STUDY PROTOCOL
NEEDLE-FREE SENSING OF BLOOD GLUCOSE

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1. Background

Diabetes is diagnosed in 5.8% of the population of New Zealand, and disproportionately affects lower socioeconomic status groups, as well as Māori, Pacific, and Asian peoples [1]. Type 1 and insulin dependent type 2 diabetics require injections of insulin multiple times per day to manage the glucose concentration in their blood. The appropriate dose of insulin is determined using a measurement of blood glucose concentration that typically requires the fingertip be pricked with a lancet. The drop(s) of blood resulting from this prick are used to perform the glucose measurement. In an effort to make this process easier for diabetics, we are investigating whether this testing can be achieved with, and integrated into, a needle-free jet injection device. Performing this process without a needle/lancet will avoid the associated issues of sharps waste, accidental needle stick, and needle-phobia [2].

Needle-free jet injection is an established method of transdermal drug delivery. The technique involves the liquid drug being formed into a high speed jet which is capable of penetrating the skin, thus removing the need for a needle. Jet injection has been shown to be an effective delivery method for vaccines, insulin, hormones, and anaesthetics, among others [2], [3]. In many studies, the pain and discomfort associated with a jet injector has been shown to be similar to that experienced with the equivalent needle based injection [2].

While blood extraction with a jet injector has not yet been trialled, one study investigated the ability of a jet injector to extract interstitial fluid [4]. This study attempted to collect the fluid back through the same small orifice through which the jet was formed, and waited less than 0.5 seconds between breaking the skin and collecting the fluid. The volume of fluid collected ranged between 10 µL and 40 µL but was found to be mainly derived from the fluid that was injected, with interstitial fluid making up between 1% and 3%. As demonstrated by this study, we anticipate that one of the major problems with obtaining a capillary blood sample using jet injection is that the resulting blood sample may be diluted with an unknown amount of the injected fluid. As part of this study we wish to investigate the extent of this dilution issue. We propose to measure the dilution by marking our injected fluid with indocyanine green (ICG), an FDA approved fluorescent marker. By exciting the retrieved blood samples with 780 nm light, and measuring the resulting fluorescence we will be able to measure the dilution of the sample.

ICG is commonly used for diagnostic purposes (e.g., cardiocirculatory, retinal and liver function diagnostic tests), typically at a dose of no more than 2 mg/kg and a concentration of 5 g/L [5]. ICG has been delivered transdermally into the subcutaneous tissue in multiple fluorescent lymphography studies, and into the dermal layer in a recent study performing fluorescent microscopy. Delivery of 20 µL of 5 g/L ICG into the dermis was described as having a "short-time tolerable burning sensation" [6], but in this study we plan to use a concentration of only 10 mg/L.

2. Research Aims

In this study we intend to use needle-free jet injection not to deliver a drug, but instead just to pierce the skin with a small volume of isotonic saline or ICG solution, and then retrieve a capillary blood sample. Ultimately we believe a single jet injection device may be able to extract blood, perform a blood glucose measurement and then, based on this measurement, deliver the ideal volume of insulin. However, this study will focus only on establishing the ability of this technique to achieve a measurement of blood glucose concentration.

This work will test the ability of a needle-free jet injection device to release capillary blood in order to measure glucose concentration. We wish to evaluate how this technique compares to the current best practice where the skin is broken with a lancet. We will compare jet injections performed with a standard circular orifice and two custom orifices that produce jets of rectangular shape ('slot' orifices). The custom slot orifices are designed to mimic the shape of the puncture made by a lancet in the skin, and we hypothesise that this may assist in obtaining a blood sample with the jet injector. The comparisons will require measurement of the volume of blood released, blood sample dilution, blood glucose concentration, and participant discomfort.

2.1. Principal Research Question

"Can a needle-free jet injection device be used to obtain a capillary blood sample for a glucose concentration measurement?"

This can be broken down into five further questions which relate directly to the measurements we will be making:

a) Does a jet injector release a similar volume of capillary blood relative to a lancet?
b) Does the use of a jet injector dilute the capillary blood sample, and, if so, how does this dilution change over time as blood is released?
c) Does a slot orifice help release more blood than a circular orifice? And is the dilution of the collected blood different for jet injection with a slot orifice versus a circular orifice?
d) Is the use of a jet injector for this purpose associated with similar, or lesser, levels of discomfort relative to a lancet?
e) Does a jet injection lead to any significantly different skin reactions at the intervention site relative to a lancet prick?

3. Research Methods

3.1. Study Design Overview

This is a pilot study aiming to assess the feasibility of using a jet injector in the place of a lancet when retrieving a capillary blood sample. Each participant in the study will receive four interventions: the current best practice for capillary blood sampling (a lancet prick), a jet injection with a standard circular orifice, and two jet injections with different custom slot orifices. This study is designed to be single-blinded and all participants will serve as their own control as each participant will receive the lancet based sampling. Participants will be aware they are receiving one of each intervention, but will be blinded to the order and identity of which intervention is occurring at which site. The study will be conducted in the ground floor laboratory space (G20) of the Auckland Bioengineering Institute (70 Symonds Street).

3.2. Population & Recruitment

We intend to recruit up to 35 healthy participants to take part in this study. These participants will be divided into two groups with up to 25 participants in the first group and up to 10 in the second. The difference between these groups relates to the measurements that will be made on their blood samples, and also in the injectate used to obtain these measurements. The samples gathered from participants in the first group, using isotonic saline for the jet injections, will be tested for glucose concentration, while those from the second group, using isotonic saline with ICG for the jet injections, will have a fluorescent based dilution measurement. Participants will be aware that the study is structured in this way, and will be aware of which group they are being recruited into.

With a minimum sample size of 20 in the first group we predict we will be able to distinguish statistically significant differences between groups whose mean dilution differs by as little as 10% ($\sigma_{glucometer}$=0.6 mmol/L, α=0.05, β=0.1). We predict we will be able to observe differences in mean dilution as low as 5% in group 2 with a sample size of at least 8 ($\sigma_{fluorimeter}$=3% , α=0.05, β=0.1). Sample volume is measured identically across both groups and we predict we will be able to observe differences in mean volume released as low as 0.84 µL ($\sigma_{BloodVolume}$=1 µL , α=0.05, β=0.1).

The study will be advertised within the Auckland Bioengineering Institute and Department of Engineering Science. This will involve flyers posted within Auckland Bioengineering House (70 Symonds Street) and an email sent to the Auckland Bioengineering Institute. These advertising materials will recommend that anybody interested in participating get in contact with the researchers for more information. In response to expressions of interest the researchers will provide a copy of the participant information sheet (PIS) and consent form (CF) to give detailed information about the study. Participants will be asked to take their time reading these forms and, if they are interested in participating, to indicate times that might suit them to come in for the study. This will also allow potential participants adequate opportunity to ask questions and seek external advice regarding participation in the study.

Participants will be required to visit the Auckland Bioengineering Institute for one 45 minute session where the interventions will take place. The participants will be asked to complete a short questionnaire 24 hours after the interventions, and send a photograph of the fingertips to the researchers.

We will aim for gender and ethnic representation in proportion with the greater New Zealand population. We imagine we will be able to get the required number of participants from within the Auckland Bioengineering Institute and Department of Engineering Science. However, if this proves more difficult than expected we will extend the recruitment to the wider University (UoA) using the same flyers and emails.

3.3. Eligibility Criteria

Inclusion and exclusion criteria will be the same for both groups in this study.

Inclusion Criteria

- Aged >20 and <60 years old. (Human skin thickness is relatively constant for people between 20 and 60 years, and is reduced outside of this range [7])
- Able to communicate in English
- Able to give full informed consent (i.e. no neurological impairment)

Exclusion Criteria

- Insulin-dependent diabetes. (Due to regular finger-prick testing, these individuals may have scarring of their fingertips, which may influence blood release.)
- Haemophilia (or other bleeding/clotting disorders)
- Carrier of blood-borne infectious agent (e.g. HIV, HBV)
- Amputation affecting a number of fingertips
- Significant peripheral circulatory reduction (e.g. Raynauds disease or beta blocker use)

3.4. Interventions

Each participant will be subjected to four interventions: a standard lancet prick, a jet injection with a standard circular orifice, and two jet injections with different custom slot orifices. These interventions will be performed on the side of the fingertip of the middle (3^{rd} finger) and ring finger (4^{th} finger) of either hand. Which finger receives which intervention will be randomised, as will the order of the interventions. The participants will be blinded by an opaque barrier which will prevent them observing the procedure but allow them to see and communicate with the practitioner. After each intervention has been applied, and the associated blood samples collected, there will be a 2 minute 'break' before performing the next intervention. The participants will be shown the jet injector and lancing device following the procedures. The interventions will be identical for both groups with the exception of the fluorescent dye (ICG, 0.01 mg/L) added to the jet injected fluid for group 2.

Lancet

Capillary blood will be sampled in accordance with the "WHO guidelines on drawing blood" [8]. This will involve a lancet piercing the skin to a depth of 2.3 mm. The collection of the blood into four sequentially-collected samples differs from the WHO guidelines, but is necessary to the study (see section 3.5), and adds no extra risk to the participant.

Jet Injection

A jet injection of less than 50 µL of sterile isotonic saline will be performed to pierce the skin. The jet will target the same depth in the dermis as the lancet (2.3 mm). Similarly to the lancet, the jet injector based blood sampling will be done as closely as possible to the WHO guidelines. However, the use of a jet injector itself is not currently within these guidelines, as this is novel to this study.

For group 2 ICG will be added to the injected saline to a concentration of (10 mg/L). An ICG concentration of 10 mg/L is well below the dose used for other diagnostic procedures (5 g/L) approved by the FDA.

3.5. Blood collection

The blood released at the intervention site will be collected at four time points following the intervention: 10 s, 20 s, 30 s and 40 s. Massaging or squeezing of fingers will be performed in accordance with the "WHO guidelines on drawing blood" before each collection. The amount of massaging or squeezing of fingers will be independent of the amount of blood observed at the intervention site. The collected blood will be separated into different sample containers based on these intervals. Hence, each intervention will be associated with four blood samples (a total of 16 blood samples per participant). We are collecting blood in this way to allow us to observe the time-related behaviour of blood release and glucose concentration following breaking the skin. This is important to research question (b) (section 2.1).

3.6. Measurements

Volume

The blood will be collected from the skin using capillary tubes. As the dimensions of these capillary tubes are known the sample volume will be measured by the length of tube that is filled with blood.

Group 1: Glucose Concentration

For the samples collected on participants from group 1 we will then measure the glucose concentration of each blood sample. This will be done using a CareSens N point of care glucometer. This approved (and Pharmac funded) device involves the blood sample being collected onto a single use test strip inserted into the glucometer. The dilution of the blood samples collected following the jet injections will be implied by comparing the glucose concentration with that measured on the control (lancet) samples. The blood samples will be disposed of as medical waste following this glucose measurement.

Group 2: Blood Dilution (Fluorescence)

For group 2 after the volume is measured, we will measure the ICG concentration in the sample using a benchtop fluorimeter that has been developed at the Auckland Bioengineering Institute specifically for this purpose. The capillary tube containing the blood sample will be placed into the fluorimeter, which will excite the sample with 780 nm light. The resulting fluorescence will be measured, indicating the concentration of ICG and hence the proportion of injectate in the blood sample.

As the fluorimeter requires a sample of 10 µL to produce a reliable measurement, and we expect our samples to typically be around 5 µL, we will pre-mix the samples with isotonic saline solution. We will mix a volume of saline rounded up to the nearest 1 µL with our sample to ensure a total volume of at least 10 µL. In samples where the initial volume is greater than 5 µL the added volume of saline will be equal to the sample volume rounded up to the nearest 1 µL. This will ensure all samples will end up in the ideal concentration range of 0 mg/L – 5 mg/L for measurement of ICG with this device. The samples will be weighed following this mixing to quantify the volume that was added. The fluorescence measurement, initial sample volume, and mixed volume will then be used to calculate the ICG concentration, and therefore the blood volume, in the initial sample.

Perceived Pain

After each intervention and its associated blood collection, the participant will be asked to score the pain associated with the intervention on a standard verbal numeric rating scale. Each participant will be asked to rate the pain experienced from 0-10, with zero representing no pain, and 10 representing extremely severe pain. After 24 hours following the interventions, the participants will be asked to complete a questionnaire to re-assess the level of pain and any swelling, redness, bruising or soreness they have perceived at each intervention site.

Site Reactions

After blood samples are collected for each intervention, a sterile cotton-wool ball will be applied until bleeding stops. A microscope image of the intervention site will be taken immediately after the bleeding has stopped. This will reveal whether each intervention site results in a visible wound. The participants will be asked to take a photograph of each of the intervention sites, and answer some questions regarding their fingertips, 24 hours after receiving the interventions. The questions will reveal any persisting discomfort or reactions at the intervention sites, and the pictures will give the researchers an indication of any swelling and/or redness.

3.7. Materials

Lancet

The lancing will be performed using a standard, commercially available tool (Accu-Check, Safe-T-Pro-Plus). This will be set to penetrate to a depth of 2.3 mm. This device shields the lancet following penetration, making accidental stick injury extremely unlikely.

Jet Injector

The injection system has been designed in accordance with medical electrical equipment safety standard IEC 60601-1.The jet injector includes an electric motor which is momentarily (<0.1 s) supplied with a voltage (<320 V) to perform the injection. All elements of the injector that are exposed to the user are made from plastic and as such, in the very unlikely event of a short circuit within the injector, it is not possible for the participant or researcher to be exposed to the driving voltage. It would require a minimum of three simultaneous faults to expose a participant or researcher to any electrical current. During use all external surfaces of the device remain well below the maximum temperature (40 °C) dictated by the IEC 60601-1 standard.

The orifices and ampoules used with the injector must be sterile as they will be in contact with the participant and the injectable fluid. The ampoules with the standard circular orifices come in sterile packaging and will be single use. The custom slot orifices and ampoules are made at the Auckland Bioengineering Institute and will be reused through the trial. This equipment will be cleaned according to AS/NZS 4187:2014, and sterilised using a class B autoclave following use on a participant (and prior to the first use). Similarly to standard medical syringes, a small amount of medical grade silicone lubricant will be used to lubricate the interior of the ampoules.

Injectate

The jet injections will be performed using a volume of less than 50 µL of sterile isotonic saline. For group 2 this will include ICG at concentration of 10 mg/L. ICG is an FDA approved, fluorescent marker which is commonly used at a concentration of 5 g/L. We will deliver ICG at a concentration five hundred times smaller than this. The ICG solution will be used as a marker that will allow us to measure the dilution of blood samples.

Blood collection/containment

The blood forming at the intervention sites will be collected using disposable capillary tubes. For samples from group 1 the blood will then be ejected from the capillary tubes onto a glucose test strip. While the capillary tubes containing the blood collected in group 2 will be placed directly into the fluorimeter. All samples and capillary tubes will be disposed as medical waste following collection and measurement.

3.8. Personnel

Our research team includes experts in needle-free drug delivery (bioengineers) and Dr Nandoun Abeysekera, who provides clinical oversight. In addition, a healthcare professional trained in phlebotomy (e.g. nurse) will be employed to oversee the blood sampling procedures.

3.9. Safety Monitoring

An internal safety monitoring committee will review the data collected during the trial looking for evidence of adverse effects or unexpected risks to participants or researchers. The committee will be particularly interested in any evidence of unexpected behaviour from the jet injections, or lasting symptoms at the intervention sites. The trial will be terminated at the discretion of the committee if evidence of these issues were to arise. The committee will consist of three members of the research team: Dr Nandoun Abeysekera, Dr Bryan Ruddy, Dr James Mckeage, as well as the health care professional hired to oversee the blood sampling procedures. This committee will meet every fortnight, assuming around 3 participants per week complete the trial, or more regularly as necessary.

Elevated pain scores, bruising, and excessive bleeding have all been identified as signs of unexpected behaviour from the jet injections. Extreme levels of pain will be measured as an average pain score of >7.5 after at least 5 participants. If evidence of unexpected adverse effects (e.g. infection, nerve damage, etc.) are observed in the participant feedback and "day after" photos the study will be terminated.

If the average glucose measurement in the control samples (lancet) suggest the participant had an extreme glucose concentration (less than 4.0 mmol/L or greater than 11.1 mmol/L) at the time of sampling, the participant will be contacted to describe this measurement, and encourage the participant to talk to their GP.

3.10. Timeline

This protocol is planned over a 12 month period (01/12/2019 – 01/12/2020). As the involvement of each individual participant is primarily just a single site visit (and a follow up questionnaire), most recruitment and data collection will occur in parallel. Data collection will likely be completed within 6 months, with the remaining 6 months set aside for data processing, reporting and publication.

4. References

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