition, the very brief application of MNs rather than keeping them embedded in the skin for a long period of time minimizes potential irritation.

Microneedles typically measure around 0.1 – 1 mm in length, which reduces the odds of reaching nerve endings and deeper skin layers making them a minimally invasive and painless technology [9]. The purpose is to create the microchannels through the stratum corneum. After applying the MNs, the diffusion of glucose through the skin is detected by the fluorescent GBP. The specific aim of this study is to conduct *in vitro* flow cell experiments on pigskin models using microneedles. This is simpler and less complicated in that the MNs do not reside the skin. Rather, the MNs serve as microlancets to make the skin more permeable to glucose diffusion. Only the outer dead layer of cells (corneocytes) is breached as microchannels of about 10 - 50 μm diameter and 20 – 400 μm in depth are formed. The sensor itself does not touch the skin but measures the rate of diffusion of transdermal glucose in the buffer.

2. Experimental

2.1. Materials

D-glucose was obtained from Sigma-Aldrich and phosphate buffered saline (PBS) solution (1x, pH 7.4) was obtained from Gibco Life Technologies. Yucatan miniature hairless female pigskin (6 months) was shipped by Sinclair BioResources (Columbia, MO).

2.2. Franz in-line Flow Cell for Glucose Sensing

Passive diffusion of glucose was demonstrated through a membrane (porcine skin) mounted on a Permegear® static type (4G-01-00-15-12) Franz cell (Perme Gear, Hellertown, PA). Franz cell was made from glass with a receptor volume of 12 mL. The area for diffusion was 1.77 cm^2. Standard glucose solutions from 2 mM (36 mg/dL) to 40 mM (720 mg/dL) were placed in the bottom reservoir while diffusion of glucose is measured in phosphate buffered saline (PBS) buffer in the top reservoir. Sample PBS buffer of 300 μL volume was placed on top and allowed to sit on the membrane until changes in the sensor reading (potential in mV) were observed. The fiber optic biosensor was placed on top part of the franz cell. Fig. 1 shows the actual franz cell set-up with fiber optic biosensor used in the experiments. Diffusion rates were measured based on the slope of potential (mV) and time to reach steady state readings using the Ni-NTA-GBP based fiber optic biosensor. The biosensor used was developed in previous studies [12].

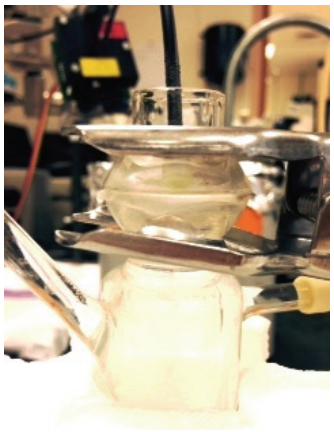

Fig. 1. *In vitro* Franz cell setup for measuring the passive diffusion of transdermal glucose with the fiber optic biosensor.

2.3. The use of Microneedles

The same procedure was repeated but after briefly pressing and withdrawing 250 µm long MNs onto the skin for 2 min. The microneedles and porcine skin were placed on top of a polydimethylsiloxane (PDMS) Sylgard® 184 Silicone Elastomer (Dow Corning) supplied as a two-part consisting of pre-polymer (base) and cross-linker (curing agent) components [13]. Fig. 2 shows the use of MNs onto the porcine skin before TG sampling.

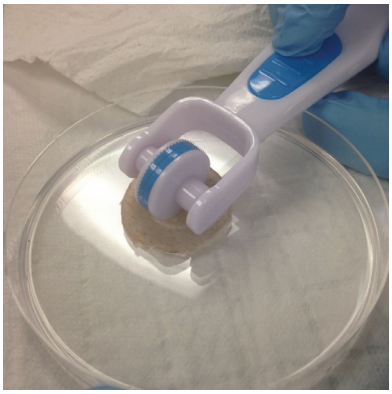

Fig. 2. Application of microneedles on porcine skin.

2.4. Transepidermal Water Loss (TEWL) Measurement

After MN application, TEWL was measured using a Delfin Vapometer (Delfin Technologies, Finland) and skin thickness was measured using a digital caliper (Mitutoyo). Three TEWL readings were taken at every time point.

3. Results

3.1. Passive Diffusion of Transdermal Glucose

Real time diffusion of transdermal glucose from our *in vitro* model was seen in tablet/computer with *LabView* software. Fig. 3 shows the diffusion rates for 2.0, 5.0 and 20 mM for both uncompromised/plain skin and skin with application of MNs. It can be seen that the diffusion rates were faster with the use of MNs and slopes were of higher value.

3.2. Transepidermal Water Loss (TEWL) Measurement

After applying MNs to the porcine skin surface, TEWL was measured. It can be seen that the TEWL increased up to 10 times, thus increasing the diffusion rate of glucose. The skin was exposed to microneedles for only about 2 min before the passive diffusion *in vitro* testing. Fig. 4 shows the TEWL measurement onto the skin with and without microneedles.

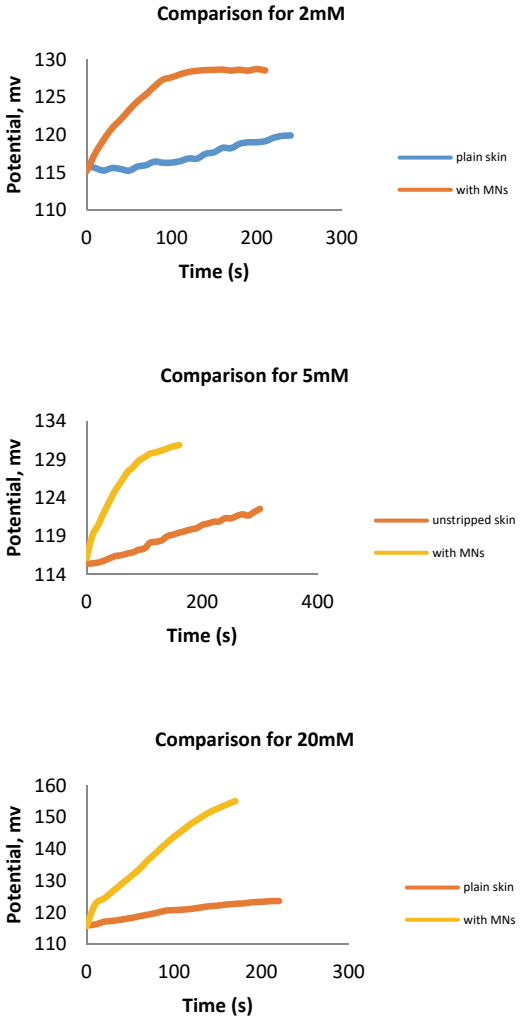

Fig. 3. Passive diffusion of glucose as detected by GBP Biosensor.

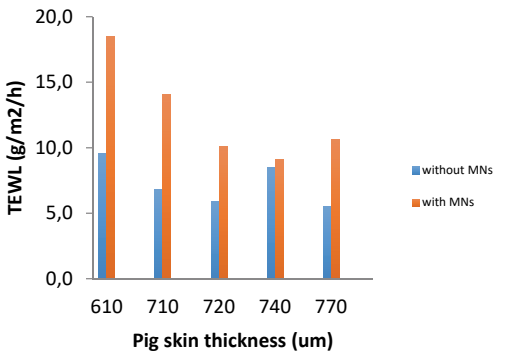

Fig. 4. TEWL measurements on porcine skin with and without application of MNs.

3.3. Correlation Between Transdermal Glucose (TG) and Glucose at the Bottom Chamber

As expected, higher slopes and faster diffusion rates were observed with the use of microneedles. Fig. 5 and Fig. 6 showed the relationship between the glucose inside the franz cell and the glucose concentration in the collected samples without and with the use of MNs. It can be seen that the glucose concentration collected in the samples is linearly correlated to the glucose inside the chamber, with $R^2 = 0.99157$ and $R^2 = 0.99344$ for without and with the use of microneedles respectively. This suggests that, by measuring the glucose on the surface of a semi-permeable membrane, the glucose concentration inside can be calculated with appropriate calibration.

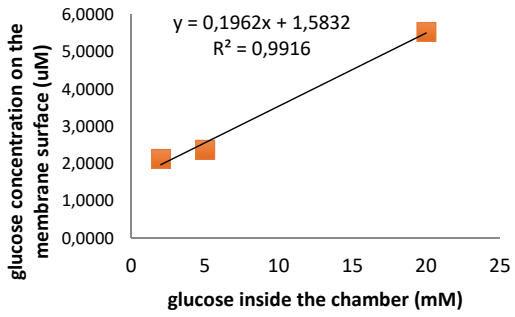

Fig. 5. Correlation between TG collected (μM) and glucose inside the chamber (mM) (without MNs) (n = 4).

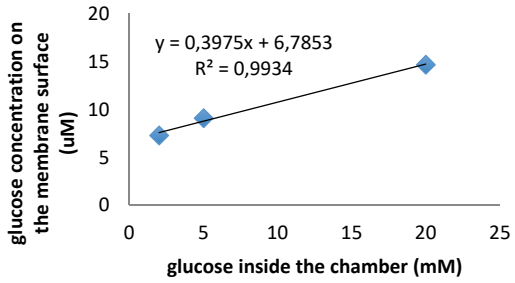

Fig. 6. Correlation between TG collected (μM) and glucose inside the chamber (mM) (without MNs) (n = 4).

4. Discussion

Microneedles are promising and powerful tool for the delivery of drugs and vaccines into the skin [9]. In this study, microneedles are used to improve the rate of diffusion of transdermal glucose. As the stratum corneum is only 10-20 μm thick, microneedles with lengths of up to a few hundreds of microns could cross the stratum corneum to enhance drug delivery [9]. The group demonstrated in *in vitro* experiments that applications of MNs (about 250 μm in length) could increase passive diffusion by up to 10 times. The MNs were only applied before *in vitro* testing and not the entire period of TG sampling. From the preliminary testing, we have found that almost no pain and only a mild feeling of pressure were experienced when the MNs were applied to the finger compared with the arm.

TEWL has been extensively used to characterize skin barrier function [14]. The finger has the thickest stratum corneum in the body yet has the highest TEWL. From passive diffusion, it is found that we can collect 10X more glucose from the finger than other parts of the skin [15]. There was also no irritation when the MNs were applied to the finger. This is probably due to the thicker stratum corneum. Nonetheless, we will ensure to prevent infection by swiping the area with an alcohol swab prior to and after application of MNs. In addition, a wide range of glucose levels as low as 13 mg/dL to as high as 1,839 mg/dL are presented in PICU. This requires a glucose-sensing device that is capable to detect such wide range.

5. Conclusions

This study demonstrated the use of microneedles to increase the diffusion rate of transdermal glucose. This could also seen through the use of fiber optic biosensor based on immobilized Ni-NTA-GBP. TEWL also increases as MNs were applied and this method of application can only cause minimal pain to almost no pain since the MNs were only applied for a few minutes. Further studies could be devoted on the use of microneedle patches.

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