CLINICAL TRIAL PROTOCOL

Clinical Assessment of a Novel Microprobe Continuous Glucose Monitor for Type 1 Diabetes
Chief Investigator: Dr N Oliver

General Information

Authors: Dr N Oliver, Professor D Johnston, Dr A El-Laboudi

Title: Clinical Assessment of a Novel Microprobe Continuous Glucose Monitor for Type 1 Diabetes

Date: **5th November 2015**

Sponsor: Imperial College London,
Exhibition Road
South Kensington
London,
SW7 2AZ

Sponsor Representative: Ruth Nicholson
510B, 5th Floor, Lab BLock,
Charing Cross Campus
Imperial College,
London,
W6 8RF
r.nicholson@imperial.ac.uk
Telephone   : 020 3311 0212
Fax Number : 020 3311 0203

Principal Investigator: Dr Nick Oliver,
Department of Medicine
Charing Cross Hospital,
Fulham Palace Road,
London,
SW6 8RF

Tel: 0203 311 1093
07957 163617
nick.oliver@imperial.nhs.uk

,

Other departments: Institute of Biomedical Engineering and Department of Chemistry
Flowers Building, South Kensington Campus,
Imperial College,
London,
SW7 2AZ

1. Background

Diabetes Mellitus represents a growing health challenge in UK with an estimated prevalence of 2.9 million representing 4.45% of the population. This is expected to soar to 5 million by 2025 in UK. The scale of the problem is similar globally with an estimated prevalence of 366 million that is expected to rise to 552 million by 2030. (1, 2)

Diabetes is associated with an increased risk of morbidity and mortality especially if poorly controlled. With increasing prevalence, health authorities in UK and worldwide are facing increasing challenges to deal with the burden of diabetes and its complications. It is currently estimated that 10% of NHS budget is spent on diabetes.(1)

Intensive treatment of diabetes and good glycaemic control has been shown to reduce the risk of complications (2, 3). A core component in a successful diabetes management strategy, particularly in subjects who are insulin treated, is glucose monitoring. It enables subjects to intensify insulin therapy with a subsequent reduction in diabetes-related complications while minimizing the risk of hypoglycaemia. There is evidence of improvement in glycaemic control associated with increased frequency of self-monitoring of blood glucose (SMBG) or with the use of continuous glucose monitoring (CGM) (4-6). Recent studies showed that the use of CGM is associated with reduced time spent in hypoglycaemia and reduction in HBA1c level in children and adults with type 1 diabetes with benefits seen even in those with HBA1c values at target (7, 8).

Since 1971, when the first glucose monitor was used, the most common method of glucose monitoring remains the use of intermittent capillary blood glucose sensors using standard finger-prick methods. This method does not provide the user with information about duration, direction and magnitude of glucose change over time. Furthermore, it is uncomfortable and painful which can result in poor compliance with the recommended frequency of glucose monitoring with subsequent negative impaction on diabetes control. Many alternative monitoring methods have been investigated to allow continuous glucose monitoring (CGM). Continuous glucose monitoring devices that have gained approval for marketing depend on amperometric electro-chemical technology using Glucose Oxidase enzyme (GOx) for detection of the interstitial fluid glucose. This has been coupled with either microdialysis or reverse iontophoresis methods for glucose extraction. However, currently the most commonly used CGM devices in clinical practice depend on an electro-chemical glucose sensor in the form of a needle that penetrates the skin with the sensor tip in the subcutaneous tissue. As these sensors use interstitial fluid (ISF) as the diagnostic fluid, they require calibration to capillary blood glucose as the ISF glucose concentration is approximately 60% of that in blood and changes in ISF glucose lag 6-7 minutes behind changes in blood (9). Despite their clinical benefits, they remain invasive, painful, costly and inaccurate, particularly in the hypoglycaemic range (10). They also require regular calibration against blood glucose values as they are susceptible to signal drift as a result of surface deposition of proteins and cells which is followed by connective tissue encapsulation (biofouling) affecting sensor's accuracy and life span (11). To overcome

these problems, emerging CGM technologies attempt to minimize the level of invasiveness without compromising on sensor accuracy or lifespan.

Human skin comprises the epidermis, dermis and subcutaneous tissue. The outer 10-15 µm of epidermis, called stratum corneum, is primarily made of dead tissue and is responsible for skin barrier characteristics. The viable epidermis, up to 50-100 µm below the stratum corneum, contains living cells, but is devoid of blood vessels and contains few nerves. Below the viable epidermis lies the dermis, 800 – 1500 µm in thickness, which contains nerves and blood vessels at approximately 400 µm depth from skin surface (12).

In order to allow minimally invasive access to ISF for CGM, research in microprobe array technology has developed in recent years. The idea of microprobe use was first suggested in 1976 by Gestel and Place to penetrate the stratum corneum layer for drug delivery. Thanks to advances in microfabrication in 1990s, manufacturing of microprobes is made possible. Since then, research in microprobe array technology has developed to explore different applications using microprobe arrays of different geometries manufactured from a range of materials including polymers, metals, glass or silicon. This novel technology provides a minimally invasive alternative to the conventional use of needles or lancets. It can be employed for therapeutic purposes by allowing or enhancing transdermal drug delivery. It can also be used diagnostically by allowing access to biofluids (ISF or blood) which can be used for analysis of physiologically relevant analytes (e.g., glucose or lactic acid). An array containing several microprobes is used to penetrate the stratum corneum. This can access ISF without impinging on the dermal nerves fibers or blood vessels. It also provides a large surface area for the enzymatic electro-chemical reaction to sense ISF glucose, potentially resulting in improved accuracy of glucose sensing, even in the hypoglycaemic range. Therefore, this new technology provides the potential for accurate continuous glucose monitoring in a less painful minimally invasive way. However, for this technology to be included into mainstream clinical medicine, microprobe arrays have to fulfill the requirements of low cost, mass production, mechanically robust design, bio-compatibility and accuracy.

Over the last ten years, several groups have been working on the development of this technology aiming to design microprobe arrays that fulfill these requirements and can be used for analysis of body fluids or drug delivery.

The mechanics of microprobe insertion into the skin is of paramount importance to device performance and safety. Successful insertion depends on microprobe geometry and material. It also depends on skin resistance to penetration. All these factors determine the force needed for insertion and risk of fracture of microprobes. Microprobes need to be of the correct geometry and physical properties to allow for a small insertion force. If the force required for insertion exceeds fracture force, needles will break or bend before insertion occurs. Several studies investigated microprobe mechanics and have demonstrated that effective microprobe insertion is achieved by a small tip radius and sufficient length to overcome skin resistance, while mechanical strength is increased with increasing wall thickness, needle base diameter and Young's modulus (measure of stiffness of material). By minimizing force of insertion and maximizing

fracture force, a large safety margin can be achieved allowing for successful skin insertion without mechanical failure (13-15).

To assess device safety, several studies were conducted to assess pain, skin irritation, skin damage, bleeding and risk of infection associated with microprobe application with comparison to hypodermic needles or conventional lancets. With the minimally-invasive nature of the device, The studies show that microprobe application provides many advantages over conventional needles and lancets including: less or no pain (16, 17), no bleeding, reduced risk of infection (18), minimal tissue trauma (17, 19), minimal skin irritation (19, 20) and rapid skin recovery (17, 19, 21). Various techniques have been employed to assess microprobe penetration to skin layers. Light microscopy or colored dye application following microprobe removal allow for *en face* visualization of the penetrated skin. Measurement of transepidermal water loss (TEWL) can also be used to confirm skin penetration. None of those techniques provide information on the depth of the created microchannels. To do so requires biopsy of the microprobe treated area and histological examination. Recent research has employed confocal microscopy and optical coherence tomography imaging techniques as an alternative to histological examination.

The use of microprobe arrays in ISF extraction for glucose monitoring has been successfully demonstrated (22). Hollow microneedles manufactured of glass (700-1500 µm in length, 15-40 µm tip radii) were used to penetrate the skin of 15 rats and 6 human subjects before applying a vacuum to extract ISF and measure glucose concentration. Skin at the insertion site was examined by bright field microscopy and multiphoton confocal microscopy. Skin histology was also examined using bright field microscopy. This confirmed skin penetration and showed similar geometry between the created hole and that of the microprobe. Analysis of extracted ISF glucose showed good correlation to capillary blood glucose with a lag time of less than 20 minutes following an insulin injection.

The Institute of Bio-medical Engineering department at Imperial College has adapted and improved the existing methodology to manufacture a novel continuous sensor platform using microprobe arrays and classical enzyme electrochemistry in order to overcome difficulties related to bio-incompatibility, biofouling, clogging and local variations in temperature, sweat and vascularity.

2. The Imperial College microprobe array continuous glucose sensor:

2.1- Device description:

The Imperial College microprobe array continuous glucose sensor consists of a three dimensional out-of-plane microprobe arrays, with 36 - 64 microprobes perpendicular to the base plate and arranged as 6x6 or 8x8 arrays. The microprobes are 800-1000µm in length with a base of 500-600µm and tip diameter of 10-50µm. The microprobe arrays are made of SU-8, an epoxy material, metallised with platinum and are functionalized with glucose oxidase (GOx) (EC 1.1.3.4) incorporated in electropolymerised polyphenols layer. The electropolymerised polyphenols layer serves also as an outer coating membrane to prolong

the linear working range (from 0 to 30mM of substrate) and increase resistance to interfering agents such as dopamine, ascorbic acid, acetaminophen, or uric acid (23).

This device is gently pushed into the skin surface using an applicator, which allows it to penetrate through the skin layers and access the interstitial fluid in a minimally invasive manner.

The materials that the device brings in contact with the layers of skin are polyphenols, platinum coating, the glucose oxidase based enzymatic system and in case of damage to the probes the underlying SU-8 epoxy layer. SU-8 is a high contrast, epoxy-based photoresist designed for micromachining and other microelectronic applications, where a thick chemically and thermally stable image is desired. The SU-8 epoxy used for research studies has viscosity of 12250cSt. A literature review on SU-8 epoxy based devices has demonstrated that SU-8 epoxy and platinum based drug delivery devices are biocompatible and showed reduced biofouling (24). Glucose oxidase and platinum are routinely used in other commercially available CE-marked predicate devices (Enlite sensor, Medtronic. Freestyle Navigator, Abbott Diabetes Care. Dexcom Seven, Dexcom). Polyphenols are natural products and are listed as inactive ingredients for approved drugs products on the FDA (25).
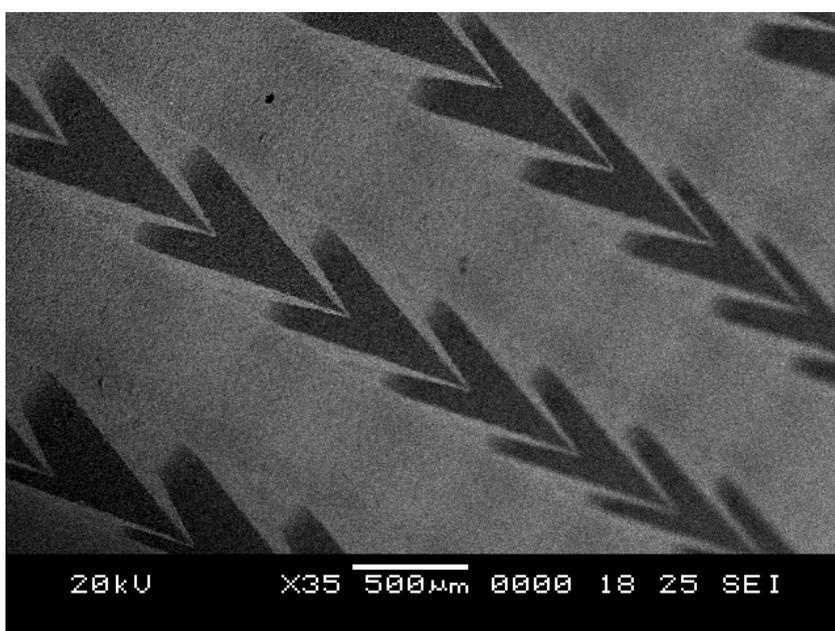
Figures 1 showing scanning electron microscopy image of the Imperial College microprobe array continuous glucose sensor.
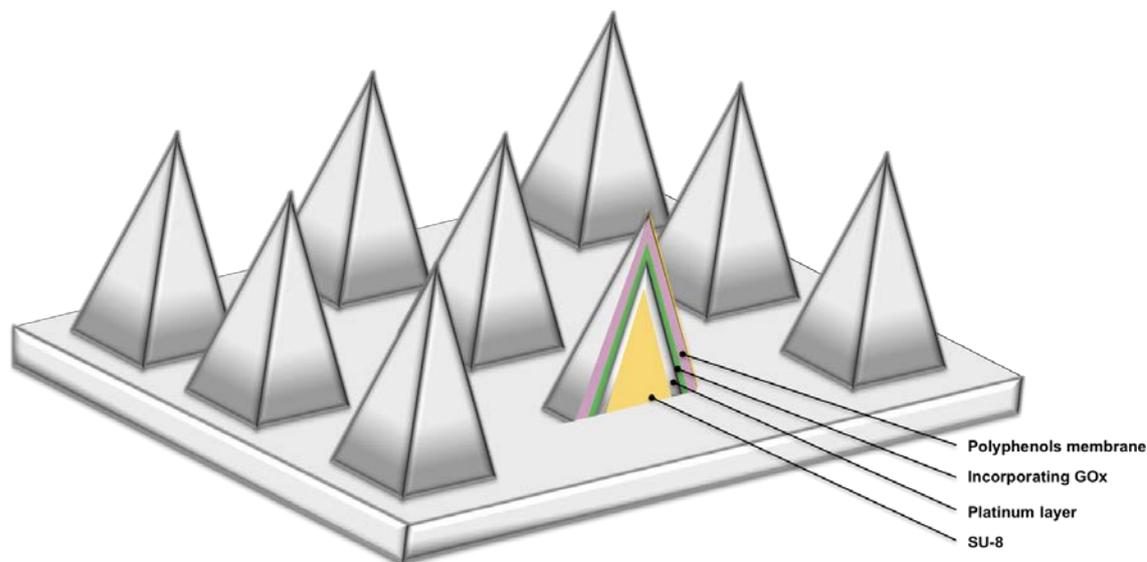
Figure 2 showing schematic of the microprobe array sensor

2.2- Fabrication process:

This involves the use of an aluminium metal master, which is used to create moulds of Polydimethoxy Siloxane (PDMS).

The master is fabricated using an Electrical Discharge Machining (EDM) technique. EDM is a controlled metal-removal process that is used to remove metal by means of electric spark erosion. The metal-removal process is performed by applying a pulsating electrical charge of high-frequency current through the electrode to the work piece. This erodes very tiny pieces of metal from the work piece at a controlled rate.

The moulds are casted with the commercially available epoxy material SU8 50 using both spinning or using vacuum. Once the epoxy is filled into the microcavities of the mould they are cross-linked by exposure to UV light at 254 nm for 30-60 minutes. The samples are cooled down at 4C and the PDMS layer is peeled off to obtain the cross-linked epoxy microprobe arrays.

An alternative to SU-8 is Injection molding of polycarbonate. For the injection molding of polycarbonate, a metal mold of aluminium is prepared using CuW microelectrodes. An injection molding setup is used to introduce molten polycarbonate at high pressure, once the polycarbonates is cooled it solidifies into bare device. Polycarbonate is a biocompatible material and is one of most commonly used and most widely tested in the medical device industry.

The bare devices are masked and sputtered with metal to obtain the working and reference electrodes. For working electrode, a platinum target is used to sputter 50 nm of Pt whilst for the reference electrode a silver target is used to sputter 100 nm of Ag.. The metallised devices are functionalised by electropolymerisation of a 50 mM of phenol solution consisting of 10 mg/mL glucose oxidase (GOx) enzyme. Electropolymerisation is done by biasing the working electrodes against a Ag/AgCl reference electrode at 0.9 V for 15 minutes.
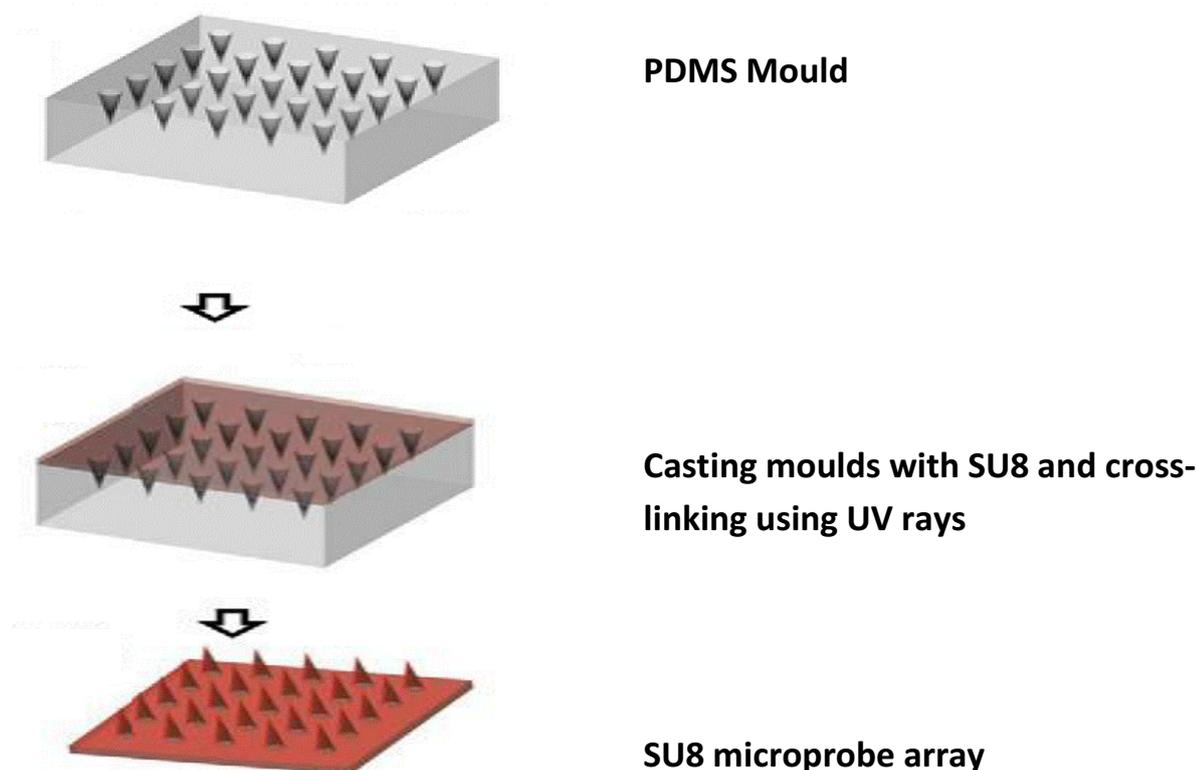

PDMS Mould

Casting moulds with SU8 and cross-linking using UV rays

SU8 microprobe array

Figure 3 showing fabrication process.

3. Validation process:

3.1-Functional Validation (in vitro):

The devices were tested using a chronoamperometric technique by subjecting the functionalised devices to varying concentrations of glucose ranging from 0 - 30 mM. Microprobe array glucose sensors reproducibly responded to changing glucose concentrations with linear responses seen in the physiological range. Currents measured were over one hundred times higher compared to those measured using the subcutaneously implanted disc electrode that is currently used in clinical practice for

CGM. This results in improvement of signal:noise ratio and thus improving accuracy and reproducibility (26).

Apart from the potential for this new technology to provide a painless accurate method for continuous sensing of ISF glucose concentration, it is also possible to partition the microprobe arrays and immobilize different enzymes across multiple sensing areas sharing a common reference electrode for continuous multi-analyte sensing. Partition may also allow multiple sensors in a voting or averaging system for the same analyte, therefore improving sensor performance.
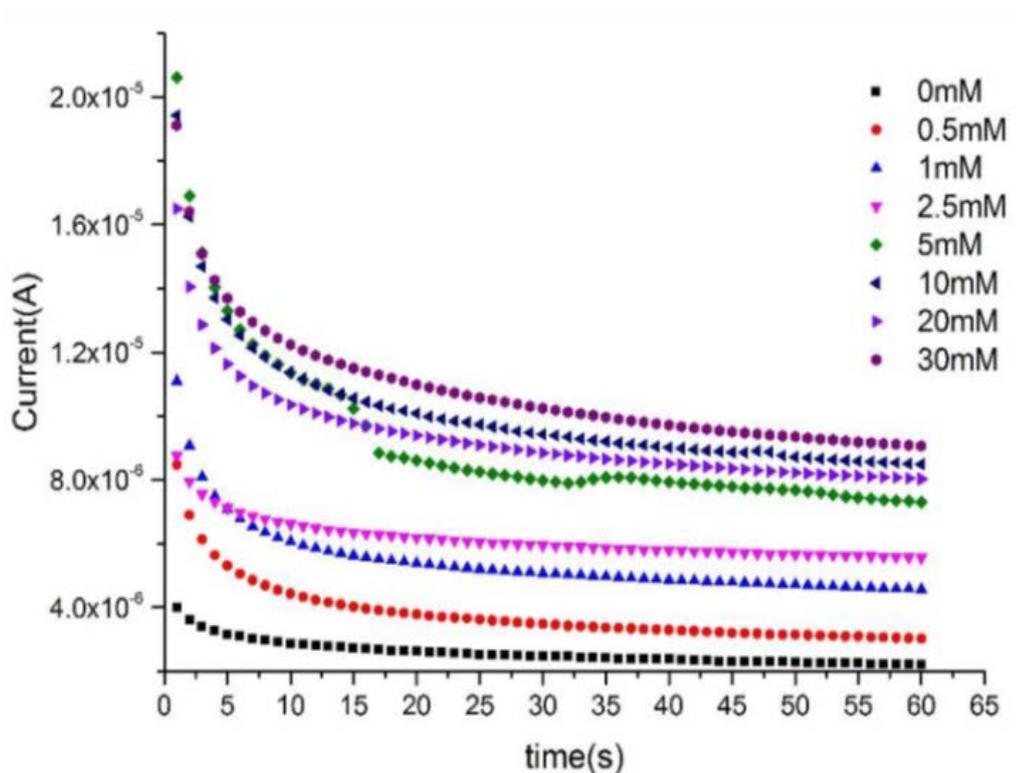

Fig. 4: Chronomamperometric measurements obtained for varying glucose concentration..

3.2. Mechanical Validation (in vitro and ex vivo):

Aim of mechanical validation study is to assess the ability of the device to penetrate stratum corneum, to access interstitial fluid and analyse its glucose content, without fracture of microprobes ex vivo through insertion tests and to assess device's mechanical robustness in vitro through fracture tests.

3.2.1. Insertion tests:

Human skin samples were obtained from plastic surgery at Charing Cross Hospital – Imperial College Healthcare NHS Trust after approval from Imperial College Human Tissue Bank (ICHTB) and patients' consent. ICHTB is approved by NRES to give "deemed ethics" for research projects that use extra samples of anonymised tissue and fluids collected specifically for research. Using Instron force-displacement test station, microprobe arrays were applied to skin surface using forces of 7, 10, 15, 20 and 25 Newton at a speed of 3 mm/s. Once reached, target force was maintained for duration of 60 seconds. Following removal of the device, methylene blue dye was applied to the microprobe array-

treated skin area for 20 minutes before it was carefully wiped using alcohol swap. Skin was then examined under digital microscopy to identify created micropores. Skin was also examined histologically to confirm penetration and assess penetration depth. Results have demonstrated the ability of microprobes to penetrate stratum corneum using forces as low as 7 Newton. Insertion ratio (number of created micropores divided by number of microprobes) was proportional to force used. Using force of 20N or above resulted in insertion ratio of almost 100%. Scanning electron microscopy examination of microprobe arrays following insertion tests showed structural integrity of the device.
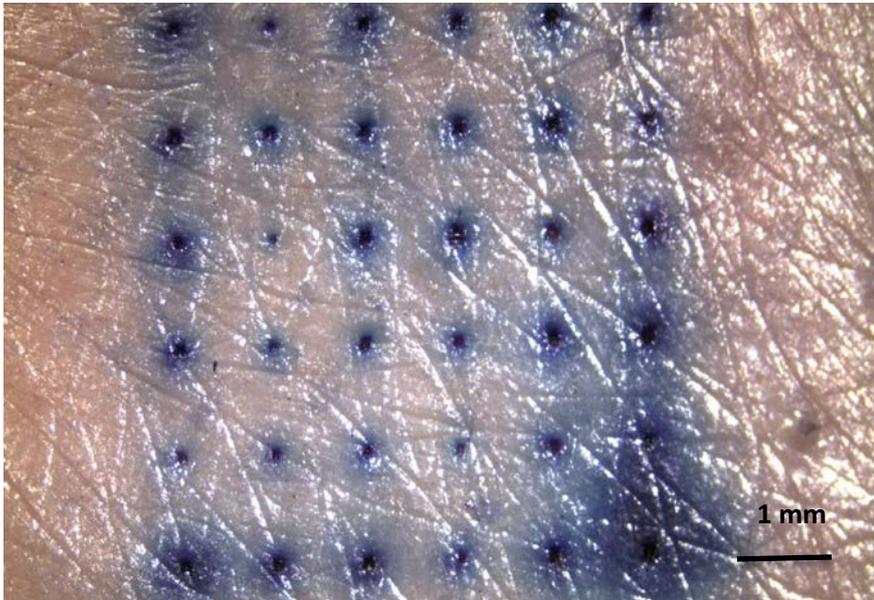

Figure 5: Digital microscopy image of a microprobe array treated human skin (*ex vivo*) following application of methylene blue dye to confirm successful penetration. This shows extravasation of the dye in deeper skin layer after penetration of stratum corneum layer.
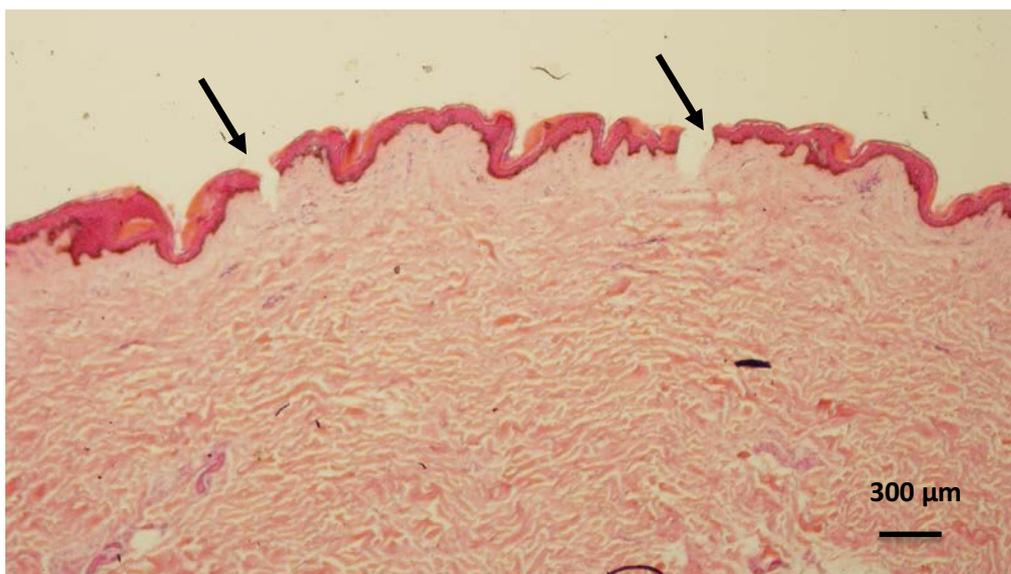

Figure 6 showing penetration of stratum corneum (arrow) with microchannels (penetration depth of 300 microns) created by microprobes.
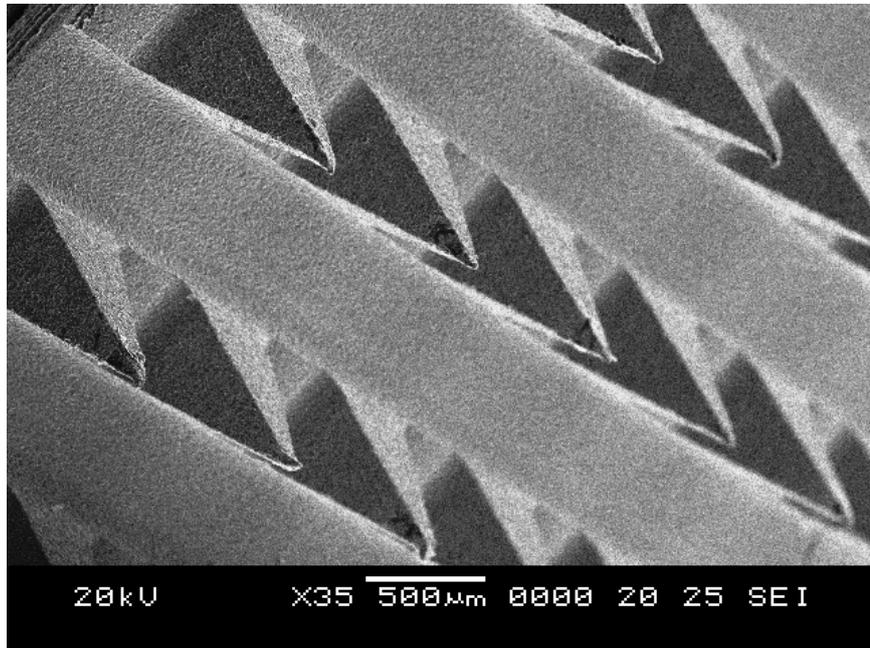
Figure 7 showing scanning electron microscopy image of microprobe array following successful skin penetration using force of 20 Newton. This shows intact integrity of the device following successful skin penetration.

3.2.1. Fracture tests:

Using Instron force-displacement test station, microprobe arrays were pressed against a metal probe using forces of 50, 100, 200, 300 and 400 Newton. Using forces up to 200 Newton only resulted in slight bending of microprobes' tips. Microprobe height reduction was observed when forces higher than 300 Newton were used. Even with the use of a force of 400N, there was no evidence of breakage of microprobes or base plate. This implies a high safety margin of the device from the mechanical aspect.
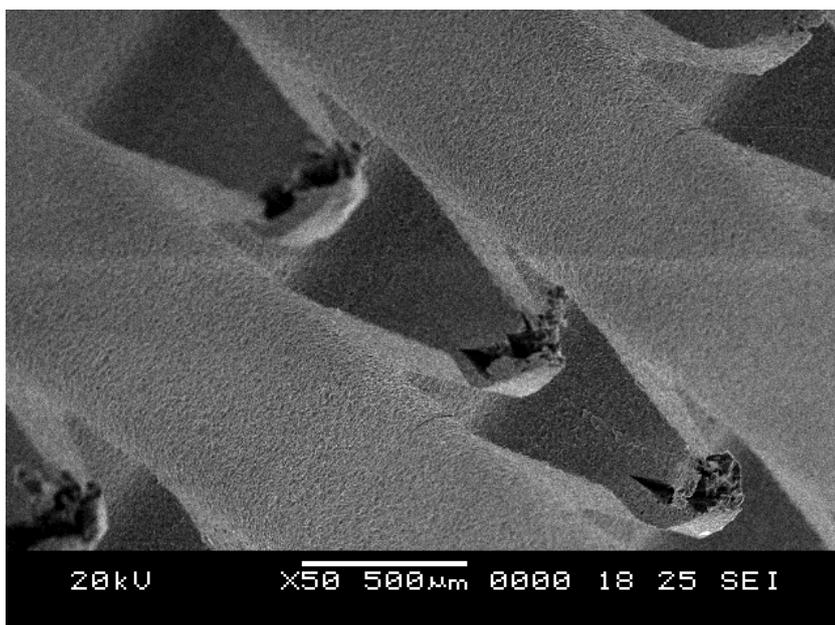

Figure 8 showing scanning electron microscopy image of a microprobe array after applying force of 400 Newton. Use of such a large force only resulted in bending of microprobes and height reduction without any fractures or collapse of the microprobes.

4. Trial Objectives and Purpose

This trial assesses the safety and efficacy of the Imperial Microprobe array biosensor for continuous monitoring of ISF glucose.

Safety will be assessed in relation to skin inflammation, skin damage, risk of fracture of microprobe tips within skin layers and pain. This will be assessed over six hours initially then over 24 hours in non-diabetic volunteers then over 24 hours in subjects with type 1 diabetes as in-patients and finally, in ambulatory situation over 5 days.

Efficacy will be assessed mechanically (ability to penetrate stratum cornuem) and functionally (ability to sense ISF analytes). Functional efficacy of microprobe arrays to continuously monitor ISF glucose will be assessed in comparison to venous blood glucose levels and ISF glucose using iPro2 in as inpatients (phase3) then in an ambulatory state (phase 4).

5. Trial Design

The principal research objective is to assess the safety and efficacy of the Imperial Microprobe array biosensor for continuous monitoring of ISF glucose. The study will be conducted over four phases:

Phase 1:

Purpose: to assess safety of the device and to demonstrate proof of concept over a period of six hours in 16 non-diabetic volunteers.

Primary outcome:

- o Skin inflammation

Secondary outcomes:

- o Skin Penetration
- o Pain score
- o Detectable signal
- o Correlation with venous blood glucose
- o Clarke error grid
- o Magnitude of current

Non-diabetic subjects will be recruited for phases 1 and 2 by poster advertisement placed at Imperial College Healthcare NHS Trust and Imperial College campuses.

Subject inclusion criteria Subjects may be recruited if:

- • Adults over 18 years of age

Subject exclusion criteria Subjects are excluded if:

- • History of upper limb neuropathy or radiculopathy
- • History of pre-existing skin condition
- • Pregnant or planning pregnancy in next 12 months
- • Breastfeeding
- • Enrolled in other clinical trials
- • uncontrolled concurrent illness

- Have active malignancy or under investigation for malignancy

Subject withdrawal criteria Subjects will be withdrawn from the study in the case of:
1. Loss of capacity to give informed consent
2. Development of skin disease or upper limb neuropathy or radiculopathy
3. Terminal illness

Withdrawal will be immediate.

Participant information sheets will be given to potential subjects and, after a minimum of 48 hours and following any questions, informed consent will be taken.

Subjects will be asked not to apply any cosmetic formulations on the non-dominant forearm for 7 days prior to the study.

Visit:

- Attend the Wellcome Trust-Sir John McMichael Clinical Research Facility at the Hammersmith Hospital campus of Imperial College between 9:00 - 10:00 am. Female subjects of childbearing age will have a urine pregnancy test performed.
- The outer margin of a 2 cm^2 skin area on the ventral aspect of the non-dominant forearm will be marked before the application of sterile microprobe array to the center of this skin area. The marked skin area will be shaved if hairy. Microprobe insertion will be achieved by applying gentle pressure. Microprobe array will be secured in place with an adhesive plaster for six hours with 5 minutes sampling as moving average of 1 minute intervals.
- An 18-G intravenous cannula will be inserted in the non-dominant upper limb for venous sampling. Throughout the 6 hours, 2.5mL venous blood will be taken at 15 minutes interval and will be distributed for venous glucose estimation using YSI analyser. Maximum blood volume collected over study visit duration is 60mL.
- Six hours after microprobe insertion (at 16:00), the microprobe array and intravenous cannula will be removed.
- Skin area around the microprobe array will be inspected at 1, 2 hours post-insertion and after device removal by a doctor for any evidence of skin inflammation using a 4-point scale (none, mild, moderate and severe).
- Following removal of the device, the microprobe treated skin area will be examined using confocal microscopy imaging technique to confirm penetration and examine the created microchannels.
- Microprobe arrays will be examined for integrity before and after application using scanning electron microscopy (SEM).
- Visual analogue scale (VAS) will be used to assess pain resulting from microprobe insertion in comparison with pain resulting from blood sampling.

Phase 2:

Purpose: to assess safety of the device over 24 hours and efficacy over 6 hours in 16 non-diabetic volunteers.

Primary outcome:

- o Skin inflammation

Secondary outcomes:

- o Skin Penetration
- o Pain score
- o Correlation with venous blood glucose
- o Clarke error grid

Same inclusion, exclusion and withdrawal criteria as phase 1

Subjects will be asked not to apply any cosmetic formulations on the non-dominant forearm for 7 days prior to the study.

Participant information sheets will be given to potential subjects and, after a minimum of 48 hours and following any questions, informed consent will be taken.

Visit:

- Attend the Wellcome Trust-Sir John McMichael Clinical Research Facility at the Hammersmith Hospital campus of Imperial College between 9:00 - 10:00 am
- The outer margin of a 2 cm^2 skin area on the ventral aspect of the non -dominant forearm will be marked before the application of sterile microprobe array to the center of this skin area. The marked skin area will be shaved if hairy. Microprobe insertion will be achieved by applying gentle pressure. Microprobe array will be secured in place with an adhesive plaster for 24 hours with 5 minutes sampling as moving average of 1 minute intervals for the first six hours.
- An 18-G intravenous cannula will be inserted in the non-dominant upper limb for venous sampling. Throughout the 6 hours, 2.5mL venous blood will be taken at 15 minutes interval and will be distributed for venous glucose estimation using YSI analyser.
- Six hours after microprobe insertion (at 16:00), the intravenous cannula will be removed and the subject will be allowed home with the implanted microprobe array.
- Subject will attend the research unit between 09:00 - 10:00 the following day. 2.5mL venous blood will be taken for venous glucose estimation using YSI analyser before removal of the array after an implantation period of 24 hours. Maximum blood volume collected over study visit duration is 62.5mL.
- Skin area around the microprobe array will be inspected at 1, 2, 6 hours post insertion and after removal of the device by a doctor for any evidence of skin inflammation using a 4-point scale (none, mild, moderate and severe).
- Following removal of the device, the microprobe treated skin area will be examined using confocal microscopy imaging technique to confirm penetration and examine the created microchannels.
- Microprobe arrays will be examined for integrity before and after application using SEM.

- Visual analogue scale (VAS) will be used to assess pain resulting from microprobe insertion in comparison with pain resulting from blood sampling.

Phase 3:

Purpose: to assess safety and efficacy of the device over 24 hours in comparison to venous blood glucose and interstitial fluid (ISF) glucose using iPro2 in 20 subjects with type 1 diabetes as inpatients.

Primary outcome:
- Correlation with venous blood glucose & ISF glucose (iPro2–Medtronic, Northridge, California)

Secondary outcomes:
- Clarke error grid
- Skin inflammation
- Skin penetration
- Pain score
- Acceptability questionnaire
- Magnitude of current

Subject inclusion criteria Subjects may be recruited if:
- Adults over 18 years of age
- Diagnosed with Type 1 diabetes for greater than 1 year
- HbA1c less than or equal to 9.0 % (75 mmol/mol)
- Registered with a GP

Subject exclusion criteria Subjects are excluded if:
- History of diabetic dermopathy or pre-existing skin condition
- History of upper limb neuropathy or radiculopathy
- Pregnant or planning pregnancy in next 12 months
- Breastfeeding
- Enrolled in other clinical trials
- uncontrolled concurrent illness
- physical or visual impairment preventing sensor's use
- Have active malignancy or under investigation for malignancy

Subject withdrawal criteria Subjects will be withdrawn from the study in the case of:
1. Loss of capacity to give informed consent
2. Development of diabetic neuropathy, dermopathy or other skin conditions
3. Terminal illness

Withdrawal will be immediate and subjects will be followed up in the appropriate out-patient diabetes clinic within 4 weeks of withdrawal.

Recruiting will be undertaken in the diabetes clinics at St. Mary's Hospital and Charing Cross Hospital campuses of Imperial College Healthcare NHS Trust. Participant information sheets will be given to

potential subjects and, after a minimum of 48 hours and following any questions, informed consent will be taken.

Usual care will be maintained for diabetes throughout the study. No concomitant medical therapies are contra-indicated.

Subjects will be asked not to apply any cosmetic formulations on the non-dominant forearm for 7 days prior to the study.

Visit 1:

- On the morning of the visit, subjects will be instructed to have their standard breakfast and to adhere to their usual practice of diabetes management, this will be maintained throughout study period.
- Attend the Wellcome Trust-Sir John McMichael Clinical Research Facility at the Hammersmith Hospital campus of Imperial College between 9:00 - 10:00 am.
- Between 10:00 – 11:00; subjects will undergo routine clinical examination, have venous blood taken for HbA1c, lipids, creatinine, liver function tests, FBC following an intravenous cannula insertion and female subjects of childbearing age will have a urine pregnancy test performed. This will be followed by insertion of microprobe array and iPro2 device in each subject.
- The outer margin of a 2 cm^2 skin area on the ventral aspect of the non-dominant forearm will be marked before the application of sterile microprobe array to the center of this skin area. The marked skin area will be shaved if hairy. Microprobe insertion will be achieved by applying gentle pressure. Microprobe array will be secured in place using adhesive plaster for 24 hours with 5 minutes sampling as moving average of 1 minute intervals.
- Medtronic iPro2 (Northridge, California) retrospective/blinded continuous glucose monitoring sensor will be implanted in the anterior abdominal wall and calibrated as per manufacturer's instructions.
- An 18-G intravenous cannula will be inserted in the non-dominant upper limb for venous sampling. 2.5 mL venous blood will be taken at 30 minutes intervals between 11:00 - 22:00 the at 60 minutes intervals between 22:00 – 7:00 then back to 30 minutes intervals between 7:00 – 11:00. Venous blood samples will be distributed for venous blood glucose using YSI analyser. Thus, in total, 40 venous samples will be withdrawn from each subject. Maximum blood volume collected over study visit duration is 100mL.
- A 60g CHO meal will be provided between 12:00 – 13:00.
- A 20 g CHO snack will be provided between 15:00 – 16:00 (optional).
- A 60 g CHO meal will be provided between 18:00 – 19:00.
- A 20 g CHO snack will be provided between 22:00 – 23:00 before sleep (optional).
- A 40 g CHO meal will be provided between 7:00 – 8:00.
- At 11:00, devices and intravenous catheter will be removed.

- Skin area around the microprobe array will be inspected at 1, 2, 6 hours post-insertion and after removal of the device at 24 hours by a doctor for any evidence of skin inflammation using a 4-point scale (none, mild, moderate and severe). This will be compared to skin area around iPro2 sensor insertion site.

- Following removal of the device at 24 hours, the microprobe treated skin area will be examined using confocal microscopy imaging technique to confirm penetration and examine the created microchannels.

- Microprobe arrays will be examined for integrity before and after application using SEM.

- Visual analogue scale (VAS) will be used to assess pain resulting from microprobe insertion in comparison with pain resulting from blood sampling and pain resulting from insertion of iPro2 sensor.

- At the end of the 24 hour period, subjects will be asked to fill a validated acceptability questionnaire.

Phase 4 (visit 2):

Purpose: to assess ambulatory efficacy and lifetime of the microprobe array sensor over 5 days in continuous ISF glucose monitoring in comparison to iPro2 in 20 subjects with type 1 diabetes

Primary outcome:
  - Correlation with ISF CGM (iPro2 – Medtronic)

Secondary outcomes:
  - Clarke error grid
  - Skin inflammation
  - Pain score
  - Acceptability questionnaire
  - Magnitude of current

Visit:
- On the morning of the visit, subjects will be instructed to have their standard breakfast and to adhere to their usual practice of diabetes management; this will be maintained throughout the study period.

- Attend the Wellcome Trust-Sir John McMichael Clinical Research Facility at the Hammersmith Hospital campus of Imperial College between 9:00 - 10:00 am

- Between 10:00 – 11:00, microprobe array and iPro2 device will be implanted in each subject.

- The outer margin of a 2 cm^2 skin area on the ventral aspect of the non-dominant forearm will be marked before the application of sterile microprobe array to the center of this skin area. The marked skin area will be shaved if hairy. Microprobe insertion will be achieved by applying gentle pressure. Microprobe array will be secured in place using adhesive plaster for 24 hours with 5 minutes sampling as moving average of 1 minute intervals.

- Medtronic iPro2 (Northridge, California) retrospective/blinded continuous glucose monitoring sensor will be implanted in the anterior abdominal wall and calibrated as per manufacturer's instructions.
- Subjects will be allowed to go home with advice to have normal daily activity with no alteration to their insulin doses or other aspects of diabetes management. Subjects will be asked to keep a food diary and a diary for self-monitored blood glucose levels.
- Subjects will attend the research unit on day 6 between 9:00-10:00. The 2 devices will be removed. Skin area around the microprobe array will be inspected by a doctor for any evidence of skin inflammation using a 4-point scale (none, mild, moderate and severe). This will be compared to skin area around iPro2 sensor insertion site.
- Following removal of the device, the microprobe treated skin area will be examined using confocal microscopy imaging technique to confirm penetration and examine the created microchannels.
- Microprobe arrays will be examined for integrity before and after application using SEM.
- Visual analogue scale (VAS) will be used to assess pain resulting from microprobe insertion in comparison with pain resulting from blood sampling and pain resulting from insertion of iPro2 sensor.
- At the end of the 5 days ambulatory period, subjects will be asked to fill a validated acceptability questionnaire.

6. Methodology
Non-randomised open label study

7. Timescale
It is anticipated that the studies will take place over 4 years. Non-diabetic subjects will be recruited to the study for phase 1 and 2 (2 visits). Subject with type 1 diabetes will be recruited for phases 3 and 4 of the study for up to 12 - 18 months, with a total of 2 visits in that timeframe.

8. Potential risks and benefits
The potential risks and burdens for research participants are as follows:

Skin inflammation may occur with the use of microprobe arrays. A literature review shows this to be minimal and transient. Subjects will be examined regularly by a doctor to assess the degree of any inflammatory reaction and the need for any intervention (ending the study or the use of local/systemic therapy if required.

Pain may result from microprobe array insertion. A literature review shows this to be absent or very minimal when compared to traditional needles or lancets. The array will be inserted with gentle manual pressure and pain score will be assessed in phases 2, 3 and 4.

In all visits (excluding phase 4) there is either a venous blood test or insertion of an intravenous cannula. These have the potential to cause discomfort. This will be minimised by experienced research personnel performing the procedure and appropriate use of equipment and aseptic technique.

In phases 3 and 4, subjects will have a continuous glucose-monitoring sensor (iPro2, Medtronic) inserted in anterior abdominal wall subcutaneous tissue. Sensor insertion can be associated with some discomfort but the insertion devices are spring-loaded with introducers, making the process rapid and often painless. Any discomfort will be minimised by adhering to manufacturer's instructions.

Assessment of efficacy of the device will be in comparison to venous glucose levels and a commercially available continuous glucose sensor over a period of twenty-four hours as in-patients. 2.5 mL venous blood will be taken at 30 minutes intervals apart from the period between 11:00 - 22:00 when the blood will be taken hourly.

During studies involving subjects with type 1 diabetes, subjects will adhere to their usual practice of diabetes management.

It is acknowledged that the clinical schedule is demanding of subject's time. This will be made clear prior to consent. This inconvenience is necessary to adequately and safely validate the device and we will minimise the burden as much as possible by providing entertainment (TV, radio, reading material, internet access) in a comfortable environment with privacy. We will also ensure make every effort to schedule visits around subject's lifestyles.

Research participants will benefit from being in the study by having increased access to diabetes professionals and more frequent visits to hospital with an emphasis on improved glycaemic control.

9. Safety

ADVERSE EVENTS INVOLVING DEVICES UNDERGOING CLINICAL INVESTIGATION

Regulation 16(10)(a) of the Medical Devices Regulations 2002 (SI 618) and Annex X of the Medical Devices Directive 93/42 require manufacturers to record fully all adverse events and report all serious adverse events occurring in all participating centres to the Competent Authority.

A "serious adverse event" is one which:

a) led to death,

b) led to serious deterioration in the health of the subject, that either resulted in;

    1) a life-threatening illness or injury, or

    2) a permanent impairment of a body structure or a body function, or

    3) in-patient or prolonged hospitalization, or

    4) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,

c) led to foetal distress, foetal death or a congenital abnormality or birth defect

NOTE Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.

All serious adverse events, whether initially considered to be device related or not, involving a device under clinical investigation coming within the scope of the Medical Devices Directive and undergoing clinical investigation, should be reported to the UK Competent Authority (Medical Devices Directive: Annex X, Para 2.3.5 and Active Implantable Medical Devices Directive: Annex 7, Para 2.3.5). Such events also include those arising out of the same investigation being carried out in other countries since such events may have a direct influence on the status of the UK investigation. These reports should initially be made as soon as possible and should not be delayed while the manufacturer attempts to gain access to, or test, the device or make a full investigation. The results of the full investigation should be made available later as appropriate.

MEDDEV 2.7/3 provides guidance on the requirements for reporting serious adverse events with timelines and provides a template form to use for this purpose.

Where an un-blinded controlled clinical investigation is being carried out using a CE marked device as the control, adverse events involving the CE marked devices should be reported to the competent authority in line with vigilance guidelines.

The Competent Authority has the right to withdraw a written notice of no objection if, in its opinion, the serious adverse events give rise to issues of public health (Medical Devices Regulations 2002: Section 16(7) and Section 29(6)).

10. Statistics

This is not a randomised study and there are no comparison or control groups. Outcomes from the study are absolutes as described above. The sample size is comparable to other initial phase CGM studies, is a realistic number for recruitment and provides robust clinical validation and safety data. The study is not powered to show a change in the primary or secondary outcomes compared with usual care but is an assessment of a new technology.

Missing, unused, and spurious data will be assessed on an individual basis and may be ignored, withdrawn or the visit may be removed from the analysis with appropriate justification adjudicated by the Principal Investigator.

11. Data

During the course of the study visits some data will be stored on laptop computers, not connected to the internet, for later statistical analysis. These data will be coded and non-identifiable. Participant data will be stored in a locked filing cabinet in a secure room in Imperial College Healthcare NHS Trust. Only the research team will have access to the filing cabinet. Electronic data will be stored by subject number only on NHS desktop computers which are in the same locked room. Only the research fellow will have access to the data. Laptop computers may be used during the study for portability and convenience. At

the end of each visit the anonymised data will be transferred immediately to the secure NHS computers and will be deleted from the laptop. Access to NHS computers is only by members of NHS staff with appropriate login privileges.

All data will be stored in an anonymised form by using study numbers for identification of participants. The NHS code of confidentiality will be followed and all activity will meet the requirements of the data protection act.

Only members of the clinical research team and those responsible for direct care will have access to subjects' data during the study. The data generated by the study will be analysed by the research team including the engineering team from Imperial College. The analysis will be on anonymised data and will take place in Imperial College Healthcare NHS Trust and in Imperial College academic buildings, both in the Faculty of Medicine and in the Faculty of Engineering.

12. Direct Access to Source Data/Documents

The investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents.

13. Regulatory Issues

Clinical Trial authorization

EC medical device directive (updated 2011) states that products manufactured in-house in a healthcare establishment and undergoing testing for proof of concept are not subject of the provisions of the Medical Devices Regulations provided that the device is being manufactured and used on patients within the sole legal entity. As such, and following confirmation from the MHRA, we are not required to submit a notification of clinical investigation to the MHRA for authorization before starting the project.

Ethics Approval

A favorable opinion from the Research Ethics Committee – London will be sought before starting the project. . The study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the ICH-GCP recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

14. Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent

should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

15. Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

16. Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

17. Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the Imperial College Healthcare NHS trust.

18. Funding

The Imperial College NIHR Biomedical Research Centre is funding this study.

19. Audits

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

20. Study Management

The day-to-day management of the study will be coordinated by Dr Oliver. Weekly research meetings and monthly data reviews will be chaired by the chief investigator or other senior researcher. Annual reports to the funder and sponsor will be written and submitted. The management team will meet at the conclusion of each visit cycle to review data and ensure that no events have occurred requiring progression to the cycle to be halted. The management team will meet again prior to commencing the next visit cycle to ensure appropriate action is taken to mitigate risk of further events. The management team includes a lay member with diabetes and a consultant diabetologist not involved with the study. A community advisory group has been established together with the local Diabetes Research Network to assist in project management. It consists of eight patients with type 1 diabetes, their family members and

carers. The steering group meets every three months to manage the research project, discuss progress and provide guidance and advice to the research group from a patient perspective

21. Publication Policy

The study will be registered on the clinicaltrials.gov system and results will be disseminated by peer reviewed scientific journals, internal report, conference presentation and publication on websites. No identifiable personal data will be published. All anthropometry and personal clinical data will be expressed as mean/ median and spread of the population in the study. All participants will be informed of the results by letter at the conclusion of the study and details of any publications that arise from the study will be disseminated to participants.

22. References:

1. Diabetes in the UK 2011/2012: Key statistics on diabetes. 2011.
2. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med. 1993 Sep 30;329(14):977-86. PubMed PMID: 8366922. Epub 1993/09/30. eng.
3. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998 Sep 12;352(9131):837-53. PubMed PMID: 9742976. Epub 1998/09/22. eng.
4. Pickup JC, Freeman SC, Sutton AJ. 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.343:d3805. PubMed PMID: 21737469. Pubmed Central PMCID: 3131116. Epub 2011/07/09. eng.
5. Evans JM, Newton RW, Ruta DA, MacDonald TM, Stevenson RJ, Morris AD. Frequency of blood glucose monitoring in relation to glycaemic control: observational study with diabetes database. BMJ. 1999 Jul 10;319(7202):83-6. PubMed PMID: 10398627. Pubmed Central PMCID: 28155. Epub 1999/07/10. eng.
6. Saudek CD, Derr RL, Kalyani RR. Assessing glycemia in diabetes using self-monitoring blood glucose and hemoglobin A1c. JAMA. 2006 Apr 12;295(14):1688-97. PubMed PMID: 16609091. Epub 2006/04/13. eng.
7. Battelino T, Phillip M, Bratina N, Nimri R, Oskarsson P, Bolinder J. Effect of continuous glucose monitoring on hypoglycemia in type 1 diabetes. Diabetes Care. Apr;34(4):795-800. PubMed PMID: 21335621. Pubmed Central PMCID: 3064030. Epub 2011/02/22. eng.
8. Beck RW, Hirsch IB, Laffel L, Tamborlane WV, Bode BW, Buckingham B, et al. The effect of continuous glucose monitoring in well-controlled type 1 diabetes. Diabetes Care. 2009 Aug;32(8):1378-83. PubMed PMID: 19429875. Pubmed Central PMCID: 2713649.
9. Boyne MS, Silver DM, Kaplan J, Saudek CD. Timing of changes in interstitial and venous blood glucose measured with a continuous subcutaneous glucose sensor. Diabetes. 2003 Nov;52(11):2790-4. PubMed PMID: 14578298. Epub 2003/10/28. eng.
10. The accuracy of the CGMS in children with type 1 diabetes: results of the diabetes research in children network (DirecNet) accuracy study. Diabetes Technol Ther. 2003;5(5):781-9. PubMed PMID: 14633343. Pubmed Central PMCID: 2249698. Epub 2003/11/25. eng.
11. Voskerician G, Anderson JM. Sensor Biocompatibility and Biofouling in Real-Time Monitoring. Wiley Encyclopedia of Biomedical Engineering. 2006.
12. Khanna P, Strom JA, Malone JI, Bhansali S. Microneedle-based automated therapy for diabetes mellitus. J Diabetes Sci Technol. 2008 Nov;2(6):1122-9. PubMed PMID: 19885301. Pubmed Central PMCID: 2769816.
13. Davis SP, Landis BJ, Adams ZH, Allen MG, Prausnitz MR. Insertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force. J Biomech. 2004 Aug;37(8):1155-63. PubMed PMID: 15212920. Epub 2004/06/24. eng.
14. Park JH, Prausnitz MR. Analysis of Mechanical Failure of Polymer Microneedles by Axial Force. J Korean Phys Soc. Apr;56(4):1223-7. PubMed PMID: 21218133. Pubmed Central PMCID: 3016089. Epub 2011/01/11. Eng.

15.   Park JH, Allen MG, Prausnitz MR. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. Conf Proc IEEE Eng Med Biol Soc. 2004;4:2654-7. PubMed PMID: 17270821. Epub 2007/02/03. eng.

16.   Gill HS, Denson DD, Burris BA, Prausnitz MR. Effect of microneedle design on pain in human volunteers. Clin J Pain. 2008 Sep;24(7):585-94. PubMed PMID: 18716497. Pubmed Central PMCID: 2917250. Epub 2008/08/22. eng.

17.   Haq MI, Smith E, John DN, Kalavala M, Edwards C, Anstey A, et al. Clinical administration of microneedles: skin puncture, pain and sensation. Biomed Microdevices. 2009 Feb;11(1):35-47. PubMed PMID: 18663579. Epub 2008/07/30. eng.

18.   Donnelly RF, Singh TR, Tunney MM, Morrow DI, McCarron PA, O'Mahony C, et al. Microneedle arrays allow lower microbial penetration than hypodermic needles in vitro. Pharm Res. 2009 Nov;26(11):2513-22. PubMed PMID: 19756972. Pubmed Central PMCID: 2900181. Epub 2009/09/17. eng.

19.   Bal SM, Caussin J, Pavel S, Bouwstra JA. In vivo assessment of safety of microneedle arrays in human skin. Eur J Pharm Sci. 2008 Oct 2;35(3):193-202. PubMed PMID: 18657610. Epub 2008/07/29. eng.

20.   Noh YW, Kim TH, Baek JS, Park HH, Lee SS, Han M, et al. In vitro characterization of the invasiveness of polymer microneedle against skin. Int J Pharm. 2010 Sep 15;397(1-2):201-5. PubMed PMID: 20619328. Epub 2010/07/14. eng.

21.   Enfield J, O'Connell ML, Lawlor K, Jonathan E, O'Mahony C, Leahy M. In-vivo dynamic characterization of microneedle skin penetration using optical coherence tomography. J Biomed Opt. Jul-Aug;15(4):046001. PubMed PMID: 20799803. Epub 2010/08/31. eng.

22.   Wang PM, Cornwell M, Prausnitz MR. Minimally invasive extraction of dermal interstitial fluid for glucose monitoring using microneedles. Diabetes Technol Ther. 2005 Feb;7(1):131-41. PubMed PMID: 15738711. Epub 2005/03/02. eng.

23.   Yu B, Ju Y, West L, Moussy Y, Moussy F. An investigation of long-term performance of minimally invasive glucose biosensors. Diabetes Technol Ther. 2007 Jun;9(3):265-75. PubMed PMID: 17561797.

24.   Voskerician G, Shive MS, Shawgo RS, von Recum H, Anderson JM, Cima MJ, et al. Biocompatibility and biofouling of MEMS drug delivery devices. Biomaterials. 2003 May;24(11):1959-67. PubMed PMID: WOS:000181720900013. English.

25.   FDA. http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm   last accessed 01/11/2015.

26.   Trzebinski J, Sharma S, Moniz AR, Michelakis K, Zhang Y, Cass AE. Microfluidic device to investigate factors affecting performance in biosensors designed for transdermal applications. Lab Chip. 2012 Jan 21;12(2):348-52. PubMed PMID: 22130554. Epub 2011/12/02. eng.

Clinical Assessment of a Novel Microprobe Continuous Glucose Monitor for Type 1 Diabetes
Chief Investigator: Dr Nick Oliver

Signatures' Page:

Signature:

Chief Investigator: Dr Nick Oliver:

Consultant and Reader in Diabetes & Endocrinology

Imperial College London

Date: 05/11/2015