DEVELOPMENT AND EVALUATION OF A NOVEL MICROPROBE ARRAY CONTINUOUS GLUCOSE MONITOR FOR TYPE 1 DIABETES

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A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE DEPARTMENT OF MEDICINE

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To Arwa, Alia, Yusuf

To my mother

& To the soul of my father
Abstract

Despite the evidence demonstrating benefits of continuous glucose monitoring (CGM), the technology has not been widely implemented in routine management of type 1 diabetes due to several challenges. Our group has developed a novel sensor for CGM based on microprobe technology. The sensor consists of an array of solid microprobes, which are functionalised for \textit{in situ} electrochemical measurement of dermal interstitial fluid glucose. The unique minimally-invasive non-extractive approach provides several avenues to enhance CGM accuracy.

In this thesis, I describe the work carried out aiming to transfer this novel technology from bench side to bedside.

To evaluate sensor’s ability to penetrate \textit{stratum corneum}, human skin was utilised \textit{ex vivo} to assess the force required for microprobes’ insertion compared to that required for their fracture. These studies guided a change in the fabrication technique and a modification in microprobes’ design.

\textit{In vitro} studies showed high sensitivity to glucose, providing the potential to enhance sensor accuracy. Further evaluation showed that neither skin insertion nor gamma ray sterilisation had impacted sensor performance.

Clinical evaluation of sensor safety and proof of concept started with phase 1. The six-hour study demonstrated that sensor use was associated with barely noticeable skin reaction and minimal pain.
The work also includes a mixed-method study aimed at capturing patients’ views in relation to CGM and describes the role of patient and public involvement in this project.

These findings have important implications for the development of an accurate, cost-effective and user-friendly CGM system. This may help in widespread implementation of CGM technology and enhance compliance with CGM use with subsequent improvement in clinical effectiveness.

Finally, a re-analysis of 448 glucose profiles from the Juvenile Diabetes Research Foundation CGM study is presented. This describes measures of glycaemic variability in type 1 diabetes and demonstrates the value of CGM in reducing these measures.
Acknowledgment

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Declaration of Contributors

The research and work described in this thesis was performed by the author. All collaboration and assistance is declared below.

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Chapter 2:
Design and dissemination of the diabetes technology questionnaire was performed with assistance of Dr Nick Oliver, Ms Daniella Neil, Mrs Lis Warren and other members of the “microprobe glucose sensor PPI advisory group”. Qualitative data analysis of the diabetes technology survey was performed with support from Dr Stella Marvoveli.

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Data processing (using Matlab and EasyGv) was performed by Dr Nick Oliver. Statistical analysis was performed under the guidance of Dr Ian Godsland.
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<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>Absolute deviation</td>
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<tr>
<td>ADRR</td>
<td>Average daily risk range</td>
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<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>AgCl</td>
<td>Silver chloride</td>
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<tr>
<td>ARD</td>
<td>Absolute relative difference</td>
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<td>Au</td>
<td>Gold</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>BG</td>
<td>Blood glucose</td>
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<tr>
<td>CBG</td>
<td>Capillary blood glucose</td>
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<tr>
<td>CG-EGA</td>
<td>Continuous glucose- error grid analysis</td>
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<tr>
<td>CGM</td>
<td>Continuous glucose monitoring</td>
</tr>
<tr>
<td>CONGA</td>
<td>Continuous overlapping net glycaemic action</td>
</tr>
<tr>
<td>%CV</td>
<td>Percentage coefficient of variation</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
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<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>DET</td>
<td>Direct electron transfer</td>
</tr>
<tr>
<td>DSP</td>
<td>Dexcom Seven Plus</td>
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<tr>
<td>EDC</td>
<td>Ethyl-dimethyl-aminopropylcarbodiimide</td>
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<tr>
<td>EDM</td>
<td>Electrical Discharge Milling</td>
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<tr>
<td>EGA</td>
<td>Error grid analysis</td>
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<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
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<td>FBR</td>
<td>Foreign body reaction</td>
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<tr>
<td>FSN</td>
<td>FreeStyle Navigator</td>
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<tr>
<td>GDH</td>
<td>Glucose dehydrogenase</td>
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<td>GOx</td>
<td>Glucose Oxidase enzyme</td>
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<tr>
<td>GRADE</td>
<td>Glycaemic risk assessment diabetes equation</td>
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<tr>
<td>GV</td>
<td>Glycaemic variability</td>
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<tr>
<td>HBGI</td>
<td>High blood glucose index</td>
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<tr>
<td>ICHTB</td>
<td>Imperial College Human Tissue Bank</td>
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<tr>
<td>ISF</td>
<td>Interstitial fluid</td>
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<tr>
<td>LBGi</td>
<td>Low blood glucose index</td>
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<tr>
<td>LGS</td>
<td>Low glucose suspend</td>
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<td>LI</td>
<td>Lability index</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
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<tr>
<td>MAG</td>
<td>Mean absolute glucose</td>
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<tr>
<td>MAGE</td>
<td>Mean Amplitude of Glycaemic Excursion</td>
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<tr>
<td>MARD</td>
<td>Mean absolute relative difference</td>
</tr>
<tr>
<td>MDI</td>
<td>Multiple daily injection</td>
</tr>
<tr>
<td>MODD</td>
<td>Mean of daily difference</td>
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<tr>
<td>NAD</td>
<td>Nicotine adenine dinucleotide</td>
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<tr>
<td>NADP</td>
<td>Nicotine adenine dinucleotide phosphate</td>
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<tr>
<td>NHS</td>
<td>N-hydroxy- succinimide</td>
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<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
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<tr>
<td>PARD</td>
<td>Precision absolute relative difference</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>PDMS</td>
<td>Polydimethoxy Siloxane</td>
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<tr>
<td>PPI</td>
<td>Patient and public involvement</td>
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<tr>
<td>PQQ</td>
<td>Pyrroloquinoline quinone</td>
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<td>PU</td>
<td>Polyurethane</td>
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<tr>
<td>QoL</td>
<td>Quality of life</td>
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<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
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<tr>
<td>RT-CGM</td>
<td>Real-time continuous glucose monitoring</td>
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<tr>
<td>SAM</td>
<td>Self-assembly monolayer</td>
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<td>SAP</td>
<td>Sensor augmented pump therapy</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SG</td>
<td>Sensor glucose</td>
</tr>
<tr>
<td>SMBG</td>
<td>Self-monitoring of blood glucose</td>
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<tr>
<td>TEWL</td>
<td>Transepidermal water loss</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<td>TMA</td>
<td>Thiomalic acid</td>
</tr>
<tr>
<td>TTF</td>
<td>tetrathiafulvalene</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>YSI</td>
<td>Yellow Springs Instrument</td>
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CHAPTER 1

INTRODUCTION & LITERATURE REVIEW
1.1. GLUCOSE MONITORING:

Glucose monitoring is a core component of a successful management strategy for people with diabetes, especially for those who are insulin-treated. It facilitates intensification of insulin therapy, with a subsequent reduction in diabetes-related complications, while minimising the risk of hypoglycaemia, (Tamborlane et al., 2008, Beck et al., 2009b). Since 1971, when the first glucose monitor was used (Hubert, 1971), the most common method of glucose monitoring has been the use of intermittent capillary blood glucose monitoring using standard finger-prick methods. It has revolutionized diabetes management in several ways. It allows patients to immediately detect and treat hyperglycaemic or hypoglycaemic excursions; it facilitates change in patients’ lifestyle by demonstrating the effect of lifestyle activities on glycaemia; and it allows therapy adjustment to achieve target HbA1c level in the long-term (Klonoff, 2007).

There are many advantages for this method of testing. It is fast, accurate, portable, simple and cost-effective (Neeser K et al., 2006, Pfutzner et al., 2014). Devices used for self-monitoring of blood glucose (SMBG) have evolved with many developments allowing improved accuracy, reduced size, memory function, reduced required blood volume, rapid analysis, ability to test for blood ketones and bolus advisor integration (Smart SMBG).

There is evidence for improvement in glycaemic control with increased frequency of SMBG in patients with type 1 diabetes (Miller et al., 2013a). However, SMBG only provides a snap shot of the glucose profile at the point of testing. Therefore, missing important information about magnitude, direction, and duration of glycaemic excursions. This can be crucial especially
at important times, from glycaemia point of view, when the patient is unable to
test like driving, exercise or sleeping. Furthermore, the procedure is invasive
and painful, which can result in reduced compliance with the recommended
frequency of monitoring with subsequent negative impact on diabetes control.

1.2. CONTINUOUS GLUCOSE MONITORING (CGM):
Emergence of CGM technology, as a complementary tool alongside SMBG,
has addressed an important drawback of SMBG technology by providing
patients and healthcare professionals with continuous information about the
glucose profile. A CGM system comprises two essential components; a body
worn glucose sensor and an electronic unit for signal processing and wireless
data transmission. Some CGM systems also comprise a unit to display
glucose values in real time.

Glucose biosensors combine a glucose recognition component with a
physiochemical detector. They can be classified according to sensing
technique, level of invasiveness or target biofluid (blood or ISF) (figure 1.1).
Figure 1.1: Classification of glucose sensors based on 1(a) invasiveness, method of access to biofluid and 1(b) sensing technique.
In general, electrochemical biosensors are the most common class of biosensors. They are based on the generation or consumption of electrons during the electrochemical reaction with subsequent generation of electrochemical signal. Currently, the only CGM systems licensed for clinical practice use Glucose Oxidase enzyme (GOx) (EC 1.1.3.4) for amperometric electrochemical detection of the interstitial fluid (ISF) glucose. These either rely on in vivo electrochemical sensing of ISF glucose or are coupled with microdialysis for glucose extraction before electrochemical analysis in vitro. Glucoday S (A. Menarini Diagnostics, Florence, Italy) is the only CGM system based on microdialysis technology.

These systems can either display glucose values in real-time (RT-CGM) or store glucose data from retrospective analysis by healthcare professionals (blinded CGM). Real-time devices display the sensor glucose value accompanied by a trend arrow to show direction and magnitude of rate of change. These devices also feature an alarm function when glucose level is outside a pre-determined range or when a hypoglycaemic event is predicted. Several RT-CGM systems are licensed for clinical use (table 1.1). Ipro2 system (Medtronic Diabetes, Northridge, California) is a blinded CGM system. FreeStyle Libre Flash Monitoring System (Abbott Diabetes Care, Alameda, California) is a new glucose monitoring system where glucose data can be accessed by scanning a reader over the sensor rather than being continuously displayed in real-time.
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>RT-CGM system</th>
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<tbody>
<tr>
<td>Medtronic Diabetes, Northridge, California, USA</td>
<td>Guardian REAL-Time system using sof-sensor, enlite sensor</td>
</tr>
<tr>
<td></td>
<td>Paradigm VEO systems using sof-sensor or enlite sensor</td>
</tr>
<tr>
<td>Abbott Diabetes Care, Alameda, California, USA</td>
<td>FreeStyle Navigator</td>
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<tr>
<td></td>
<td>FreeStyle Navigator II</td>
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<tr>
<td>DexCom Inc., San Diego, California, USA</td>
<td>DexCom SEVEN PLUS</td>
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<tr>
<td></td>
<td>DexCom G4 PLATINUM</td>
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<tr>
<td></td>
<td>DexCom G4 PLATINUM with software 505 (G4AP)</td>
</tr>
<tr>
<td>A. Menarini Diagnostics, Florence, Italy</td>
<td>Glucoday S</td>
</tr>
<tr>
<td></td>
<td>GlucoMen Day (not approved)</td>
</tr>
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</table>

Table 1.1: List of RT-CGM systems that are currently licensed for clinical use.

On the basis of available evidence, as will be discussed later in this chapter, RT-CGM can be used therapeutically for further optimisation of subcutaneous continuous insulin pump therapy regimen if the target HbA1c has not been achieved. It can also be used for protection against recurrent disabling hypoglycaemia, those with hypoglycaemia unawareness or debilitating fear of hypoglycaemia (Hammond et al., 2010).

1.2.1. Structure and operation of electrochemical glucose sensors:
Amperometric electrochemical sensing of ISF glucose is based on a cascade of redox reactions, resulting in generation of an electrical current that is proportionate to ISF glucose concentration and the transfer of electrons from the reaction site (redox centre of the enzyme) to the electrode surface to measure the generated current.
The redox reaction cascade in first generation glucose biosensors

$$\text{GOx}(\text{FAD}) + \text{glucose} \rightarrow \text{GOx}(\text{FADH}_2) + \text{gluconolactone}$$

$$\text{GOx}(\text{FADH}_2) + \text{O}_2 \rightarrow \text{GOx}(\text{FAD}) + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$$

The redox reaction cascade in first generation glucose biosensors

An amperometric enzymatic electrochemical glucose sensor designed for in vivo CGM typically consists of a three-electrode system, Enzyme – electron acceptor system and membrane coating.

1- Three electrode system:

A three-electrode system is required for the redox reactions’ cascade and measurement of the generated current. It consists of a working electrode (rendered specific to glucose via immobilisation of GOx enzyme), a counter electrode and a reference electrode. A constant potential is maintained at the working electrode, with respect to the reference electrode, to complete the cascade of redox reactions (e.g. oxidation of hydrogen peroxide in first generation sensors), whilst current flows between the working electrode and the counter electrode. If current densities are low (< 2μA/cm²), the reference and counter electrodes can be combined into one electrode (two-electrode system) (Bartlett, 2008). The nature of the working electrode and its surface activity plays an important role in defining sensor sensitivity. The noble metals (e.g. gold, silver or platinum) can considerably reduce the overpotential required for hydrogen peroxide oxidation. Determining the operating potential is important since relatively high applied potential can lead to oxidation of other electroactive ISF analytes, such as uric acid, ascorbic acid, acetaminophen, causing interference with sensor performance (Vaddiraju, 2009). In general, the lower the working electrode potential, the lower the
sensor’s susceptibility to interferent species with subsequent enhancement of sensor’s selectivity.

2- Enzymes and electron acceptors:
Rendering the working electrode specific to glucose requires immobilisation of glucose oxidoreductase enzyme such as GOx or glucose dehydrogenase (GDH). GOx is a structurally rigid glycoprotein with a molecular weight of ca. 160 kDa and consists of two identical polypeptide subunits, each containing a flavin adenine dinucleotide (FAD) redox centre (Kotanen et al., 2012). GOx catalyses the oxidation of glucose to gluconolactone. In this process, the enzyme gets converted to its reduced form GOx(FADH₂). Conversion of the enzyme back to its oxidised form GOx(FAD) requires an electron acceptor, which defines the generation of the biosensors. Electron acceptors can be either oxygen (physiological electron acceptor) in first-generation sensors or redox mediator (synthetic electron acceptor) in second-generation sensors. Release of electrons from the reduced electron acceptors requires the application of a constant voltage between working and reference electrodes with subsequent generation of an electrical current that is proportionate to ISF glucose.

GDH enzymes are defined as oxidoreductases that are unable to utilise oxygen as the electron acceptor and instead transfer electrons to various natural and artificial electron acceptors. Glucose dehydrogenases are classified according to their redox cofactors, which are the essential non-protein component that act as the primary electron acceptor, mainly into nicotine adenine dinucleotide dependent GDH (NAD-GDH) (EC 1.1.1.118),
nicotine adenine dinucleotide phosphate dependent GDH (NADP-GDH) (EC 1.1.1.119), pyrroloquinoline quinone dependent GDH (PQQ-GDH) (EC 1.1.5.2), flavin adenine dinucleotide dependent GDH (FAD-GDH) (EC 1.1.99.10), which is further subdivided into bacterial and fungal FAD-GDHs (Ferri et al., 2011).

Use of GDH enzymes instead of GOx for amperometric biosensing of glucose provides higher catalytic activity, enabling rapid glucose sensing, and independence of measurements from the oxygen concentration. However, this can be at the expense of specificity. A clear example was the detection of non-glucose sugar maltose by PQQ-GDH glucose monitors, resulting in falsely elevated blood glucose levels. In one case, maltose interference led to 13 reported deaths when patients were mistakenly treated for hyperglycaemia diagnosed on the basis of false high glucose meter readings (Felice et al., 2013). Also, the thermal stability of PQQ-GDH is inferior to that of GOx (Bartlett, 2008).

3- Membrane coating:

As the concentration of oxygen in the ISF is only 0.1 mM (Vaddiraju, 2009) compared to 5.6 mM for glucose in the physiological conditions, a glucose diffusion limiting membrane covering the enzyme layer is required to modulate the glucose diffusion in comparison to oxygen diffusion. This allows glucose oxidation process to “keep up” with glucose diffusion, with subsequent increase in the linear range of glucose concentration, without being limited by oxygen deficit in the tissues (Heller and Feldman, 2008). Permselective membrane coating also plays an important role in resisting interference from other electroactive ISF analytes based on interferents’ size (e.g. cellulose
Membrane coating also helps to confine GOx enzyme close to electrode surface. Furthermore, it provides a biocompatible interface between the sensor and the tissues and enhances sensors’ stability by protecting their surface from surface-active macromolecules and reducing foreign body reaction (FBR). This, however, comes at the expense of reduced sensor sensitivity and increased sensor response time. Examples of biocompatible membrane coatings include Nafion, polyurethane and cellulose acetate (Wang, 2008).

1.2.2. Classification of electrochemical glucose sensors:

The transfer of electrons between the GOx active site and the electrode surface is an important limiting factor in the operation of amperometric glucose biosensors. GOx does not directly transfer electrons to conventional electrodes because of a thick protein layer surrounding its FAD redox centre and introducing an intrinsic barrier to direct electron transfer (Wang, 2008).

According to the method of electron transfer between redox centre and electrode surface, electrochemical glucose sensors are classified into 3 generations:

a. First-Generation Glucose Biosensors

First-generation glucose sensors rely on the use of oxygen as a physiological electron acceptor.

The biocatalytic reaction starts with reduction of the FAD redox centre of the GOx enzyme by reaction with glucose to give the reduced form of the enzyme (FADH$_2$) and gluconolactone. This is followed by re-oxidation of the enzyme by oxygen to regenerate the oxidised form of the enzyme GOx(FAD) and
generate hydrogen peroxide. Hydrogen peroxide is then oxidised at the electrode surface, upon the application of an electrical potential of around +700 mV (vs Ag/AgCl reference electrode), giving a current signal (proportional to the ISF glucose concentration) whilst regenerating oxygen.

Both Medtronic and Dexcom use first generation glucose sensors. These have the advantage of being simpler, especially when miniaturised devices are concerned. However, these devices suffer from oxygen dependence (as they rely on oxygen as a physiological electron acceptor) and are more susceptible to interference from other endogenous or exogenous electroactive analytes (as they require a relatively large potential to oxidise hydrogen peroxide, which can also oxidise other electroactive ISF analytes).

b. Second-Generation Glucose Biosensors

In 1984, Cass et al first demonstrated the use of ferrocene derivatives (Cass et al., 1984), as synthetic electron acceptors, replacing oxygen in carrying electron between the redox centre of the enzyme and electrode surface, through the following reaction cascade:

\[
\text{GOx(FAD) + glucose} \rightarrow \text{GOx(FADH}_2\text{)} + \text{gluconolactone} \\
\text{GOx(FADH}_2\text{)} + 2 \text{ Mediator}_{\text{oxidised}} \rightarrow \text{GOx(FAD) + 2 Mediator}_{\text{reduced}} + 2\text{H} \\
2 \text{ Mediator}_{\text{reduced}} \rightarrow 2 \text{ Mediator}_{\text{oxidised}} + 2\text{e}^- 
\]

The redox reaction cascade in second generation glucose biosensors

Examples of redox mediators include ferrocene derivatives, ferricyanide, and osmium complexes.
The use of synthetic electron acceptors (mediators) avoids issues related to oxygen dependence and also allows the use of relatively small electrical potential with subsequent reduction in the risk of interference from other electroactive ISF analytes. However, as mediators are small molecules, they can diffuse out of the coating membrane immobilised on the electrode surface, which results in a loss of catalytic activity. In addition, potential leaching and toxicity of most of redox mediators have restricted their use to SMBG devices (Bott, 2005).

“Wired” enzyme technology, developed by Heller’s group in 1987 (Degani and Heller, 1987), provides a solution to prevent diffusion of the mediators out of the coating membrane by connecting the redox centre of the enzyme to the electrode surface using a long flexible hydrophilic polymer backbone [poly(vinylpyridine) or poly(vinylimidazole)] having a dense array of covalently linked osmium-complex electron relays (mediators). The created 3-dimensional network facilitates electron transfer between redox centre and electrode surface thereby allowing; oxygen independence, the use of low electrical potential and large current densities with subsequent improvement in sensors’ selectivity and specificity. Enzyme wiring technology is currently used by Abbott’s FreeStyle Navigator and FreeStyle Libre systems, which uses an operating potential of only +40 mV (Feldman et al., 2003).

c. Third-Generation Glucose Biosensors

Direct electron transfer (DET) from the redox centre of the enzyme to the electrode surface, which eliminates the need for an electron acceptor (whether physiological or synthetic), allows the use of very low operating
potential with subsequent very high selectivity. Recently, Sode’s group has reported the development of a DET glucose sensor using engineered FAD-dependent GDH from *Burkholderia cepacia* (Yamashita et al., 2013). However, their use of a relatively large operating potential (+250 mV (vs Ag/AgCl)) raises questions as to whether the sensor truly relies on DET.

**Figure 1.2:** A schematic representation of the principles of first-, second-, and third-generation glucose sensors. Electrons from the glucose oxidation reaction are first taken up by the enzyme’s cofactor (primary electron acceptor) and transferred to either oxygen (first generation), an electron mediator (second generation), or directly to the electrode (third generation).

<table>
<thead>
<tr>
<th>Sensor Generation</th>
<th>FreeStyle Navigator (Liu et al., 2013)</th>
<th>Medtronic (Van Antwerp, 1998)</th>
<th>DexCom (Brister et al., 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron acceptor</td>
<td>Osmium complex</td>
<td>Oxygen</td>
<td>Oxygen</td>
</tr>
<tr>
<td>Operating potential</td>
<td>+40 mV</td>
<td>+500-700 mV</td>
<td>+700 mV</td>
</tr>
<tr>
<td>Working electrode</td>
<td>Carbon</td>
<td>Platinum</td>
<td>Platinum</td>
</tr>
<tr>
<td>Reference electrode</td>
<td>Ag/AgCl</td>
<td>Ag/AgCl</td>
<td>Ag/AgCl</td>
</tr>
<tr>
<td>Counter electrode</td>
<td>Carbon</td>
<td>Platinum</td>
<td>Combined reference/counter</td>
</tr>
<tr>
<td>Membrane</td>
<td>poly(vinylpyridine-co-styrene) copolymer</td>
<td>Diisocynate/polyurethane/polyurea/polyethylene glycol/polyethylene glycol copolymer</td>
<td>polyurethane/polyethylene glycol copolymer</td>
</tr>
</tbody>
</table>

**Table 1.2:** Comparison between commercially available continuous glucose sensors with regards to sensor structure and operation.

### 1.2.3. Technical challenges facing implantable electrochemical sensors for *in vivo* CGM:

There are several challenges that face implantable electrochemical glucose sensors resulting in reduced sensor accuracy and reduced sensor life span. These include:
1.2.3.1. Interferences (selectivity):

Application of relatively high potential, for oxidation of hydrogen peroxide at the working electrode, can result in oxidation of other endogenous or exogenous ISF electroactive analytes. This can cause an overestimation of the ISF glucose concentration. Strategies employed to reduce interference include the use of a permselective membrane to minimize access of interferents to the working electrode based on size or charge. An alternative approach is the use of a secondary working electrode to measure the background current (generated by interferents) with subsequent subtraction of the background current from the current measured by the primary working electrode (Vaddiraju et al., 2010). Advances in sensor technology, which allow the use of low potential, can also help in reducing the risk of interference.

1.2.3.2. Oxygen deficit:

Since oxygen concentration in ISF is significantly lower in comparison to physiological glucose concentration (0.1 mM in comparison to 5.6 mM), oxygen can be the limiting factor in generation of hydrogen peroxide in the redox reaction cascade in first generation glucose sensors. This results in a generated current that is proportional to oxygen rather than glucose concentration. In addition, a change in tissue oxygen level (e.g. in case of anaesthetics or hypoxia) can affect sensor performance.

To overcome this problem, glucose sensors employ a mass transport limiting membrane that increases the oxygen/glucose permeability ratio and limits glucose diffusion to the electrode surface allowing glucose concentration to be the limiting factor in hydrogen peroxide generation. An alternative way to
overcome the problem of oxygen deficit is the use of synthetic electron acceptor (mediators) as in second generation glucose sensors.

1.2.3.3. Foreign body reaction (FBR):

Glucose sensor implantation in subcutaneous tissue results in initiation of wound healing cascade and the development of FBR. Proteins rapidly adhere to the biomaterial surface in a process referred to as biofouling, which represents an integral part of subsequent development of FBR. The FBR is composed of three main layers. The innermost layer is composed generally of macrophages and foreign body giant cells (the barrier cell layer). The intermediate layer is a wide zone (30-100 µm) composed primarily of fibroblasts and fibrous matrix (the fibrous zone). The outermost layer is loose connective granular tissue containing new blood vessels (the vascular zone) (Brauker et al., 2004). FBR has a significant impact on the implanted glucose sensor and its function. Other than chemical and physical alterations (e.g. local drop in pH and resistance to glucose mass transfer by fibrous tissue) that happen at the tissue-sensor interface, FBR can also result in degradation of sensor layers (Nichols et al., 2013).

Various strategies have been employed to mitigate the effect of FBR. Considerable efforts have been paid to employ more biocompatible elements for sensor fabrication and the use of biocompatible and inert membrane coating (e.g. Nafion, polyurethane, polyethylene glycol, and hydrogels). Another strategy is the release of locally active molecules to modulate local FBR by either; suppressing inflammation (e.g. dexamethasone), inducing neovascularization (e.g. vascular endothelial growth factor) or causing
vasodilatation (e.g. nitric oxide) (Wisniewski et al., 2000). Also, reducing the size of the sensor was shown to be effective in reducing FBR as will be discussed later in this chapter.

1.2.3.4. Lag time between blood and ISF:
Currently the most commonly used CGM devices in clinical practice use a needle-type electrochemical glucose sensor with the sensor tip in the subcutaneous tissue to measure ISF glucose. Glucose concentration in the ISF is dependent on blood flow, metabolic rate and the rate of change of glucose concentration in the blood (Oliver et al., 2009). Comparison of glucose levels in subcutaneous ISF and finger capillary blood showed high correlation with estimated lag time between 0 and 45 minutes (Cengiz and Tamborlane, 2009). The time lag between plasma and ISF glucose appears to differ depending on the CGM system used (sensor size, sensor type, algorithm) and whether plasma glucose values are rising or falling (Basu et al., 2014). A study that evaluated the time lag between blood glucose and subcutaneous ISF, measured by a commercially available electrochemical sensor, following an oral meal in 14 subjects with type 1 diabetes suggested that the mean lag time is 6-7 minutes (Boyne et al., 2003). This was supported in recent studies that reported a mean lag of 5.3-6.2 minutes in healthy subjects and a median time lag of 6.8 minutes in subjects with type 1 diabetes (Basu et al., 2013, Basu et al., 2014). In these studies, time lag was estimated by direct measurement of the transport of glucose tracers from the vascular to the subcutaneous ISF compartment in subjects under overnight fasting condition. Glucose tracers were administered intravenously and measured in the abdominal subcutaneous tissue using 4 microdialysis
catheters.

In comparison to subcutaneous ISF glucose, there are limited data on dermal ISF glucose content and dynamics. Using a microdialysis technique to compare dermal and subcutaneous ISF glucose against blood glucose showed that dermal ISF glucose concentration is similar to that of the blood (99.1%), while subcutaneous ISF glucose concentration is only 50% in comparison to blood glucose (Petersen et al., 1992, Boschmann et al., 2001). It also demonstrated that dermal ISF glucose has identical peak concentration to blood glucose and suffers from less lag in comparison to subcutaneous ISF glucose. This is consistent with cutaneous blood supply, where blood flow reaches the dermis first then the subcutaneous tissue (Petersen et al., 1992, Boschmann et al., 2001, Stout et al., 2001).

Commercially available electrochemical sensors provide an estimate of ISF glucose every 5 minutes (288 values per 24 hours) and are licensed for use for up to 7 days, with the exception of FreeStyle Libre, which measures ISF glucose every minute and is licensed for use for up to 14 days.

As these sensors use ISF as the diagnostic biofluid and because they are prone to signal drift post-implantation, resulting from FBR and alteration in sensor chemistry, they require calibration to capillary blood glucose. The aim of calibration is to convert the electrical signal obtained from the sensor, which is proportional to ISF glucose, into a glucose value by using a capillary blood glucose value. The calibration process can be a significant source of error and needs to be carried out with care. A single error in calibration will get carried forward until it is corrected by an appropriately performed calibration.
Several requirements need to be fulfilled to fabricate a calibration-less continuous ISF glucose sensor. These include: reproducible manufacturing allowing minimal variation between sensors; stable sensor sensitivity *in vitro* and *in vivo*; lack of significant difference in *in vivo* sensor sensitivity between different insertion sites and different persons; and for the *in vivo* sensor sensitivity to be inferable from *in vitro* sensitivity. Two clinical studies were conducted to evaluate the performance of the FreeStyle Libre sensor over 5 days and 14 days in a total of 88 subjects with type 1 and type 2 diabetes. The studies reported: minimal inter-sensor variability (the coefficient of variation between sensors was only 2.9%); minimal change in sensor sensitivity (decline by 0.25% per day *in vitro* and by 0.66% *in vivo*); and that the difference between sensor sensitivity between subjects and between sensors inserted in the abdomen or those inserted in the arm was not statistically significant. This implies reproducible manufacturing, minimal FBR and stable sensor performance in different subjects, regardless of insertion site, over 14 days. To establish the relationship between *in vitro* and *in vivo* sensitivity, the authors compared a standard finger-stick calibration algorithm to a simulation of factory calibration using one universal calibration factor applied to all sensors in the study. This single sensor calibration factor was calculated for each sensor from the median of the individual sensitivity values (sensor signal divided by capillary blood glucose). All paired blood glucose reference points following the first hour post sensor insertion, up to the end of the sensor wear period, were used for this calculation. The analytical and clinical accuracy of the two calibration algorithms showed that the single calibration algorithm was superior in one study but inferior in the other,
compared to the standard calibration algorithm (Hoss et al., 2013, Hoss et al., 2014). The FreeStyle Libre system is currently marketed as a calibration-less glucose monitoring system.

1.2.3.5. **Stability:**

Implantable glucose sensors can fail secondary to membrane degradation, biofouling of electrode surface, loss of enzyme activity or enzyme leaching. To overcome these problems, different strategies can be employed for effective membrane coating and effective enzyme immobilisation techniques. Examples of immobilisation techniques include fixation of the enzyme onto the electrode surface by electrostatic interactions generated by polyelectrolytes or cross-linking of the enzyme with bovine serum albumin with glutaraldehyde (Nichols et al., 2013).

From the technical aspect, glucose sensors need to fulfill several requirements including specificity, accuracy, wide linear range, sensitivity, rapid response, and resistance to interferents, biocompatibility and stability. Therefore design and optimisation of a glucose biosensor requires careful choice of working electrode material, enzyme load and its immobilisation method, electron acceptor, type and thickness of the coating membrane and the operating potential. While any of these components serves an important function, there is usually a trade off between them. For example while the use of a thick membrane coating enhances sensor’s selectivity by reducing interference from other electroactive ISF analytes, this comes at the expense of reduced sensitivity to glucose and increased response time.
1.2.4. Assessment of CGM system accuracy:

Several recommendations exist for performance evaluation of monitoring devices for SMBG. According to International Organization for Standardization (ISO) 15197:2013 guidelines, ≥95% of sensor glucose (SG) results should be within ±0.8 mmol/L (15 mg/dL) or ±15% of the blood glucose (BG) reference results for samples with glucose concentrations <5.6 mmol/L (<100 mg/dL) and ≥5.6 mmol/L (≥100 mg/dL), respectively (Pfutzner et al., 2014). Manufacturers and academic researchers have extended the use of these guidelines to evaluate CGM system performance. Bland-Altman plot (bias plot) compares glucose meter accuracy against a reference method. In this method, the difference between sensor glucose and reference glucose is plotted against reference glucose values. Therefore, it provides information on sensor accuracy over the range of absolute glucose values, as defined by the reference method. It also displays the bias (mean difference between sensor glucose and reference glucose measurements) and limits of agreement (bias ± 1.96 x standard deviation of the bias) to show the 95% confidence interval in agreement between the two measurement methods.

Another method to evaluate numerical accuracy of CGM systems is by using mean and median absolute relative difference (ARD). Mean absolute relative difference (MARD) is more commonly reported, as it is more sensitive to outliers. ARD is calculated using paired SG and reference BG values in the equation ([SG – BG]/BG) x 100. In addition to reporting the overall aggregated MARD when evaluating accuracy of a CGM system, MARD can be categorised according to glycaemic range, day of sensor implantation or for individual sensor performance. For evaluation of clinical accuracy of
glucose meters, Clarke error grid analysis (EGA) is used to categorise paired SG - BG values according to the consequences of treatment decisions (Clarke et al., 1987). Zone A represents the clinically accurate zone with SG within 20% of reference BG and/or SG reading <3.9 mmol/L (<70 mg/dL) when BG is <3.9 mmol/L (<70 mg/dL); Zone B, benign errors, SG difference >20% but error would not lead to either serious hypo or hyperglycaemia; Zone C, overcorrection errors, SG above or below the target range of 3.9–10 mmol/L (70-180 mg/dL) when reference BG is within target, and treatment would result in either hypo- or hyperglycaemia; Zone D, failure to detect (hypo- or hyperglycaemia) errors, SG values within target range when reference BG is either above or below target; and Zone E, erroneous treatment errors, SG is either above or below target and reference BG is in the opposite extreme, resulting in insulin being given when BG is <3.9 mmol/L (<70 mg/dL) or rapid-acting glucose being given when reference is >10 mmol/L (>180 mg/dL) (figure 1.3(A)). A limitation to the Clarke EGA is related to the transition between adjacent zones (for example zone D is contiguous to zone A, skipping zones B and C). Therefore two results with the same amount of error can result in different clinical outcomes. To overcome this limitation, Parkers EGA was proposed (figure 1.3(B)).
Figure 1.3: Clarke (A) and Parkers (B) error grid analyses designed to evaluate clinical point accuracy of glucose meters, divided into zones A-E.

However, all these measures reflect point accuracy. As CGM systems are capable of glucose measurement as a process of time, Continuous Glucose-EGA (CG-EGA) has been proposed to evaluate trend accuracy of CGM systems to allow assessment of accuracy of rate and direction of BG change. CG-EGA combines point EGA with rate EGA (figure 1.4) by expanding the point clinical accuracy of EGA to incorporate rapidly changing glucose values.
based on the rate and direction of change and the assumed lag time between BG and ISF glucose (Clarke and Kovatchev, 2009). MARD can also be categorised according to the rate and direction of change.

![Rate error grid analysis](image)

**Figure 1.4:** Rate error grid analysis divided into zones A-E for SG rate vs BG rate (presented in mg/dL/min). [l, lower; R, rate; u, upper].


Whilst the MARD evaluates CGM system’s accuracy by examining the relationship between SG and reference BG, the precision absolute relative difference (PARD) has been proposed to evaluate precision of CGM systems by examining the relationship between two CGM sensors running simultaneously, but independently, in one patient. This avoids limitations related the limited number of paired data points that are used to calculate MARD, which may lead to inability to detect certain issues (e.g. transient signal drop due to tissue compression resulting in erroneous errors)
Similar to MARD, PARD can be categorised according to range of glycaemia, day of sensor implantation and rate and direction of glucose change (Zschornack et al., 2013).

A novel metric was proposed for evaluating CGM inaccuracies (Leelarathna et al., 2012). To evaluate the severity of CGM inaccuracy, 3 levels of sensor error were defined. Sensor error was estimated using absolute deviation (AD) for plasma glucose levels < 6 mmol/L or MARD for plasma glucose levels ≥ 6 mmol/L. Level 1 (least severe) is defined as \( \text{AD} \geq \pm 2.4 \text{ mmol/L} \) or \( \text{MARD} \geq \pm 40\% \), level 2 is \( \text{AD} \geq \pm 3 \text{ mmol/L} \) or \( \text{MARD} \geq \pm 50\% \), and level 3 (most severe) is \( \text{AD} \geq \pm 3.6 \text{ mmol/L} \) or \( \text{MARD} \geq \pm 60\% \). This metric quantifies the incidence and duration of each error level. The incidence of each error level is further categorised into sensor over-reading or sensor under-reading.

Performance of existing CGM systems has been evaluated in several studies. In a study evaluating the performance of the Dexcom G4AP against that of G4 PLATINUM, G4AP showed improved accuracy particularly at day 1 of sensor implantation with MARD (measured against YSI venous blood glucose) of 14.7% compared to 16.8% for the G4 PLATINUM. The new CGM system employs the same sensor and transmitter as the G4 PLATINUM but contains updated de-noising and calibration algorithms. The new algorithm was applied to raw sensor data of 72 subjects (108 sensors). This resulted in improvement in the overall MARD (for 7 days of sensor use) from 13.2% to 11.7% for a glucose range of 2.2-22.2 mmol/L. Similarly, the overall MARD for a glucose range of 2.2-4.4 mmol/L improved from 11.1% to 9.5%. However, G4AP did
not reduce the proportion of large errors (levels 1-3) lasting for more than 60 minutes when compared to G4 PLATINUM (Garcia et al., 2013).

A clinical study evaluating the performance of the Abbott Diabetes Care FreeStyle Libre glucose monitoring system in 62 subjects with type 1 diabetes and type 2 diabetes reported a MARD (measured against capillary blood glucose) of 13.9% over 14 days of sensor use. However, the use of a single calibration factor that was applied retrospectively to the raw sensor data, rather than standard calibration (1 hour, 2 hours, 10 hours, 3, 5, 7, 9, 11 and 13 days since sensor insertion), resulted in improvement of sensor accuracy with a MARD of 12.2%. Without recalibration, there was no significant difference in MARD between days of sensor wear. Analysis using point-EGA showed that 88% of results were in zone A, 10.6% in zone B, 1.4% in zone C (Hoss et al., 2013).

Evaluating the accuracy of the Medtronic Enlite Veo 6-day CGM system reported an overall MARD of 13.89% versus YSI glucose. System accuracy was worse on day 1 of sensor wear (MARD of 14.9%) and in glucose range between 2.2-4.4 mmol/L (MARD of 18.4%). MARD at day 1 of sensor wear in glucose range between 2.2-4.4 mmol/L was reported as 20% (Keenan et al., 2012).

However, comparison between different CGM systems, based on these studies, is made difficult by differences in experimental design between different studies. For example, the difference in the method of calculation of MARD (YSI venous blood versus capillary blood glucose), number and frequency of paired CGM-reference blood points, number of participants or whether the study was conducted as an inpatient or in ambulatory settings. A
head-to-head comparison between three CGM systems was performed in six subjects with type 1 diabetes under closed-loop blood glucose control. Each subject wore the FreeStyle Navigator (FSN), Dexcom Seven Plus (DSP) and Medtronic Guardian-sofsensor system simultaneously in two 48-hour experiments in the hospital. Results from the 12 experiments showed that the FSN had the best overall accuracy with an average MARD of 11.8 ± 3.8%. In comparison, the DSP and Guardian produced an average MARD of 16.5 ± 6.7% and 20.2 ± 6.8%, respectively. Similarly, the FSN showed the best performance across different ranges of glycaemia, except in glucose level >13.9 mmol/L (> 250 mg/dL) (Damiano et al., 2013).

More recently, the same group has reported the results of their head-to-head comparison study including new generations of CGM systems. In a similar study design of 24 experiments, subjects simultaneously wore FSN, G4 PLATINUM and Medtronic Enlite Veo CGM systems. Results revealed that G4 PLATINUM had the best accuracy and precision, followed closely by FSN. The two CGM systems significantly outperformed the Enlite Veo system. The mean (SD) of all 24 individual 48-hour MARDs were 12.3 ± 4.7%, 10.8 ± 2.8% and 18.3 ± 8% for the FSN, G4 PLATINUM and Enlite, respectively. As the data were collected as part of a closed-loop study, there were relatively few points <3.9 and >13.9 mmol/L (<70 and >250 mg/dL). Aggregate MARDs reported for glucose range between 2.8-3.9 mmol/L (50-70 mg/dL) were 36%, 19% and 23% for the FSN, G4 PLATINUM and Enlite, respectively. Frequencies of very large errors (MARD ≥ 50%) were 1.4% for the FSN, 0.5% for G4 PLATINUM and 4.4% Enlite. Most of these very large errors were due to reduced sensitivity for detection of hypoglycaemia. Similar to the previous
study, the FSN showed tendency to underestimate glucose values at the hyperglycaemia range (plasma glucose >13.9 mmol/L). CGM reliability (defined as the percentage of glucose values reported by the CGM relative to the total number possible) were 99.7% for the FSN, 99.5% for G4 PLATINUM and 97.1% for the enlite (Damiano et al., 2014). This study highlights the progress of CGM technology resulting in improvement in accuracy, precision and reliability of current CGM systems. It also highlights the rapid pace of technology development. Currently, there are two more recent CGM generations that have not yet been assessed in head-to-head comparison (FSN II and G4AP). Similarly, Abbott’s Freestyle Libre glucose monitoring system has not been evaluated so far in a head-to-head comparison.

1.2.5. Evidence for the use of CGM:

Research evaluating the effectiveness of CGM technology is extensive. It has studied the effect of CGM on several glycaemic outcomes including effect on HbA1c, hypoglycaemia measures and glycaemic variability measures. It has also studied non-glycaemic outcomes including effect on quality of life. The effectiveness of CGM has been evaluated in different settings (ambulatory, inpatient and in intensive therapeutic unit (ITU)) and in different types and subgroups of diabetes. However, several confounding factors need to be considered while evaluating the CGM evidence. As the CGM is a diagnostic tool, its effectiveness relies on effective translation of the CGM data into an effective therapeutic intervention that will eventually impact the outcome. This effective translation depends on patient’s training, skills and compliance. It also depends on the experience of the diabetes team and the level of support
provided to patients. Therefore, some of the CGM studies might not only evaluate the use of CGM and its accuracy, but also evaluate factors related to patient and diabetes team interaction with the CGM. Furthermore, CGM cannot be investigated in a double-blind manner. Therefore, the best possible evidence can be obtained from large-scale open-label randomised controlled crossover studies, where subjects act as their own control. Another important factor to consider when evaluating the CGM evidence is the rapid development in CGM technology. The continuous development in CGM sensor fabrication and algorithms used for glucose data analysis has resulted in significant improvement in CGM accuracy. Therefore, studies conducted few years ago using older generations of CGM systems might have shown different results if they were conducted using newer generations of CGM with enhanced accuracy.

The effectiveness of blinded CGM in guiding therapeutic interventions was assessed in a number of studies. In an open-label randomised controlled trial (RCT) of 71 pregnant women with type 1 and type 2 diabetes, the use of blinded-CGM (CGMS Gold – Medtronic) for up to 7 days at intervals of 4-6 weeks between 8-32 weeks gestation was compared against standard antenatal care. Subjects in the intervention arm showed lower HbA1c between 32-36 weeks, lower mean infant birth weight and reduced risk of macrosomia, compared to the control group (Murphy et al., 2008).

The effectiveness of RT-CGM devices has also been demonstrated in a number of studies. The landmark Juvenile Diabetes Research Foundation (JDRF) CGM study evaluated the use of RT-CGM in 322 subjects with type 1 diabetes with baseline HbA1c ≥ 7%. Subjects were randomised to either
continuous RT-CGM or standard care and were offered the choice of Medtronic, Abbott or DexCom devices. At 26 weeks the HbA1c fell significantly only in subjects aged ≥25 years from 7.6 to 7.1% but not in those aged 8–14 years or 15–24 years (Tamborlane et al., 2008). In all three age groups, factors that were associated with a greater reduction in HbA1c from baseline to 6 months were sensor use of 6 days or more per week and higher baseline HbA1c (Beck et al., 2009a). The study has also demonstrated that reduction in HbA1c was not associated with increased risk of hypoglycaemia, which did not change at 26 weeks. The JDRF CGM study also evaluated the potential benefits of RT-CGM use in subjects with HbA1c<7%. Although the reduction in time spent with glucose levels <3.9 mmol/L was not significantly different between the CGM and control groups at 26 weeks, total time out of range (<3.9mmol/L or >10mmol/L) was significantly lower in the CGM group and between-group difference in HbA1c was 0.34% favoring the CGM group (Beck et al., 2009b). The 26-week extension of the JDRF study in the adult group, combining subjects with HbA1c<7% and those with HbA1c ≥ 7%, showed the added benefits of RT-CGM in reducing rates of severe hypoglycaemia, reducing time spent in hypoglycaemia and increasing time spent in glucose level between 4-10 mmol/L while sustaining reduction in HbA1c (Bode et al., 2009).

These findings were supported by a meta-analysis, which showed an overall mean HbA1c reduction of 0.3% in type 1 diabetic subjects using RT-CGM in comparison to SMBG. This meta-analysis also highlighted the correlation between compliance with the frequency of CGM device use and its
effectiveness; for every one day increase in sensor usage per week there was an additional HbA1c reduction of 0.15% (Pickup et al., 2011).

Combining the diagnostic benefits of CGM with the therapeutic benefits of subcutaneous continuous insulin pump therapy and the development of sensor augmented pump therapy (SAP) was evaluated in several studies. The STAR3 study was a 1-year RCT with 485 subjects who were randomised to either SAP or standard care (multiple daily injection and SMBG). At the end of the study there was HbA1c reduction from 8.3% to 7.5% in the intervention group with significant between-group difference in HbA1c of 0.6% favoring the intervention group. There was no statistically significant difference in the rate of severe hypoglycaemia between the two groups. However, the study failed to demonstrate the effect of CGM or insulin pump therapy alone compared to SAP therapy to determine the beneficial contribution of each component of the system (Bergenstal et al., 2010). At the end of 1-year in the STAR3 study, subjects from the control group were crossed over to SAP group for 6 months. This resulted in reduction of HbA1c from 8% to 7.6%. Subjects already in the intervention group who continued with SAP therapy showed sustained reduction in HbA1c. The study also highlighted the role of compliance with sensor use in the effectiveness in SAP therapy. Subjects in the SAP group were able to maintain HbA1c if they used the sensor >40% of the time, whereas in the crossover cohort the maximum reduction in HbA1c was observed with sensor wear times>60% (Bergenstal et al., 2011).

To evaluate the contribution of CGM to SAP therapy, the SWITCH study was conducted with 153 children and adults already on insulin pump therapy. In this randomised controlled crossover study, subjects were randomised to
CGM “sensor on” or “sensor off” groups for six months before washout period of four months followed by crossover for another six months. The study showed HbA1c reduction from 8.47% to 8.04% (difference of 0.43%, \(P<0.001\)) in the SAP group. The study also demonstrated that the SAP group spent less time in hypoglycaemia and that stopping CGM led HbA1c to revert back to baseline (Battelino et al., 2012).

While SAP still relies on appropriate therapeutic interventions from patients and/or physicians to impact outcomes, it represents an important step towards realisation of the closed loop insulin delivery system. Another important step in this direction was the development of SAP with low glucose suspend (LGS) feature by Medtronic. The Paradigm Veo System with the LGS feature allows suspension of insulin delivery for 2 hours when a prespecified glucose threshold setting is reached by the associated continuous glucose monitoring. The ASPIRE In-Clinic study evaluated this LGS feature in 50 subjects with type1 diabetes. In this randomised crossover study subjects fasted overnight and exercised whilst using SAP, with LGS feature either turned on or turned off, until their sensor glucose value was <4.7 mmol/L. LGS feature was set to suspend insulin therapy when CGM-detected glucose concentration <3.9mmol/L. The mean hypoglycaemia duration was lower and mean nadir glucose value was higher in the LGS-On sessions, compared to LGS-Off sessions, without causing rebound hyperglycaemia (Garg et al., 2012).

Similarly, the ASPIRE In-Home study evaluated the ambulatory use of LGS feature over three months in 121 subjects with documented nocturnal hypoglycaemia. The study showed that use of LGS feature resulted in
reduction in nocturnal hypoglycaemia without increasing HbA1c and was not associated with ketoacidosis (Bergenstal et al., 2013b).

Evidence for the benefit of CGM technology in subjects with type 2 diabetes is relatively limited by paucity of data. In an RCT of 100 subjects with type 2 diabetes not treated with prandial insulin, subjects were randomised to either RT-CGM or SMBG for 3 months before they were follow-up for 40 weeks. During the intervention phase, RT-CGM occurred in the intervention group in four cycles (2 weeks on /1 week off). At 12 weeks, there was significant reduction in HbA1c by 1% in the intervention group compared with 0.5% in the control group ($P = 0.04$) without increasing incidence of hypoglycaemia. This reduction in HbA1c was sustained, in contrast to the subjects with type 1 diabetes in the SWITCH study. As in type 1 diabetes, the study also demonstrated the positive correlation between compliance with the frequency of CGM device use and effectiveness (Vigersky et al., 2012, Ehrhardt et al., 2011).

Glycaemic variability has also been the focus of interest in several CGM studies (this is discussed in chapter 6 – Glycaemic Variability and The Effect of RT-CGM).

An evolving area for research is the use of CGM in the ITU setting. CGM can potentially allow the use of intensive insulin therapy to achieve tight glycaemic control in critically ill patients without increasing the risk of hypoglycaemia. It may also reduce the need for frequent blood sampling for glucose estimation with subsequent reduction in ITU staff workload. However, the alteration in blood flow, metabolic rate and capillary permeability in the subcutaneous tissue in critically ill patients represents a concern with regards to accuracy
and reliability of ISF glucose measurement using a subcutaneous implanted glucose sensor. This was assessed in a study of 50 critically ill subjects on intensive insulin therapy. The study demonstrated that circulatory shock requiring epinephrine did not affect the accuracy and reliability of subcutaneous CGM systems (Holzinger et al., 2009). Another study that evaluated two subcutaneous CGM systems in postoperative cardiac surgery patients during ITU stay reported numerical accuracy of the sensors that is better compared to reported data for outpatient use. The median ARD was 11% and 14% with a lag of 0-4 minutes compared to reference arterial blood glucose values (Siegelaar et al., 2011a). In an RCT with 124 critically ill subjects, the effect of RT-CGM (Guardian CGMS) use on glycaemic control and risk of hypoglycaemia was compared to standard care. Glucose data were used to feed an algorithm to guide insulin infusion rate in the two groups. The control group also had a blinded CGM. The study demonstrated that RT-CGM reduced risk of severe hypoglycaemia by 9.9% in the intervention group but had no effect on mean glycaemia (Holzinger et al., 2010). A recent RCT of 177 subjects in a mixed ITU was conducted to compare subcutaneous CGM (FreeStyle Navigator) to intermittent capillary blood glucose (CBG) testing to guide insulin treatment. In the intervention group, glucose data were obtained every 10 minutes from CGM and used to feed a computerised glucose regulation algorithm to guide IV insulin infusion rate, with a target glucose of 5-9 mmol/L. Subjects in the control group had blinded CGM and the same algorithm was fed by intermittent CBG measurement every two hours. In both groups, arterial reference blood glucose samples were collected six times daily and used for calibration. The study demonstrated significant reduction in
ITU staff workload in the intervention group but no difference in incidence of severe hypoglycaemia or percentage of time within target range between the two groups. The study highlighted the reduced accuracy of subcutaneous CGM in comparison to CBG testing device, with a median ARD of 13.7% vs 7.1% respectively, which is comparable to published data on ambulatory CGM. There were also technical difficulties with CGM device use (related to calibration and temporary signal loss) despite training of all ITU nurses. Furthermore, CGM device failure occurred in 21 subjects (Boom et al., 2014). Further studies are required for full assessment of the effect of CGM on different glycaemic measures, morbidity and mortality in ITU setting.

Despite the extensive research in the field of CGM technology, there remain important gaps in our knowledge. The impact of CGM use on risk of severe hypoglycaemia remains unclear (Choudhary et al., 2013). This is largely secondary to the lack of RCTs that have been specifically designed to evaluate CGM use in this population. Moreover, subjects with history of severe hypoglycaemia are often excluded from interventional studies. Another area of controversy in this field is the impact of CGM use on quality of life, with conflicting evidence emerging from different RCTs (Langendam et al., 2012, Hommel et al., 2014, Polonsky and Hessler, 2013). The cost-effectiveness of CGM use in patients with type 1 diabetes is another important area for future research (McQueen et al., 2011). The National Institute for Health and Care Excellence has conducted a de novo cost-effective analysis for its recent updated guidelines, which has found that existing CGM systems are not cost-effective even in people with type 1 diabetes who suffer from
impaired awareness of hypoglycaemia. However, these systems could be of some value in adults with type 1 diabetes with elevated HbA1c (NICE, 2015). However, despite the clear benefits of continuous glucose monitoring, it has not been widely implemented in the routine management of type 1 diabetes due to several challenges. This is evident from T1D exchange data demonstrating that CGM technology is being used by only 6.5% of people with type 1 diabetes in United States, despite reimbursement (Miller et al., 2013b). The devices remain invasive and are associated with discomfort. This can negatively affect compliance and therefore the effectiveness of CGM, and is a potential limitation to continuous use. This is corroborated by the high dropout rate in clinical studies (Hermanides et al., 2011) and in routine clinical practice, as demonstrated in the T1D exchange data showing that among individuals who have used a CGM, two-thirds stopped using it (Miller et al., 2013b). Moreover, data for CGM sensor accuracy has demonstrated clinically relevant reduced accuracy in the critical hypoglycaemic range (Mader et al., 2010, Keenan et al., 2012, Weinstein et al., 2007, Mastrototaro et al., 2008). The complexity of CGM devices, the need for ongoing calibration against blood glucose values and the limited life span of glucose sensors can negatively impact patient’s experience when using CGM (Voskerician and Anderson, 2006). Other important limitations to CGM use could be related to high cost and clinicians’ reluctance due to either lack of experience or lack of resources required for effective management of patients on CGM. To overcome some of the challenges facing CGM, emerging technologies, including microneedles, aim to minimise the level of invasiveness and enhance sensor’s accuracy.
1.3. USE OF MICRONEEDLE-ARRAY TECHNOLOGY IN CGM:

1.3.1. Background

Microneedle use was first suggested in 1976 by Gestel and Place who proposed the use of multiple needle shaped projections with a needle length of 5-100 µm to penetrate the stratum corneum layer for drug delivery. Thanks to advances in microfabrication in 1990s, microneedle manufacture was feasible. In 1998, Henry et al showed that using microneedles of 150 µm length to penetrate the stratum corneum can dramatically increase skin permeability to a model drug, calcein (Henry et al., 1998). Since then, research in microneedle array technology has developed rapidly, particularly in the field of transdermal drug delivery. Microneedles can be manufactured as in-plane or out-of-plane where the needles are either parallel or perpendicular to the fabrication surface respectively. Out-of-plane microneedles can be either solid or hollow. Microneedles of different geometries, designs and materials (including polymers, metals, glass, carbohydrates or silicon) have been manufactured and microneedle technology has been extensively investigated as a therapeutic tool for transdermal drug delivery. Other applications for microneedle technology have been investigated including for biopsy (Byun et al., 2005), light delivery to deeper skin layers for diagnosis and treatment of epithelial cancers (Kosoglu et al., 2010) and for measurement of electrical potentials (for example in electrocardiography) (Yu et al., 2009). Several research groups have investigated the use of microneedle technology as a diagnostic tool allowing access to biofluids (ISF or blood) for analysis of variable analytes including glucose. For CGM using ISF, an array containing several
microneedles can be employed to penetrate the stratum corneum and access ISF without impinging on the dermal nerve fibers or blood vessels. ISF can then be either extracted to the surface (using hollow microneedles) or analysed *in situ* using microneedle sensors.

![Microneedle Image](image)

**Figure 1.5:** A scanning electron microscopy image of a 500 µm tall microneedle next to the tip of a 27-gauge hypodermic needle.


1.3.2. Potential advantages of microneedle technology for CGM:

1.3.2.1 *Minimal invasiveness*

Human skin comprises the epidermis, dermis and subcutaneous tissue. The outermost layer of epidermis, called stratum corneum, is primarily made of dead tissue and is responsible for skin barrier characteristics. The viable epidermis, 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 (Khanna et al., 2008). (Figure 1.6)

**Figure 1.6:** A schematic diagram showing a cross section of skin layers and a microprobe array applied to skin surface with the microprobes’ tips in the upper dermis.

One of the main aims of microneedles accessing the dermal ISF compartment, to measure its glucose content, is to penetrate the layer of stratum corneum without impinging on dermal microcirculation or dermal nerve fibers.

The stratum corneum represents the main mechanical barrier to microneedle insertion (Park et al., 2005). Knowledge of stratum corneum thickness is therefore essential to determine microneedle length and ensure adequate penetration. There is a marked site variation in mean stratum corneum thickness; ranging from 22.6+/-4.33 µm for volar forearm, 29.3+/-6.84 µm for back of the hand, and 173.0+/-36.96 µm for palm. The stratum corneum thickness tends to become age-dependently thicker at the forearm (Egawa et al., 2007). Similarly, variation in the total epidermal thickness largely depends
on body sites, with no correlation to either age or skin type (Sandby-Moller et al., 2003).

In comparison to the smallest needle-type glucose sensor used in clinical practice for CGM which has a length of 9mm and a width of 420 μm (27-gauge), microneedles employed for the same purpose have a length of less than 2000 μm and a tip diameter of less than 50 μm. Thanks to its minimally invasive nature, microneedles' application results in less skin inflammation, less pain, reduced risk of infection or bleeding and rapid skin recovery following removal.

1.3.2.1.1. Skin reaction

Skin irritation is a reversible local inflammatory reaction that leads to erythema and oedema. It can also be associated with local heat and pain and may be immediate or delayed. The ISO, responsible for setting the requirement for medical device manufacture and design, defines irritation as a "localised inflammatory response to single, repeated, or continuous application of the test substance, without involvement of an immunological mechanism." If irritation occurs, the resulting erythema can be assessed clinically or measured by different methods including chromametry or laser doppler imaging methods. Chromametry measures superficial colour change while laser doppler measures blood flow deeper in the skin (Noh et al., 2010).

The minimally invasive nature of microneedle arrays suggests that they are likely to be associated with less skin irritation when compared to invasive devices provided that a biocompatible material is used. The safety of microneedles with regards to skin irritation has been demonstrated in a double-blinded, sham-controlled, randomised study in 54 human subjects. An
8-point skin reaction score was employed to assess skin irritation following application of 35, pyramidal, 700 µm long, solid microneedle to facial skin or a sham device. Microneedle application was associated with minimal self-limiting facial skin irritation that rapidly resolved in less than 1 hour (Hoesly et al., 2012).

The relationship between different variables of microneedle array manufacture and skin irritation has been evaluated in a number of studies. The relationship between microneedle geometry and skin redness was investigated using metal microneedles of variable length (200 – 550 µm) and shapes in eighteen human subjects. Skin redness was measured by assessment of skin colour and laser Doppler imaging. This showed that microneedles with sharper tips resulted in less skin irritation compared to solid ones and that skin blood flow increased with longer microneedles. For all microneedles studied, irritation was minimal and lasted less than 2 hours (Bal et al., 2008).

To study the effect of microneedle application time on skin irritation, polymer microneedles of 500 µm length were inserted for 2, 10, 60, 120 and 240 minutes in five human subjects. Skin redness was measured by reflectance spectrophotometer as a determinant of the degree of skin irritation. Redness score increased for all microneedles with the highest initial value noted for microneedles applied only for 2 minutes, and was maintained for 30 minutes indicating that redness was greater when application time was shorter. Regardless of the duration of microneedle application, redness was maintained for 30 minutes then rapidly resolved between 30 minutes and 2 hours after removal (Noh et al., 2010; Bal et al., 2008) (table 1.3).
<table>
<thead>
<tr>
<th>Author</th>
<th>Microneedle Details</th>
<th>Human/animal</th>
<th>Assessment</th>
<th>Results</th>
</tr>
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</table>
| Bal (2008)  | Stainless steel     | Human in vivo | To assess: | • Increasing MN length increased TEWL and skin irritation  
|             | Arrays of 4x4 solid MN: 200 µm wide 200, 300, 400 µm long 1250 µm pitch Arrays of 4x4 assembled hollow MN: 300 µm wide 300 and 550 µm long 1250 µm pitch | (18)          | 1 - Skin penetration using TEWL  
2 - Skin irritation using chromameter and LDI  
3 - Pain sensation | Assembled MN induced higher TEWL but less skin irritation  
Irritation was minimal and lasted less than 2 hours for all MNs  
Pain was similar and minimal |
| Noh (2010)  | Solid Polymer       | 5 human subjects (in vivo) Rats in vivo | To assess relationship between application time and :  
• skin irritation using reflectance spectrophotometer  
• microchannel depth using confocal microscopy  
• penetration using electrical conductance | • Electrical conductance values were sharply increased dependent on application time. Values reached baseline 420 minutes after MN removal (rats)  
• Skin redness increased and was maintained for 30 minutes before it decreases rapidly between 30 and 420 minutes (human)  
• Microchannel depth increases as the application time increases (rats) |
| Donnelly (2009) | Solid silicon array of 6x5 MN | In vitro using silicon membrane (Silescol) and neonatal porcine skin | The number of C. albicans, P. aeroginosa, S. epidermedis crossing Silescol membrane or skin layers following MN application compared to a 21-G hypodermic needle | • MN puncture resulted in significantly less microorganism penetration compared to hypodermic needle.  
• No organisms crossed the viable epidermis in MN-punctured skin |

**Table 1.3**: Studies investigation skin irritation / infection following microneedle application. [MN, microneedle, TEWL, transepidermal water loss, LDI, laser Doppler imaging]
1.3.2.1.2. Pain

Needle insertion, whether for drug administration or diagnostic fluid sampling can cause undesirable pain and trauma. This is particularly relevant in diabetes where frequent needle use is part of the daily routine for people with diabetes for either insulin administration or for monitoring. This can result in declining treatment adherence and is particularly challenging in people with needle phobia and in children. Microneedle array technology represents a minimally invasive alternative to traditional needles and lancets, resulting in reduced pain and tissue trauma.

The first study to evaluate pain related to microneedle application was carried out in 12 healthy human subjects challenged by silicon microneedle arrays containing 400 solid microneedles (length of 150 μm, base diameter of 80μm and tip radius of 1 μm). A 26-gauge hypodermic needle was used as a positive control (inserted to 2 mm) and a smooth silicon surface as a negative control. Pain was assessed using a 100 mm visual analogue scale (VAS). The mean score was 0.67 mm for microneedles compared to that of 0.42 mm for the negative control (P=0.09) and 23.9 mm for the positive control (P=0.001) (Kaushik et al., 2001).

The relationship between microneedle geometry and the degree of pain resulting from microneedle insertion has also been evaluated. An expected positive correlation between pain and microneedle length was demonstrated in a number of studies (Kaushik et al., 2001, Gill et al., 2008, Gupta et al., 2011). As microneedle length increases, the chances of stimulating dermal nerve fibers lying at an approximate penetration depth of 400 μm increases. However, even with long microneedles, the resulting pain seems to be
minimal, presumably due to the small diameter of the microneedles. In a study using 2 mm long microneedle to extract blood for glucose monitoring, the insertion was described as barely noticeable by the majority of subjects (Smart and Subramanian, 2000). In another study, single microneedles with variable geometry (variable tip angles, thickness, width and lengths that ranged from 480–1450 µm) and arrays containing 5 or 50 microneedles were compared to a negative control Teflon rod and a 5 mm insertion of a 26-gauge hypodermic needle as a positive control in ten human subjects. Pain intensity was assessed using a 100mm VAS. The study showed that all microneedle arrays were significantly less painful than the hypodermic needle with a pain score varying from 5-40% of the pain from the hypodermic needle use. Microneedle length has the strongest effect on pain with a three-fold increase in length increasing pain score seven-fold. The number of microneedles also affects pain score but to a lesser extent, with a ten-fold increase in number of microneedles increasing pain score 2.5 fold (Gill et al., 2008).

Pain and sensation were assessed in twelve human subjects in response to microneedle platinum-coated silicon arrays of two different lengths (180 µm and 280 µm microneedle) and a 25-gauge hypodermic needle. Pain intensity was assessed using VAS and sensory perception was determined by using the McGill Pain Questionnaire short form. Microneedles were perceived to be significantly less painful compared to hypodermic needles. Subjects described a pressing and heavy sensation rather than a sharp or stabbing one in response to microneedle application (Haq et al., 2009).
1.3.2.1.3. Infection

The skin acts as the main barrier against environmental organisms and any breach in the skin barrier carries an infection risk. Because of the advantage of being minimally invasive, microneedles may be expected to be associated with less damage to skin barrier characteristics and therefore lower risk of local infection.

The ability of three different microorganisms to traverse microneedle-induced holes was investigated in vitro using porcine skin and using a silicon membrane that mimics the stratum corneum. A 21-gauge hypodermic needle was used as a positive control. The study showed that microorganisms could traverse microchannels created following microneedle application. However, microbial penetration was significantly less in the microneedle created holes and no microorganism crossed the viable epidermis. The study also demonstrated that microorganisms could adhere to microneedle arrays, which reflects the importance of microneedle sterilization during manufacturing and safe disposal after use (Donnelly et al., 2009).

Fabrication of antimicrobial microneedles has been demonstrated by the use of composite materials that contain biocompatible polymer and gentamicin (Gittard et al., 2010) or by coating microneedles with a thin film of silver (Gittard et al., 2009).

Despite the lack of studies that were designed to investigate the risk of infection in subjects with diabetes or those who are immunocompromised, none of the animal or human studies investigating microneedles has reported microneedle-related infection.
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<th>Author</th>
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<tbody>
<tr>
<td>Kaushik (2001)</td>
<td>Solid 3x3 mm² arrays containing 400 silicon MN</td>
<td>Human <em>in vivo</em></td>
<td>12 Human subjects. Each challenged by microneedle, positive and negative control. 100-mm visual analogue scale</td>
<td>Pain scale mean values: 0.67 microneedle 0.42 for negative control (<em>P</em>=0.09) 23.9 positive control (<em>P</em>&lt;0.001)</td>
</tr>
</tbody>
</table>
| Gill (2008)   | Solid stainless steel single MN of variable geometry:            | Human *in vivo* | Effect of needle geometry on pain in 10 human subjects compared to positive and negative control using 100-mm VAS | • Pain score of MN was 5-40% of 26-G hypodermic needle (positive control). 
• 3-fold increase in length increased pain score 7-fold while 10-folds increase in MN number increased it only 2.5 fold. |
| Haq (2009)    | Solid Silicon pyramidal 6x6 microneedles array                  | Human *in vivo* | Effect of MN application on pain, sensation, penetration and skin recovery in 12 human subjects compared to 25-G hypodermic needle using: 10-mm VAS, McGill pain questionnaire short form, staining dye, TWEL | • Pain: significantly less with MN 
• Sensation: as heavy or pressing 
• Penetration confirmed 
• Recovery: TEWL values normalised over 24 hours |
| Smart (2000)  | Single Hollow Silicon                                           | Human *in vivo* | Assessment of blood extraction for SMBG and pain | • Successful blood extraction for SMBG 
• Average pain perception scale: 1.2 for MN applied to the arm 1.7 for lancet applied to the arm 2.3 for lancet applied to fingertip |

**Table 1.4:** *Studies investigation the relationship between pain and microneedle application.*
1.3.2.1.4. **Bleeding**

The most superficial skin microcapillaries are located in the dermis at a depth of approximately 400 µm from skin surface. Therefore, microneedle arrays resulting in penetration below 400µm can impinge on cutaneous microcirculation and cause bleeding. However, despite the use of longer microneedles, bleeding is rarely observed in human studies (Prausnitz et al., 2009).

In one study, tiny droplets of blood (e.g. 1 µl) were observed at the insertion site of 1450 µm long microneedle. Shorter needles (400 µm and 700 µm) did not result in bleeding (Gill et al., 2008).

1.3.2.1.5. **Skin recovery**

Disruption of skin barrier characteristics following microneedle application can be assessed by several markers including optical imaging. Since disruption of skin barrier results in increased epidermal water loss, measurement of transepidermal water loss (TEWL) can also be used as a marker of successful microneedle penetration of stratum corneum. Markers used to assess disruption of skin barrier characteristics can be also employed to assess recovery of those characteristics. In general, recovery of the barrier characteristics of the stratum corneum is rapid following its disruption (Fluhr et al., 2002).

Several studies have assessed skin recovery following microneedle application. In 12 human subjects, TEWL increased significantly following insertion of microneedles of 180 µm and 280 µm length and a 25-gauge hypodermic needle. In each case, TEWL recovered to baseline within 24
hours with no significant difference between devices (Haq et al., 2009). Similar results were obtained in animal studies (Yan et al., 2010).

The relationship between skin recovery and microneedle length was also demonstrated using TEWL measurement in human subjects. TEWL declined rapidly to reach baseline in 30 and 60 minutes following application of solid microneedle arrays of 300 µm and 400 µm length respectively (Bal et al., 2008).

Electrical impedance spectroscopy has been employed to analyse the resealing of skin barrier characteristics following microneedle insertion in human subjects. Stainless steel microneedles of five different geometries were used in the absence and presence of occlusive covering to the microneedle-treated areas. In the absence of occlusion, skin barrier characteristics recovered within 2 hours for all insertion sites regardless of microneedle geometry. This extended up to 40 hours in the presence of occlusion (Gupta et al., 2011). Optical coherence tomography (OCT) was used to assess microchannel closure rate. Silicon microneedle arrays of 280 µm length were manually inserted in human subjects in the forearm and fingernail folds, after 85 minutes of microneedle removal the microchannel depth had reduced from 158 +/- 20 µm down to a depth of 76 +/- 13 µm (Enfield et al., 2010).

**1.3.2.2. Enhanced accuracy:**

Microneedle technology also provides the potential to enhance accuracy of electrochemical sensing of ISF glucose through different strategies. *In vivo* analysis of ISF using multiple microsensors compared to one macrosensor
provides a larger surface area for the enzymatic electrochemical reaction resulting in increased current with potential to improve accuracy of glucose sensing even in the hypoglycaemic range by improving the signal:noise ratio. This was demonstrated in an in vitro study that showed that the current generated from an array of solid microneedles was in the microampere domain (100 times larger than the current generated from gold disc electrode of comparable “footprint”) (Radomska-Botelho Moniz et al., 2012).

It has been demonstrated that the simultaneous use of multiple glucose sensors improves accuracy and precision of continuous glucose monitoring (Castle et al., 2012). Partitioning a microneedle array (designed for non-extractive in vivo measurement) into separately addressable electrodes allows for multiple glucose oxidase sensors, as well as providing a reference electrode. Other than allowing simultaneous multiple glucose sensing, this important feature provides redundancy for technical failure of single sensors as a single malfunctioning sensor can be “voted out” on the basis of divergent results.

Another avenue for potential enhancement of accuracy of ISF glucose sensing using microneedle technology is related to the measurement of glucose concentration in dermal ISF rather than subcutaneous ISF. Using microdialysis technique to compare dermal and subcutaneous ISF glucose against blood glucose showed that dermal ISF glucose concentration is similar to that of the blood (99.1%), while subcutaneous ISF glucose concentration is only 50% in comparison to blood glucose. It also demonstrated that dermal ISF glucose has identical peak concentration to
blood glucose and suffers from less lag in comparison to subcutaneous ISF glucose (Petersen et al., 1992, Boschmann et al., 2001).

1.3.2.3. Reduced biofouling and FBR:
Sensor size and geometry are important factors that affect FBR to implanted biosensors with less significant FBR to smaller sensors. This was supported by a study that demonstrated that subcutaneous implantation of 300 µm thick polyurethane substrate in rats for seven weeks resulted in capsule formation that is 20% thinner in comparison to the one that surrounded a 2000 µm thick implant (Ward et al., 2002). Another study, investigating the effect of needle size on tissue inflammation in pigs, has demonstrated that reduction in needle size significantly reduced hemorrhage, fibrin exudation, cell infiltration and FBR (Kvist et al., 2006)

1.3.2.4. Multi-analyte sensing:
Partitioning of a microneedle array (designed for non-extractive in vivo measurement) can also permit continuous multi-analyte sensing by immobilisation of different enzymes across multiple sensing areas sharing a common reference electrode. Simultaneous continuous monitoring of lactate, glycerol, bicarbonate and ß-hydroxybutyrate could, in theory, be beneficial in daily management of type 1 diabetes.
1.3.3. Use of microneedle technology for glucose sensing:

As previously discussed, glucose sensors vary with regards to degree of invasiveness, sensing technique and type of analysed biofluid (blood or ISF) (Oliver et al., 2009). Microneedle technology has been investigated for detection of physiologically relevant analytes such as glucose, lactic acid, hydrogen peroxide or glutamate in biological fluids (Windmiller et al., 2011a, Trzebinski et al., 2012, Windmiller et al., 2011b). Microneedle arrays can be employed for either extraction of biological fluid followed by offline analysis or as sensors for in situ analysis. This will impact microneedle array geometry, material and therefore mechanics of insertion. For example, microneedle array-based sensors that target ISF should be able to penetrate the stratum corneum layer and achieve a penetration depth of less than 400 µm to access ISF without impinging on cutaneous microcirculation or nerve fibers. Those targeting blood for analysis of its glucose content should be able to achieve penetration depth of 400–900 µm (Khanna et al., 2008, Moon et al., 2006). Microneedle arrays used for biofluid extraction will also need to have a specific design to allow for this.

1.3.3.1. Microneedles for blood extraction:

The concept of "e-Mosquito" blood sampling system consisting of silicon hollow microneedles, microactuators and microsensors has been described (Gattiker et al., 2005). A mechanism for biofluid extraction depending on volume change of a copolymerised gel caused by temperature and pH changes has been described. This resulted in a pressure change in a flow...
channel connected to a hollow microneedle to withdraw fluid for analysis by a glucose micro-sensor. The system was not upgraded to a practical level due to challenges in miniaturisation and system integration (Suzuki et al., 2002, Suzuki et al., 2004). Another blood extraction system incorporates titanium hollow microneedles (1 mm long, 25 µm internal diameter and 60 µm external diameter), micropumping system and glucose sensor. The system has not been tested in vivo (Tsuchiya et al., 2005). A 2 mm microneedle blood extraction system operated using a microprocessor to advance the microneedle into the skin has been assessed with blood drawn from the dermal microcirculation by capillary force to fill a microcuvette containing glucose assay reagent allowing one-step testing without the need to transfer blood from skin surface to a testing machine. Challenges to the system include the reagent formulation, sterilisation procedure, shelf life and integration of an electrochemical glucose sensor into their system (Smart and Subramanian, 2000).

1.3.3.2. Microneedles for ISF extraction:

The use of microneedle arrays has also been demonstrated for ISF extraction. Single microneedles manufactured from glass (700-1500 µm in length, 15-40 µm tip radii) were inserted and removed 10 times in a 1 cm² area of the skin of 15 rats and 6 human subjects before applying a vacuum to extract ISF and measure glucose concentration compared to that of capillary blood. Penetration was confirmed by the mean of confocal microscopy and histology. 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.
(Wang et al., 2005). In another study, a device containing an array of hollow silicon microneedles with internal diameter of 10-15 µm and length of 200-350 µm and microfluidic channels was used for ISF extraction and subsequent glucose measurement from human skin (Mukerjee et al., 2004). The complexity in obtaining the ISF sample, extraction time, erythema at the treated skin site, biocompatibility of glass or silicon and possibility of tip clogging when using hollow microneedles represent challenges to clinical use of these extraction systems.

A novel microneedle based system for ISF extraction has been demonstrated in vivo for measurement of glucose area under the curve (AUC) 2 hours following a glucose load. Two hydrogel patches were applied for 2 hours to microneedle-treated skin area to extract ISF. The hydrogel was then placed in water overnight for glucose extraction. The study showed a good correlation between ISF and plasma glucose AUC. The complex procedure of ISF collection and glucose measurement represent a challenge for this system to be used clinically. Moreover, glucose AUC has not been widely used as an index for hyperglycaemia and does not replace the need for glucose monitoring (Sakaguchi et al., 2012).
<table>
<thead>
<tr>
<th>Author</th>
<th>Microneedle</th>
<th>Human/animal</th>
<th>Assessment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smart (2000)</td>
<td>Single Hollow Silicon Length 2000 µm Thickness 100 µm</td>
<td>Human <em>in vivo</em> (41 subjects with diabetes, 21 healthy volunteers)</td>
<td>Assessment of blood extraction for SMBG and pain</td>
<td>• Successful blood extraction for SMBG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Average pain perception scale: 1.2 for MN applied to the arm</td>
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<td>• 1.7 for lancet applied to the arm</td>
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<td></td>
<td></td>
<td>• 2.3 for lancet applied to fingertip</td>
</tr>
<tr>
<td>Mukerjee (2004)</td>
<td>Hollow silicon array of 20x20 MN Length 200-350 µm Base diameter 120 µm Bitch 300 µm Internal diameter 10-15 µm</td>
<td>Human <em>In vivo</em></td>
<td>To assess penetration and ISF extraction in 1 human subject (the author) using confocal microscopy and staining dye</td>
<td>Successful penetration and ISF extraction</td>
</tr>
<tr>
<td>Wang (2005)</td>
<td>Solid tip/hollow bore Glass Single MN Length 1500 µm Tip 15-40 µm</td>
<td><em>6 healthy Human subjects (in vivo)</em></td>
<td>Single MN inserted and removed ten times in 1 cm² area then applying -200 mm Hg vacuum for 5-20 min. To Assess: 1- Penetration using confocal microscopy and histology 2- ISF extraction and comparing its glucose concentration to capillary blood glucose</td>
<td>• Successful penetration and ISF extraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rats</td>
<td></td>
<td>• Local erythema for few hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Generally painless procedure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• ISF glucose correlated well with CBG</td>
</tr>
<tr>
<td>Sakaguchi (2012)</td>
<td>Polycarbonate 305 MN/array Length 300 µm</td>
<td>Human <em>in vivo</em> (37 subjects with diabetes, 10 healthy volunteers)</td>
<td>Assessment of ISF extraction for estimation of glucose area under the curve.</td>
<td>Successful ISF extraction and good correlation between ISF and plasma glucose AUC.</td>
</tr>
</tbody>
</table>

*Table 1.5: In vivo studies investigating the use of microneedles for biofluid extraction.*
Figure 1.7: Scanning electron microscopy image showing a 500 µm high microneedle array. “Reprinted from El-Laboudi et al., Use of microneedle array devices for continuous glucose monitoring: a review. Diabetes Technol Ther. 2013 Jan;15(1):101-15. Copyright (2013), with permission from Mary Ann Liebert, Inc”

1.3.3.3. Microneedles for CGM:

To employ microneedle array technology for continuous monitoring of biofluid analytes including CGM, microneedles need to be incorporated in a system that extracts ISF continuously for subsequent in vitro analysis or to be modified for use as sensors for non-extractive in situ analysis.

Microdialysis techniques used for CGM involves the use of a semipermeable microdialysis membrane inserted into the subcutaneous tissue, allowing diffusion of glucose from ISF into a dialysate that is collected for glucose analysis. The use of microdialysis-based microneedles for CGM has been reported (Zahn et al., 2005, Zimmermann et al., 2003). In one study, the microdialysis system consisted of an array of eight 200 µm hollow silicon microneedles, an integrated porous poly-silicon dialysis membrane and an integrated GOx-based flow-through glucose sensor. Following penetration of
stratum corneum, the microneedles fill with ISF by capillary force. Glucose diffuses through the integrated dialysis membrane into a dialysate that is pumped past the integrated glucose sensor for glucose measurement. The system recalibrates automatically through periodic pumping of reference glucose solution past the sensor avoiding the need for finger-prick testing. The estimated sensor operation time was 72 hours with an instrumental lag of only 2 minutes. However, device assessment showed capillary forces are not sufficient to maintain a constant flow of ISF past the sensor (Zimmermann et al., 2003).

Several in vitro studies into the use of microneedle-based sensors for sensing of ISF analytes in situ have been recently published. In one study, the use of microneedle array-based carbon paste amperometric sensors was demonstrated for simultaneous detection of pH, glucose and lactate. An acrylate-based polymer hollow microneedle array was aligned with wells placed on a flexible flat cable. Each well had been filled with carbon paste material that was tailored to detect pH, glucose or lactate. The microneedle sensors were able to selectively detect changes in any of those analytes when tested in complex solutions and showed suitable performance when tested over physiologically relevant concentration range for each analyte (Miller et al., 2012). Our group has also demonstrated the performance of our microneedle array based glucose and lactate biosensors in vitro. Using covalent coupling chemistry, solid microneedle arrays were modified with glucose oxidase or lactate oxidase. An epoxy-polyurethane based membrane was used to extend the linear working range of the biosensors. Glucose biosensors performance was optimum over glucose concentrations ranging
from 0-25 mmol/L and was consistent for over 48 hours. The study has highlighted the potential to improve sensor accuracy by demonstrating that a glucose-dependent signal over one hundred times that of commercially available CGM, even in the hypoglycaemic range. This in turn improves signal:noise ratio and therefore sensor accuracy (Radomska-Botelho Moniz et al., 2012). Safety and performance (including accuracy, precision and life span) of these systems are yet to be demonstrated in vivo in human studies, which is an objective of this project.

Recently, in vivo assessment of a microneedle-based CGM system was described (Jina et al., 2014). The system consists of an array of 200 hollow microneedles that, upon application to skin surface and penetration of stratum corneum, allows passive diffusion of ISF to a glucose collection chamber. Glucose level is measured using an external amperometric first generation glucose sensor. The clinical study involved 10 subjects with diabetes wearing four devices simultaneously for 48 - 72 hours. Comparison of the system’s performance and calibration were made using capillary blood glucose measured using FreeStyle blood glucose meter (Abbott Diabetes Care, Alameda, CA, USA). The microneedle CGM systems were calibrated using a reference blood glucose value after an initial 2-hour warm-up period and then once daily with a morning finger-stick glucose value. The authors concluded that the device is accurate with MARD of 15% and 98.4% of paired points in the A+B region of the Clarke error grid. However, comparative capillary blood glucose testing (done every 20 minutes) was only done when subjects reported to the study site. Graphs published in the paper demonstrate that microneedle CGM glucose measurements reached as high as 33.3 mmol/L.
(600 mg/dL) without having a comparative capillary blood glucose measurement at the time. Lag time was estimated as 17 minutes and minimal skin reaction that resolved completely without treatment in several days was reported.

1.4.  RESEARCH MOTIVATION:

The growing evidence from CGM studies correlating compliance with frequency of device use and effectiveness has highlighted the need for a painless, non-invasive (or minimally invasive), accurate continuous glucose monitor. This is particularly important in view of recent advances in diabetes technology, including the use of sensor-augmented pump therapy and rapid progress in closed-loop research.

The use of microneedles as biosensors for continuous glucose monitoring shows great potential, overcoming a number of disadvantages related to the currently available sensors. The minimally invasive nature of the technology allows for pain-free continuous monitoring with minimal tissue trauma and inflammation, and allows for rapid skin recovery. By providing a large surface area for the enzymatic electrochemical reaction and measuring dermal (rather than subcutaneous) ISF glucose, functionalised microneedles provide the potential to improve the accuracy of glucose sensing, even in the hypoglycaemic range. It is also possible to partition microneedle arrays, that are designed for non-extractive sensing, into multiple subarrays to sense glucose simultaneously or providing a multiple analyte platform. The use of multiple simultaneous glucose sensors improves accuracy and precision of glucose monitoring (Castle et al., 2012). Advances in microfabrication allow mass production of microneedles at low cost with economies of scale. This is
particularly important as the high cost of clinically available CGM devices may represent one of the important limitations in approved clinical uses (Burge et al., 2008).

To be considered for clinical use, electrochemical glucose biosensors need to fulfill several requirements including specificity, accuracy, wide linear range, sensitivity, rapid response, resistance to interferents, biocompatibility and stability. Therefore design and optimisation of an electrochemical glucose biosensor requires careful choice of working electrode material, enzyme load and its immobilisation method, electron acceptor, type and thickness of the coating membrane and the operating potential. Despite the large efforts invested by various academic teams and the industry in the field of electrochemical continuous glucose sensors, there are several technical challenges and important barriers facing this technology.

The growing interest in microneedle-array technology for continuous analyte sensing is promising. However, further work is required before microneedle-based sensors are embedded in clinical practice. Despite the description of several microneedle-based glucose monitoring systems in the literature, only few systems have managed to reach human studies (El-Laboudi et al., 2012). Apart from general technical challenges facing electrochemical glucose sensors, microneedle-based electrochemical glucose sensors also face the challenges of miniaturisation, system integration and lifespan extension.

This research aims to complete the work that started in Cass’s group on microprobe array sensors (Trzebinski et al., 2012, Radomska-Botelho Moniz et al., 2012) and move it from bench side to bedside through optimisation, pre-clinical characterisation and clinical assessment of the novel microprobe
array continuous glucose sensor. In this work, we have optimised sensor design for clinical use from mechanical and functional aspects. At an early stage of this project, we have established a patient and public involvement group to help in identifying users’ requirements with regards to the device’s design and functionality and in gauging the problems that patients feel affect them day-to-day. We have also designed a questionnaire aimed at people with diabetes to find out their views on different aspects of existing diabetes technology and how it can help them in diabetes management.

To describe our solid microneedle array that are designed for non-extractive in vivo measurement of dermal ISF glucose, we have adopted the term “microprobes” to distinguish it from hollow microneedles that are usually employed for ISF extraction for in vitro glucose measurement. We have characterised the device mechanically by assessing the force required to penetrate the stratum corneum skin barrier and the force required to fracture microprobes in the axial and transverse planes. This is to ensure that microprobes are capable of reaching the dermal skin compartment to perform its intended function in measuring ISF glucose and to ensure that the risk of microprobes failing mechanically as they are penetrating skin layers is minimum. The work also includes electrochemical validation of the sensor in vitro and ex vivo and data on eight subjects from the first phase of the clinical study to assess safety of the device in healthy volunteers. Finally, we present retrospective analysis of the JDRF CGM randomised controlled study dataset to evaluate different measures of glycaemic variability in subjects with type 1 diabetes and the effect of RT-CGM on these measures.
CHAPTER 2

CONTINUOUS GLUCOSE MONITORING:

PATIENTS’ PERSPECTIVE
2.1. INTRODUCTION:

Despite the extensive research in the field of diabetes technology, research conducted to capture the views of the eventual end-users (patients or caregivers) is relatively limited. In recent years, there has been increasing awareness of the importance of patient and public involvement (PPI) in healthcare and healthcare research. In the UK, PPI is a core component in transforming health services towards patient-centered, collaborative and individualised care (NICE, 2012). Similarly, academic researchers, funding bodies and the pharmaceutical industry now consider PPI as an essential part of clinical research for the several benefits it brings throughout the different stages of the research process (Staley, 2009). By providing a different perspective to that of the researchers, a patient and public perspective offers a unique and invaluable insight that can help to correctly set research priorities, enhance research strategy and improve research outcome. It also helps in reaching potential subjects for recruitment and in dissemination and implementation of research findings. In diabetes technology research, active involvement of patients at an early stage of the research can correctly identify users' priorities and reveal potential limitations of the technology from users' perspectives. This will help to ensure that the end product is more acceptable to patients.

2.2. AIM:

This chapter describes the work conducted to capture patients' and public views and concerns in relation to continuous glucose monitoring (CGM) and the use of microprobe technology in CGM. It also describes PPI in our project.
to develop a novel continuous glucose monitoring system based on microprobe technology.

2.3. METHOD:

2.3.1. Microprobe Glucose Sensor focus group:

A partnership was created with the North West London Diabetes Clinical Research Network. A focus group of people with type 1 diabetes, their family members or caregivers, was convened at Imperial College London. As a moderator, I briefly presented: a) advantages and challenges facing existing CGM technology; and b) described microprobe technology and the potential advantage of using microprobe arrays to access interstitial fluid compartment in a less invasive manner, compared to existing needle-type sensors. Images were shown to participants, to illustrate microprobe arrays and show the difference between microprobes and needle-type sensors. Also, a prototype of the microprobe glucose sensor (36 microprobes in 6x6 rows) was passed around the group. Discussions were transcribed and open-ended questions were used to facilitate the 2 hours session. The aim of the focus group was to capture patients' and public views on CGM technology and the potential benefits or concerns related to the use of microprobe technology in CGM. We also aimed to invite interested participants to establish the “Microprobe Continuous Glucose Monitoring PPI Advisory Group” to present patients' and public perspectives throughout the different stages of the research project.

2.3.2. Diabetes Technology Questionnaire:

With help from the PPI group, an online questionnaire was designed to explore views and concerns of people with type 1 diabetes regarding diabetes
technology. The questionnaire covered the two main areas in diabetes technology: glucose monitoring (intermittent and continuous) and Insulin delivery (using insulin pens and subcutaneous insulin pump). It also addressed sensor augment pump therapy. Each area of diabetes technology was assessed using ranking questions (quantitative) related to potential advantages, potential challenges and features that should be added to make the technology more acceptable for patients. As subjects were allowed to choose more than one answer as the top priority, some of the results can add up to more than 100%. At the end of each section and at the end of the whole questionnaire, participants were asked an open-ended question to elaborate on their views and the reasons behind these views and their answers to the ranking questions. The questionnaire was designed using Google Forms and was distributed electronically, using social media and emails, by members of the PPI group, North West London Diabetes Clinical Research Network and Imperial Clinical Research Facility.

Questionnaire analysis of CGM data is presented in this chapter. Quantitative data were analysed using the Excel software (Microsoft Office Excel 2011 for Mac) and SPSS 21.0 for Mac (SPSS Inc., Chicago, IL). Qualitative data were analysed according to the principles of thematic analysis using Atlas.ti software (ATLAS.ti Scientific Software Development GmbH, London, UK) (Kitzinger, 1995, Pope et al., 2000).

2.4. RESULTS:

2.4.1 Focus Group:

The group consisted of 9 participants; 4 females and 5 males with an age range of 23 – 70 years. The group comprised 7 participants with type 1
diabetes and 2 family members. 3 participants were current users of insulin pump therapy and 1 participant was a current user of a continuous glucose monitor as part of sensor augment pump therapy.

We identified the following themes:

1- Advantages of CGM technology:

The group members identified benefits related to CGM use. Continuous knowledge of glucose fluctuations and how glucose levels are impacted by daily activities (e.g. diet and exercise), improvement in glycaemic control, “peace of mind” and the possibility of reducing the frequency of finger prick testing were all cited as advantages for using RT-CGM.

- “I would like to know constantly what my BG [blood glucose] is to help maintain control”
- “….Finger prick testing only lets me know what my BG [blood glucose] is for a segment of time….“
- “…I am tired of pricking my fingers so anything that helps me avoid finger prick testing would be good….“

2- Limitations to CGM use:

Cost was identified as the main reason for preventing CGM being widely available through the NHS.

- “….The cost of these devices needs to come down significantly before they can be funded by the NHS.”

The need to carry the device was also recognised as a limitation to continuous CGM use.
• “My only concern would be that it would constantly be in me.”

One participant, who was a current user of a CGM system, highlighted the issue of lack of CGM accuracy and reliability.

• “…The problem is that it [CGM] doesn't recognise whether I am high or low ….”

Important features for further development of CGM systems that participants identified, were related to; sensor life span, pain related to sensor application, data transmission, display and accessibility by healthcare professionals:

• “What size would the screen be? Where would you look for a reading?”

• “I would prefer to have the reading transmitted to my mobile phone. I’d also like my consultant to be able to access the data online.”

• “What is the range of readings on the device?”

• “If it is not painful then I don’t mind changing it every day.”

• “Will it be affected by extremes in temperature?”

• “Will it be waterproof?”

3- The potential advantages for the use of microprobe arrays in continuous glucose monitoring were identified from comments raised by the participants. These were mainly related to the small size and painless application.

• “Sounds excellent….I like the idea of it being small and not too invasive”

• “The new sensor sounds to be small enough not to be noticeable or a hindrance”

• “Brilliant idea….very useful for needle phobics”

• “The size is so much better – I would be able to take up rugby again”
4- Potential limitations to microprobe technology in CGM:

However, participants also raised a number of concerns related to microprobe arrays. These were related to cost, life span and the method of sensor application.

- “It’s a lovely idea but I hope it is not too expensive.”
- “How would you ensure that the company that will eventually buy this technology, if your research proves it works, won’t market it at a very high price similar to current continuous glucose monitors?”
- “What is the lifespan of the patch? How often would you need to replace it?”
- “How would you apply the patch? Would you need a device to put it on?”

Interestingly, all group members expressed the importance of designing an appropriate applicator for microprobe insertion. Since microprobe application is likely to be painless, the user will have no indication to know that the microprobes have been applied correctly. This might prompt some users to unnecessarily continue applying large force on the device, if applied manually, which can carry higher risk of microprobes fracture.

2.4.2 Microprobe Glucose Sensor PPI Advisory group:

Following the focus group meeting, an advisory group was established with membership of 8 participants (6 participants with type 1 diabetes and 2 family members of people with type 1 diabetes). Communication between group members and the research group was through email communication and
regular meetings (2-3 annually). The group participated in the development of research protocols and participant information leaflets for the clinical study, to evaluate safety and performance of a microprobe array continuous glucose sensor. They also participated in reviewing and editing grant applications. Furthermore, the group also helped in design and dissemination of the diabetes technology questionnaire. This active partnership between the research team and the patient and public group that was established at an early stage of research had many positive impacts. Other than improving the research design, protocol and clarifying participant information material, it was also positively viewed by funding bodies and proved pertinent in the success of obtaining a grant from the National institute for Health Research (NIHR) – Invention for Innovation (i4i) programme.

PPI also had a clear impact on participants. It resulted in the promotion of a well-informed and motivated group who developed a sense of ownership of the project and gained new skills in diabetes management. An example was a participant who found her participation in the PPI advisory group as a motivation to explore diabetes technology. She was suffering from frequent hypoglycaemia and impaired awareness, and therefore qualified for NHS funding for an insulin pump and she self-funded a Dexcom CGM system. She has also become actively involved in diabetes technology and education in her local community and through different online forums. For the research team, the interaction with the group members was useful in understanding patients’ priorities in diabetes management.
2.4.3 Diabetes Technology Questionnaire:

Participation in the online questionnaire was open for a period of 8 weeks to people with diabetes or their caregivers, regardless of diabetes type or geographical location. Social media was a very useful tool in disseminating the questionnaire to potential participants. Figure 1 demonstrates the interaction of potential subjects to reminders sent through social media by one of the members of the microprobe glucose sensor advisory PPI group who helped in dissemination of the questionnaire.

![Graph showing number of daily responses and effect of social media on increasing response rate](image)

**Figure 2.1:** The number of daily responses to the survey and the effect of the use of social media on increasing response rate (arrows represent points of interaction with the social media to invite potential participants to take part in the questionnaire).
2.4.3.1 Participants’ characteristics:

A total of 214 responses were received. As shown in tables 1 and 2, the majority of responses were from patients or caregivers of people with type 1 diabetes (92.5%), living in the UK (74.8%), treated with insulin pump therapy (69.6%) and either current or previous CGM users (57%).

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th>Children</th>
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</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>178 (83%)</td>
<td>36 (17%)</td>
</tr>
<tr>
<td><strong>CGM use</strong></td>
<td></td>
<td></td>
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<tr>
<td>Current CGM user</td>
<td>93 (52%)</td>
<td>29 (80%)</td>
</tr>
<tr>
<td>Previous CGM user</td>
<td>53 (30%)</td>
<td>22 (61%)</td>
</tr>
<tr>
<td>CSII treatment</td>
<td>40 (22%)</td>
<td>7 (19%)</td>
</tr>
<tr>
<td>SAP treatment</td>
<td>117 (65%)</td>
<td>32 (89%)</td>
</tr>
<tr>
<td>Gender (females)</td>
<td>126 (71%)</td>
<td>20 (56%)</td>
</tr>
<tr>
<td><strong>Age Groups (% of total participants)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-25 years:</td>
<td>16 (9%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>26-40 years:</td>
<td>47 (27%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>41-65 years:</td>
<td>74 (42%)</td>
<td>21 (58%)</td>
</tr>
<tr>
<td>&gt;65 years:</td>
<td>61 (34%)</td>
<td>11 (31%)</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>140 (79%)</td>
<td>20 (56%)</td>
</tr>
<tr>
<td>North America</td>
<td>29 (16%)</td>
<td>14 (39%)</td>
</tr>
<tr>
<td>rest of Europe</td>
<td>2 (1%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (4%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Type of diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>162 (93%)</td>
<td>36 (100%)</td>
</tr>
<tr>
<td>Type 2</td>
<td>10 (6%)</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>2 (1%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Duration of diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>5 (3%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>1-5 years</td>
<td>23 (13%)</td>
<td>21 (58%)</td>
</tr>
<tr>
<td>6-10 years</td>
<td>18 (10%)</td>
<td>9 (25%)</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>132 (74%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td><strong>SMBG frequency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 times/week</td>
<td>10 (6%)</td>
<td>-</td>
</tr>
<tr>
<td>2-4 times per day</td>
<td>42 (24%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>&gt;5 times per day</td>
<td>125 (70%)</td>
<td>32 (89%)</td>
</tr>
</tbody>
</table>

**Table 2.1:** Characteristics of the 214 respondents to the diabetes technology questionnaire (divided according to age group).
Table 2.2: Characteristics of the 214 respondents to the diabetes technology questionnaire (divided according to age group and CGM use).

The ability to monitor fluctuations and trends in glucose levels, in response to various daily activities, was cited as the indication for CGM use by 50% of respondents. Other indications included: improving glycaemic control (32%), prevention of hypoglycaemia (25%), monitoring glycaemic fluctuations overnight (21%), hypoglycaemic unawareness (16%), reassurance (11%), flexible lifestyle (mainly around exercise and children’s activities) (10%). In 2 subjects “the need to avoid frequent finger pricking” was reported as the indication for CGM use.
When asked to rank potential advantages obtained from CGM use, the two groups ranked the ability to monitor glucose fluctuations and detects trends as the most important advantage for CGM use. The value of CGM in improving metabolic control was less important compared to the ability to gather information about glucose fluctuations when unable to test CBG (e.g. nighttime) in the adult group. Similarly, it was less important compared to prevention of hypoglycaemic events in the children group (table 2.3).

<table>
<thead>
<tr>
<th>Response</th>
<th>Adults</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provides info on glucose fluctuation</td>
<td>119</td>
<td>26</td>
</tr>
<tr>
<td>Provides info at time when unable to check</td>
<td>109</td>
<td>22</td>
</tr>
<tr>
<td>Helps in improving control</td>
<td>107</td>
<td>22</td>
</tr>
<tr>
<td>Helps in avoiding hypos</td>
<td>91</td>
<td>25</td>
</tr>
<tr>
<td>Reassurance by alarm function</td>
<td>87</td>
<td>21</td>
</tr>
<tr>
<td>Helps with hypo awareness</td>
<td>79</td>
<td>19</td>
</tr>
<tr>
<td>Flexibility with daily activity</td>
<td>70</td>
<td>18</td>
</tr>
<tr>
<td>Reduces the need for repeated SMBG</td>
<td>61</td>
<td>14</td>
</tr>
<tr>
<td>Helps in modifying behavior around food</td>
<td>55</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2.3: Advantage of CGM as viewed by respondents.

With regards to limitations to CGM use, the majority of adult patients with current or previous experience in CGM (64%) ranked cost as the top challenge facing CGM technology. This was followed by lack of accuracy (21%). The order was reversed for the children group, where CGM inaccuracy was ranked as the most important challenge (43%) followed by cost (28%). As expected, pain related to the use of CGM was more important for the children
group (ranked the forth) compared to the adult group (ranked the 13\textsuperscript{th}). 10\% of participants in the children group considered pain as the biggest challenge facing the use of CGM.

Out of respondents with current or previous experience in CGM use, 89\% had positive views about CGM technology. Only 4 subjects (3 adults and one child) stated that they would not use CGM in the future. Lack of accuracy was cited by the three adult respondents, while the child cited pain as the reason. Other reasons were the bulky size of current CGM devices (in comparison to insulin pumps), false alarms, discomfort and lag time.

![Figure 2.2: Limitations to the use of continuous glucose monitoring technology from participants’ perspectives.](image)
Consistent with responses to limitations of current CGM technology, the majority of adult respondents ranked improved accuracy and affordability as the most important features for future development of CGM technology and to improve its acceptance. Longer sensor life, which could have an impact on the overall cost of CGM and also minimises the frequency of exposure to pain related to sensor insertion, was ranked third for both groups.

The majority of respondents (96% of adults and 94% of children) expressed a preference for RT-CGM compared to blinded CGM. Similarly, 73% of adults and 81% of children preferred the use of continuous CGM compared to intermittent CGM use.

**Table 2.4: limitations to continuous glucose monitoring use.**
Almost 80% of respondents expressed positive views to CGM use. However, 3% felt negative about CGM:

- “Found it inaccurate and too stressful”
- “I didn't like having to insert and wear another thing on my abdomen and having to change it so frequently “
- “Thought it would aid in meter accuracy and cut back on testing “

Analysing respondents’ narratives (169 responses) identified several themes and subthemes (table 2.6). To enhance the reader’s understanding of each subtheme, some quotations were selected based on clarity.

1- Limitations to CGM use:
   a. **Cost:** 32.5% of respondents identified cost as the main barrier to the use or continuous use of CGM. 87% of those who mentioned cost as a barrier to CGM use were from the UK. One participant stated:
• “….CGM needs to be funded by the NHS. I now eat once a day or sometimes every other day so I can afford the sensors”.

<table>
<thead>
<tr>
<th>A. Limitations to CGM use:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cost</td>
<td></td>
</tr>
<tr>
<td>2 Accuracy and reliability including impact of lag time and the importance of proper calibration</td>
<td></td>
</tr>
<tr>
<td>3 Pain/discomfort</td>
<td></td>
</tr>
<tr>
<td>4 Education and support by healthcare professionals</td>
<td></td>
</tr>
<tr>
<td>5 Others:</td>
<td></td>
</tr>
<tr>
<td>Adhesion problems</td>
<td></td>
</tr>
<tr>
<td>Sensor life</td>
<td></td>
</tr>
<tr>
<td>More devices to carry around</td>
<td></td>
</tr>
<tr>
<td>Data interpretation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Benefits of CGM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ability to see fluctuations and trends in glycaemia</td>
<td></td>
</tr>
<tr>
<td>2 Metabolic control</td>
<td></td>
</tr>
<tr>
<td>3 Quality of life and the value of CGM in empowering and motivating patients</td>
<td></td>
</tr>
<tr>
<td>4 Hypoglycaemia</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Features for future development of CGM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Improved accuracy</td>
<td></td>
</tr>
<tr>
<td>2 Integration with insulin pumps</td>
<td></td>
</tr>
<tr>
<td>3 Integration with smartphones and remote monitoring</td>
<td></td>
</tr>
<tr>
<td>4 User-friendly and compatible PC software</td>
<td></td>
</tr>
<tr>
<td>5 Waterproof CGM</td>
<td></td>
</tr>
<tr>
<td>6 Smaller size</td>
<td></td>
</tr>
<tr>
<td>7 Improved look and display.</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.6: themes and subthemes of participants’ responses**
b. **Accuracy and reliability of CGM.** The issue of sensor accuracy was highlighted by 20% of participants. In the majority of cases, poor accuracy resulting in increased frequency of false alarms was considered as a barrier to CGM system’s use and an important area for future development of CGM technology, as highlighted in this response:

- “……I have totally disabled the Low suspend feature on my pump because of lack of accuracy. Particular problems with pump thinking I am hypo overnight when I am in target (e.g. 5 [mmol/L] but pump thinks I am 4[mmol/L]) - sometimes this leads me to turn off alarms overnight in order to avoid continued alarms disturbing sleep - but this kind of defeats point of CGM!”

However, four responses have highlighted difference of accuracy between different sensors:

- “My daughter loves it when it is accurate, but is easily frustrated when it is out of sync. She knows they are expensive and doesn’t feel they are accurate enough for her – she is using medtronic/enlites. I would like to try Dexcom now because they seem to be more accurate and last longer. The accuracy is the biggest thing that is stopping us using the enlite, cost of restarting on dexcom is stopping us going that route. When she was using sensors full time her HBA1c was around 6.5 [%], Now it is creeping to 6.9 [%] and will be higher still next time, She is 11.”
c. **Pain**: pain or discomfort related to sensor insertion was highlighted by 2 adults and 3 children:
   
   • “I want insertion to be less painful and less damaging to skin - ideally entirely non-invasive - so that we do not have tears at bedtime every third day”
   
   • “My daughter found CGM painful to wear and due to this will not wear one again”.

d. Few respondents highlighted issues related to lack of education and support from healthcare professionals:
   
   • “New users should ALWAYS get support in first weeks of use (should be mandatory) and on going education to ensure it’s being used effectively. Seen far to many users online who don’t understand even basic principles…..”.

2- **Benefits of CGM:**

a. **Glycaemic control** was mentioned by 15%. This was almost always linking the use of CGM to good control. However, few respondents added that good control was not adequate and the technology needs to develop further to reduce disease burden.

   • “I want a safe, effective technology that will fit in with life while ensuring good control”.

b. **Improved quality of life (QoL) and reducing disease burden** was mentioned by 21% of respondents as the most important benefit for CGM use. Improved QoL resulted from effect of CGM on providing “peace of mind”, reassurance and sense of safety
related to help in detection and prevention of hypoglycaemia and flexible lifestyle related to diet and exercise. Words like “love” and “importance” of CGM was mentioned by 22% of the respondents:

• “In my opinion CGMs are an essential tool for managing diabetes. Even more important than a pump. Once you have become used to monitoring blood glucose in real time it is very difficult to go back to finger stick tests alone. Rather like driving a car with no speedo or fuel gauge….”

• “….we have both an insulin pump (Animas Ping) and a CGM. As parents of a young (9 yr old) active boy, we would give up the pump before the CGM. The CGM gives him the ability to be a child, to play, to do sports and to 'forget' about diabetes for even a short while. We don't need to be constantly bugging him, we have the information at our fingertips…”

Nevertheless, few responses reflected the frustration that can result from CGM use and how it can negatively impact QoL and increase stress:

• “I find the CGM isn't super accurate. I calibrated it but found that the CGM was telling me I was hypo and I was actually high when I checked on my glucometer. This was quite alarming and frustrating….”.

• “The alarms drove me mad - I ripped out CGM in middle of night due to waking me repeatedly w/false highs or lows-not acceptable! It was like having a baby crying and waking you
up off & on at night- didn't work well enough for me to want this on 24/7.”

Further developments were suggested by some participants to further reduce disease burden …… “I need a system that takes all the maths from my diabetes control”.

c. The value of CGM in visualising glycaemic fluctuations, trends, showing effect of life activities on glycaemia and guiding insulin adjustments was important for 12%:
   • “I thank the big guy upstairs for the CGMS daily. It's a no brainer IMO [in my opinion] that every diabetic should have one. Finger pokes are like driving in the dark and turning your lights on every 10 miles, the CGMS is having the lights on always. With children it's a must!”

d. Prevention and reducing risk of hypoglycaemia especially at nighttime was reported by 8% of respondents:
   • “CGM would give my family peace of mind as well as myself. I do tend to run low in my sleep and an alarm would give my husband peace of mind as he checks I'm ok during the night.”

3- Requirements for future development of CGM:

a. 25% of respondents wanted CGM to be integrated with insulin pumps. Other than providing the advantage of carrying less devices, interaction between the CGM and pump to allow low glucose suspend was also highlighted:
• I am very angry that open loop and closed loop integration is not already available for my Animas Vibe plus Dexcom G4. It is insulting and patronising and a wasted opportunity because the technical capability is there. I am really angry about this. The pump has a Chinese wall [firewall] to pretend it doesn't know the CGM readings. I have to enter them manually, I don't even get prompted with the sensor values….”.

• “…At work, I have to carry a cell phone, and an oxygen monitor in my pocket, and with a pump and CGM besides, my hips look three miles wide!”

b. 12% of responses addressed the issue of smartphone integration and ability for remote monitoring.

• “Better integration between multiple devices and compatibility with Macintosh computers, or even smartphone apps is important”.

However, potential challenges to the use of smartphone, as an alternative to a dedicated CGM display unit, was highlighted by some respondents:

• “Please don't make mobile phones the default receiving device. This would mean students taking exams could not wear their CGM (unable to have mobile phone in exam hall). There is also a greater risk of a phone being lost/stolen than another receiving device. Data integrated on the pump screen (for insulin pump users) is by far the best option”.
• “If parents or other nominated carer could remotely view the pump and sensor screen it would be hugely helpful both in terms of peace of mind and allowing more independence for my son. Ideally the system would involve text alerts to parents/carers of impending lows/highs and if a GPS location were incorporated I would be much less worried about my son being out on his own as I could remotely view glucose trends to flag up potential problems and be able to locate him quickly in an emergency”.

c. **Alarms** were mentioned by 8%. This included the need for louder alarms, ability to customise alarms and false alarm:

- “ability to personalise alarms, cycle different alarms as after a while brain gets used to alarms and you don't hear them as they become familiar background noise. Different alarms for different things, really annoying if driving and alarm goes, is it hypo alarm or too high alarm. One needs dealing with more urgently than the other”.

d. Other important themes included: the need for smaller CGM devices; the need for waterproof CGM devices; the need for better looking CGM devices; the need for sensors with longer life; and the importance of user-friendly compatible PC software.

### 2.5. DISCUSSION:

There is a gap between our knowledge of the potential benefits of CGM technology, and the implementation of this technology in the general
management of people with diabetes. Identifying the reason(s) for this gap is particularly important, since research evidence has linked CGM effectiveness to compliance with the frequency of its use (Tamborlane et al., 2008, Pickup et al., 2011). Moreover, evidence from RCTs showed that CGM use was not associated with improved QoL (Langendam et al., 2012, Hommel et al., 2014). Capturing patients’ views will not only help to identify limitations related to diabetes technology, but could also help assessing the psychological aspects of the technology and help to improve patient selection and training in the use of this technology. This in turn could lead to improvement in patient satisfaction, QoL, compliance and glycaemic control. For CGM technology, which is the focus of this project, cost has always been cited as the major culprit for this gap. However, data from the T1D exchange demonstrate that, despite reimbursement, CGM technology is being used by only 6.5% of people with type 1 diabetes in the United States and that among individuals who have used CGM, up to two-thirds stopped using it (Miller et al., 2013b). The high drop out rate among subjects recruited to blinded, as well as real-time, CGM clinical studies suggest that reduced accuracy and reliability of existing CGM systems might not be the only reason for reduced tolerability to CGM (Hermanides et al., 2011).

By capturing the views of patients, qualitative research and PPI can provide invaluable insights to researchers prior to conducting quantitative research and helps to improve users’ acceptability of the end product (Kitzinger, 1995). In the field of diabetes research, the value of PPI in setting research priorities was highlighted through setting up a partnership of patients, carers, health professionals and diabetes organisations to develop a top 10 list of priorities.
in type 1 diabetes research. The James Lind Alliance Type 1 Diabetes Priority Setting Partnership (including Juvenile Diabetes Research Foundation, Insulin Dependent Diabetes Trust, Diabetes Research Network, Diabetes UK, Scottish Diabetes Research Network, UK Database of Uncertainties in the Effects of Treatments and NHS Evidence - Diabetes) has identified that the ability to constantly and accurately monitor blood glucose levels, in people with type 1 diabetes, with a discrete device represents the top research priority for both patients and clinicians (Gadsby et al., 2012).

Aiming to identify the reasons for the existence of the gap between CGM technology and its adoption by end-users, we conducted this mixed-method study to capture the views and concerns of patients with diabetes and their family members or caregivers in relation to CGM.

As shown, participation was from highly motivated subjects with the majority having current or previous exposure to diabetes technology. This might be considered as one of the study limitations, since the sample does not represent the general population with diabetes, for whom exposure to the diabetes technology might be relatively limited, and therefore does not address potential misconceptions related to the use of diabetes technology. However, it can also be considered as one of the strengths of this study having obtained its results from those with real life experience with diabetes technology. Another limitation is relying exclusively on electronic method of distribution. This excludes a significant proportion of people with diabetes (target population) who are not regular users of Internet or social media.

The study shows that respondents had overwhelmingly positive views and experience related to the use of CGM. They have identified the positive
impact on their QoL, reassurance by the alarm function, the ability to monitor glucose fluctuations and trends through day and night, detection of hypoglycaemia and impact in improving glycaemic control as important advantages of CGM. “Peace of mind” related to the use of CGM has been frequently cited as a key CGM benefit.

Concerns related to existing CGM technology were mainly related to cost, lack of accuracy, the sensor life span and having to carry another electronic device. Despite these concerns, 80% of participants viewed existing CGM technology positively. Also, most of the participants (96%) had positive views in relation to overall diabetes technology and what it can provide to them to reduce the burden of their, or their children’s, disease.

Enhancement in CGM accuracy with subsequent reduction in false alarms, alarm fatigue and improvement in reliability could result in improvement in QoL, satisfaction related to sensor use, compliance and glycaemic control (Polonsky and Hessler, 2013). Reducing the cost has also been identified as a key issue in future development in CGM technology.

Further development in CGM should also consider CGM-pump integration, enhancement of user interface, improving alarm function, data display, ease of data interpretation, user friendly PC software and smartphone integration. Smartphone integration could help in reducing the number of devices carried by the patient (by mitigating the need to carry a dedicated CGM display unit) and might enhance interaction with the CGM through the use of a more familiar user-friendly interface provided by the smartphone. Integration between CGM systems and other devices (pump or smartphones) can also reduce the initial cost of a CGM system by mitigating the need to buy a
dedicated CGM display unit. Furthermore, smartphone integration can allow remote monitoring, which was shown to reduce the risk of prolonged nocturnal hypoglycaemia (DeSalvo et al., 2014). Another important development in CGM, from patients’ perspective, would include longer sensor life.

Results from our survey are consistent with results of recent qualitative research that used framework analysis of narratives of 100 adults with type 1 diabetes using RT-CGM. Lack of sensor accuracy and reliability was highlighted by most of the participants. Issues related to lag time, calibration and sensor life were also highlighted. Other barriers to CGM use included insertion discomfort or pain, sensitivity to tape, more kit to wear, difficulty in interpreting data, cost and issues related to support by healthcare professionals (Pickup et al., 2014). Participants in our survey had different background with regards to age group and exposure to CGM.

Our results are also consistent with those of a study that evaluated views of children and young adults (mean age 15+/- 4.8 years) in relation to CGM use. 58 responses from subjects with type 1 diabetes showed that providing continuous data on glucose level and improvement in glycaemic control were the top two reported beneficial features for RT-CGM use. Pain was the most disliked aspect of RT-CGM use with 14% of subjects discontinuing RT-CGM as a result. In subjects who stopped using RT-CGM, problematic equipment and inaccuracy accounted for 64% of reasons for discontinuation of RT-CGM use. This was followed by intrusion in life and insurance issues. Despite the reported problems 52% of subjects continued to use RT-CGM (Ramchandani et al., 2011).
Another limitation to this study is that it did not address views or concerns of healthcare professionals in relation to CGM or other aspects of diabetes technology. The reluctance of clinicians to adopt CGM devices is another important challenge preventing widespread implementation of the CGM technology. This can be related to lack of the necessary skills required to identify patients who are likely to benefit from CGM or for interpretation of CGM data (Bergenstal et al., 2013a). It could also be related to lack of resources or due to inadequate accuracy of existing CGM systems affecting its acceptance by clinicians (Christiansen et al., 2013). Including the views of healthcare professionals in this study or in a parallel study would have been useful to find out the reasons, views, concerns or misconceptions contributing to the gap between diabetes technology and its adoption by clinicians. This is particularly important as patients and clinicians might have different reasons for adopting CGM technology. While clinicians might mainly focus on reduction in HbA1c and risk of hypoglycaemia, patients’ top priority, as shown in the result of this survey, seems to largely focus on reduction of disease burden.

The use of microprobe technology in CGM was assessed through a focus group. The potential benefits identified by the participants included reduced pain and the small size of the sensor. However, concerns raised included the need for a method to ensure proper application and the potential cost. A qualitative study that assessed public and healthcare professionals views in relation to the use of microneedle technology for transdermal drug delivery demonstrated the potential advantages of this technology in comparison to
conventional needles. These included reduced pain, tissue damage, and risk of infection (Birchall et al., 2011).

This study also highlights the value of social media in healthcare research and as an important platform to reach potential participants. It also highlights the impact of social networking on diabetes management. Not only does it provide a platform to gain knowledge and exchange experiences, it can also be an important motivational tool through online peer support and engagement. Furthermore, it can serve as a method to disseminate information, improve doctor-patient communication or as a valuable research tool (Chretien and Kind, 2013). A qualitative analysis of posts and discussion forum topics on the 10 largest Facebook groups focused on diabetes management revealed that patients, as well as family members and friends, share personal clinical information, receive emotional support, and request diabetes management guidance from other group members. However, the study has also highlighted that promotional activity and requests for personal data collection were also common (Greene et al., 2011). A clear example highlighting the impact of social networking and the importance of communication with patients to capture their views and demands in relation to CGM technology is “The Nightscout Project”. The project aims at allowing real-time remote monitoring of the CGM data for Dexcom users. It requires a cable to connect the Dexcom receiver to an android smartphone and an open source software that transmits the CGM data to the smartphone and then to a server (cloud platform), where it can be accessed remotely (http://www.nightscout.info/).
Finally, this chapter highlights the positive impact of PPI on the research project, participants and researchers and the value of establishing this involvement at an early stage of the project.

2.6. CONCLUSION:

This study provides important insight into patients’ views, concerns and demands in relation to CGM technology. Cost and reduced accuracy represent important barriers to adoption and widespread implementation of this technology in general management of people with diabetes. Efforts to enhance CGM accuracy and reduce cost could have a significant impact in users’ perception of CGM technology with subsequent improvement in satisfaction, QoL, cost effectiveness, compliance and glycaemic control. PPI in diabetes technology research can play an important role throughout the different stages of the research and can potentially positively impact research design, conduct and outcome and has the potential to help in enhancing users’ acceptability to the end product.
CHAPTER 3

FABRICATION AND MECHANICAL CHARACTERISATION OF A NOVEL MICROPROBE ARRAY CONTINUOUS GLUCOSE SENSOR
3.1 INTRODUCTION

3.1.1 Skin microanatomy:

As previously highlighted, human skin comprises the epidermis, dermis and subcutaneous tissue. The outermost layer of epidermis, stratum corneum, is primarily made of dead keratinocytes and is responsible for skin barrier characteristics. The viable epidermis, below the stratum corneum, is around 100 µm and mainly consists of 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 (Khanna et al., 2008).

One of the main aims of microneedles accessing the dermal interstitial fluid (ISF) compartment is to penetrate the layer of stratum corneum without impinging on dermal microcirculation or dermal nerve fibers.

The stratum corneum represents the main mechanical barrier to microneedle insertion (Park et al., 2005). Knowledge of stratum corneum thickness is therefore essential to determine microneedle length and ensure adequate penetration. There is a marked site variation in mean stratum corneum thickness; ranging from 22.6 +/-4.33 µm for volar forearm, 29.3 +/- 6.84 µm for back of the hand, and 173.0 +/- 36.96 µm for palm. The stratum corneum thickness tends to become age-dependently thicker at the forearm (Egawa et al., 2007).

3.1.2 Mechanics of microneedle insertion:

The mechanics of microneedle insertion into skin is of paramount importance to device performance and safety. The mechanics of microneedle insertion were first investigated by Davis et al (Davis et al., 2004) who measured the
force required for fracture, the force required for insertion, and their ratio (termed the margin of safety). To determine the effect of microneedle geometry on the force of insertion, individual hollow metal microneedles with tip radii of 30 – 80 µm, wall thicknesses of 5 – 58 µm and a constant length of 720 µm were pressed against human subjects’ skin using a displacement-force test station until a preset maximum load was reached. Measured insertion forces ranged from 0.1 – 3 N, which is sufficiently low to permit insertion by hand. Forces of insertion showed an approximately linear dependence on the interfacial area of the needle tip but are independent of wall thickness. To determine the effect of microneedle geometry on the force of fracture, individual hollow microneedles were pressed against a hard surface using an axial load test station until a preset maximum displacement of 500 µm was reached. Fracture force increased significantly with increasing wall thickness and increased modestly with increasing wall angle and tip radius. The ratio of the fracture force to the insertion force was described as “the margin of safety”; values greater than one identify needles that will insert into the skin without breaking. The largest margin of safety was achieved using needles with small tip radius to reduce insertion force and large wall thickness to provide strength and increase fracture force. A similar method has been used to determine the margin of safety of microneedle arrays, rather than individual microneedles, has shown a safety margin of 6 - 9 when arrays of silicon solid microneedles, pyramidal in shape, with a tip radius of 5.5 µm, a base diameter of 250 µm and 308 µm high are assessed (Forvi et al., 2010).

In another study to evaluate the relationship between microneedle geometry and fracture force, the force required to cause microneedle failure by axial
loading increased with decreasing microneedle length, increasing base diameter or using microneedles of larger Young’s modulus (a measure of stiffness of materials). The force required to cause microneedle failure by a transverse load was also measured. This showed that the transverse-load failure force is smaller than the axial-load failure force for microneedles of the same geometry and material. This indicates that if microneedles experience significant transverse load due to incorrect axial insertion, microneedles could fail by bending (Park et al., 2005). Microneedles with geometry below a 12:1 aspect ratio of length-to-equivalent diameter and a polymer with more than 3 GPa of Young’s modulus were recommended to avoid microneedle mechanical failure by axial force (Park and Prausnitz, 2010).

The effect of microneedles’ distribution on skin insertion was evaluated by Donnelly’s group. The study has demonstrated that microneedle interspacing only begins to increase insertion force at low interspacing (<150 µm interspacing at microneedle base) (Olatunji et al., 2013).

3.1.3 Skin penetration:

Several methods have been employed to assess the ability of microneedles to successfully penetrate stratum corneum (micropore formation) and to further penetrate the underlying tissue (microchannels formation). Application of a staining dye to the insertion site allows en face visualisation of the created micropores. Alternatively, as intact stratum corneum is a barrier against water diffusion, interruption of this layer results in increased water loss and measurement of transepidermal water loss (TEWL) can be used as a measure of successful skin penetration by microneedles. Infra-red spectroscopy and electrical impedance spectroscopy have also been used
(Gupta et al., 2011). Although those techniques confirm whether the stratum corneum has been penetrated, they do not provide information on microchannel characteristics. Assessment of depth of penetration and its relation to microneedle array geometry require transverse visualisation of microchannels, which can be obtained by biopsy of the microneedle-penetrated skin for histological examination. However, this may alter the biomechanical characteristics of the punctured skin and alter macro- or microscopic appearance. The use of confocal laser scanning microscopy for in vivo imaging is limited to a penetration depth of only 200 µm (Enfield et al., 2010, Coulman et al., 2011). Optical coherence tomography (OCT) has emerged as a powerful tool to obtain in vivo transverse images of microneedles in situ and of the created microchannels after device removal. OCT is a non-invasive optical imaging technique analogous to ultrasound, mapping the variations of reflected light rather than sound from biological samples. The major advantage of OCT is that it is capable of penetrating to a depth of 2 mm, providing cross-section imaging of epidermis and upper dermis for accurate in vivo assessment of microneedle penetration and of the created microchannels without the need for skin biopsy. Furthermore, no prior sample preparation is needed as with histological examination. When compared to histology, OCT images showed that the histological techniques overestimate the dimensions of the created microchannels (Coulman et al., 2011).

Apart from microneedle geometry, skin resistance is another factor influencing the mechanics of microneedle insertion. Due to the elasticity of skin, application of microneedles results in skin indentation at the insertion site prior
to penetration of the stratum corneum layer which may result in incomplete or even failure of penetration (Martanto et al., 2006, Verbaan et al., 2007, Verbaan et al., 2008). The effect of skin elasticity and deflection on microneedle insertion was shown in a study that used trypan blue staining and TEWL to evaluate the relationship between microneedle length and skin penetration. It showed that manual application of short microneedles (300 µm long) was not successful in penetrating the stratum corneum and microneedles of 550 µm or more are needed to overcome skin elasticity and resistance (Verbaan et al., 2007). Subsequently, the use of an electrical applicator at a velocity of 1 or 3 m/s showed successful insertion of the short microneedles (300 µm long) (Verbaan et al., 2008).

Skin resistance to microneedle insertion was also demonstrated by OCT images showing stratified skin tissue is compressed during microneedle application leading to partial penetration (700 µm long microneedles created 300 µm long microchannels when inserted in palmar skin of human subjects). This skin resistance to microneedle insertion seems to increase as microneedle length increases, as shown in data demonstrating that the distance between the lower microneedle array base plate and stratum corneum was significantly lower with microneedle arrays of relatively short length (250 and 350 µm) compared to those of greater length (600 and 900 µm) (Donnelly et al., 2010a).

Various approaches have been used to reduce skin deformation at the microneedle insertion site and to overcome skin resistance including insertion using rotation (Martanto et al., 2006), vibration (Yang and Zahn, 2004) or at high velocity (Verbaan et al., 2008).
3.1.4 Microchannel characteristics:

Assessment of the characteristics of the resulting microchannels following successful insertion and establishing the relationship to microneedle geometry and insertion mechanics is equally important. For example, with the use of microneedles as biosensors for *in situ* monitoring of ISF glucose, the larger the surface area in contact with ISF, the better signal:noise ratio and this can lead to more accurate results being obtained.

OCT has been employed to evaluate the effect of microneedle geometry and force of application on skin penetration characteristics. A spring activated applicator has been used to insert silicon microneedle arrays into neonatal porcine skin *in vitro* using defined insertion forces showing that increasing the microneedle length or the force of application results in a significant increase in the depth of penetration. Moreover, alteration of the microneedle interspacing (density) had no effect on penetration depth achieved at a constant microneedle length and force of application (Donnelly et al., 2010a).

OCT was also used to evaluate the diameter of the created microchannels. It showed that following removal of microneedles, the created microchannels collapsed secondary to the elastic properties of human skin suggesting that microneedles are possibly even less invasive than previously suggested (Coulman et al., 2011).

Previous studies employing TEWL to evaluate the relationship between microneedle geometry and skin penetration characteristics show a positive correlation between microneedle length and TEWL (Badran et al., 2009, Verbaan et al., 2007). Measuring the TEWL of rat skin before and after treatment with silicon microneedles of variable length and density showed that
TEWL increased when arrays with longer microneedles and lower microneedle density were employed (Yan et al., 2010). This supports the hypothesis that increasing microneedle density reduces their skin piercing capacity, due to proportional pressure reduction at the tips of individual microneedles, referred to as the “bed of nails” effect (Stoeber and Liepmann, 2005). However, other studies showed no relation between microneedle array density and skin penetration characteristics (Donnelly et al., 2010a, Verbaan et al., 2008). Subsequently, the relationship between microneedle interspacing and skin penetration was evaluated suggesting that force required to insert microneedles starts to increase when interspacing distance at the base is <150 μm (Olatunji et al., 2013).

3.2 DESCRIPTION OF THE IMPERIAL COLLEGE MICROPROBE ARRAY CONTINUOUS GLUCOSE SENSOR:

The microprobe array continuous glucose sensor consists of a three dimensional out-of-plane microprobe arrays, with 64 microprobes (solid microneedles) perpendicular to the base plate and arranged as 8x8 arrays. The pyramidal-shaped microprobes are 1000 μm in length with a base of 600 μm, a tip diameter of 25 μm and pitch (distance between 2 adjacent microprobes) of 1200 μm (centre to centre). The microprobe arrays are made of SU-8, a biocompatible (Voskerician et al., 2003) negative photoresist epoxy material, coated with gold and are functionalised with glucose oxidase (GOx) enzyme (EC 1.1.3.4) to detect glucose electrochemically. Subsequently these microprobes were coated with a biocompatible (Wang et al., 2013) epoxy-polyurethane (epoxy-PU) membrane to prolong their linear working range
(figures 3.1 and 3.2). The epoxy-PU membrane is known to limit mass transport, reduce biofouling and increase resistance to interfering agents such as dopamine, ascorbic acid, acetaminophen, or uric acid (Yu et al., 2007, Wang et al., 2013).

This device is intended to be applied to the skin surface and inserted using thumb pressure or a specially-designed applicator allowing its microprobes to penetrate through the epidermis and access the dermal ISF and sense its glucose content electrochemically.

Figure 3.1: A scanning electron microscopy image of the Imperial College microprobe array continuous glucose sensor.
Figure 3.2: A schematic of the functionalised microprobe array demonstrating different layers of the glucose sensor.

3.3 AIM:

The aim of this chapter is to describe process and methods for optimisation of sensor fabrication and mechanical characterisation of the sensor to assess its ability to access dermal ISF without microprobes’ fracture.

Mechanical characterisation of the sensor aimed at assessing the force required to penetrate stratum corneum (insertion force) and the force required to fracture microprobes (fracture force) in the axial and transverse planes.

3.4 METHOD:

3.4.1 Materials:

SU-8 photoresist was obtained from Chestech Ltd, UK. Methylene blue was obtained from VWR International Ltd, UK. Masters used for fabrication of Polydimethoxy Siloxane (PDMS) moulds were initially obtained through collaboration with Centre for NanoHealth, Swansea University and
subsequently fabricated at Imperial College London. PDMS was obtained from Sigma-Aldrich, UK.

3.4.2 Fabrication of the microprobe array glucose sensor:
Several methods for microneedle/microprobe array fabrication have been described in the literature (Donnelly et al., 2010b). In our attempt to obtain a device with the correct geometry, we have explored two fabrication techniques (photolithography and micromoulding) using SU-8. SU-8 is a biocompatible negative photoresist viscous epoxy polymer (Voskerician et al., 2003). On exposure to ultraviolet (UV) rays, SU-8’s long molecular chains cross-link leading to the solidification of the material. SU-8 is available in several varieties with different densities and viscosities. Examples include SU-8 2, SU-8 25, SU-8 50 and SU-8 100 (higher numbers indicating higher viscosity).

3.4.2.1 Photolithography:
The Imperial College microprobe arrays were first fabricated using method reported by Kim et al (Kabseog Kim, 2004). An SU-8 mesa was formed on a Pyrex glass substrate. A second SU-8 layer was then deposited on to the first SU-8 and exposed to UV rays through the backside of the glass substrate using a mask that has circular openings of a preset diameter. When exposed to UV, SU-8’s long molecular chains cross-link, causing the solidification of the material and formation of an array of SU-8 tapered pillar structures (figure 3.3). Geometry of microneedles is controlled by the thickness of SU-8 layers and the diameter of the mask’s circular openings.
Apart from the high technical complexity and the high cost of the photolithography fabrication technique, it was difficult to obtain reproducible microprobe arrays of the correct geometry (figure 3.4). Therefore, we moved towards micromoulding technique for device fabrication.
Figure 3.4: A scanning electron microscopy image of an attempted fabrication of a microprobe array using photolithography.

3.4.2.2 Micromoulding technique:
This manufacturing method involves the use of a metal masters. The masters are used to create moulds of PDMS. Alternatively, moulds can be created from PDMS casts by directly punching holes into the cast using a laser ablation system.

To create PDMS moulds, masters were initially obtained through collaboration with Centre of NanoHealth, Swansea University. As the geometry obtained was suboptimal (as shown from initial skin insertion studies that will be discussed later in this chapter), we replaced these masters with aluminium masters, fabricated using an Electrical Discharge Milling (EDM) technique at Imperial College London.

The PDMS moulds were cast with SU-8 (50) using vacuum and spinning to ensure SU-8 is filling mould’s microcavities. The PDMS casts were subjected to 10 mm Hg vacuum pressure for 2 minutes and spun in petri dish at 4000 rpm for 30 minutes in a centrifuge (Eppendorf). The SU-8 was then cross-
linked by exposure to UV light at 365 nm for 30 - 60 minutes (figure 3.5). The samples were cooled at 4°C and the PDMS layer peeled off to obtain the cross-linked epoxy microprobe arrays. Bare microprobe arrays were then coated with an adhesion layer of titanium (15 nm) followed by gold (135 nm) using conformal sputtering (DC sputter system, JLS MPS 500) in a clean room.

![PDMS Mould](image1)

![Cross-linking of SU-8](image2)

![SU-8 microprobe array](image3)

**Figure 3.5:** Fabrication of microprobe array using micromoulding.

### 3.4.3. Mechanical validation

#### 3.4.3.1. Measurement of insertion force in human skin ex vivo:

Insertion force, (the force required to insert microprobes into human skin ex vivo), was determined using an Instron 5866 instrument with a 50 N load cell and Bluehill software (Norwood, MA). Full thickness human skin samples (breast or abdomen) were obtained from elective surgery at Imperial College.
Healthcare NHS Trust after approval from Imperial College Human Tissue Bank (ICHTB) and patients’ consent. ICHTB is approved by National Research Ethics Service in England to give “deemed ethics” for research projects to use extra samples of anonymised tissue and fluids collected specifically for research. Skin was either transported immediately in physiological saline solution, to the laboratory for mechanical testing or stored at –20°C. All skin samples were used within two weeks from date of surgery.

The subcutaneous fat was removed, using sharp dissection, and the skin stretched and mounted, dermis side down, on a cork dissection board covered with parafilm. The skin surface was wiped with a 70% ethanol swab to remove any subcutaneous fat remnants. Microprobe arrays were attached to the Instron’s movable probe, face down, using double-sided adhesive tape. The probe was lowered onto the skin at a speed of 3 mm/s until the required force was exerted. Forces of 7, 10, 15, 20 and 25 Newton (N) were applied (figure 3.6). Once reached, the target force was maintained for duration of 60 seconds. Following removal of the device, methylene blue solution, which selectively stains the sites of stratum corneum penetration, was applied to the upper surface of the skin for 20 minutes to visualise created micropores (Donnelly et al., 2011). Methylene blue dye was then carefully wiped using alcohol swap before skin examination using digital microscopy (Leica EZ4D) to count created micropores and determine insertion ratio (number of created micropores : number of microprobes) (Donnelly et al., 2011). Skin was then fixed in formaldehyde for 24 hours at room temperature. Skin samples were subsequently examined by the histology department (Imperial College Healthcare NHS Trust). This involved the skin being sectioned into 4 µm
sections. Sections were then stained with haematoxylin and eosin and examined by light microscopy to confirm penetration of stratum corneum layer.

**Figure 3.6:** A schematic showing method of measurement of insertion force in human skin ex vivo.

### 3.4.3.2. Measurement of microprobe axial compression force *in vitro:*

The effect of applying axial compression load (Donnelly et al., 2011) to the microprobe array was assessed using an Instron 5866 instrument with a 500 N load cell and Bluehill software (Norwood, MA). Microprobe arrays were placed on a fixed metal plate with the microprobes facing upwards before applying the desired force through the movable probe of the Instron compression system. The Instron instrument pressed the microprobe arrays using an axial force (parallel to the microprobes’ axes) at a rate of 1mm/s until the required force was exerted. Forces ranging from 50N to 400 N (50, 50 repeated, 100, 200, 300 and 400 N) were tested (figure 3.7). Microprobe arrays were examined before and after application of the compression load.
using scanning electron microscopy (SEM) (JEOL JSM 5610 SEM) to detect the presence and mode of any mechanical failure. The height of each microprobe was measured after testing and the percentage change in microprobe height calculated.

![Diagram of axial force and microprobe array]

**Figure 3.7:** A schematic showing method of measurement of microprobes’ axial compression force.

3.4.3.3. Measurement of transverse fracture force of microprobes *In vitro*:

The transverse failure forces of microprobe arrays were measured with the same force station as described above. A T-slot was used to clamp a thin probe (Agar Scientific) (0.5 mm thickness at the tip). The probe was adjusted to ensure that it pressed orthogonally against a row of eight microprobes (figure 3.8). The probe was moved at a speed of 1 mm/min. The force required to fracture a single microprobe was determined by dividing the transverse force required to fracture one row by the number of microprobes in each row (eight). The microprobe arrays were examined by SEM prior to and after fracture testing to assess mechanical failure.
3.5 RESULTS

3.5.1 Insertion tests - ex vivo:

Initially, six ex vivo tests were performed using devices fabricated using masters obtained from Swansea University. Histological examination revealed that none of the tests have shown consistent penetration of stratum corneum. Examination of the device using SEM revealed that microprobes had large tip diameter. Although the microprobes' target tip diameter is 10 - 50 µm, microprobes used for ex vivo tests had a tip diameter in the range of 100 - 140 µm (figure 3.9(a)). As discussed in the introduction, the efficiency of penetration depends inversely on microneedles' tip diameter (Davis et al., 2004), which is probably the reason for failure of microprobes with larger tips to perform adequately.

Figure 3.8: A schematic showing method of measurement of microprobes’ transverse fracture force.
Figure 3.9(a): A scanning electron microscopy image of one microprobe with tip diameter of 100 µm in a device fabricated using master obtained from University of Swansea. 9(b) A scanning electron microscopy image of aluminum master fabricated using electrical discharge milling at Imperial College London showing the pyramidal shaped microprobes with tip diameter of 25 µm.

Figure 3.10: Histological examination of microprobe array treated skin area showing indentation (a) and penetration (b) of stratum corneum.
Following the replacement of these masters with masters fabricated at Imperial College London using EDM, we managed to fabricate devices with pyramidal shaped microprobes that had tip diameter of 25 µm (figure 3.9(b)). Testing devices fabricated using new masters, the insertion ratio, as determined from the tests (n=10) on excised human skin, was 83% for moderate forces (7 N). Using forces of 10 N or above resulted in insertion ratio of over 90%. Above this threshold, the insertion ratio was proportional to force used (slope 0.5N⁻¹, R² = 0.93) (table 3.1). SEM images of the microprobe arrays following insertion tests confirmed the structural integrity of the device.

Figure 3.11(a): A 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. Figure 11(b): Histological examination of microprobe array treated skin area showing penetration of stratum corneum (arrow) with microchannels (penetration depth of 300 microns) created by microprobes.
Table 3.1: Relationship between axial compression force tested and insertion ratio.

<table>
<thead>
<tr>
<th>Axial Force</th>
<th>Mean insertion ratio (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>7N</td>
<td>83</td>
<td>11.64</td>
</tr>
<tr>
<td>10N</td>
<td>90.36</td>
<td>8.25</td>
</tr>
<tr>
<td>15N</td>
<td>91.68</td>
<td>8.44</td>
</tr>
<tr>
<td>20N</td>
<td>93.83</td>
<td>8.91</td>
</tr>
<tr>
<td>25N</td>
<td>98.22</td>
<td>2.14</td>
</tr>
</tbody>
</table>

3.5.2 Axial compression tests

Axial compression tests (n=10) have demonstrated the ability of microprobes to tolerate large forces without fracture of microprobes or base plate. The reduction of microprobe height was proportional to the applied force. This ranged between 4.1% for 50 N and 18 % for 400 N axial pressures (figures 3.12 and 3.13 - table 3.2).

Figure 3.12(a): A scanning electron microscopy image of a microprobe array following successful skin penetration using force of 20 N. Figure 12(b): A scanning electron microscopy image of a microprobe array after applying axial force of 400 N.
### Table 3.2: Relationship between axial compression force tested and microprobes’ final height.

<table>
<thead>
<tr>
<th>Axial Force (N)</th>
<th>Mean Final Height (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>95.81</td>
<td>2.72</td>
</tr>
<tr>
<td>50N repeated</td>
<td>93.69</td>
<td>4.01</td>
</tr>
<tr>
<td>100</td>
<td>89.76</td>
<td>5.05</td>
</tr>
<tr>
<td>200</td>
<td>85.63</td>
<td>3.96</td>
</tr>
<tr>
<td>300</td>
<td>83.81</td>
<td>2.86</td>
</tr>
<tr>
<td>400</td>
<td>81.91</td>
<td>6.74</td>
</tr>
</tbody>
</table>

3.5.3 Transverse fracture tests

Transverse fracture tests (n=10) showed that the force required to fracture one row of eight microprobes was $25.18 \pm 2.81$ N ($\approx 3$ N per microprobe) (figures 3.14 and 3.15).
Figure 3.14: A scanning electron microscopy image showing effect of transverse force application on the first row of microprobes.

Figure 3.15: Instron generated graphs of 10 transverse fracture tests [as the Instron’s probe gets in contact with the shaft of microprobes, the force starts to increase. This continues until the microprobes fracture, which is identified by the drop in the recorded compressive force].

3.6 DISCUSSION:
Mechanical characterisation is an important step in pre-clinical validation of microneedle-based devices to assess the ability of microneedles to reach target compartment without mechanical failure. Successful insertion depends on microneedles' geometry (microneedle length, wall thickness, tip radius,
base diameter, wall angle) and distribution. It also depends on the mechanical properties of the skin and its resistance to penetration (Olatunji et al., 2013). All these factors, together with type of microneedles’ material, determine the force needed for insertion or fracture of microneedles. Microneedles of the correct geometry and physical properties allow for a small insertion force. If the force required for insertion exceeds fracture force, needles will break or bend before insertion occurs.

The term “safety margin” has been used in microneedle literature to describe the device’s mechanical safety (Davis et al., 2004). It describes the relationship between the force required to fracture microneedles and that required for skin insertion, by dividing the fracture force by insertion force. The higher the safety margin, the lower the risk of mechanical failure and the safer the device. To ensure successful skin insertion of microneedles without breaking, appropriate microneedle geometry, distribution and materials are required to minimise insertion force and to maximise fracture force achieving the largest possible safety margin. Insertion is best achieved by small tip radius, interspacing of >150 μm at the base and sufficient microneedle length to overcome skin resistance, while mechanical strength is increased by increasing wall thickness (for hollow microneedles), base diameter and using materials of larger Young’s modulus (a measure of stiffness of materials) (Davis et al., 2004, Park et al., 2005, Olatunji et al., 2013). Examples of safety margins reported in the microneedle literature include a safety margin (for axial load) of up to five for a single hollow polymer microneedle with small tip and large wall thickness (Davis et al., 2004) and up to 23 for solid microneedle array fabricated from dissolvable polymer (Donnelly et al., 2011).
Mechanical characterisation of our sensor has demonstrated a safety margin, when an insertion force of 15 - 25 N is used, to range between 16 - 26 for axial load and 8 - 13 for transverse load, implying a high safety margin of the device (table 3.3). The higher safety margin for our device compared to typical microneedles is likely related to the microprobes’ geometry (low aspect ratio), being solid and the pyramidal shape, which was shown to have stronger mechanical properties than conical microneedles of comparable geometry (Hoa et al., 2014, Lee et al., 2008). It also reflects the relatively small insertion force achieved by small tip radius and microprobes’ height that is sufficient to overcome resistance of the stratum corneum without compromising microprobes’ fracture force.

<table>
<thead>
<tr>
<th>Insertion force (N)</th>
<th>Mean insertion ratio (%)</th>
<th>Safety margin (Axial load)</th>
<th>Safety margin (Transverse load)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>83</td>
<td>56</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>90.36</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>91.68</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>20</td>
<td>93.83</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>25</td>
<td>98.22</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

*Table 3.3: Relationship between insertion force, insertion ratio, safety margin for axial compression force [obtained by dividing 400 N (which was the maximum axial force tested and only resulted in 18% reduction in microprobes’ height) by insertion force] and safety margin for transverse fracture force [obtained by dividing 192 N (transverse fracture force was 3N per microprobe X total number of microprobes (64)) by insertion force].*

Another important factor for successful stratum corneum penetration by microneedles (apart from microneedle geometry and distribution) is the
application force. This can be delivered by using either a manual pressure or an applicator. In a recent study, the mean force delivered by human volunteers to insert microneedles, when they were asked to use the force they would use to press an elevator button or a press a stamp on an envelope, was estimated to be 20 N (Larraneta et al., 2014). The use of an applicator enables the delivery of a defined insertion force or velocity to microneedle arrays. Several studies have highlighted the importance of the application method in microneedle insertion (Donnelly et al., 2010a). The use of manual self-application of microneedles simplifies the application processes and avoids the cost and training related to the use of applicators. However, the use of an applicator allows for reproducible skin penetration by limiting inter- and intra-individual variability in skin resistance to penetration and force applied for insertion (Singh et al., 2011). It can also result in reduction of insertion force with subsequent increase in the device’s safety margin (Olatunji et al., 2013). Another potential advantage to the use of a microneedle applicator device is that it provides the user (and the healthcare professional) with the assurance that a successful penetration has occurred, even in the absence of any associated pain or sensation, by producing a marker such as an audible click (Singh et al., 2011).

### 3.7 CONCLUSION:

Mechanical characterisation studies have demonstrated the ability of microprobes to penetrate the stratum corneum layer to reach its target dermal compartment for ISF glucose measurement. This was achieved using small insertion force with low risk of fracture either at the axial or transverse planes.
CHAPTER 4

SENSOR FUNCTIONALISATION AND ASSESSMENT OF SENSOR PERFORMANCE IN VITRO AND EX VIVO
4.1. INTRODUCTION:

4.1.1 Sensor functionalisation

As discussed in chapter one, amperometric electrochemical glucose biosensors based on glucose oxidase (GOx) enzyme has been widely used for continuous glucose monitoring (CGM) systems. In these systems, the sensor consists of a three-electrode system, where a potential is applied between the working and reference electrodes and the generated current is measured between the working and counter electrodes. GOx enzyme is used as a biorecognition element to catalyse the reaction with glucose, with subsequent production of electroactive hydrogen peroxide. Application of a specific potential between working electrode and reference electrodes (which is typically +700 mV in first generation sensors) oxidise hydrogen peroxide. This results in generation of a current that is proportional to glucose concentration. This process requires an electron acceptor, which can be either oxygen (as in first-generation sensors) or synthetic mediators (in second generation sensors). The latter have the advantages of avoiding the issues of oxygen depletion following in vivo sensor implantation and the ability to use a lower potential to oxidise hydrogen peroxide, which reduces the risk of oxidising other electroactive analytes (interferents). However, a limitation to the use of mediators in implantable biosensors is lack of biocompatibility and the concern that leakage of mediators from the sensor to host tissues can be potentially harmful (Wang, 2008).

Several factors need to be considered for the design of an enzymatic glucose biosensor including the detection element (electrode) material, the
biorecognition element (enzyme), the enzyme immobilisation technique, the redox couple and the permselective membrane.

4.1.1.1 **Glucose oxidase enzyme immobilisation**

Rendering the working electrode specific to glucose requires immobilisation of a glucose oxidoreductase enzyme such as GOx or glucose dehydrogenase (GDH). Enzyme immobilisation on an electrode surface may enhance the communication between the active site of the enzyme and the electrode and prevents enzyme leaching (Mani et al., 2014).

Several strategies have been used for GOx enzyme immobilisation on the electrode surface. GOx can be physically entrapped between the electrode surface and the permselective membrane or it can be covalently coupled within a polymer film on top of the electrode surface (Arica and Hasirci, 1993). The advantage of the latter method is that it allows entrapping large quantities of GOx which results in higher current density and can enhance the enzyme activity. A commonly used method is to cross-link the enzyme to bovine serum albumin with gluteraldehyde (Crespilho et al., 2006). An alternative method is the deposition of an enzyme using self-assembly monolayer (SAM) technique. This involves functionalisation of gold electrode with thiomalic acid to provide a carboxylic group modified surface leading to an amide bond formation to amino groups on the GOx enzyme and its covalent attachment to the electrode surface (Li et al., 2007, Trzebinski et al., 2012).

In this work, GOx sourced from *Aspergillus niger* (E.C.1.1.3.4) was used as the functional part of the biosensors and was immobilised to the gold electrode surface using the SAM technique.
4.1.1.2 **Epoxy-polyurethane membrane**

An important step in the design of an enzymatic glucose biosensor is the choice of the permselective membrane (also referred to as mass transport limiting or flux limiting membranes) as it serves several important functions. Its primary function is to extend the glucose detection range of the sensor by limiting glucose flux to electrode surface and enzyme layer. This serves two functions. First, it prevents GOx saturation at high glucose concentrations. Secondly, it avoids the problem of oxygen deficit (in first generation glucose sensors) by maintaining a balance between oxygen and glucose fluxes. It also acts as a protective coating providing a biocompatible interface between the electrodes and the tissues enhancing sensor biocompatibility and protecting sensor components (e.g. electrode, enzyme or mediators) from degradation or leaching. Furthermore, permselective membranes limit electroactive interferents from reaching the electrode. However, use of permselective membranes comes at the expense of sensor sensitivity. Also, small variations in steps of permselective membrane fabrication can lead to high variability in sensor performance (Trzebinski et al., 2011).

Several materials such as Nafion, cellulose acetate, polycarbonate and polyurethane (PU) have been used as permselective membranes. Addition of epoxy resins reinforces the membrane with subsequent improvement in its mechanical integrity and durability (Wang et al., 2013). In this work, PU membrane reinforced with epoxy has been chosen as a permselective membrane.
4.1.2 Electrochemical techniques

Electrochemical evaluation of implantable electrochemical glucose sensors, both in vitro and in vivo, is an essential step in the design of a CGM system and a pre-requisite by regulatory bodies before approval for clinical studies (Agency, 2013). It aims at assessment of sensor functionality including glucose detection range and its linearity, sensitivity, effect of sterilisation, skin insertion and potential interferents on sensor performance.

Several methods have been established to study electrochemical reactions. In this work, we have used cyclic voltammetry and chronoamperometry for electrochemical characterisation of the microprobe array sensor.

4.1.2.1 Cyclic voltammetry

Cyclic voltammetry (CV) is a potentio-dynamic technique that provides information about the electrode and the thermodynamics and kinetics of redox processes. It involves the measurement of the current of a sensor immersed in a redox solution. In this technique, the potential applied between the working electrode and the reference electrode is swept linearly against time at a fixed rate until it reaches a set point, after which the potential is swept in the opposite direction to return to the initial potential. This cycle can be repeated several times (figure 4.1). Plotting the generated current ($I$) against the applied potential ($E$) gives the cyclic voltammogram trace (figure 4.2).
Figure 4.1: An example of potential versus time plot for a cyclic voltammetry experiment. A full cycle potential triangular waveform consists of a forward scan and a reverse scan.

The initial potential is usually chosen where no redox reactions occur. As the oxidation potential increases, the anodic current increases until a peak oxidation current ($I_{p\text{ ox}}$) is reached, after which a steady state current is reached. At that point, the current is mass transport limited not potential limited. During the reverse scan (reducing potential), the accumulated oxidised species are reduced which results in a cathodic current until a peak reduction current is reached ($I_{p\text{ red}}$), followed by a steady state current.
4.1.2.2 Chronoamperometry

This technique is used to study the sensor response to a change of substrate concentration, which is referred to as titration. In chronoamperometry a constant potential is applied to the working electrode and the resulting current is measured as a function of time. The applied potential is usually chosen (based on the CV experiments) such that the resulting current is mass transport limited thus it reflects the concentration of the electroactive analyte of interest. Chronoamperometry involves measurements of the output current of a sensor under constant polarisation immersed in a buffer solution, while changing the analyte concentration (stepwise). The results are plotted on a current versus time curve (figure 4.3).

Figure 4.2: An example of a potential versus current plot for a cyclic voltammetry [$I_{p\text{ ox}}$, peak oxidation current; $I_{p\text{ red}}$, peak reduction current].
Figure 4.3: An example of chronoamperometry demonstrating current output of a sensor in relation to increasing analyte concentration.

4.2. AIM:

This chapter aims at a description of the process of functionalisation of the microprobe array sensor, the evaluation of its electrochemical characteristics, sterilisation validation and biocompatibility of sensor components.

4.3. METHOD:

4.3.1 Materials:

SU-8 photoresist was obtained from Chestech Ltd, UK. Ferrocene carboxylic acid, Thiomalic acid (TMA), ethyl-dimethyl-aminopropylcarbodiimide (EDC), N-hydroxy-succinimide (NHS), glucose oxidase (GOx, EC 1.1.3.4), from Aspergillus niger, lyophilized, powder, ~200 units/mg), ATACS5104 epoxy adhesive, Brij 30, polyurethane (PU), tetrahydrofuran (THF) and potential interferents (ascorbic acid, acetaminophen and uric acid) were obtained from Sigma-Aldrich, UK. Methylene blue was obtained from VWR International Ltd,
UK. Silver conductive paint “RS 186-3600”, copper tape and PVC tape were obtained from RS Components Ltd, UK.

4.3.2 Sensor fabrication and wire bonding:

Following sensor fabrication and metallisation (as described in chapter 3), the microprobe array was wire bonded using silver paint and ATACS5104 epoxy adhesive to enable sensor functionalisation and electrochemical testing. The base of the metallised microprobe array was then encapsulated by spinning SU-8 photoresist followed by crosslinking using UV rays. The edges of the array were also encapsulated by applying a layer of SU-8 followed by crosslinking. Encapsulation is done to avoid electrochemical reactions occurring at the base or edges of the array. Subsequently, the wire connection and the electrode surface of the microprobe array were evaluated by CV using ferrocene carboxylic acid as a redox probe before starting the functionalisation protocol. Copper tape and PVC tapes were later also used as an alternative to silver paint and ATACS5104 epoxy adhesive for wire bonding.

4.3.3 Functionalisation and membrane coating:

Metallised microprobe arrays were functionalised with GOx enzyme to obtain the electrochemical glucose biosensors. Immobilisation of GOx on gold microprobe electrodes was achieved by employing self-assembled monolayers of TMA. Using drop coating, 40 mM TMA in deionised water was deposited on the microprobe array for 90 minutes. Subsequent treatment with EDC and NHS generates an activated ester coating, which immobilises the GOx through amide bond formation to amino groups on the enzyme. The
procedure involved deposition of an aqueous solution composed of 40 mM EDC and 100mM NHS in 10 mM phosphate buffered saline (PBS) solution, using drop coating, for 45 minutes at room temperature. This was followed by overnight incubation at 4 °C in 0.1M PBS (pH 7.4) containing 10mg/ml of GOx (figure 4.4). Finally an epoxy-PU membrane was conformally deposited by dip coating in a solution of 17.8 mg each of Part A and Part B of ATACS5104 epoxy adhesive and 26.7 mg of PU in 4 ml of THF and 1 ml of Brij 30 (Wang et al., 2013). The sensors were stored at 4°C until further use.

**Figure 4.4**: A schematic diagram showing steps of glucose oxidase (GOx) immobilisation using self-assembled monolayer (not to scale).

### 4.3.4 *In Vitro* tests:

#### 4.3.4.1 **Biosensor characteristics**

Electrochemical measurements were performed with a CHI 1003b potentiostat (CHI Instruments, Llanelli, UK) running a general-purpose electrochemical software (GPES v4). The microprobe array glucose biosensors acted as the working electrode with a commercially available Ag/AgCl reference/counter electrode (World Precision Instruments). Before
chronoamperometry measurements, the sensor was polarised at +700 mV versus the Ag/AgCl reference/counter electrode, unless otherwise stated, until a stable current was observed. A magnetic stirrer (Fischer Scientific) was used to provide the convective transport for the electrochemical measurements. Calibration plots were obtained by measuring the current in 10 mM PBS under constant stirring with stepwise glucose concentration increments of 2.5 mM every 125 seconds in the range of 0 – 20 mM. The resulting plot was used to evaluate linearity of the detection range and sensor sensitivity. All experiments were done at room temperature (22+/−0.5 °C).

4.3.4.2 *Interference studies*

Metallised microprobes were conformally covered with PU membrane of different thicknesses by adjusting the duration of dip coating (5,10, 20 seconds). Using chronoamperometry, interference studies were performed by polarising the metallised sensors at either +700 mV or at +530 mV in the presence of uric acid, ascorbic acid or acetaminophen. The sensors were calibrated in 10 mM PBS under constant stirring by stepwise uric acid concentration increments of 0.1mM in the range of 0.1–1.0 mM. For ascorbic acid, the studied range was 0.02-0.2 mM using concentration increments of 0.02 mM. For acetaminophen the tested range was 0.01-2.0 mM using concentration increments of 0.02 mM.

4.3.4.3 *Gamma ray sterilisation and its effect on sensor performance*

Functionalised sensors were packed in falcon tubes (VWR, Sussex, UK) and sent to TCM Associates (Essex, UK) for repackaging. Following repackaging,
they were sent to Synergy Health for Cobalt-60 gamma ray irradiation, assay of the bioburden levels on the microprobe arrays and subsequent estimation of the dose for sterilisation. (These studies were done in accordance with ISO 11137-2:2012, Sterilisation of health care products -Radiation- Part 2: Establishing the Sterilisation dose). As described above, calibration curves were obtained before and after sensor sterilisation to assess the effect of gamma ray sterilisation on sensor performance.

4.3.5 *Ex vivo tests:*

4.3.5.1 *Effect of skin insertion*

To evaluate the effect of skin insertion on sensor performance, the electrochemical response of one sensor was characterised before and after skin insertion. Full thickness human skin samples (breast or abdomen) were obtained from elective surgery at Imperial College Healthcare NHS Trust after approval from Imperial College Human Tissue Bank and patients’ consent. Skin insertion was achieved using an Instron 5866 instrument, running Bluehill software (Norwood, MA), with a 50 Newton (N) load cell to apply a force of 15 N at a speed of 3 mm/s. Following device removal, methylene blue solution was applied to the microprobe array-treated skin area to confirm penetration.

4.3.5.2 *Comparison of generated current against a commercially available needle-type electrochemical sensor ex vivo*

Similarly, sensor performance was assessed *ex vivo* using fresh skin sample. In this experiment, full thickness human skin samples were transported immediately, after surgical removal, in physiological saline solution to the electrochemistry laboratory and were stretched and mounted, dermis side
down, on a cork dissection board covered with parafilm. An 8x8 solid microprobe array, metallised then functionalised with GOx and coated with epoxy-PU membrane (working electrode) and a 1x8 microprobe array of Ag/AgCl (reference/counter electrode) were inserted into the skin using a force of 15 N with an Instron compression system. A commercial needle-type sensor (Enlite, Medtronic, Northridge, CA) was inserted nearby using the sensor’s inserter device (figure 4.5). The current output from the sensors was then measured.

**Figure 4.5:** A digital image showing 2 microprobe arrays (8x8), a reference electrode (1x8) and a needle-type sensor inserted in fresh full-thickness human skin ex vivo.

4.4. RESULTS:

4.4.1 Wire bonding:

Difficulty in controlling the epoxy adhesive thickness and its hygroscopic nature, that leads to its swelling following exposure to water or moisture, resulted in the formation of a thick layer at the point of wire bonding (figure
While wire bonding using epoxy adhesive enables sensor functionalisation and in vitro electrochemical testing, its thickness at the point of wire bonding raised concerns of interference with microprobes’ skin insertion during clinical studies (figure 4.6).

Figure 4.6: showing the thick epoxy adhesive layer used to bond electrical wire to a microprobe array.

Therefore, alternative methods for wire bonding were explored. These included replacing epoxy adhesive with either copper tapes or PVC tapes. Due to copper tape corrosion during the functionalisation process and poor adhesion of PVC tapes, wire bonding was finally achieved by drilling two holes in the edge of the array to allow threading of the wire into one hole, looping it back through the other hole before twisting the wire end around the wire to create a loop.
4.4.2 *In Vitro* tests:

4.4.2.1 **Biosensor characteristics**

Using 6x6 microprobe arrays, the current generated ranged from 20 – 150 nanoampere (nA) with a sensitivity of 7 nA/mM. The response was linear in the glucose range tested (0-20 mM) (figure 4.7).

![Graph showing output current plotted against time to demonstrate the current response to a stepwise glucose concentration (calibration curve).](image)

**Figure 4.7(a):** Graph showing output current plotted against time to demonstrate the current response to a stepwise glucose concentration (calibration curve). **Figure 4.7 (b):** Graph showing output current plotted against time to demonstrate linearity and sensitivity of glucose measurement.
4.4.2.2 Interference studies

For metallised microprobes dip coated in PU for more than 10 seconds, interference was seen only at an applied potential of +700 mV with acetaminophen concentrations higher than 0.1 mM (therapeutic concentration 0.03 - 0.13 mM) (figure 4.8). However, no interference was seen from uric acid or ascorbic acid using a potential of +700 mV. Also, no interference was seen from any of the three tested interferents when the metallised sensors were polarised with +530 mV (figure 4.9).

**Figure 4.8:** A graph showing acetaminophen Interference in vitro study at an applied potential of +700 mV using microprobe arrays coated with variable thickness of PU obtained by dip coating for 15 seconds (blue) and 20 seconds (orange).
**Figure 4.9:** A graph showing acetaminophen Interference in vitro study at an applied potential of +530 mV using microprobe arrays coated with variable thickness of PU obtained by dip coating for 15 seconds (blue) and 20 seconds (orange).

### 4.4.2.3 Gamma ray sterilisation and its effect on sensor performance

Based on $V_{D_{\text{max}}}^{25}$ study for sterilisation validation, a gamma irradiation dose of 25 kilogray (kGy) was accepted. Ten microprobe arrays were subjected to 25 kGy of gamma ray irradiation and eight were used for subsequent bioburden measurements. Two microprobe arrays were used for functional and performance studies. The microprobe array sensor was calibrated before and after gamma ray sterilisation using a dose of 25 kGy. The electrochemical response of the microprobe sensor was the same before and after gamma ray sterilisation (figure 4.10).
**Figure 4.10:** A graph showing the effect of gamma irradiation (a dose of 25 kGy) on sensor's functionality.

4.4.3 Ex vivo tests:

4.4.3.1 Effect of skin insertion

Similarly, chronoamperometry before and after skin insertion showed similar results, with no negative impact caused by skin insertion on sensor's functionality (figure 4.11).
4.4.3.2 Comparison of generated current against a commercially available needle-type electrochemical sensor ex vivo

The relative currents from the microprobe sensor were initially at least 2 times higher than those from the enlite sensor. In both sensors the currents decreased over the duration of the study period likely reflecting the decline in glucose concentration in devitalised tissue (figure 4.12). Assessment of correlation between the outputs of the two sensors showed an $R^2$ of 0.98 (figure 4.13).

Figure 4.11: A graph showing the effect of skin insertion ex vivo on sensor’s functionality.
Figure 4.12: A graph showing relationship between output current from microprobe sensor and the enlite sensor in human skin ex vivo.

Figure 4.13: A graph showing correlation between output current from microprobe array sensor and a commercially available needle-type-sensor.
4.5. DISCUSSION:

In addition to in vitro mechanical evaluation of implantable glucose sensors, in vitro assessment of functionality, biocompatibility and sterilisation is required prior to in vivo evaluation of sensor’s performance and safety in clinical trials. Characterisation of performance of electrochemical sensors includes evaluation of linear range, sensor sensitivity, background current, response time and effect of potential interferents. Commercially available CGM systems have a working glucose range of 2.2 - 22.2 mmol/L (40 - 400 mg/dL). In this work, we have demonstrated that microprobe array sensors coated with epoxy-PU membrane demonstrated a linear response to changing glucose concentrations covering a glucose range similar to that of existing CGM systems. More importantly, the current generated and sensor sensitivity in vitro were higher than needle-type sensors (7 nA/mM compared to 0.96 nA/mM for the latest needle-type sensor from Abbott’s diagnostics (Freestyle Libre) for example) (Hoss et al., 2014). This implies the potential of microprobe array sensors to have better signal: noise ratio and therefore better accuracy, particularly at the hypoglycaemic range. Furthermore, microprobe array sensors allow for further enhancement of the functional performance through adjustments of the microprobe array geometry by increasing the number of microprobes per array or increasing microprobes’ length or base diameter.

Several exogenous and endogenous substances can interfere with electrochemical glucose sensor performance and compromise its accuracy. The ISO 15197:2013 standards and EP7-A2 guidelines from the Clinical & Laboratory Standards Institute list 22 potential interferents (McEnroe et al.,
Common electroactive interferents include acetaminophen, ascorbic acid and uric acid, which represent major specificity issues for CGM systems. One of the important functions of sensor’s coating membrane is to block interferents from reaching electrode surface by either electrostatic repulsion based on charge (e.g. Nafion) or mechanically based on interferents’ size (e.g. cellulose acetate film or PU). Interference from acetaminophen represents a challenge for most of electrochemical glucose sensors as the low oxidation potential of acetaminophen makes it electroactive during amperometric measurements of hydrogen peroxide. Furthermore, acetaminophen cannot be blocked based on electrostatic repulsion, as the molecule is uncharged (Lucarelli et al., 2012). Acetaminophen is reported to affect the enlite sensor and the G4 PLATINUM systems, while Ascorbic acid is reported to have minor effects on the FreeStyle Navigator and the enlite. Salicylate is reported to be the main interferent for FreeStyle Navigator (FDA, 2008). In addition the coating membrane, current generated from electroactive interferents can be accounted for by the use of a non-functional electrode (not functionalised by GOx enzyme but coated with an outer membrane). Subtraction of the current measured by the non-functional electrode from that measured by the working electrode will account for background current and current resulting from interferents. Portioning of the microprobe array into several subarrays allows for integration of a “subtraction electrode” with the potential to further enhance device accuracy and specificity. In this work, we used metallised microprobe arrays (without functionalisation with GOx enzyme) coated with variable thickness of PU membrane. As GOx enzyme renders electrodes specific to glucose, use of non-functionalised electrodes
represents the worst-case scenario with the electrodes being more prone to the effect of electroactive interferents. Neither of ascorbic acid or uric acid showed interference in the tested range. However, acetaminophen showed interference at levels of ≥0.1 mM (therapeutic range 0.07 - 0.13 mM/L), regardless of PU membrane thickness, when a potential of +700 mV was used.

In view of the labile nature and thermal instability of the GOx enzyme, the main challenge facing sterilisation of GOx-based glucose biosensors is the ability to ensure that sterility assurance is met without impacting the functionality of the sterilised sensor. Several methods have been evaluated for sterilisation of GOx-based sensors (Ahmed et al., 2000). Gamma radiation is frequently applied in medical sterilisation. The sterilising effect of gamma radiation is based on the generation of breaks in nucleic acid chains, preventing cell division and causing microorganism destruction. A dose of 25–35 KGy irradiation is generally required to kill most commonly occurring microorganisms with an adequate safety factor and so achieve successful sterilisation of medical devices. Sensor evaluation is required post-sterilisation to ensure that sterility standards are met and sensor functionality is maintained. Sterilisation studies of the microprobe array sensor showed that a dose of 25 kGy was sufficient to meet sterility standards. Functional assessment showed that sensor functionality was maintained post-sterilisation using 25 kGy. This is consistent with the result of one study that evaluated the effect of two doses of gamma radiation (25 kGy and 30.6 kGy) on functionality of GOx-based biosensor and showed that sensor functionality was only maintained with the use of the smaller radiation dose (Ahmed et al., 2000).
The presence of the epoxy-PU membrane resulting in mass transport limited current, rather than enzyme kinetic limitations means that sensors should be less sensitive to loss of enzyme activity as a consequence of sterilisation. Similarly, skin insertion did not impact sensor functionality. The aim of this study was to ensure that the process of sensor insertion into the skin does not impact sensor layers, particularly the outer epoxy-PU membrane layer. However, this study does not provide information on the effect of duration of sensor implantation on sensor layers and performance. An attempt to assess this in another ex vivo study looked at the performance of the microprobe arrays over 15 hours of implantation in comparison to a commercially available needle type sensor. The performance of the microprobe sensors inserted into excised human skin was compared with that of a commercially available continuous glucose sensor. With a devitalised excised skin sample, glucose concentrations declines over time and this makes it difficult to separate the effects of longer-term implantation per se from those of declining glucose concentration. However, the study demonstrated that the current output from the microprobe array sensor was at least 2 times higher compared to output from the commercially available sensor and showed an excellent relationship ($R^2=0.98; y=1.93x-8\times10^{-8}$) (figure 4.13). This study also does not provide information on the effect of the method of insertion (application speed or force) on the integrity of sensor layers.

In vitro and in vivo assessment of the biocompatibility of sensor components is an essential step in sensor evaluation and is a requirement by regulatory bodies before evaluation in clinical studies. The standards specify that biocompatibility testing should be done on the end product after it has
undergone all washing, packaging and sterilisation steps (Koschwanez and Reichert, 2007). One method for *in vitro* evaluation of cytotoxicity of material used in sensor fabrication and functionalisation is examination of mouse fibroblast cells (L-929) after being exposed to sensor materials for several days. The cage implant system allows *in vivo* evaluation of sensor’s materials biocompatibility. In this method, the material of interest is inserted in a stainless steel mesh cage, which is then implanted into a test animal. Exudate from within the cage can be collected over the course of the experiment to examine for inflammatory and immune responses and cell-material interactions in comparison to an exudate from a control cage (Koschwanez and Reichert, 2007). In moving the microprobe array glucose sensor technology from bench side to bedside, several factors came into play including biocompatibility of components used in sensor fabrication and functionalisation. Initial work on the use of microprobe technology for CGM that was conducted in Cass’s group used tetrathiafulvalene (TTF) mediator (Trzebinski et al., 2012). As the biocompatibility of TTF has not been previously demonstrated for implantable glucose sensors, we decided to use unmediated first generation glucose sensors. The devices described in this chapter had already had their components assessed by this method and the results are available in the literature. The biocompatibility of SU-8 photoresist was evaluated *in vivo* using the cage implant method (Voskerician et al., 2003). SU-8 was placed into a stainless steel cage and implanted subcutaneously in a rodent model. Exudates within the cage (sampled at 4, 7, 14, and 21 days) did not show any significant difference between the stages of inflammatory response elicited by SU-8 and the empty cage control over the
duration of the study. The biocompatibility of glucose oxidase and epoxy-PU has also been established (Wang et al., 2008), and these two components are routinely used in other commercially available CE-marked predicate devices.

4.6. CONCLUSION:

Functional evaluation of the microprobe array glucose biosensor, fabricated using biocompatible materials, showed high glucose-dependent current and sensitivity and that were not adversely affected by skin insertion or gamma ray sterilisation at a dose of 25 kGy. Interference studies showed that the device is liable to interference from acetaminophen at therapeutic concentration when the desired operating potential of +700 mV is used. These important observations, together with mechanical evaluation data showing the device mechanical robustness (chapter 3), were essential in obtaining regulatory bodies’ approval to proceed to clinical studies for evaluation of safety and efficacy of the sensor in human subjects.
CHAPTER 5

CLINICAL EVALUATION OF SAFETY OF THE MICROPROBE ARRAY CONTINUOUS GLUCOSE SENSOR
5.1. INTRODUCTION:
For clinical evaluation of sensor’s safety and proof of concept of its performance, a non-randomised open-label prospective feasibility study has been planned to take place over four phases in healthy volunteers and in subjects with type 1 diabetes. The aim of this chapter is to describe the work done so far for clinical evaluation of device’s safety in non-diabetic subjects in phase 1 of the clinical study.

5.2. METHOD:
Regulatory approval from the National Research Ethics Committee and Medicines and Healthcare Products Regulatory Agency (letter of no objection) were obtained. Non-diabetic subjects were recruited through poster adverts in Imperial College London campuses. Inclusion criteria were 18–75 years of age. Subjects were excluded if there was a history of upper limb neuropathy or radiculopathy, history of pre-existing skin condition, pregnant or planning pregnancy in next 12 months, breastfeeding, enrollment in other clinical trials, uncontrolled concurrent illness, have active malignancy or under investigation for malignancy.
Subjects’ withdrawal from the study was in the case of loss of capacity to give informed consent, development of skin disease or upper limb neuropathy or radiculopathy or terminal illness.
Participant information sheets were given to potential subjects and, after a minimum of 48 hours and following any questions, informed written consent was taken. Subjects were asked not to apply any cosmetic formulations on the non-dominant forearm for 7 days prior to the study.
Primary outcome was safety of the microprobe array glucose sensor (skin inflammation, pain score, bleeding, infection, fracture of microprobes). Secondary outcomes were presence of a detectable signal, magnitude of the generated current and correlation with venous blood glucose.

5.2.1. Sensor design:

Each microprobe array was partitioned into four subarrays. One subarray functioned as Ag/AgCl reference/counter electrode. The other three subarrays were metallised with gold, functionalised with glucose oxidase enzyme and coated with epoxy-PU membrane to function as three independent working electrodes allowing for multiple simultaneous glucose sensing (figure 5.1).

The disposable sensor housings were sealed in foil package (TCM Associates Ltd, Essex, UK) and sterilised using gamma irradiation at a dose of 25 kilogray (Synergy Health, Swindon, UK). The packaged, sterilised sensors were stored at +4C ° until use.

Figure 5.1: A microprobe array partitioned into 4 subarrays (three gold working electrodes and one Ag/AgCl reference/counter electrode).
5.2.2. Study design:

Subjects attended the NIHR/Wellcome Trust Imperial Clinical Research Facility at 09:30 am non-fasting. After revisiting inclusion and exclusion criteria and subject’s consent to start the clinical study, a 20-G intravenous cannula was inserted in the forearm for venous sampling. The microprobe sensor was applied to the upper part of the ventral aspect of the non-dominant forearm. Microprobe insertion was achieved by applying thumb pressure for 60 seconds. The sensor was secured in place with a transparent adhesive plaster (Elastoplast, Beiresdorf, Hamburg, Germany) for six hours. For measurement of output current, the sensor was directly connected to CHI 1003b potentiostat (CHI Instruments, Llanelli, UK) running a general-purpose electrochemical software (GPES v4) (figure 5.2).

![Figure 5.2: showing the microprobe array glucose sensor applied to subject’s forearm and connected to a potentiostat for measurement of the output current.](image)

Every 15 min throughout the six-hour study, a venous blood sample was taken and analysed for glucose using the YSI 2300 glucose and lactate analyser (Yellow Springs Instrument, Yellow Springs, OH). Subjects had one
main meal (lunch) during the six-hour study and were allowed to eat and drink throughout the study.

5.2.3. Assessment of skin inflammation:
The skin area around the microprobe array was inspected at 1 and 2 hours post-insertion and after device removal to determine degree of skin inflammation. Based on guidelines of the International Contact Dermatitis Research Group and the North American Contact Dermatitis Group, skin inflammation was rated as either: a negative reaction, a faint erythema (doubtful reaction), a mild erythema with possible papules (weak positive reaction), a moderate-intense erythema, papules or vesicles (strong positive reaction) or an intense erythema and infiltration and coalescing vesicles (extreme positive reaction) (Boone et al., 2015).

In the case of persistent intense skin inflammation over 30 minutes following device removal, a 2 mm punch biopsy was planned.

5.2.4. Assessment of integrity of microprobe arrays:
Microprobe arrays were examined before and after application using scanning electron microscopy (SEM). This aimed at assessment of the structural integrity of microprobes and whether skin insertion has resulted in fracture of microprobe tips or resulted in damage to the epoxy-PU coating membrane.

5.2.5. Visual analogue scale (VAS) pain scores:
A 100mm-VAS was used to assess pain resulting from microprobe array (at insertion and throughout the six-hour study) in comparison to pain resulting from insertion of a 20-G intravenous cannula. The pain intensity score was measured immediately after sensor and intravenous cannula application at the beginning of the study, and immediately after sensor removal. The VAS
was determined by asking the participant to mark the degree of perceived pain on a 100 mm line. The line had word descriptors at each end (“No Pain = 0 mm” at one end and “Worst Pain = 100 mm” at the other end). The VAS score is determined by measuring in millimeters from the left hand end of the line to the point that the participant’s mark. Participants were also asked to describe the sensation related to sensor application (e.g. sharp, pricking, heavy, pressure).

5.2.6. Statistical analysis:

Pain score data were analysed using Wilcoxon-Rank test. Spearman correlation was used to assess the relationship between sensor outputs whenever data on multiple simultaneous sensing where available. Similarly, the relationship between sensor output and venous blood glucose was evaluated using Spearman correlation. For this purpose, correlation was evaluated for each hour of the six-hour study. Analysis was performed using SPSS 21.0 for Mac (SPSS Inc., Chicago, IL). In all cases $P < 0.05$ indicated significance.

5.3. RESULTS:

5.3.1. Subjects’ characteristics:

Eight subjects (6 females, 2 males) were enrolled and studied at the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital. 3 subjects were of White race, 3 of Asian race, 1 Middle Eastern and 1 of mixed race. Subjects were 34.6 ± 6.9 years old (range 23-48 years).
5.3.2. Skin inflammation:

None of the eight subjects had skin reaction in the area adjacent to the microprobe array when assessed at 1 and 2 hours post-insertion. The skin response at the microprobes’ insertion site, assessed immediately following sensor’s removal, was graded as barely noticeable (7 subjects) or mild erythema (in 1 subject). In comparison, none of the subjects had skin reaction to the adhesive plaster (Elastoplast, Beiresdorf, Hamburg, Germany) used to secure the microprobe array in place. In all subjects, skin reaction completely disappeared within 1 hour of device removal.

Figure 5.3: A digital image of microprobe array treated skin area immediately following removal of the microprobe array.

5.3.3. Pain scores:

In only one subject, sensor insertion was described as painful. The remaining 7 subjects reported insertion as painless. Sensation associated with sensor insertion was described as either “pricking” or “pressure” sensation. Median (interquartile range) pain scores for microprobe insertion on 100 mm VAS were 10 (1.25 – 17.5) compared to 30 (20 – 37.5) for the insertion of a 20-G
The difference between pain scores related to microprobe insertion in comparison to 20 G intravenous cannula insertion was statistically significant ($P=0.017$). Following sensor insertion, none of the subjects reported pain or sensation, in relation to the sensor, for the duration of the six-hour study.

![Figure 5.4: A box plot showing median and interquartile range of pain scores on 100 mm visual analogue scale for microprobe array insertion and the insertion of a 20 gauge intravenous cannula.](image)

### 5.3.4. Infection:

Subjects were contacted the following day of the study to find out if there has been any delayed skin reaction or any signs of infection at the area that was treated with the microprobe array. None of the 8 subjects reported persistent or new dermatological symptoms/signs at the site of microprobe application.

### 5.3.5. Bleeding:

There was no evidence of bleeding at the site of microprobe array insertion in any of the 8 subjects.
5.3.6. Structural integrity of microprobes:

SEM of microprobes following removal of the sensors, showed no evidence of fracture of microprobe tips as result of skin insertion. However, there was evidence of disruption of the epoxy-PU membrane (figure 5.5).

Figure 5.5: A scanning electron microscopy image showing a top view of a microprobe following its removal from one of the subjects in phase 1. This shows intact microprobe structure but the epoxy-PU coating membrane was disrupted.

5.3.7. Sensor performance:

A total of 189 paired YSI-sensor data points were available from the 8 subjects. Mean glucose concentration (whole blood glucose estimated using YSI) was 5.19 ± 0.95 mmol/L (range 3.03 – 7.85 mmol/L). The average time between start of the study and lunch was 3:07 h ± 0.4h.

From the total 8 microprobe array sensors (representing 24 working electrodes) placed, 9 working electrodes did not provide data. The main reason for this was malfunction of the electrical connection caused by
corrosion at the point of contact between the electrical wire and the working electrode.

In subjects where more than one glucose sensor (working electrode subarray) were functioning, significant correlation existed between the simultaneously functioning glucose sensors (table 5.1).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number of functioning glucose sensors</th>
<th>Spearman Correlation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3</td>
<td>3</td>
<td>Sensor 1 vs 2: 0.71</td>
<td>&lt;0.001</td>
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<tr>
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<td></td>
<td>Sensor 1 vs 3: 0.92</td>
<td>&lt;0.001</td>
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<tr>
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<td></td>
<td>Sensor 2 vs 3: 0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>0.5</td>
<td>0.013</td>
</tr>
<tr>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0.86</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 5.1:** Number of functioning glucose sensors for each of the 8 microprobe arrays used in phase 1 of the clinical study so far.

However, there was no correlation between sensor output and YSI venous blood glucose (Figure 5.6).
Subject 3

Subject 4
### Subject 5

- **Sensor 1 (NanoAmp)**: 
  - 1:00: 2400 
  - 1:30: 3800
  - 2:00: 5200
  - 2:30: 6700
  - 3:00: 9000
  - 3:30: 11800
  - 4:00: 14000
  - 4:30: 16800
  - 5:00: 20000

- **Sensor 2 (NanoAmp)**: 
  - 1:00: 2500 
  - 1:30: 4000
  - 2:00: 5500
  - 2:30: 7000
  - 3:00: 8500
  - 3:30: 10000
  - 4:00: 11500
  - 4:30: 13000
  - 5:00: 14500

- **YSI Glucose (mmol/L)**: 
  - 1:00: 4.3 
  - 1:30: 5.8
  - 2:00: 6.3
  - 2:30: 6.8
  - 3:00: 7.3
  - 3:30: 7.8
  - 4:00: 8.3
  - 4:30: 8.8
  - 5:00: 9.3

### Subject 6

- **Sensor 1 (NanoAmp)**: 
  - 1:00: 2300 
  - 1:30: 3800
  - 2:00: 5300
  - 2:30: 6800
  - 3:00: 8300
  - 3:30: 9800
  - 4:00: 11300
  - 4:30: 12800
  - 5:00: 14300

- **Sensor 2 (NanoAmp)**: 
  - 1:00: 2400 
  - 1:30: 3900
  - 2:00: 5400
  - 2:30: 6900
  - 3:00: 8400
  - 3:30: 9900
  - 4:00: 11400
  - 4:30: 12900
  - 5:00: 14400

- **YSI Glucose (mmol/L)**: 
  - 1:00: 4.2 
  - 1:30: 5.7
  - 2:00: 6.2
  - 2:30: 6.7
  - 3:00: 7.2
  - 3:30: 7.7
  - 4:00: 8.2
  - 4:30: 8.7
  - 5:00: 9.2

---

**Snack**: 3:15

**Lunch**: 3:30

---

**Snack**: 4:30

**Lunch**: 4:45

---

**Snack**: 5:30

**Lunch**: 6:00
Figure 5.6: Graphs showing venous blood glucose measured by YSI and output current of microprobe sensors for each of the eight subjects in phase 1 of the clinical study.
5.4. DISCUSSION:

Initial results of the phase 1 of the clinical study, obtained from 8 subjects, demonstrate safety of the microprobe array glucose sensor. Skin inflammation was barely noticeable and transient. Pain scores related to sensor insertion were low. There was no evidence of bleeding or infection at the insertion sites. As discussed earlier (chapter 2), one of the barriers to the use of CGM technology is pain, particularly in children. In the DirectNet study, pain during sensor insertion was cited as one of the reasons for discontinuation of CGM in children with type 1 diabetes (Weinzimer et al., 2009). Thanks to the minimally invasive nature of the micropores, their use is associated with less risk of pain, skin reaction, bleeding or infection (El-Laboudi et al., 2013).

In our study, partitioning of the microprobe array, allowing simultaneous multiple glucose sensing, provides the potential to improve CGM system accuracy (Castle et al., 2012). However, sensor performance, the secondary outcome of this phase 1 clinical study, showed no correlation between sensor output current and venous blood glucose. Failure mode and effect analysis (table 5.2) has highlighted potential source(s) of failure and recommended action(s). Failure of penetration of stratum corneum layer could result from use of sensors with blunt microprobe tips secondary to error in fabrication. This can avoided by the use of fresh new PDMS micomolds on fabrication of each sensor patch and SEM examination of the fabricated microprobe sensors. Errors in steps of sensor metallisation, functionalisation or membrane coating could result in poor performance or non-functioning sensors. Electrochemical assessment of at least one sensor per patch following these steps could identify these errors and guide any necessary
<table>
<thead>
<tr>
<th>Process</th>
<th>Potential Failure Mode</th>
<th>Potential Effect(s) of Failure</th>
<th>Potential Cause(s)/Mechanism(s) of Failure</th>
<th>Recommended Action(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabrication</td>
<td>Microprobe tips are not of the correct geometry (blunt)</td>
<td>Inconsistent penetration of stratum corneum</td>
<td>Recurrent use of PDMS micromolds</td>
<td>SEM of at least one sensor per patch to ensure correct geometry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Use of new PDMS micromold for each patch</td>
<td>Use of new PDMS micromold for each patch</td>
</tr>
<tr>
<td>Metallisation</td>
<td>Gold layer of poor quality or contaminated</td>
<td>Poor sensor function and low sensitivity</td>
<td>Use of other metals (potential contaminants) in the conformal sputtering system prior to metallisation of the sensor</td>
<td>To use a new gold source in the conformal sputtering system for sensor metallisation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Assessment of gold quality by performing cyclic voltammetry of at least one sensor per patch</td>
<td>Assessment of gold quality by performing cyclic voltammetry of at least one sensor per patch</td>
</tr>
<tr>
<td>Connections</td>
<td>Corrosion at points of contact</td>
<td>Non-functioning of the sensor</td>
<td>Exposed contact point</td>
<td>Isolation of point of contact using SU-8 or epoxy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Test quality of connection by performing cyclic voltammetry of at least one sensor per patch</td>
<td>Test quality of connection by performing cyclic voltammetry of at least one sensor per patch</td>
</tr>
<tr>
<td>Functionalisation</td>
<td>Reduced activity of glucose oxidase enzyme</td>
<td>Poor sensor function and low sensitivity</td>
<td>Wrong storage of glucose oxidase enzyme</td>
<td>Assessment of functionalised sensors by performing glucose titration curve (amperometry) of at least one sensor per patch</td>
</tr>
<tr>
<td>Coating Membrane</td>
<td>Rupture during skin insertion</td>
<td>Sensor malfunction</td>
<td>Ratio of epoxy (which is responsible for the mechanical robustness of the coating membrane) to the polyurethane is relatively low</td>
<td>Adjusting epoxy:polyurethane ratio through further in vitro and ex vivo studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not using an applicator to apply the sensor using a pre-set velocity and force</td>
<td>Design of an applicator</td>
</tr>
<tr>
<td>Process</td>
<td>Potential Failure Mode</td>
<td>Potential Effect(s) of Failure</td>
<td>Potential Cause(s)/Mechanism(s) of Failure</td>
<td>Recommended Action(s)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
<td>-------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Gamma Ray Sterilisation</td>
<td>Use of a higher dose than the 25kgy determined by sterilisation studies</td>
<td>Non-functioning sensors secondary to inactivation of glucose oxidase enzyme</td>
<td>Factory error</td>
<td>Performance assessment through in vitro glucose titration curve (amperometry) of at least one sensor per patch following sterilisation</td>
</tr>
<tr>
<td>Storage</td>
<td>Wrong storage</td>
<td>Non-functioning sensors secondary to inactivation of glucose oxidase enzyme</td>
<td>Error in setting thermostat</td>
<td>Performance assessment through in vitro glucose titration curve (amperometry) of at least one sensor per patch immediately before the clinical study</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>Long time period between sensor functionalisation and clinical study</td>
<td>Non-functioning sensors secondary to inactivation of glucose oxidase enzyme</td>
<td>Error in study design (delay in recruitment)</td>
<td>Performance assessment through in vitro glucose titration curve (amperometry) of at least one sensor per patch immediately before the clinical study</td>
</tr>
<tr>
<td>Application</td>
<td>Failure to achieve penetration or consistent penetration of microprobes</td>
<td>Poor sensor function and low sensitivity</td>
<td>Application using thumb pressure rather than using a specially designed applicator</td>
<td>Design of an applicator to deliver pre-set force and velocity</td>
</tr>
<tr>
<td>Process</td>
<td>Potential Failure Mode</td>
<td>Potential Effect(s) of Failure</td>
<td>Potential Cause(s)/Mechanism(s) of Failure</td>
<td>Recommended Action(s)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------</td>
<td>---------------------------------------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Potentiostat</td>
<td>Faulty Instrument</td>
<td>Failure to correctly measure sensor output</td>
<td>Faulty Instrument</td>
<td>Test potentiostat before clinical study</td>
</tr>
<tr>
<td>Clinical Study</td>
<td>Movement of the sensor during clinical study</td>
<td>Dislodgment of some of the microprobes resulting in poor sensor function</td>
<td>Movement of subjects during clinical study (e.g. going to the toilet)</td>
<td>Securing sensor and sensor connections to minimise effect of movement Use of portable potentiostat rather than a desktop potentiostat Use of skin imaging modality to ensure consistent penetration</td>
</tr>
<tr>
<td></td>
<td>Sweeting at sensor site</td>
<td>Interference with sensor function</td>
<td>Sweeting</td>
<td>Isolation of sensor base and edges</td>
</tr>
</tbody>
</table>

*Table 5.2: Failure mode and effects analysis for the different steps of sensor fabrication and sensor use in phase 1 of the clinical study.*
actions. Inactivation of the glucose oxidase layer could result from wrong storage, wrong shelf life or wrong dose of gamma ray. Electrochemical assessment of at least one sensor per patch following these steps and before immediately before starting clinical studies could identify these errors. Another potential failure mode is disruption of the epoxy-PU membrane (figure 5.5). Although previous ex vivo tests showed that skin insertion did not affect the integrity of the coating membrane, these tests were performed using an Instron machine that allowed the use of preset speed and force to insert the microprobe arrays. In the in vivo studies performed so far, sensor was inserted by the use of thumb pressure. The speed of sensor insertion into skin can allow skin insertion using minimal force and this is known to affect the amount of local tissue trauma (Schmelzeisen-Redeker et al., 2013). The need to apply larger force, when inserting the microprobes using thumb pressure, could result in larger friction between skin and the coating membrane resulting in its rupture. The risk of membrane disruption can be minimised by altering the ratio between epoxy and PU in the membrane to enhance its robustness. The use of an applicator to use preset speed and force for sensor insertion could also minimise the risk of disruption of the coating membrane.

While manual application of the sensor simplifies the insertion processes and avoids the cost and training related to the use of applicators, the use of an applicator allows for reproducible skin penetration by limiting inter- and intra-individual variability in skin resistance to penetration and force applied for insertion (Singh et al., 2011). Another potential advantage to the use of an applicator device, which was highlighted by participants in the focus group, is that it can provide the user with the assurance that successful penetration has
occurred, even in the absence of any associated pain or sensation (Singh et al., 2011).

Equally important to the use of an applicator is evaluation of characteristics of skin penetration following sensor application. Other than confirming penetration and establishing the insertion ratio (number of inserted microprobes in relation to the total number of microprobes), it can also help in evaluating the relationship between depth of insertion and sensor’s output current. As discussed earlier, several methods have been described in the microneedle literature to confirm penetration of the stratum corneum and study the characteristics of the created microchannels. Evaluation using non-invasive optical imaging techniques, such as confocal microscopy or optical coherence tomography (OCT), avoids the need for skin biopsy that, other than being invasive, may alter the biomechanical characteristics of the punctured skin and alter macro- or microscopic appearance. In our efforts to pursue an optical imaging tool for in vivo in situ evaluation of effect of microprobe application to the skin, an OCT imaging system (Thorlabs Telesto II) was used to image transparent non-metallised and gold metallised microprobe arrays (figure 5.7). While OCT was able to image through the non-metallised arrays and visualise structures underneath it, it was not able to do the same with gold metallised arrays. This implies that the OCT system cannot be used for in vivo in situ imaging of the metallised sensor. Alternatively, it can be used to confirm skin penetration and study characteristics of the created microchannels following sensor removal. We are currently planning further studies using an applicator and an OCT machine. In the studies conducted so far, post-removal in vitro electrochemical evaluation of the sensors was considered. However,
because of corrosion of contacts in vitro calibration was not possible due to the high background current. In future studies, contacts will be isolated during fabrication to allow in vitro electrochemical evaluation of sensors before and after clinical studies if required.

Figure 5.7: *En face video and optical coherence tomography (OCT) images (cross section and 3D) showing microprobes of a non-metallised array (A) and of a microprobe array metallised with gold (B). OCT can only show the underlying structures when used to image the transparent microprobe array (A2, A3). [OCT imaging was done at Thorlabs Laboratory using Thorlabs Telesto II OCT imaging system].*

5.5. CONCLUSION:

The novel microprobe array glucose sensor has been tested in 8 subjects (out of 16 subjects planned for phase 1 of the clinical study). Initial results show that the sensor was well tolerated. Pain scores during sensor insertion were low and there was no sensation related to the sensor over the six-hour study
period. Similarly, skin inflammation was barely noticeable and transient. There was no evidence of bleeding at sensor site or infection.

Further optimisation of sensor functionalisation is required to address the issue of disruption of the epoxy-PU membrane. Design of an applicator for sensor insertion should allow insertion without disrupting sensor layers. It will also allow delivering preset force and may reduce intra- and inter-subject variability in relation to percentage of microprobes penetrating stratum corneum and penetration depth. Assessment of skin insertion using optical imaging may help in evaluating the relationship between penetration depth, sensor output current and performance.

Finally, further clinical studies in subjects with type 1 diabetes are required to assess sensor performance at different glycaemic ranges and at difference rates and directions of glucose change.
CHAPTER 6

GLYCAEMIC VARIABILITY AND THE EFFECT OF REAL-TIME CONTINUOUS GLUCOSE MONITORING
6.1. INTRODUCTION

6.1.1. Definition of glycaemic variability:

There is growing interest, supported by evidence and the increased availability of CGM technology, in the role of glycaemic variability (GV) in the development of diabetes related complications, independent of HbA1c. However, there are many aspects related to GV that are potential source of confusion for both researchers and clinicians. This begins with the definition of GV. The term has been used to describe several concepts of variation in glycaemia. Based on studies evaluating different forms of glucose variations, the term GV has been used in the literature to describe (Frontoni et al., 2013):

1- Day to day variations in fasting glucose
2- Post-prandial hyperglycaemia
3- HbA1c variation
4- Short-term Interday and intraday variability

The confusion is further complicated by controversy related to the effect of GV on diabetes-related complications, the plethora of GV measures with the lack of a “gold standard” measure, and the limited accuracy of existing CGM systems, all of which may affect results obtained for GV measures.

6.1.2. Measures of glycaemic variability:

Glycated Haemoglobin (HbA1c) has been the gold standard for clinical assessment and monitoring of glycaemic status in people with diabetes. As several clinical studies (including the landmark Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes
Study (UKPDS)) have demonstrated the positive relationship between HbA1c level and the risk of vascular complications (UKPDS, 1998, DCCT, 1993), HbA1c has been used by clinicians as a treatment target and for assessment of clinical response to therapeutic interventions. More recently, HbA1c has also been used for diagnosis of diabetes and impaired glucose tolerance.

Despite its value being the glycaemic marker most linked to diabetes-related complications, HbA1c measures glycaemic exposure over 2-3 months, therefore it may not provide adequate information on frequency, duration or magnitude of short-term fluctuations in glycaemia and is limited in people with haemoglobinopathies, renal disease, and iron deficiency anemia. Two patients with the same HbA1c can have completely different daily glucose profiles with different patterns of glycaemic excursions and hypoglycaemia. Furthermore, HbA1c is a poor predictor of hypoglycaemic episodes accounting for only 8% of risk of future severe hypoglycaemia (Kovatchev et al., 2006). DCCT has also demonstrated that the three fold increased risk of hypoglycaemia in the intensive treatment group could not be completely explained by difference in HbA1c between the two groups (DCCT, 1997).

With the growing interest in GV and its role in the pathogenesis of diabetes-related complications, independent from HbA1c level, several measures have been proposed to describe GV (table 6.1). Some of these measures can be calculated from SMBG data, while others require CGM data. GV measures can be broadly subdivided into (Rodbard, 2009a, Rodbard, 2012):
A. Measures based on glucose distribution: examples include:
- Standard deviation (SD)
- Mean amplitude of glycaemic excursion (MAGE)
- Continuous overlapping net glycaemic action (CONGA)
- Mean of daily difference (MODD)
- Lability index (LI)
- Mean absolute glucose (MAG)

B. Measures based on quality of glycaemic control that are also sensitive to GV: examples include:
- M-value
- J-index

C. Risk measures: examples include:
- High blood glucose index (HBGI)
- Low blood glucose index (LBGI)
- Average daily risk range (ADRR)
- Glycaemic risk assessment diabetes equation (GRADE)

SD is the amount of spread around the mean. While it is easy to calculate and it is possibly the simplest tool to assess GV, the lack of Gaussianness of glucose profile data (being more skewed towards hyperglycaemic range) means that SD is more sensitive to hyperglycaemic excursions and not sensitive to hypoglycaemia. Furthermore, SD is dependent on mean glucose and there is a potential for widely different glycaemic curves having the identical numerical value of SD. However, SD remains a robust measure of GV because a linear relation has been established between interquartile range and SD (DeVries, 2013).

Several methods to assess interday and intraday GV based on SD have been proposed (Rodbard, 2009a), such as total SD (SD_T), SD within days (SD_w), SD within corresponding time points of multiple days (SD_{hh:mm}), SD
between multiple days within corresponding time points \(SD_{bh:mm}\) and SD between daily means \(SD_{dm}\).

Percentage coefficient of variation \(\%CV\) is the ratio of SD to mean. This parameter allows for standardised comparisons between patients with different levels of mean glycaemia. It was proposed that \(\%CV\) may be the best parameter to characterise GV since it avoids dependency of SD and GV measures on mean glucose and HbA1c (Rodbard, 2011).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
<th>Formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Measure of spread around the mean</td>
<td>[SD = \sqrt{\frac{\sum (G-x)^2}{N-1}}]</td>
</tr>
<tr>
<td>CONGA</td>
<td>It is the SD of the differences between glucose values that are (n) hour(s) apart ((n) can vary from 1 to 24 hours).</td>
<td>[CONGA = \sqrt{\frac{\sum_{t=t_1}^{t_k} (D_i - \bar{D})^2}{k-1}}]</td>
</tr>
<tr>
<td>LI</td>
<td>Measure of severity of GV and hypoglycaemia. Was proposed for candidates of islet cell transplantation</td>
<td>[LI = \sum_{n=1}^{N-1} \frac{(G_n - G_{n+1})^2}{(t_{n+1} - t_n)}]</td>
</tr>
<tr>
<td>Measure</td>
<td>Description</td>
<td>Formulae</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>J-Index</td>
<td>A measure of both variability and mean glycaemia.</td>
<td>$J=0.324 \times (MBG+SD)^2$&lt;br&gt;MBG=mean glucose levels&lt;br&gt;SD=SD of glucose levels</td>
</tr>
<tr>
<td>GRADE</td>
<td>A measure that provides the risk attributable to a certain glucose level (hypoglycaemia or hyperglycaemia and its degree of severity)</td>
<td>GRADE=median $(425 \times {\log[\log(G_n)]+0.16})^2$&lt;br&gt;G=glucose measured</td>
</tr>
<tr>
<td>LBGI/HBGI</td>
<td>Measures of hypoglycaemia and hyperglycaemia. The formulae convert glucose values into risk scores. If the risk score is below 0, then the measure is labelled as LBGI. If the risk score is above 0, the measure is labelled as HBGI.</td>
<td>$LBGI = \frac{1}{N} \sum_{i=1}^{N} rl(x_i)$&lt;br&gt;$HBGI = \frac{1}{N} \sum_{i=1}^{N} rh(x_i)$&lt;br&gt; $N=$number of readings&lt;br&gt; $rl=$risk value associated with a low glucose (if $x&lt;0$)&lt;br&gt; $rh=$risk value associated with a high glucose (if $x&gt;0$)&lt;br&gt; $x=$nonlinear transformation of glucose measured</td>
</tr>
<tr>
<td>MODD</td>
<td>A measure of inter-day variability. It is the mean of the absolute difference between paired glucose values from consecutive 24-hour periods.</td>
<td>$MODD = \frac{\sum_{i=t_1}^{t_k}</td>
</tr>
<tr>
<td>MAGE</td>
<td>A Measure of major glucose fluctuations, by measuring average amplitude of upwards or downwards glucose fluctuations with magnitudes greater than 1SD.</td>
<td>$MAGE = \sum_{x} \frac{\lambda}{x}$ if $\lambda &gt; \nu$&lt;br&gt; $\lambda=$blood glucose changes from peak to nadir&lt;br&gt; $x=$number of valid observations&lt;br&gt; $\nu=1$ SD of mean glucose for a 24-h period</td>
</tr>
<tr>
<td>Measure</td>
<td>Description</td>
<td>Formulae</td>
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<tr>
<td>---------</td>
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</tr>
<tr>
<td>ADRR</td>
<td>A measure that combines information from both LBGI and HBGI.</td>
<td>$ADRR = \frac{1}{N} \sum_{i=1}^{N} [LR + HR]$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$N=\text{total number of readings}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$LR=\text{risk value attributed to low glucose}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$HR=\text{risk value attributed to high glucose}$</td>
</tr>
<tr>
<td>M-value</td>
<td>A measure of both variability and mean glycaemia. The formula incorporates a reference value, representing ideal glucose level, that is typically set to 6.67 mmol/L.</td>
<td>$M = \frac{\sum_{i=1}^{k} \left</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$G=\text{glucose measured}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$IGV=\text{ideal glucose value}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k=\text{total number of observations}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$N=\text{total number of readings}$</td>
</tr>
<tr>
<td>MAG</td>
<td>A measure for assessment of intraday GV. It calculates the sum of the differences between successive glucose values (typically 60 minutes apart) divided by the total time measured in hours.</td>
<td>$MAG = \frac{\sum_{n=1}^{N-1} (G_n - G_{n+1})}{T}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$G=\text{glucose measured}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$N=\text{number of glucose measurements}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$T=\text{total time (in h)}$</td>
</tr>
</tbody>
</table>

**Table 6.1:** Description and formulae of evaluated glycaemic variability measures (adopted from (Hill et al., 2011)).

MAGE was first described by Service et al (Service et al., 1970) using SMBG data of 48 hours to assess major glucose swings and exclude minor ones. It is calculated by estimation of the SD before identifying the direction of the first glycaemic excursion that exceeds 1SD. Subsequent qualifying excursions (exceeds 1SD and in the same direction of the first excursion) are then identified and the arithmetic mean of glucose changes from peak to nadir is calculated. Despite its popularity as a measure of GV, MAGE has several limitations. It discards minor excursions (smaller than 1SD), which
can potentially be clinically relevant. It does not take frequency of excursions or the absolute value of each excursion into account. There is also a difference in outcome when upwards or downwards excursions are used for calculation of MAGE. Moreover, its analysis is time-consuming and operator dependent. Four computer algorithms have been described to standardise MAGE calculation but they show poor correlation (Sechterberger et al., 2014). A modified method (MAGE-CGM) has been proposed to select a peak or trough based on direction of change (rising or falling) of the preceding and succeeding data points. The MAGE-CGM formula also contains a 15-min lag window for the direction of change based on the delay between interstitial fluid glucose measurement and plasma glucose concentrations. It also contains an algorithm that eliminates short-term fluctuations related to sensor inaccuracies (Hill et al., 2011).

CONGA is a GV measure that was developed for CGM (McDonnell et al., 2005). It is the SD of the differences between glucose values that are \( n \) hour(s) apart. \( n \) can vary from 1 to 24 hours. Analysis requires at least 24 hours CGM trace. Comparison between subjects with type 1 diabetes and non-diabetic subjects showed mean CONGA\(_1\) value in the group with diabetes of 2.5 (range 1.7–3.2) compared with 0.7 (range 0.4–1.2) in the control non-diabetic group. The mean CONGA\(_4\) value in the diabetes group was 4.6, compared with 1.0 in the control group (McDonnell et al., 2005).

MODD is a measure of inter-day variability. It is the mean of the absolute difference between paired glucose values from consecutive 24 hour periods. Therefore, it requires at least 48 hours of CGM (Molnar et al., 1972).
MAG is a measure for assessment of intraday GV. It was originally developed to assess the impact of GV on mortality in intensive care unit. It calculates the sum of the differences between successive glucose values (typically 60 minutes apart) divided by the total time measured in hours (Hermanides et al., 2010).

LI was proposed to assess the severity of GV and hypoglycaemia before and after islet cell transplantation (Ryan et al., 2004). The LI is calculated based on the change in glucose level over a four-week period using SMBG with glucose measurements 1-12 hours apart. It was reported that a LI ≧ 24 mmol/L²/h.week⁻¹ indicates high risk of hypoglycaemia. A modified LI was proposed since LI is dependent on the number of glucose measurements used (Kim et al., 2011, Rodbard, 2009b). In a study using four glucose measurements daily, correlation between LI and modified LI (LI divided by number of daily glucose measurements) was strong (r = 0.87, P < 0.01) (Kim et al., 2011).

M-value is a hybrid measure of both variability and mean glycaemia. It is calculated on each glucose value using a formula and then is divided by the total number of values to produce a mean. The formula incorporates a reference value that is typically set to 6.67 mmol/L (120 mg/dL). The formula gives greater emphasis to hypoglycaemia than hyperglycaemia. To use M-value for comparison between studies, the same reference value needs to be used (Service, 2013).

The J-index is also a measure of both variability and mean glycaemia. It was originally derived from SMBG but has been adapted for CGM data. It is
calculated from the equation: $J = 0.324 \text{ (mean blood glucose + SD)}^2$. $J$-index > 30 indicates poor control, while $J$-index between 10-20 indicates optimum control (Hill et al., 2011).

Hypoglycaemia has been identified as a significant barrier to optimisation of glycaemic control and has been linked to morbidity and mortality in people with diabetes (Seaquist et al., 2013). Various approaches have been adopted to assess risk and severity of hypoglycaemia including low HbA1C, history of severe hypoglycaemia, hypoglycaemic awareness questionnaires, and HYPO score, which is a score based on the frequency, severity, and degree of unawareness of hypoglycaemia recorded over a four-week glucose monitoring period and from self-reported episodes over the previous year (Ryan et al., 2004). However, HbA1c is a poor predictor of hypoglycaemic episodes accounting for around 8% of risk of future severe hypoglycaemia (Kovatchev et al., 2006). On the other hand, measures of GV like SD are less sensitive to hypoglycaemia because of the skewness of glucose data towards the hyperglycaemic range.

Several measures of GV have been proposed to assess future risks of hypoglycaemia, hyperglycaemia or both. As glucose profile is skewed towards the hyperglycaemic range, the numerical centre of glucose data (17 mmol/L) is different from the clinical one (6-7 mmol/L). Three risk measures were proposed based on log transformation of the glucose profile to give equal weights to hypoglycaemic and hyperglycaemic ranges. By symmetrisation of glucose data around zero (equivalent to 6.25 mmol/L), HBGI and LBGI were computed to assess the risk of hyperglycaemic and hypoglycaemic excursions, respectively (Kovatchev et al., 1997). HBGI and
LBGI were originally computed from SMBG but have been adapted for use with CGM. HBGI>15 indicates high risk of hyperglycaemic excursions, while HBGI≤4.5 indicates low risk. Correlation between HBGI and HbA1c has been reported (Kovatchev et al., 1997). On the other hand, LBGI>5 indicates high risk of hypoglycaemia, while LBGI<2.5 indicates low risk. In a study evaluating LBGI in adults with type 1 diabetes, the frequency of future severe hypoglycaemia was predicted by the LBGI and history of severe hypoglycaemia, while HbA1c, age, duration of diabetes, and BG variability were not significant predictors (Kovatchev et al., 1998).

ADRR was proposed to give a predictive value of both hypoglycaemic and hyperglycaemic excursions. It is computed from SMBG data collected typically over one month (at least 14 days) with a typical frequency of 3-5 glucose measurements per day. An ADRR > 40 indicates high risk for GV, while ADRR<20 indicates low risk (Kovatchev et al., 2006).

Another GV measure that assesses the risk of hypoglycaemia and hyperglycaemia is GRADE. It was created based on 50 questionnaires completed by healthcare professionals, who were asked to assign a risk value to each glucose concentration. The lowest score was assigned to glucose value of 5 mmol/L and a logarithmic formula was used to transform every glucose value into a GRADE value. GRADE score is then obtained by calculating the mean of all GRADE values (Hill et al., 2009). GRADE score is reported as overall score, GRADE_{hypo}, GRADE_{hyper} and GRADE_{eu} to express the contribution of hypoglycaemia, hyperglycaemia and euglycaemia in the assessed glucose profile, respectively.
6.1.3. Tools to calculate glycaemic variability:

SD is usually reported as a measure of GV in CGM reports, however calculation of other measures of GV is a complicated and time-consuming task, representing another barrier to the use of these GV measures in clinical practice. Several computer programs are currently available to compute several GV parameters. EasyGV is an excel-enabled workbook that is available free for non-commercial use at www.easygv.co.uk. It allows calculation of SD, mean, M-Value, MAGE, LI, ADRR, J-Index, LBGI, HBGI, CONGA, MODD, GRADE and MAG. The main excel sheet has a number of options that allows the user to set up the sampling interval, CONGA length, LI interval, reference value of M-value and whether SMBG or CGM is used for MAGE calculation (Hill et al., 2011).

Glyculator is another tool for calculation of GV measures. It is available as a web-based application or as a more complex Windows-based programme. In both versions available, variability parameters are mean, median, SD, SD\(_w\), SD\(_b\)\(_{hh:mm}\), SD\(_d\)\(_{mm}\), %CV, M-Value, J-index, MAGE, MODD, CONGA (for 1, 2, 4, 6, and 24 h time intervals), and percentage of hyperglycaemia (levels between 7 and 10 mmol/L) and hypoglycaemia (levels below 3.9 mmol/L) episodes (Czerwoniuk et al., 2011).

Two tools were specifically developed for computation of MAGE aiming to replace the time-consuming graphical estimation method (Fritzsche et al., 2011, Baghurst, 2011). However, a study that compared MAGE values obtained from these two tools, EasyGV and Glyculator, showed poor correlation (Sechterberger et al., 2014).
6.1.4. Impact of GV:

6.4.1. Impact of GV on hypoglycaemia:

The DCCT study has demonstrated that the three fold increase in the risk of severe hypoglycaemia in the intensive treatment group compared to the conventional treatment group could not be simply explained by the difference in HbA1c between the two groups, with HbA1c accounting for only 8% of risk of severe hypoglycaemia (Kilpatrick et al., 2007). To study the relationship between GV and hypoglycaemia, a re-analysis of the DCCT study has demonstrated that HbA1c, mean blood glucose, and GV measurements (assessed as SD) each have an independent role in determining an individual’s risk of severe hypoglycaemia in type 1 diabetes (Kilpatrick et al., 2007). The Diabetes Outcomes in Veterans Study assessed hypoglycaemia risk in 195 stable, insulin-treated subjects with type 2 diabetes, and found that mean blood glucose and SD were stronger predictors of hypoglycaemia than HbA1c (Murata et al., 2004). Similarly, a retrospective analysis of data from 24 week insulin trials including more than 2000 subjects with type 2 diabetes (who only started insulin therapy in the trial), demonstrated that intraday and interday GV are significantly associated with the risk of hypoglycaemia, independent of mean glycaemia. In this study, out of all the evaluated GV measure, intraday % CV at baseline and interday % CV at 24 weeks were the strongest predictors of risk of hypoglycaemia between 12 and 24 weeks (Qu et al., 2012). In a study aiming to define the relative contribution of GV and mean glycaemia to the incidence of asymptomatic hypoglycaemia in 222 adults with type 2 diabetes, GV (assessed by SD and MAGE using CGM) and the 48 hour
mean glucose were significant independent predictors of frequency of asymptomatic hypoglycaemia. Whereas HbA1, age, gender, duration of diabetes and treatment modality were not (Monnier et al., 2011). In this study, the analysis was limited to asymptomatic hypoglycaemia to exclude the rebound effect resulting from correction of symptomatic hypoglycaemia on both glucose concentration and GV. This suggests that high GV is the cause rather than the consequence of hypoglycaemia.

6.4.2. Impact of GV on mortality in ITU:

It was also demonstrated that GV is a significant independent predictor of ITU and hospital mortality in diabetic and non-diabetic adults. In a retrospective analysis that evaluated the relationship between GV (measured as SD and %CV) and ITU or hospital mortality in more than 7000 critically ill subjects, both mean glucose and GV were significant independent predictors of ITU and hospital mortality (Egi et al., 2006, Krinsley, 2008). Similarly, a retrospective analysis of 5728 subjects (12.2% known to suffer from diabetes) showed that high GV (measured as MAG and SD) was a significant predictor of ITU and hospital mortality, independent of mean glycaemia (Hermanides et al., 2010).

6.4.3. Impact on micro- and macro-vascular complications:

As discussed earlier, the term “glycaemic variability” has been used to describe different forms of glucose variation, particularly when evaluating the effect of glucose variation on diabetes-related vascular complications.

The relationship between variation in fasting plasma glucose (FPG) and diabetes-related microvascular complication, was evaluated in several studies. In a study of 170 subjects with type 2 diabetes (treated with either
diet, oral hypoglycaemic agents or insulin) and mean follow-up of 33 years, the SD of FPG was reported to be a significant risk factor for the development of proliferative diabetic retinopathy, independent of mean FPG or HbA1c (Takao et al., 2011). Post-prandial glucose (PPG) and its relationship to cardiovascular disease and mortality was also evaluated in several studies. The San Luigi Gonzaga Diabetes Study reported that post-prandial hyperglycaemia is a strong predictor of cardiovascular disease and mortality, independent of HbA1c. In this study, the relationship between 5 glycaemic control parameters (fasting blood glucose, blood glucose 2 h after breakfast, blood glucose 2 h after lunch, blood glucose before dinner, and HbA1c), and cardio-vascular events and all-cause mortality were examined in 505 subjects with type 2 diabetes with mean follow up of 14 years. Both HbA1c and blood glucose 2 hours post lunch, but not FPG, were strong independent predictors of cardio-vascular events and of all-cause mortality (Cavalot et al., 2006, Cavalot et al., 2011). This is supported by findings of the STOP-NIDDM study, a double-blind randomised controlled trial (RCT) that evaluated the effect of targeting PPG using acarbose on the risk of development of major cardiovascular events in more than 1000 subjects with impaired glucose tolerance, who were followed up for a mean of 3.3 years. Compared to placebo, targeting PPG using acarbose resulted in significant reduction in development of cardiovascular events (Chiasson et al., 2003).

In contradiction, the HEART2D study has demonstrated that a significant difference in PPG values between two insulin regimens, while achieving comparable HbA1c and FPG values, was not associated with a difference in cardiovascular outcome. In this RCT, the effects of a prandial and a basal
insulin regimen with respect to cardiovascular outcomes was examined in
more than 1000 subjects with insulin-treated type 2 diabetes after acute
myocardial infarction who were followed for a mean of 963 days (Raz et al.,
2009).

The role of HbA1c variability in the development of diabetes-related
complications was also addressed in several studies. In type 1 diabetes, a
re-analysis of the DCCT reported that HbA1c variability (after the first 6
months to allow stabilisation in glycaemia) was an independent and stronger
predictor, compared to mean HbA1c, of nephropathy or retinopathy, while
short-term GV was not (Kilpatrick et al., 2008). This was supported by a
more recent study that evaluated the relationship between HbA1c variability
(measured as SD of HbA1c) and proliferative diabetic retinopathy in more
than 2000 subjects with type 1 diabetes (Hietala et al., 2013).

The A1c-Derived Average Glucose (ADAG) study, a multi-centre study that
was designed to assess the relationship between HbA1c and mean glucose
in 507 subjects (268 subjects with type 1 diabetes, 159 subjects with type 2
diabetes and 80 non-diabetic subjects) using periodic CGM and seven
points SMBG over 16 weeks, evaluated the relationship between different
indices of glycaemia. While measures of intraday GV (SD, MAGE and
CONGA) showed strong intercorrelation, their correlation with indices of
fasting, postprandial, mean glucose and HbA1c was relatively weaker.
Therefore, authors suggested that measures of intraday GV may convey
different information to other measures of glycaemia (Borg et al., 2010).

Several studies were conducted using different measures of interday and
intraday GV to examine the relationship between short-term GV and diabetes-related vascular complications. The relationship between oxidative stress, which is believed to be the putative mediator for diabetes-induced vasculopathy, and GV, has been demonstrated both in vitro and in vivo. Intermittent exposure to hyperglycaemia in vitro had more evident effect on increasing oxidative stress compared to sustained hyperglycaemia (Risso et al., 2001, Quagliaro et al., 2003, Quagliaro et al., 2005, Piconi et al., 2004, Schiekofer et al., 2003). In human studies, Monnier et al examined the relationship between oxidative stress, as measured by urine 8-iso prostaglandin F$_{2\alpha}$ excretion, short-term GV (measured as MAGE), PPG (measured as mean postprandial incremental area under the curve), FPG and HbA1c levels in 21 subjects with poorly controlled type 2 diabetes, who were not insulin-treated. In this case-control study, only MAGE and PPG showed significant correlation with oxidative stress (Monnier et al., 2006). Consistent with this, Ceriello et al demonstrated that glucose oscillation between 5 and 15 mmol/L every 6 hours for a period of 24 hours have more deleterious effect on endothelial function and oxidative stress compared to constant hyperglycaemia of 10 or 15 mmol/L. The study was conducted in 27 subjects with diet- controlled type 2 diabetes and 22 non-diabetic subjects. In this study, flow mediated dilatation was used as a marker for endothelial function, whereas plasma 3-nitrotyrosine and 24-h urinary excretion rates of free 8-iso prostaglandin F$_{2\alpha}$ served as markers for oxidative stress. The effect of glucose oscillation on endothelial function and oxidative stress was counterbalanced by vitamin C infusion (Ceriello et al., 2008).

In an 11-year follow up study, while HbA1c was an independent predictor of
nephropathy, GV (assessed as SD of blood glucose) was a predictor of peripheral neuropathy and a highly significant predictor of hypoglycaemic unawareness in subjects with type 1 diabetes (Bragd et al., 2008). GV has also been linked to coronary artery calcification in men with type 1 diabetes, independent of HbA1c (Snell-Bergeon et al., 2010). In 75 subjects with type 1 diabetes, a gender-dependent significant relationship was noted between measures of intraday and interday GV and coronary artery calcification, a surrogate marker of cardiovascular disease.

The relationship between GV (measured as MAGE) and severity of coronary artery disease (CAD) was investigated in 286 subjects who were referred for coronary angiogram following stable angina or angina-like chest pain and were found to have newly diagnosed type 2 diabetes. The study reported that MAGE was significantly higher in patients with CAD compared to those without and that high MAGE (≥3.4 mmol/L) was an independent predictor (and stronger predictor compared to HbA1c) of prevalence and severity of CAD (Mi et al., 2012). These findings are similar to those of a study conducted by the same research group in 344 subjects with type 2 diabetes. 35-40% of subjects were on insulin and subjects who had their antihyperglycaemic therapy changed between coronary angiogram and CGM were excluded from the study. While the study showed the significant relationship between intraday GV (expressed as MAGE) and prevalence and severity of CAD, interday GV (expressed as MODD) failed to show any significant relationship to CAD (Su et al., 2011).
Despite the above evidence, the effect of such a complex phenomenon as short-term interday and intraday GV on diabetes-related complications remains controversial. In contrast to the study by Monnier et al that showed correlation between GV and markers of oxidative stress, a similar study failed to establish this relationship in subjects with either type 1 diabetes or well-controlled type 2 diabetes (Wentholt et al., 2008, Siegelaar et al., 2011b). The DCCT has demonstrated that HbA1c accounted for almost 96% of the variation in risk of retinopathy between the intensive and conventional control groups, concluding that GV may only have small contribution towards the risk of retinopathy between the two groups over time (Lachin et al., 2008). This was supported by another re-analysis of DCCT data, which reported that only variability in HbA1c, rather than that of GV (using SD or MAGE) predicted the development of retinopathy or nephropathy (Kilpatrick et al., 2006, Kilpatrick et al., 2008, Kilpatrick et al., 2009). An observational study aiming to evaluate the relationship between diabetic retinopathy and GV (assessed using SD, MAGE, CONGA$_2$ and HBGI) was conducted in 35 subjects with type 1 diabetes and 33 subjects with type 2 diabetes. Although univariate analysis showed significant relationship between diabetic retinopathy and each of SD, CONGA$_2$ and HBGI, multivariate analysis showed that diabetes duration was the only significant parameter correlating with diabetic retinopathy (Sartore et al., 2013). In a reanalysis of HEART2D, the effect of GV on cardiovascular disease was assessed using MAG, MAGE and SD. Despite an 18% reduction in MAG in the prandial insulin group, there was no difference between the two groups with regards to cardiovascular events (Siegelaar et al., 2011c). The ADAG study group reported that while mean glucose and HbA1c have
stronger associations with cardiovascular disease risk factors than fasting glucose or postprandial glucose in subjects with type 1 and type 2 diabetes, measures of intraday GV did not have a significant effect (Borg et al., 2011). Furthermore, a recent systematic review concluded that GV was associated with increased risk of development of microvascular complications only in type 2 diabetes and that the relationship between GV in type 1 diabetes and either microvascular or macrovascular complications was less clear (Smith-Palmer et al., 2014).

6.4.4. Impact of GV on psychological function:
GV has also been linked to psychological function, quality of life (QoL) and patients’ satisfaction. In a randomised, controlled, crossover study, 388 subjects with type 1 diabetes and insulin treated type 2 diabetes were randomised to either basal-bolus or twice-daily insulin regimens for 12 weeks before crossover. Reduction in GV (measured as SD using CGM) and improved mean glycaemia in the basal-bolus group were both independent predictors of patients’ satisfaction (Testa et al., 2012). In another study that examined the correlation between GV and health-related QoL in 23 women with type 2 diabetes, 24 hour SD and CONGA showed significant correlation with QoL after adjustment for age and weight (Penckofer et al., 2012).

6.2. AIMS:
The present analysis aims to describe several indices of GV and quality of glycaemic control in a large cohort of subjects with type 1 diabetes in comparison to normal reference ranges defined in people without diabetes. It also aims to examine the intercorrelation between these measures and the
correlation with HbA1c. Furthermore, it studies the effect of RT-CGM on these indices and the baseline factors predicting change, aiming to identify GV measures that are more sensitive to overall glycaemic status of people with type 1 diabetes.

6.3. METHOD:

We used data from the JDRF sponsored “Randomized Clinical Trial to Assess the Efficacy of RT-CGM in the Management of Type 1 Diabetes”. The publically accessible data obtained from “Jaeb Center for Health Research” website (http://diabetes.jaeb.org/Dataset.aspx) were processed using Matlab (Mathworks) and EasyGV (v8.8.2.R2).

Study design and intervention:

The JDRF CGM study is a randomised, parallel group, efficacy and safety study (Group, 2008). The study included subjects meeting the following criteria: aged 8 years old or older with a diagnosis of type 1 diabetes for at least 1 year, HbA1c ≤10% and being able to use blinded RT-CGM during the assessment period. Exclusion criteria were the presence of a significant psychiatric or medical disorder that in the judgment of the investigator would adversely affect conduct of the study, home use of a RT-CGM systems in the preceding 6 months, participation in another intervention study in the preceding 6 weeks or pregnancy.

Subjects started to enroll in the study, that took place in 10 clinical centers, in early 2007. As shown in Figure 6.1, subjects with type 1 diabetes who meet eligibility criteria were randomised 1:1 to either standard SMBG alone or use of RT-CGM as a supplement to SMBG. At completion of the 6-month RCT,
subjects in the RT-CGM group continued to use RT-CGM for another 6 months. In addition, subjects in the SMBG control group were offered use of RT-CGM for 6 months with less intensive contact than was provided at initiation of RT-CGM use in the RT-CGM group.

On enrollment in the study, subjects were allowed to choose from the Medtronic (Northridge, CA), DexCom (San Diego, CA), or Abbott Diabetes Care (Alameda, CA) RT-CGM systems.

To qualify for randomisation, subjects must have used the RT-CGM system for at least 6 out of the 7 days assessment period, have a total of at least 96 hours of RT-CGM glucose values with at least 24 hours between the hours of 10 p.m. and 6 a.m., and have performed at least three meter glucose tests each day.

![Figure 6.1: Diagram showing JDRF CGM study design.](image)


According to baseline HbA1c, the study cohort was separated into two different cohorts. The main study cohort included subjects with baseline
HbA1c between 7-10% inclusive. The other cohort included subjects with the same eligibility criteria but with baseline HbA1c <7%.

Outcomes and measures of GV and quality of glycaemic control:
The primary outcome of the JDRF CGM study was between group differences in HbA1c at 26 weeks.

SMBG control group subjects were blinded to baseline CGM data, and subsequent blinded CGM profiles were obtained at 13 and 26 weeks post-randomisation. These data were collected for comparison against unblinded sensor data obtained in the RT-CGM group at the same time points of study, to detect between group differences in GV indices at 26 weeks.

Glucose profile data collected at 52 weeks from subjects in the control group who crossed-over to RT-CGM (unblinded control), were compared with data collected at 26 weeks in the same group (blinded control), to evaluate the effect of unmasking of CGM on changes of GV indices.

Statistical analysis:
Data were examined for normality. Non-normally distributed variables (baseline LI, baseline J-index, baseline LBGI, baseline M-value and baseline MAG) were logarithmically transformed with the use of geometric mean, SD and 95% CI for descriptive statistics of baseline data (n=448). Pearson correlation was used to examine the relationship between HbA1c and indices of GV and quality glycaemic control at baseline (n=448). Independent t-test was used to analyse the effect of RT-CGM in comparison to blinded control on the change of GV indices at 26 weeks. To study the effect of unmasking CGM in the control group, we used paired t-tests to compare GV indices at 52 weeks (unblinded CGM, n=207) with GV indices
at 26 weeks (blinded CGM, n=214). The associations of baseline demographic and clinical factors with change in GV indices from baseline to 6 months were evaluated in the RT-CGM group (intervention, n=231) using regression models. The analysis was constructed using the following predictor variables: age, gender, race, education level of care giver, Insulin modality (pump or multiple daily injections (MDI)), frequency of daily self-reported blood glucose monitoring, occurrence of one or more episodes of severe hypoglycaemia in the preceding 6 months, diabetes duration, baseline HbA1c and baseline GV. Categorical variables were included as dummy variables. Baseline factors with \( P \leq 0.2 \) in the univariate analysis were carried forward to multivariate analysis. Data are presented as means (SD), unless otherwise stated. Statistical tests were two tailed with statistical significance at \( P < 0.05 \) and performed using SPSS 21.0 for Mac (SPSS Inc., Chicago, IL).

6.4. RESULTS:

6.4.1. Participants’ characteristics:

CGM profile data were available for 448 subjects at baseline (54.9% females, 94.4% white race), following exclusion of 3 subjects due to missing HbA1c data at baseline. Mean age was 25.1 years (SD 15.8) and mean diabetes duration 13.6 years (SD 11.7). 231 subjects were randomised to the RT-CGM group and 217 subjects to the control group. At 26 weeks, CGM profile data were available for all the 231 subjects in the RT-CGM group and 214 subjects in the control group (blinded CGM). At 52 weeks, data were available for 207 subjects in the control group (unblinded control). Baseline characteristics were similar between the 2 groups (table 6.2).
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RT CGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>217</td>
<td>231</td>
</tr>
<tr>
<td>Female sex – no. (%)</td>
<td>124 (57.1%)</td>
<td>122 (52.8%)</td>
</tr>
<tr>
<td>Age – no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 – 14 yrs</td>
<td>68 (31.35%)</td>
<td>74 (32%)</td>
</tr>
<tr>
<td>15 – 24 yrs</td>
<td>70 (32.25%)</td>
<td>71 (30.7%)</td>
</tr>
<tr>
<td>≥ 25 yrs</td>
<td>79 (36.4%)</td>
<td>86 (37.2%)</td>
</tr>
<tr>
<td>White race – no. (%)</td>
<td>205 (94.4%)</td>
<td>218 (94.3%)</td>
</tr>
<tr>
<td>Duration of diabetes – yr</td>
<td>13.42 (11.54)</td>
<td>13.85 (11.96)</td>
</tr>
<tr>
<td>Insulin administration – no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pump</td>
<td>175 (80.6%)</td>
<td>190 (82.2%)</td>
</tr>
<tr>
<td>MDI</td>
<td>42 (19.4%)</td>
<td>41 (17.8%)</td>
</tr>
<tr>
<td>HbA1c - %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7.0% - no. (%)</td>
<td>61 (28.1%)</td>
<td>67 (29%)</td>
</tr>
<tr>
<td>7.0 – 8.0% - no. (%)</td>
<td>109 (50.2%)</td>
<td>109 (47.2%)</td>
</tr>
<tr>
<td>8.1 – 8.9% - no. (%)</td>
<td>39 (18%)</td>
<td>44 (19%)</td>
</tr>
<tr>
<td>≥ 9.0% - no. (%)</td>
<td>8 (3.7%)</td>
<td>11 (4.8%)</td>
</tr>
<tr>
<td>≥ one episode of severe hypoglycaemia in last 6 months – no. (%)</td>
<td>17 (7.8%)</td>
<td>21 (9.1%)</td>
</tr>
<tr>
<td>Self-reported daily SMBG – no./day</td>
<td>6.26 (2.73)</td>
<td>6.11 (2.42)</td>
</tr>
<tr>
<td>College graduate (patient or primary care giver) – no. (%)</td>
<td>194 (89.4%)</td>
<td>197 (85.3%)</td>
</tr>
</tbody>
</table>

Table 6.2: Baseline characteristics of participants in the control and intervention groups.
6.4.2. Measures of GV and glycaemic control at baseline:

A normative range for 13 measures for GV and quality of glycaemic control, computed using EasyGV, was previously described by analysing CGM profiles of 70 non-diabetic subjects (table 6.3) (Hill et al., 2011). Figure 6.2 shows a comparison between these measures in subjects with type 1 diabetes, obtained by analysis of the glycaemic profiles of the 448 subjects enrolled in the JDRF CGM study, and the normal range in non-diabetic subjects. The mean values of GV indices at baseline in the 448 subjects with type 1 diabetes were all outside the normal range ($P<0.001$). Measures that showed the largest difference between the two groups were ADRR (33.35 vs 0.4, $P<0.001$), LI (6.17 vs 0.4, $P<0.001$), HBGI (12.03 vs 0.2, $P<0.001$), GRADE (8.37 vs 0.4, $P<0.001$) and J-index (52.72 vs 14.3, $P<0.001$). On the other hand, differences in SD and MAG were relatively smaller, (3.73 vs 1.5, $P<0.001$) and (2.7 vs 1.3, $P<0.001$), respectively. Mean glucose in type 1 diabetes subjects was 9.27 mmol/L vs 5.1 mmol/L for the non-diabetic subjects.
Table 6.3: Descriptive statistics (mean, 95% CI) for GV indices in 70 subjects without diabetes representing normal reference range (Hill et al., 2011) and in the 448 subjects with type 1 diabetes at baseline. Difference between measures of GV and glycemic control in the two groups is statistically significant (P<0.001).

<table>
<thead>
<tr>
<th>GV measure</th>
<th>Reference (mean, 95%CI)</th>
<th>Type 1 DM (mean, 95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.10 (4.98, 5.22)</td>
<td>9.27 (9.10, 9.43)</td>
</tr>
<tr>
<td>SD</td>
<td>1.50 (1.34, 1.66)</td>
<td>3.73 (3.64, 3.82)</td>
</tr>
<tr>
<td>CONGA</td>
<td>4.60 (4.48, 4.72)</td>
<td>8.29 (8.13, 8.44)</td>
</tr>
<tr>
<td>LI</td>
<td>0.40 (-0.12, 0.92)</td>
<td>6.17 (5.84, 6.51)</td>
</tr>
<tr>
<td>J index</td>
<td>14.30 (13.20, 15.40)</td>
<td>52.72 (50.83, 54.67)</td>
</tr>
<tr>
<td>LBGI</td>
<td>3.10 (2.65, 3.55)</td>
<td>4.10 (3.79, 4.43)</td>
</tr>
<tr>
<td>HBGI</td>
<td>0.20 (-0.69, 1.09)</td>
<td>12.03 (11.53, 12.53)</td>
</tr>
<tr>
<td>GRADE</td>
<td>0.40 (-0.09, 0.89)</td>
<td>8.37 (7.98, 8.76)</td>
</tr>
<tr>
<td>MODD</td>
<td>0.80 (0.47, 1.13)</td>
<td>4.06 (3.95, 4.16)</td>
</tr>
<tr>
<td>MAGE-CGM</td>
<td>1.40 (1.24, 1.56)</td>
<td>6.76 (6.54, 6.98)</td>
</tr>
<tr>
<td>ADRR</td>
<td>0.40 (-0.56, 1.36)</td>
<td>33.35 (32.21, 34.48)</td>
</tr>
<tr>
<td>M-value</td>
<td>4.70 (3.81, 5.59)</td>
<td>14.70 (13.83, 15.62)</td>
</tr>
<tr>
<td>MAG</td>
<td>1.30 (1.21, 1.39)</td>
<td>2.70 (2.61, 2.79)</td>
</tr>
</tbody>
</table>
Figure 6.2: Relationship between various measures of glycaemic variability and quality of glycaemic control in subjects with type 1 diabetes and non-diabetic subjects (mean and 95% confidence interval). *Geometric mean.
6.4.3. Intercorrelation between measures of GV at baseline and correlation with HbA1c:

Statistically significant intercorrelation between measures of GV and quality of glycaemic control was common. ADRR showed the strongest intercorrelation with other measures with r>0.7 in 9 out of 12 intercorrelations. Other measures of intraday GV (e.g. SD, CONGA, GRADE and M-Value) and interday GV (e.g. MODD) also showed strong intercorrelation. By contrast, LBGI showed the weakest intercorrelation. Similarly, all these measures, apart from LBGI, correlated significantly with HbA1c, as shown in table 6.4.

The relationship between percent of time spent in hypoglycaemia (glucose level ≤3.9 mmol/L (≤70mg/dL), ≤3.3 mmol/L (≤60 mg/dL) and ≤2.8 mmol/L (≤50 mg/dL)), LBGI and HbA1c was analysed using spearman correlation. As shown in table 6.5, only LBGI correlated strongly with time spent in hypoglycaemia at glucose levels of ≤3.9 mmol/L, ≤3.3 mmol/L or ≤2.8 mmol/L (r>0.88, P<0.001).
<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>CONGA</th>
<th>LI</th>
<th>J-index</th>
<th>LBGI</th>
<th>HBGI</th>
<th>GRADE</th>
<th>MODD</th>
<th>MAGE</th>
<th>ADRR</th>
<th>M-value</th>
<th>MAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>.679**</td>
<td>.514**</td>
<td>.664**</td>
<td>.402**</td>
<td>.676**</td>
<td>-0.07</td>
<td>.659**</td>
<td>.655**</td>
<td>.483**</td>
<td>.446**</td>
<td>.613**</td>
<td>.548**</td>
<td>.345**</td>
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<tr>
<td>Mean</td>
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<td>.976**</td>
<td>.515**</td>
<td>.956**</td>
<td>-.192**</td>
<td>.890**</td>
<td>.922**</td>
<td>.620**</td>
<td>.547**</td>
<td>.843**</td>
<td>.725**</td>
<td>.431**</td>
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<tr>
<td>SD</td>
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<td>.768**</td>
<td>.833**</td>
<td>.378**</td>
<td>.888**</td>
<td>.674**</td>
<td>.936**</td>
<td>.710**</td>
<td>.844**</td>
<td>.892**</td>
<td>.658**</td>
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<td></td>
</tr>
<tr>
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<td>.423**</td>
<td>.954**</td>
<td>-.173**</td>
<td>.900**</td>
<td>.897**</td>
<td>.636**</td>
<td>.511**</td>
<td>.803**</td>
<td>.741**</td>
<td>.325**</td>
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<tr>
<td>LI</td>
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<td>.358**</td>
<td>.680**</td>
<td>.577**</td>
<td>.741**</td>
<td>.728**</td>
<td>.806**</td>
<td>.710**</td>
<td>.926**</td>
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<td>.911**</td>
<td>.788**</td>
<td>.654**</td>
<td>.921**</td>
<td>.860**</td>
<td>.546**</td>
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<td>.360**</td>
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</tr>
<tr>
<td>HBGI</td>
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<td>.892**</td>
<td>.841**</td>
<td>.691**</td>
<td>.913**</td>
<td>.917**</td>
<td>.570**</td>
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<td></td>
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<tr>
<td>GRADE</td>
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<td>.826**</td>
<td>.820**</td>
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<tr>
<td>MODD</td>
<td>-</td>
<td>.683**</td>
<td>.798**</td>
<td>.836**</td>
<td>.640**</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>MAGE</td>
<td>-</td>
<td>.721**</td>
<td>.701**</td>
<td>.623**</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADRR</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.844**</td>
<td>.727**</td>
</tr>
<tr>
<td>M-value</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>.615**</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 6.4: Correlation matrix examining the relationship between various measures of glycaemic variability and quality of glycaemic control in 448 subjects with type 1 diabetes at baseline.
Table 6.5: Correlation matrix examining the relationship between time spent in various glucose ranges, HbA1c, SD and LBGI in 448 subjects with type 1 diabetes at baseline.

<table>
<thead>
<tr>
<th></th>
<th>≤2.8 mmol/L</th>
<th>≤3.3 mmol/L</th>
<th>≤3.9 mmol/L</th>
<th>4-10 mmol/L</th>
<th>10.1-13.9 mmol/L</th>
<th>≥14 mmol/L</th>
<th>SD</th>
<th>LBGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>-.101**</td>
<td>-.213**</td>
<td>-.285**</td>
<td>-.627**</td>
<td>.507**</td>
<td>.663**</td>
<td>.514**</td>
<td>-0.066</td>
</tr>
<tr>
<td>≤2.8 mmol/L</td>
<td></td>
<td>.888**</td>
<td>.822**</td>
<td>-.119*</td>
<td>-.281**</td>
<td>-0.016</td>
<td>.306**</td>
<td>.894**</td>
</tr>
<tr>
<td>≤3.3 mmol/L</td>
<td></td>
<td></td>
<td>.965**</td>
<td>0.033</td>
<td>-.430**</td>
<td>-.166**</td>
<td>.217**</td>
<td>.923**</td>
</tr>
<tr>
<td>≤3.9 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td>.130**</td>
<td>-.522**</td>
<td>-.247**</td>
<td>.158**</td>
<td>.889**</td>
</tr>
<tr>
<td>4-10 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-.684**</td>
<td>-.897**</td>
<td>-.75**</td>
<td>-.170**</td>
</tr>
<tr>
<td>10.1-13.9 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.554**</td>
<td>.266**</td>
<td>.254**</td>
<td></td>
</tr>
<tr>
<td>≥14 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.842**</td>
<td>0.042</td>
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<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.378**</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

6.4.4. Effect of RT-CGM on GV measures in the intervention group:

At 26 weeks, results available from all subjects in the intervention group and from 214 subjects in the control group, showed significant reduction in the majority of measures of GV and quality of glycaemic control from baseline in the intervention group in comparison to the control group (table 6.6 - figure 6.3). There was a significant reduction of HbA1c in the intervention group from 7.41% to 7.18% ($P<0.001$). Correspondingly, the largest reductions in measures of GV were observed in M-value, LBGI and GRADE (measures of quality of glycaemic control) with a relative reduction of 25.7%, 24.9% and 16.5%, respectively ($P<0.001$). Similarly, a reduction in MODD, MAGE and
SD of 8.3%, 6.7% and 6.6%, respectively was observed. In contrast, there was no statistically significant reduction in any of the measures of GV in the control group at 26 weeks compared to baseline.

<table>
<thead>
<tr>
<th>Δ at 26 weeks</th>
<th>RT-CGM group (n=231) Mean (SD)</th>
<th>Control Group (n=214) Mean (SD)</th>
<th>Difference in Mean</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>-0.24 (0.68)</td>
<td>-0.01 (0.56)</td>
<td>-0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>-0.34 (1.31)</td>
<td>-0.04 (1.29)</td>
<td>-0.30</td>
<td>0.016</td>
</tr>
<tr>
<td>SD</td>
<td>-0.24 (0.67)</td>
<td>0.001 (0.74)</td>
<td>-0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CONGA</td>
<td>-0.43 (1.3)</td>
<td>-0.06 (1.27)</td>
<td>-0.36</td>
<td>0.003</td>
</tr>
<tr>
<td>LI</td>
<td>-0.17 (2.87)</td>
<td>0.09 (3.68)</td>
<td>-0.27</td>
<td>0.382</td>
</tr>
<tr>
<td>J-index</td>
<td>-5.58 (16.4)</td>
<td>-0.72 (15.67)</td>
<td>-4.86</td>
<td>0.002</td>
</tr>
<tr>
<td>LBGI</td>
<td>-1.32 (3.3)</td>
<td>-0.19 (4.48)</td>
<td>-1.12</td>
<td>0.003</td>
</tr>
<tr>
<td>HBGI</td>
<td>-1.76 (3.9)</td>
<td>-0.38 (3.9)</td>
<td>-1.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GRADE</td>
<td>-1.38 (3.2)</td>
<td>-0.31 (3.33)</td>
<td>-1.06</td>
<td>0.001</td>
</tr>
<tr>
<td>MODD</td>
<td>-0.33 (0.82)</td>
<td>0.37 (1.12)</td>
<td>-0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAGE</td>
<td>-0.45 (1.66)</td>
<td>0.2 (2)</td>
<td>-0.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADRR</td>
<td>-2.03 (8.6)</td>
<td>6.65 (12.6)</td>
<td>-8.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>M-value</td>
<td>-4.5 (8.5)</td>
<td>-0.9 (9.17)</td>
<td>-3.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAG</td>
<td>0.05 (0.89)</td>
<td>0.11 (1.05)</td>
<td>-0.07</td>
<td>0.451</td>
</tr>
</tbody>
</table>

Table 6.6: Comparing the change in mean of HbA1 and glycaemic variability indices in both the intervention (n=231) and control (n=214) groups at 26 weeks.
**Figure 6.3:** The effect of RT-CGM at 26 weeks on relative change in the mean of various measures of glycaemic variability and quality of glycaemic control in comparison to control group. *** P<0.001, ** P<0.01, *p<0.05.
6.4.5. Effect of unmasking of CGM in the control group:

The effect of unmasking CGM in the control group at 26 weeks was evaluated by comparing GV measures at 52 weeks to those at 26 weeks (immediately prior to unmasking CGM). Despite the non-significant change in HbA1c at 52 weeks, there was significant reduction in most of the measures of GV and quality of glycaemic control from baseline (26 weeks). The largest reduction was observed in LBGI (21.8%, \(P<0.001\)). Correspondingly, ADRR, GRADE and HBGI (representing other risk measures) were reduced by 21.2%, 6% and 9.5% respectively \((P<0.001)\). Other significant large reductions were observed in M-value, MODD and MAGE with a reduction of 17.6%, 16% and 12% respectively \((P<0.001)\). (figure 6.4 - table 6.7).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (26 weeks)</th>
<th>Mean (52 weeks)</th>
<th>Paired Differences</th>
<th>% Difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>7.44</td>
<td>7.42</td>
<td>-0.02</td>
<td>-0.27</td>
<td>0.63</td>
</tr>
<tr>
<td>Mean</td>
<td>9.27</td>
<td>9.16</td>
<td>-0.11</td>
<td>-1.22</td>
<td>0.07</td>
</tr>
<tr>
<td>SD</td>
<td>3.85</td>
<td>3.56</td>
<td>-0.28</td>
<td>-7.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CONGA</td>
<td>8.29</td>
<td>8.05</td>
<td>-0.24</td>
<td>-2.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LI</td>
<td>7.62</td>
<td>7.55</td>
<td>-0.07</td>
<td>-0.94</td>
<td>0.72</td>
</tr>
<tr>
<td>J-INDEX</td>
<td>57.23</td>
<td>53.66</td>
<td>-3.56</td>
<td>-6.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LBGI</td>
<td>5.34</td>
<td>4.17</td>
<td>-1.16</td>
<td>-21.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HBGI</td>
<td>11.99</td>
<td>10.85</td>
<td>-1.14</td>
<td>-9.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GRADE</td>
<td>8.04</td>
<td>7.55</td>
<td>-0.48</td>
<td>-6.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MODD</td>
<td>4.52</td>
<td>3.80</td>
<td>-0.72</td>
<td>-15.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAGE</td>
<td>7.41</td>
<td>6.52</td>
<td>-0.89</td>
<td>-12.04</td>
<td>&lt;0.001</td>
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<tr>
<td>ADRR</td>
<td>41.14</td>
<td>32.42</td>
<td>-8.72</td>
<td>-21.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>M-VALUE</td>
<td>17.30</td>
<td>14.25</td>
<td>-3.04</td>
<td>-17.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAG</td>
<td>3.08</td>
<td>3.05</td>
<td>-0.04</td>
<td>-1.19</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**Table 6.7:** showing mean values of measures of GV at 52 weeks compared to baseline (26 weeks) in the control group following unmasking of CGM. The table also shows the absolute and relative change in the mean values.
Figure 6.4: The effect of unmasking CGM in the control group at 52 weeks on relative change in the mean of HbA1c and measures of glycaemic variability. *** P<0.001, ** P<0.01, *P<0.05.
6.4.6. Factors predictive of response in the RT-CGM group:

As shown in table 6.8, baseline GV was found to be a significant predictor of change in measures of GV at 26 weeks in the intervention group, with higher baseline GV associated with greater reduction in GV at 26 weeks. Baseline HbA1c was also a significant predictor of change in GV in the majority of the evaluated GV measures (except LBGI, LI and MAG). Treatment with insulin pump and frequent use of SMBG predicted a reduction in some of the evaluated GV measures.
<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>SD</th>
<th>CONGA</th>
<th>LI</th>
<th>J-index</th>
<th>LBGI</th>
<th>HBGI</th>
<th>GRADE</th>
<th>MODD</th>
<th>MAGE</th>
<th>ADRR</th>
<th>M-value</th>
<th>MAG</th>
</tr>
</thead>
<tbody>
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<td>Frequency of SMBG use</td>
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<td></td>
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<tr>
<td>Baseline HbA1c</td>
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<td>0.5</td>
<td>5.8</td>
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<td>1.27</td>
<td>0.241</td>
<td>0.266</td>
<td>2.7</td>
<td>1.98</td>
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<tr>
<td>Baseline GV</td>
<td>-0.49</td>
<td>-0.69</td>
<td>-0.4</td>
<td>-0.65</td>
<td>-0.68</td>
<td>-0.59</td>
<td>-0.702</td>
<td>-0.554</td>
<td>-0.69</td>
<td>-0.53</td>
<td>-0.76</td>
<td>-0.29</td>
</tr>
<tr>
<td>Education level of care-giver (College graduate)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Insulin modality (pump)</td>
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<td></td>
<td></td>
<td></td>
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<td>-1.09</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td>-0.57</td>
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</tr>
<tr>
<td>Severe hypoglycaemia in the preceding 6 months</td>
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<td></td>
<td>-3.74</td>
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<td></td>
</tr>
</tbody>
</table>

*Table 6.8: showing coefficients of significant predictors of change in measures of GV at 6 months in the RT-CGM group. Predictor variables included in the multiple linear regression model included: age, gender, race, education level of care giver (college vs non-college graduates), Insulin modality (pump vs MDI), frequency of daily self-reported blood glucose monitoring, frequency of severe hypoglycaemia in last 6 months, diabetes duration, baseline HbA1c and baseline GV.*
6.5. Discussion:

The development and advances in CGM have paved the way for researchers to investigate short-term GV and its potential role in the pathogenesis of diabetes-related complications and ITU mortality, even in non-diabetics. However, there remain controversies particularly related to the role of post-prandial hyperglycaemia and short-term GV as a risk factor for diabetes-related vascular complications particularly in type 1 diabetes. Apart from clinical studies that were conducted in very specific subgroups (e.g. HEART2D in type 2 diabetes subjects post-MI and STOP-NIDDM that was conducted in subjects with impaired glucose tolerance), the evidence is largely based on pathophysiological studies and observational studies with conflicting results. Most of the evidence evaluating role of short-term GV in development of diabetes-related vascular complications in type 1 is from analysis of the DCCT. It was previously estimated that only around 20% of the total type 1 diabetes population would meet the selection criteria of the DCCT, affecting the ability to generalise findings based on DCCT data to the overall type 1 diabetes population (Kilpatrick et al., 2007). Furthermore, as highlighted by Mounnier et al, the role of exogenous insulin as an anti-inflammatory in neutralising the deleterious effect of GV on oxidative stress can be a confounding factor when evaluating the relationship between GV and the risk of development of diabetes-related vascular complications in insulin-treated diabetic subjects (Monnier et al., 2012).

Several GV measures have been proposed, with a lack of consensus on a gold standard measure, causing increasing confusion with regards to the significance of some of these measures and whether they add any further
clinically relevant information for glucose profile assessment (Rodbard, 2012). An ideal GV measure should accurately reflects and predicts risks of both hypoglycaemic and hyperglycaemic excursions in relation to both frequency and severity. An ideal measure should also result in clinical improvement once targeted by therapeutic interventions and should have prognostic value. As existing GV measures assign different weights and importance to hypoglycaemic and hyperglycaemic components of the glucose profile, it might be important, as shown in this analysis, to identify two “gold standard” GV measures that can accurately assess each component of the glycaemic profile.

The data reported here are from the largest continuous glucose dataset with evaluation of GV, quality of glycaemic control, the effect of RT-CGM on these measures and predictors of changes to variability. Since the normal range of several GV measures was not identified when these measures were proposed, several studies were conducted aiming to establish the range of GV measures in non-diabetic subjects. The normal ranges for the measures of GV evaluated in this analysis have been previously described by analysing CGM profiles of 70 non-diabetic subjects of mixed ethnicities using EasyGV (Hill et al., 2011). Another study assessed the normal range of MAGE and SD in 434 non-diabetic Chinese subjects recommended a MAGE level of <3.9 mmol/L and SD<1.4 mmol/L as the normal reference ranges for GV in Chinese adults (Zhou et al., 2011). Our analysis demonstrates the magnitude of variability in people with type 1 diabetes and the distinct difference between measures of GV at baseline between subjects with type 1 diabetes and non-diabetic subjects. These findings are consistent with the findings of
the ADAG study that reported MAGE, CONGA₄ and SD of its cohort of 268 subjects with type 1 diabetes, 159 subjects with type 2 diabetes and 80 non-diabetic subjects (Borg et al., 2010). However, our data also extend the description to other measures of GV in a larger cohort of subjects with type 1 diabetes.

It was previously reported that GV measures show strong intercorrelation in non-diabetic subjects, whereas the intercorrelation between these measures in 48 subjects with type 1 diabetes was relatively weaker (Cameron et al., 2010). In contrast to this, the present analysis shows there is a large level of agreement between the evaluated measures with 75 out of 78 correlations being significant at the $P<0.05$ level. Although it is expected that measures that assess quality of glycaemic control will show stronger intercorrelation compared to their correlation with measures of GV, that was not the case with ADRR showing close correlation with 9 out of 12 measures. Similarly, SD, J-index, M-value and HBGI showed close intercorrelation with both measures of GV and quality of glycaemic control. This suggests that these measures convey similar information and it might be sufficient, clinically, to rely on only one of them. The measure that showed the weakest correlation with other measures was LBGI. The likely explanation for this is while LBGI is designed to be sensitive to hypoglycaemia alone, other measures are more influenced by hyperglycaemic excursions.

Despite the evidence suggesting that GV plays a role in the pathogenesis of development of diabetes-related complications, the issue remains controversial. To date, there has been no prospective randomised clinical
trial to examine the relationship between GV and diabetes-related complications independent of HbA1c. Examining the relationship between HbA1c and measures of GV at baseline, we identified significant correlations between HbA1c at baseline and most of these measures (with the exception of LBGI). This is consistent with results from the ADAG study, where MAGE, CONGA and SD showed significant intercorrelation alongside significant correlation with HbA1c (Borg et al., 2010). This implies that GV measures convey similar information to HbA1c for assessment of glycaemic status. These intercorrelations between markers of variability and their close relationship with HbA1c in a large cohort suggest that there may only be a very limited role for the metrics over and above HbA1c.

However, LBGI, designed to be sensitive to hypoglycaemia alone, correlated poorly with other GV measures and not at all with HbA1c. This support the hypothesis that HbA1c may have a limited role in reflecting or predicting the risk of hypoglycaemia (Kovatchev et al., 2006) and suggests that LBGI may offer additional information to HbA1c when assessing CGM data. This observation is supported by further correlation analysis between time spent in hypoglycaemia, LBGI and HbA1c, which demonstrated that only LBGI correlates strongly with time spent in hypoglycaemia (glucose level ≤3.9 mmol/L (≤70mg/dL), ≤3.3 mmol/L (≤60 mg/dL) and ≤2.8 mmol/L (≤50 mg/dL)). However, the relationship between time spent in hypoglycaemia and LBGI may suggest that LBGI can be replaced by direct assessment of the percent of time spent in hypoglycaemia, which is routinely reported when downloading CGM data.
This analysis also demonstrates the positive effect of RT-CGM on GV measures. RT-CGM results in significant improvement in the majority of the evaluated measures of GV and quality of glycaemic control. After 26 weeks of RT-CGM in the intervention group, there was a statistically significant reduction in all measures of quality of glycaemic control. These findings are consistent with previous studies that demonstrated the effect of RT-CGM on HbA1c reduction and increasing the time spent in target glucose range (Tamborlane et al., 2008). The findings are also consistent with study that looked at the effect of unmasking CGM in participants with type 1 diabetes over a period of three weeks (Rodbard et al., 2009). The largest reductions were in M-value (25.71, \( P<0.001 \)) and in LBGI (24.91, \( P<0.001 \)). All measures of GV (with the exception of LI and MAG) showed significant reduction in the range between 5.21\% and 8.29\% \( (P<0.001) \). Similarly, unmasking of CGM in the control group resulted in statistically significant reduction in LBGI, ADRR and M-value (all measures of quality of glycaemic control) by 22\%, 21\% and 18\% respectively.

On assessing factors predictive of change in HbA1c at 6 months in the intervention group, the JDRF CGM group included only subjects with baseline HbA1c \( \geq 7\% \) (162 subjects). A univariate analysis has demonstrated that only age, baseline HbA1c and number of days per week of sensor use during six months showed significant relationship. In a multivariate analysis, age lost its significance when corrected for number of days of sensor use per week during the six months. The study has also demonstrated that factors associated with sensor use of \( \geq 6 \) days per week during the sixth months were age, frequency of self-reported prestudy daily blood glucose meter
measurements, CGM use during the first months and percentage of sensor glucose values between 4 – 10 mmol/L during first month (Beck et al., 2009a).

In our analysis, we used data from all subjects in the intervention group (231 subjects) to study the potential predictors of change in GV measures at 6 months. Using regression models to study baseline clinical and demographic factors as potential predictors of change in GV measures at 26 weeks in the CGM group showed baseline GV as a negative predictor for all GV measures, with higher GV at baseline predicting greater reduction of GV at 26 weeks in the intervention group. Further analysis showed the effect to be independent from the effect of regression to the mean. Baseline HbA1c predicted change in GV in the majority of the evaluated GV measures. However, although a higher HbA1c was a significant predictor of reduction in GV at 26 weeks in the CGM group according to univariate analysis, its predictive value changed after adjustment for baseline GV in a multivariate model. This indicates that the negative univariate association between baseline HbA1c and change in GV was dependent on baseline GV and that when variation in baseline GV is taken into account, increasing HbA1c is associated with less of a reduction in GV. Interestingly, education level of caregiver did not predict change in any of the GV measures.

Limitations of this analysis include the reliability of some of the evaluated GV measures. ADRR requires data collected typically over one month (at least 14 days) with typical frequency of 3-5 glucose measurements per day, whereas CGM data used to compute ADRR in this analysis were collected for 96 hours. Also, calculation of MAGE using EasyGV software (as in this analysis) was shown to have weak correlation with the other three tools to
calculate MAGE. However, none of the four calculators, evaluated in that paper, was evaluated against the “gold standard” graphical “ruler and pencil” approach (Sechterberger et al., 2014). EasyGV allows the use of modified method (MAGE-CGM) for MAGE calculation (Hill et al., 2011). Accuracy of CGM systems used in the study, particularly at the hypoglycaemic range, can be considered as another limitation as it can affect GV measurements. Since the JDRF CGM study was conducted, accuracy of available CGM systems has improved significantly and the large volume of data analysed here mitigates much of this problem.

6.6. **CONCLUSION:**

Despite the evidence supporting a role for GV, its contribution to diabetes-related complications remains controversial and its value as an additional marker to HbA1c remains unclear. This data analysis suggests that the evaluated GV measures are of limited value in addition to HbA1c but that a measure of hypoglycaemia is a useful adjunct to HbA1c. Very importantly this analysis describes the clear beneficial impact of RT-CGM on glucose variability, which is a significant source of distress to people with diabetes.

A large-scale longitudinal interventional study involving subgroups of subjects with different types of diabetes and treatment modalities is required to identify the HbA1c-independent role of GV in development of diabetes-related vascular complications, role of insulin therapy, effect of targeting GV and identification of a “gold-standard” GV measure.
CHAPTER 7

CONCLUSION AND

RECOMMENDATIONS FOR FUTURE

STUDIES
7.1. **CONCLUSION:**

Despite the advances in the field of continuous glucose monitoring (CGM) and the improvement of CGM systems’ accuracy in recent years, there remain several challenges facing the CGM technology. The development of an accurate, safe, cost effective and user-friendly CGM system may help improvement in compliance with CGM use, clinical effectiveness and widespread implementation in management of people with diabetes. It may also allow realisation of non-adjuvant use of CGM, which is an important aspect in the development of closed-loop systems.

The use of microneedle technology for CGM provides the potentials to overcome some of these challenges. It provides a minimally invasive access to dermal interstitial fluid (ISF) which is associated with less risk of pain, skin reaction, bleeding or infection. The novel system also offers the potential to enhance sensor accuracy through simultaneous multi-glucose sensing in the dermal compartment. While existing CGM technology targets the subcutaneous tissue for measurement of its ISF glucose content, current evidence suggests that measurement of glucose content of dermal ISF is superior to that of the subcutaneous tissue.

This thesis describes the work done towards the development and evaluation of a novel microprobe array sensor for continuous glucose monitoring aiming to move the technology from bench side to bedside. It describes work done to capture patient’s views regarding continuous glucose monitoring and microneedle technology aiming to identify barriers for its use and how to make the technology more acceptable from patients’ perspective. This has highlighted the importance of perceived CGM accuracy and cost. The thesis
also highlights the impact of effective patient and public involvement at an early stage in diabetes technology research.

Detailed description of steps of fabrication, functionalisation and pre-clinical evaluation of the sensor are presented. To ensure that microprobes are of the correct geometry to penetrate the stratum corneum and reach dermal ISF, the fabrication technique was changed from photolithography to micromoulding. In addition, this technique allowed for more reproducible, lower cost and scalable production. *In vitro* and *ex vivo* mechanical characterisation studies of the pyramidal shaped microprobes showed that they were able to access the dermal compartment without mechanical failure. The microprobe arrays are fabricated using SU-8 polymer and sputtered with gold to serve as electrodes for electrochemical glucose sensing. The gold surface is functionalised with glucose oxidase. To protect the enzyme layer, reduce interference and to increase the linear working range of the sensor, epoxy-polyurethane (epoxy-PU) membrane was used. Electrochemical studies of the sensor showed a linear detection range (0-20 mmol/L), sensitivity of 7 nA/mmol and no negative impact related to gamma ray sterilisation or skin insertion. However, similar to existing CGM sensors, acetaminophen interference was present.

To assess safety and performance of the device in a clinical study, approval from regulatory bodies was obtained to conduct the clinical study over four phases in healthy volunteers and in people with type 1 diabetes. The thesis describes work done so far in phase 1 aiming to assess safety of the device in healthy volunteers. Sensor application for six hours was not associated with
skin reaction, bleeding, infection or pain. Most of the subjects described a “pricking” sensation only during device application. Finally, the thesis describes several indices of glycaemic variability (GV) and the effect of RT-CGM on the evaluated indices. The analysis of glucose profiles of 448 subjects with type 1 diabetes that were recruited in the landmark Juvenile Diabetes Research foundation CGM study showed that GV is significantly higher in subjects with type 1 diabetes than in non-diabetic subjects. Use of RT-CGM reduces GV and improves measures of glucose risk compared with SMBG in children, adolescents and adults with type 1 diabetes. There were significant inter-correlations with HbA1c suggesting a limited role for GV indices in clinical practice. However, the lack of correlation between either low blood glucose index or time spent in hypoglycaemia with HbA1c suggest a potential place for an additional metric assessing risk of hypoglycaemia.

7.2. FUTURE WORK AND RECOMMENDATION FOR FUTURE STUDIES:

7.2.1. Further clinical evaluation:

Phase 1 of the clinical study is in progress. The second phase is planned to assess sensor’s performance over the first six hours, as a primary outcome, and safety over 24 hours in healthy volunteers. In the first six hours, sensor output will be compared to venous blood glucose measured every 15 minutes using YSI to evaluate the device’s performance. During these six hours, the development of skin reaction, bleeding or pain during insertion or throughout its use will be evaluated to assess safety of the device. For the remaining 18
hours, subjects will be allowed to go home with the sensor to further assess sensor safety over the whole 24 hour period.

The sensor performance and safety in people with type 1 diabetes will be assessed in phases 3 and 4. The third phase is planned to take place in 20 subjects with type 1 diabetes over 24 hours. Performance will be assessed against venous blood glucose measured using YSI and against ISF glucose measured using a commercially available CGM system (iPro®2–enlite CGM system - Medtronic, Northridge, California). Phase 4 will assess sensor performance and safety in 20 subjects with type 1 diabetes in an ambulatory circumstance over 5 days in comparison to ISF glucose measured using iPro®2–enlite CGM system.

Initial results from phase 1 of the clinical study have highlighted the need for further optimisation of the epoxy-PU coating membrane to reduce the risk of its disruption during skin insertion. Findings from phase 1 of clinical study also suggest the potential benefit of the use of a skin imaging tool (e.g. optical coherence tomography or confocal microscopy) for confirmation of stratum corneum penetration by microprobes and visualisation of the created microchannels. This could be used for evaluation of the relationship between length of created microchannels, application force used and the magnitude of measured current. In addition, the use of an applicator for microprobe array insertion could be beneficial. Other than enhancing the ability of microprobes to penetrate stratum corneum, it could also ensure consistent penetration and reduce inter-person variability. The use of an applicator for microprobe insertion was one of the themes identified by patients in our focus group.
Further electrochemical evaluation, following any required modification in the sensor guided by findings of clinical studies, will be required to assess sensor stability and effect of other potential interferents.

**7.2.2. Automation and Scalability**

Currently the microprobe array sensor is hand fabricated which, other than being time- and resource-intensive, can result in higher sensor-to-sensor variability. The use of injection-moulding for device fabrication and automation of the functionalisation process allow the possibility to scale up sensor fabrication and functionalisation, minimise cost and reduce sensor to sensor variability.

**7.2.3. Development of other components of microprobe array CGM system**

While the novelty of the proposed CGM system is mainly related to the microprobe array sensor, several factors need to be considered in relation to other components of the CGM system for the realisation of our vision in developing an accurate minimally invasive and cost-effective “smart patch CGM”. These include miniaturisation of other components of the CGM system, power supply, connectivity, algorithm development and data display.

**7.2.4. Human factor analysis**

An important part for the design of the microprobe continuous glucose-monitoring device is human factor studies. These need to assess the physical component (e.g. use of the applicator to apply the sensor, the adhesive used to fix the sensor at the insertion site, the process of connecting the sensor to
the transmitter unit). It also evaluates the informational component (e.g: interaction with data displayed). This ensures that the final device is user-friendly, effective and safe.

7.2.5.Multi-analyte sensing in dermal ISF:
Other than providing the potential to enhance accuracy of ISF glucose measurement while reducing invasiveness, the microprobe array provides the potential for simultaneous multi-analyte sensing. This requires characterisation of dermal ISF analytes’ content and dynamics.

7.2.6.Further research in glycaemic variability:
While there is mounting evidence supporting the pathophysiological effect of GV, a large-scale longitudinal intervention study is required to evaluate the HbA1c-independent role of GV in development of diabetes-related vascular complications, role of insulin therapy, effect of targeting GV and identification of a “gold-standard” GV measure.
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APPENDIX
Appendix I

Diabetes Technology Questionnaire

We are diabetes technology researchers from Imperial College and would like to hear from people with diabetes (and their carers) about their views on new technology and how it can help people with diabetes. This will help us and others to focus our research on what would be most helpful to you. You can answer this survey either as yourself, or on behalf of someone else with diabetes. The survey is anonymous and the results will be kept confidential.

The survey contains questions about: glucose monitors including continuous glucose monitors (CGM), insulin pumps and pens, and CGM enabled pumps. At the end of the survey, there is a space for additional comments about any issues not covered in the questions.

We estimate that this survey will take 20 minutes to complete. Thank you for your help.

* Required

1. I am: *
   
   Mark only one oval.
   
   ☐ a person with diabetes. Skip to question 2.
   
   ☐ a carer for a child with diabetes. Skip to question 9.

2. I am: *
   
   Mark only one oval.
   
   ☐ Male
   
   ☐ Female

3. I am aged: *
   
   Mark only one oval.
   
   ☐ 16-25
   
   ☐ 26-40
   
   ☐ 41-65
   
   ☐ over 65

4. I live in: *
   
   Mark only one oval.
   
   ☐ UK
   
   ☐ Europe
   
   ☐ North America
   
   ☐ Other: .................................................................
5. I have: *
   Mark only one oval.
   
   □ Type 1 diabetes
   □ Type 2 diabetes
   □ Other: .................................................................................................................................................

6. I have been diagnosed with diabetes for: *
   Mark only one oval.
   
   □ less than 1 year
   □ 1-5 years
   □ 6-10 years
   □ more than 10 years

7. To manage my diabetes I use: *
   Tick ALL that apply
   Check all that apply.
   
   □ Lifestyle measures (diet & exercise)
   □ Glucose-lowering tablets (like metformin, sulfonylurea...etc)
   □ Injections that are not insulin (like Byetta, Victoza or Lyxumia)
   □ 1-2 insulin injections daily
   □ 3-5 insulin injections daily
   □ Insulin pump
   □ Other: .................................................................................................................................................

8. How many times per week do you exercise 30 minutes or more? *
   Check all that apply.
   
   □ I don't exercise regularly
   □ 1-3 times per week
   □ 4-7 times per week

Skip to question 14.

9. My child is: *
   Mark only one oval.
   
   □ Male
   □ Female

10. My child is aged: *
    Mark only one oval.
    
    □ 0-5
    □ 6-15
    □ Over 15 years old
11. **We live in:** *
   *Mark only one oval.*
   - UK
   - Europe
   - North America
   - Other: .................................................................

12. **My child has been diagnosed with diabetes for:** *
   *Mark only one oval.*
   - less than 1 year
   - 1-5 years
   - 6-10 years
   - more than 10 years

13. **My child manages diabetes using mainly:** *
   Choose from the list below.
   *Check all that apply.*
   - 1-2 insulin injections daily
   - 3-5 insulin injections daily
   - Insulin pump
   - Other: .................................................................

   *Skip to question 16.*

**Monitoring your blood glucose levels.**

14. **How often do you carry out finger prick glucose monitoring?** *
   *Mark only one oval.*
   - Never
   - 1 - 5 times per week
   - 2 - 4 times per day
   - more than 5 times daily
15. Where 1 is most important, and 9 is least important, please rank the challenges you may have faced using finger prick glucose testing. *

Mark only one oval per row.

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Skip to question 18.

Monitoring your child's blood glucose levels.

16. How often does your child carry out finger prick glucose monitoring? *

Mark only one oval.

☐ Never
☐ 1 - 5 times per week
☐ 2 - 4 times per day
☐ more than 5 times daily

17. Where 1 is most important, and 9 is least important, please rank the challenges you / your child may have faced using finger prick glucose testing. *

Mark only one oval per row.

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Continuous Blood Glucose Monitoring

The following questions are about the use of continuous blood glucose monitors. Typically, a continuous glucose monitoring system (CGM) consists of a sensor sensor that is applied on the body to measure glucose concentrations in the interstitial fluid, a transmitter that is attached to the sensor, and a monitor that displays glucose levels. The sensor needs to be replaced every 5 - 7 days.

18. **Have you ever used a Continuous Glucose Monitor (CGM)?** *

   *Mark only one oval.*
   - ☐ Yes, I currently use CGM
   - ☐ Yes, I have used CGM in the past
   - ☐ No

19. **If you are a current or previous user of a CGM, what is the reason you started using one?**

   (Please type in your answer below.)

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20. **How do you view CGM technology?** *

   *Mark only one oval.*
   - ☐ I feel positive about it.
   - ☐ I am undecided/neutral
   - ☐ I feel negative towards it.
21. **In order of importance, please rank potential advantages a continuous glucose monitor might offer for you and for people with diabetes:**

1 is most important, and 9 is least important

*Mark only one oval per row.*

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22. In order of importance, please rank features that you think an ideal CGM should have * 
1 is most important, and 12 is least important 
*Mark only one oval per row.*

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23. If you are a current or previous CGM user, please rank challenges of using CGM device in order of importance
1 is most challenging, and 13 is least challenging
Mark only one oval per row.

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<td>Makes me anxious by reminding me about diabetes all the time</td>
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<td>Alarms disturb my life</td>
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<td>Need to do finger pricks to calibrate or to confirm glucose readings</td>
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24. How often do you think you would like to use a CGM? *
(Please choose one of the options below.)
Mark only one oval.

- Intermittently (one week every few months to get an insight into my glucose levels)
- Continuously
- I don’t want to use a CGM device

25. What type of CGM device do you prefer to use? *
Mark only one oval.

- One that displays glucose level on a monitor (real time CGM)
- One that does not display glucose levels but stores the data for a later review with my diabetes team
- I don’t want to use a CGM device
26. **Do you have any other comments about CGM?**  
(Please type your answer in the box below.)

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*Skip to question 36.*

**Continuous Blood Glucose Monitoring and your child.**

The following questions are about the use of continuous blood glucose monitors and your child. Typically, a continuous glucose monitoring system (CGM) consists of a sensor sensor that is applied on the body to measure glucose concentrations in the interstitial fluid, a transmitter that is attached to the sensor, and a monitor that displays glucose levels. The sensor needs to be replaced every 5 - 7 days.

27. **Has your child ever used a Continuous Glucose Monitor (CGM)?**  
*Mark only one oval.*

- [ ] Yes, my child is currently using a CGM  
- [ ] Yes, my child used CGM in the past  
- [ ] No

28. **If your child is a current or previous user of a CGM, what is/was the reason they started using one?**  
(Please type in your answer below.)

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29. **How do you or your child view CGM technology?**  
*Mark only one oval.*

- [ ] feel positive about it.  
- [ ] undecided/neutral  
- [ ] feel negative towards it.
30. In order of importance, please rank potential advantages a continuous glucose monitor might offer for your child and for people with diabetes: *

1 is most important, and 8 is least important

*Mark only one oval per row.*

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<th>Advantage</th>
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<td>Provides information on glucose level when it is difficult to not use finger prick glucose monitor (while asleep, playing or at school)</td>
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31. **In order of importance, please rank features that you think an ideal CGM should have**

1 is most important, and 12 is least important

*Mark only one oval per row.*

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32. **If your child is a current or previous CGM user, please rank challenges of using CGM device in order of importance**
   1 is most challenging, and 13 is least challenging
   *Mark only one oval per row.*

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<td>Increases anxiety by reminding about diabetes all the time</td>
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<td>Alarms disturb my child's life and mine</td>
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<td>Need to do finger pricks to calibrate or to confirm glucose readings</td>
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<td>Another electronic device to manage</td>
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</table>

33. **How often do you think you and your child would like to use a CGM?**
   * (Please choose one of the options below.)
   *Mark only one oval.*

- Intermittently (one week every few months to get an insight into my glucose levels)
- Continuously
- We don’t want to use a CGM device

34. **What type of CGM device would you and your child prefer to use?**
   * (Mark only one oval.)

- One that displays glucose level on a monitor (real time CGM)
- One that does not display glucose levels but stores the data for a later review by my doctor
- We don’t want to use a CGM device
35. **Do you have any other comments about CGMs?**
(Please type your answer in the box below.)

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Skip to question 45.

**Insulin pumps**
The following questions are about insulin pens and pumps.

36. **In order of importance, please rank the challenges you may have faced using insulin injection pens.** *
1 is most important, and 7 is least important

*Mark only one oval per row.*

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<tbody>
<tr>
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<tr>
<td>Using in places with other people near</td>
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<td>Sometimes I forget taking insulin</td>
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<tr>
<td>I find estimating insulin doses based on carbohydrate content of the meal very difficult</td>
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<td>Time consuming</td>
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<td>Need to carry the insulin pen wherever I go</td>
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</table>

37. **Have you ever used an insulin pump?** *

*Mark only one oval.*

- ☐ Yes, I am currently using an insulin pump
- ☐ Yes, I used an insulin pump in the past
- ☐ No

38. **If you are a current or previous user of an insulin pump, what was the reason for this?**

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39. If you are a previous pump user, why you are no longer using one?

40. How do you view insulin pump technology? *
   *Mark only one oval.*
   
   ☐ I am positive about it.
   ☐ I am undecided/neutral
   ☐ I am negative towards it.

41. In order of importance, please rank potential advantages an insulin pump might offer for you and for people with diabetes
   1 is most important, and 6 is least important
   *Mark only one oval per row.*

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<table>
<thead>
<tr>
<th>Advantage</th>
<th>1</th>
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<th>3</th>
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<th>5</th>
<th>6</th>
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<tr>
<td>Discrete</td>
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<tr>
<td>Avoids discomfort of repeated insulin injections</td>
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<tr>
<td>Provides more flexibility with daily</td>
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<tr>
<td>activities like diet and exercise</td>
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<tr>
<td>Helps me to estimate insulin doses at meal time</td>
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<td>more accurately</td>
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<tr>
<td>Helps me to avoid hypos (low blood sugar)</td>
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<tr>
<td>Helps me to manage my diabetes</td>
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</table>
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42. In order of importance, please rank features that you think an ideal insulin pump should have
   1 is most important, and 10 is least important
   *Mark only one oval per row.*

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<table>
<thead>
<tr>
<th>Feature</th>
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<td>Remote control</td>
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<td>Alarms early if malfunctions</td>
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<td>Ability to transfer my data to my computer or the</td>
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<td>Compatibility with a CGM</td>
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<td>Nice-looking</td>
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</table>
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43. **If you are a current or previous pump user, please rank challenges of using insulin pump in order of importance**

1 is most challenging, and 9 is least challenging

*Mark only one oval per row.*

<table>
<thead>
<tr>
<th>Challenge</th>
<th>1</th>
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<tbody>
<tr>
<td>Pain at insertion site</td>
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<td>Tubing between pump and cannula gets in the way sometimes</td>
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<td>Interfering with daily activities</td>
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<tr>
<td>I find the pump too complicated to use</td>
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<td>Is a reminder of my diabetes</td>
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<td>Alarms disturb my life</td>
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<td>Another electronic device to manage</td>
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<td>Getting access to training and support</td>
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44. **Do you have any further comments about the use of insulin pumps?**

*(Please type in your answer in the box below.)*

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*Skip to question 54.*

**Insulin pumps and your child.**

The following questions are about insulin pumps and your child.

45. **In order of importance, please rank the challenges your child may have faced using insulin injection pens.**

1 is most challenging, and 7 is least challenging

*Mark only one oval per row.*

<table>
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<tr>
<th>Challenge</th>
<th>1</th>
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<tr>
<td>Pain</td>
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<td>Difficult to use</td>
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<td>Using in places with other people near</td>
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<tr>
<td>Sometimes I forget injecting insulin at the right time.</td>
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<tr>
<td>I find estimating insulin doses based on carbohydrate content of the meal very difficult</td>
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<td>Time consuming</td>
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<tr>
<td>Need to carry the insulin pen wherever we go</td>
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</table>
46. **Has your child ever used an insulin pump?**

*Mark only one oval.*

- [ ] Yes, my child is currently using an insulin pump
- [ ] Yes, my child has used an insulin pump in the past
- [ ] No

47. **If your child is a current or previous user of an insulin pump, what is the reason for this?**

- [ ]
- [ ]
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48. **If your child no longer uses an insulin pump, why is this?**

- [ ]
- [ ]
- [ ]
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- [ ]

49. **How do you and your child view insulin pump technology?**

*Mark only one oval.*

- [ ] Positively
- [ ] Undecided/neutral
- [ ] Negatively

50. **In order of importance, please rank potential advantages an insulin pump might offer for your child and for people with diabetes**

1 is most important, and 7 is least important

*Mark only one oval per row.*

<table>
<thead>
<tr>
<th>Advantage</th>
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<tr>
<td>Avoids discomfort from repeated insulin injections</td>
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<tr>
<td>Provides more flexibility with daily activities like diet and exercise</td>
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<tr>
<td>Helps me to estimate insulin doses at meal time more accurately</td>
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<td>Helps to avoid hypos (low blood sugar)</td>
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<tr>
<td>Helps in management of my child's diabetes</td>
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</table>
51. In order of importance, please rank features that you think an ideal insulin pump should have
1 is most important, and 10 is least important
Mark only one oval per row.

<table>
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<tr>
<th>Feature</th>
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<td>Simple and easy to use</td>
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<td>Ability to transfer data to my computer or the web</td>
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<td>Compatibility with a CGM</td>
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<td>Nice-looking</td>
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52. If your child is a current or previous pump user, please rank challenges of using insulin pump in order of importance
1 is most challenging, and 9 is least challenging
Mark only one oval per row.

<table>
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<tr>
<th>Challenge</th>
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<tbody>
<tr>
<td>Pain at insertion site</td>
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<tr>
<td>Tubing between pump and cannula gets in the way sometimes</td>
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<td>Interfering with daily activities</td>
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<td>I find the pump too complicated to use</td>
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<td>Is a reminder of diabetes</td>
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<td>Alarms disturb my child's life and mine</td>
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<td></td>
</tr>
<tr>
<td>Visible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Getting access to training and support</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

53. Do you have any further comments about the use of insulin pumps?
(Please type in your answer in the box below.)

........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

Skip to question 57.

SENSOR AUGMENTED PUMP THERAPY (SAP)
This is where information about blood glucose levels from the CGM is sent wirelessly to be displayed on your pump’s monitor, rather than having a separate monitor for the CGM. This
reduces the number of devices carried by the patient. Also, establishing a communication between the CGM and the insulin pump can allow pump suspension if hypoglycaemia is detected by the CGM.

54. If you are a current or previous user of SAP, what is/was the reason for this?

........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

55. In order of importance, please rank potential advantages a sensor augmented pump might offer for you and for people with diabetes:
1 is most important, and 6 is least important
Mark only one oval per row.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provides more flexibility with daily activity like diet and exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reassures me by providing alarms if it is too high or too low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helps me to avoid hypos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improves hypo awareness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helps me to control my diabetes and therefore reduce risks of complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ability to switch off insulin delivery if I am low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

56. How do you view the use of insulin pump and CGM simultaneously?
Mark only one oval.

☐ I am positive about it.
☐ I am undecided/neutral
☐ I am negative towards it.

Skip to question 60.

SENSOR AUGMENTED PUMP THERAPY (SAP) and your child.
This is where information about blood glucose levels from the CGM is sent wirelessly to be displayed on your child's pump's monitor, rather than having a separate monitor for the CGM. This reduces the number of devices carried by the patient. Also, establishing a communication between the CGM and the insulin pump can allow pump suspension if hypoglycaemia is detected by the CGM.

57. If your child is a current or previous user of SAP, what is/was the indication for this?

........................................................................................................................................
58. In order of importance, please rank potential advantages a sensor augmented pump might offer for your child and for people with diabetes:
1 is most important, and 6 is least important
*Mark only one oval per row.*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provides more flexibility with daily activity like diet and exercise</td>
<td>□□□□□□</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reassures by providing alarms if it is too high or too low</td>
<td>□□□□□□</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helps to avoid hypos (low blood sugar)</td>
<td>□□□□□□</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improves hypo awareness by providing alarms</td>
<td>□□□□□□</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helps me to control my child's diabetes and therefore reduce risks of complications</td>
<td>□□□□□□</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ability to switch off insulin delivery if I am low</td>
<td>□□□□□□</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

59. How do you view the use of insulin pump and CGM simultaneously, in relation to your child?
*Mark only one oval.*

☐ I am positive about it.
☐ I am undecided/neutral
☐ I am negative towards it.

*Skip to question 62.*

60. How do you view diabetes technology? *Mark only one oval.*

☐ I am positive about it.
☐ I am undecided/neutral
☐ I am negative towards it.

61. Please use the box below to write what you want from technology in the future to help you manage diabetes

........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

*Stop filling out this form.*
62. **How do you and your child view diabetes technology?** *

*Mark only one oval.*

- [ ] I am positive about it.
- [ ] I am undecided/neutral
- [ ] I am negative towards it.

63. **Please use the box below to write what you want from technology in the future to help your child manage diabetes**

...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
...........................................................................................................................................
Appendix II

TCM ASSOCIATES

VD_{\text{max}}^{25} \text{ STUDY}

STERILIZATION VALIDATION STUDY TO
ISO 11137-2:2012, USING METHOD VD_{\text{max}}^{25}
"STERILIZATION OF HEALTH CARE PRODUCTS –
RADIATION – Part 2: Establishing the sterilization dose"

GLUCOSE SENSORS
LOT: 014AT, 015AT, 016AT, 002AT.

Goods Receipt Number 0558999

February – March 2013

Report Prepared By: ...................... Senior Laboratory Administrator

Report Reviewed By: ..................... Laboratory Manager

GRN Ref: 0558999
CONTENTS

- Summary
- Introduction
- Validation of bioburden methodology
- Assessment of bioburden
- Verification dose determination
- Sterility testing
- Test of sterility result form
- Media formulae
- Environmental monitoring data sheets
- Certificates of irradiation
SUMMARY

This study was undertaken in accordance with ISO 11137-2:2012, Sterilization of Health Care Products – Radiation - Part 2: Establishing the sterilization dose. The \( VD_{\text{max}}^{25} \) method was followed for substantiation of 25kGy as a Sterilization Dose.

Samples of TCM Associates, Glucose Sensors were assayed for bioburden levels. The overall average for the 30 samples tested was 2.3 CFU/Device. As none of the lot averages was greater than twice the overall average bioburden, 2.3 CFU was used to calculate the sub-process verification dose. With reference to Table 9 of the ISO11137 standard, the nearest value listed equal to or greater than the bioburden level is 2.5 CFU. Therefore the dose required was 5.5 kGy ± 10% kGy (4.95 kGy – 6.05 kGy).

Therefore 10 devices were gamma irradiated to receive a dose of 5.5 kGy and subsequently individually tested for sterility. After the full incubation period zero tests gave a positive result, therefore substantiation of 25 kGy as a sterilization dose is accepted.
INTRODUCTION

Sterility is an absolute term, but the assurance that any given item is sterile is a probability function. The sterility assurance level (SAL) is defined as the probability of any given unit being non-sterile after exposure to a validated sterilization process.

The $V_{D_{\text{max}}}^{25}$ method is based on a knowledge of the number and radiation resistance of contaminating micro-organisms that occur naturally in/on product, and a prediction of the dose needed to achieve a predetermined standard of sterility or Sterility Assurance Level (SAL).
VALIDATION OF BIOBURDEN TEST METHODOLOGY

Goods Receipt Number Ref: 0557692
Purchase Order No: 3461
Product: Glucose Sensors
Batch No: 002AT
Date Received: 18.01.13
Date on Test: 31.01.13
Date off Test: 01.02.13
Tested By: CF

Introduction
The objective of this study was to validate the recovery methodology used to estimate the bioburden levels on the devices. The product was inoculated with known numbers of Bacillus atrophaeus spores, dried, then rinsed in a sterile diluent. The eluate was filtered through a 0.45 micron nitro-cellulose membrane filter and the filter placed on a Tryptone Soya Agar plate. After incubation the number of CFU were enumerated and the recoveries were analysed to determine the ‘Recovery Factor’:

Recovery Factor = \frac{\text{Number of CFU recovered from product}}{\text{Number of CFU in inoculum}}

Ref: ISO11737-1 Sterilization of medical devices – Microbiological methods –
Part 1: Estination of population on micro-organisms on products.

Method
The samples were opened under laminar flow using sterile instruments and placed into a sterile container. A spore suspension was prepared in sterile de-ionised water (DIW) and 100μl of this suspension was distributed over the samples, then left to dry under laminar flow. 100ml of sterile of Maximum Recovery Diluent (MRD) plus 0.1% Tween 80 was added. The samples were placed in a sonic bath for 1 minute and agitated for 2 minutes. The eluate was filtered through a 0.45μm membrane filter and then flushed with 100ml of MRD three times. This procedure was repeated a further 4 times. The filter was placed onto a sterile Tryptone Soya Agar (TSA) plate. Samples of spore suspension were filtered to act as recovery controls. All the plates were incubated at 30°C ± 2°C for a minimum of 24 - 48 hours, before enumeration of the colonies.

Media Used: TSA 12113032, MRD 10234, MRD + 0.1% Tween 10229.
DIW 10191.

Spores: Bacillus atrophaeus Lot: 0342

GRN Ref: 0558999
Results – Glucose Sensors

<table>
<thead>
<tr>
<th>Control</th>
<th>Plate 1</th>
<th>Plate 2</th>
<th>Plate 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU</td>
<td>83</td>
<td>76</td>
<td>85</td>
<td>81.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rinse</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>67</td>
<td>35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total (5 rinses used)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum Count</td>
<td>81.3</td>
<td>81.3</td>
<td>81.3</td>
</tr>
<tr>
<td>Recovery Factor</td>
<td>0.39</td>
<td>0.82</td>
<td>0.43</td>
</tr>
</tbody>
</table>

The average Recovery Factor for the three samples = 0.55

This Recovery Factor could be applied to samples tested routinely using 5 rinses.

All calculations are carried out using Microsoft Excel software.
BIOBURDEN ASSESSMENT

Glucose Sensors
Date on test: 28.02.13/01/03/13
Date off test: 04.03.13
Tested by: CF/TO

Method
Each unit was treated individually, the sample was transferred into a sterile container with 100ml of sterile of Maximum Recovery Diluent (MRD) plus 0.1% Tween 80. The samples were placed in a sonic bath for 1 minute and agitated for 2 minutes. The eluate was filtered through a 0.45μm membrane filter and then flushed with 100ml of MRD three times. This procedure was repeated a further 4 times and the filters were placed onto TSA (Tryptone Soya Agar) plates. All of the plates were incubated at 30°C ± 2°C for 3 - 5 days, before enumeration of the resulting Colony Forming Units (CFU).

Media Used: TSA 13020637, MRD 10263, 10264, 10265, MRD + 0.1% Tween 10253, 10275.

Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lot: 014AT</th>
<th>Lot: 015AT</th>
<th>Lot: 016AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
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<td>3</td>
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<tr>
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<td>0</td>
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<tr>
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<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Average</td>
<td>0.8</td>
<td>1.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Application of 0.55 RF: 1.45, 2.54, 2.91

Presumptive Types: *Bacillus sp.*, *Staphylococcus sp.*

The overall average is \( \frac{1.45 + 2.54 + 2.91}{3} = 2.3 \text{CFU} \)

GRN Ref: 0558999
VERIFICATION DOSE DETERMINATION

As none of the lot averages was greater than twice the overall average bioburden, 2.3 CFU was used to calculate the sub-process verification dose.

10 samples were irradiated at 5.5 kGy +/- 10% (4.95 kGy – 6.05 kGy).

Dose selected from Table 9 of Ref: ISO11137-2:2012 Sterilization of Health Care Products – Radiation - Part 2: Establishing the sterilization dose (Values of VD_{max}^{25}).
STERILITY TESTING

Product: Glucose Sensors          Lot: 016AT

Method

All work was carried out in a cleanroom under a laminar flow, operated in accordance with SOP 3459.000, which was monitored in accordance with SOP 3401.

The sample was aseptically transferred from into a sterile container with 1000ml of sterile Tryptone Soya Broth (TSB). The samples were incubated at 30°C ± 2°C for a minimum of 14 days and inspected for signs of microbial growth.

Results

Please see Test of Sterility Result Forms.

Zero positive results were noted after the full incubation period, therefore substantiation of 25 kGy as a sterilization dose is accepted.
TEST OF STERILITY RESULT FORM

Customer Name: TCM Associates
Test Number: 0558999
Test Product: Glucose Sensors
Lot No: 016AT
Date test carried out: 14.03.13 PM1
Date test complete: 28.03.13

10 test items irradiated at the \( VD_{\text{max}}^{25} \) dose.

TSB Media No: 10278
Negative control: All clear
Positive control: Reference B&F Test GRN: 0559021

14 days at 30°C ± 2°C

Result: 10 negative

<table>
<thead>
<tr>
<th>Day</th>
<th>Number Negative</th>
<th>Number Positive</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0</td>
<td>15.03.13</td>
</tr>
<tr>
<td>2</td>
<td>SAT</td>
<td>SAT</td>
<td>16.03.13</td>
</tr>
<tr>
<td>3</td>
<td>SUN</td>
<td>SUN</td>
<td>17.03.13</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0</td>
<td>18.03.13</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0</td>
<td>19.03.13</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0</td>
<td>20.03.13</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>0</td>
<td>21.03.13</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>0</td>
<td>22.03.13</td>
</tr>
<tr>
<td>9</td>
<td>SAT</td>
<td>SAT</td>
<td>23.03.13</td>
</tr>
<tr>
<td>10</td>
<td>SUN</td>
<td>SUN</td>
<td>24.03.13</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>0</td>
<td>25.03.13</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>0</td>
<td>26.03.13</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>0</td>
<td>27.03.13</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>0</td>
<td>28.03.13</td>
</tr>
</tbody>
</table>

TEST PASSED/FAILED

Signature: [Signature]

GRN Ref: 0558999

10 of 12
### MEDIA FORMULAE

<table>
<thead>
<tr>
<th>Tryptone Soya Agar</th>
<th>gm/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>15.0</td>
</tr>
<tr>
<td>Soya Peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>pH 7.3 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tryptone Soya Broth</th>
<th>gm/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>17.0</td>
</tr>
<tr>
<td>Papaic Digest of Soya bean meal</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Dibasic Potassium Phosphate</td>
<td>2.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.5</td>
</tr>
<tr>
<td>pH 7.3 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maximum Recovery Diluent + 0.1% Tween 80</th>
<th>gm/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>8.5</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maximum Recovery Diluent</th>
<th>gm/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>8.5</td>
</tr>
<tr>
<td>pH 7.0 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>
# CLEAN ROOM ENVIRONMENTAL MONITORING FORM

<table>
<thead>
<tr>
<th>Customer Details</th>
<th>TCM Associates</th>
<th>Date Exposed</th>
<th>14.03.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product</td>
<td>Glucose Sensors</td>
<td>LAF ID</td>
<td>CR1</td>
</tr>
<tr>
<td>Session / Area</td>
<td>PM1</td>
<td>Initial</td>
<td>MG</td>
</tr>
<tr>
<td>Settle Plate Lot No</td>
<td>13020637</td>
<td>Contact Plate Lot No</td>
<td>13021135</td>
</tr>
</tbody>
</table>

### Manipulation Area

- **S1**
- **S2**
- **S3**
- **S4**

<table>
<thead>
<tr>
<th>Before Test</th>
<th>Sample Packaging</th>
<th>Gloves</th>
<th>Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. CFU</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Organism (s)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>During Test</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. CFU</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Organism (s)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After Test</th>
<th>Operator 1</th>
<th>Base</th>
<th>Air (1000L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. CFU</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Organism (s)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Presumptive Key:**

- S = *Staphylococcus sp.*
- B = *Bacillus sp.*
- M = *Micrococcus sp*
- E = Environmental Fungus
- Y = Yeast
- GNB = Gram negative bacilli.

All plates must be incubated for a minimum of 72 hours prior to reading.

<table>
<thead>
<tr>
<th>Date Read</th>
<th>Initial</th>
<th>Reviewed By</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.03.13</td>
<td>MG</td>
<td>2. S. Cheeth</td>
<td>04 - Apr - 13</td>
</tr>
</tbody>
</table>

GRN Ref: 0558999

12 of 12
## Certificate of Irradiation

**Customer Name & Address**
Isotron Laboratories  
Thornhill Road  
South Marston  
Swindon  
Wiltshire  
SN3 4TA

**Site Name:** Synergy Health plc  
**Address:** Moray Road  
Elgin Industrial Estate.  
Swindon  
Wiltshire SN2 8XS  
**Telephone:** 01793 601004  
**Fax:** 01793 601040  
**Website:** www.synergyhealthplc.com

**Customer Account Number:** ISO001  
**Customer P.O. Number:** ISO LABS ISO 11137  
**Date Rec:** 8/03/13  
**Date of C.O.I.:** 13/03/13

<table>
<thead>
<tr>
<th>Item Code / Synergy Batch</th>
<th>Item Specification</th>
<th>Quantity</th>
<th>Additional Details</th>
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<tr>
<td>S3ISO0010177</td>
<td>ISO11137 VD MAX TCM ASSOCIATES LTD</td>
<td>10</td>
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<tr>
<td>S21303T037</td>
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**Total:** 10

This is to certify that the above items have been irradiated as specified above.

Authorising Signature:  
For and behalf of SYNERGY HEALTH PLC
Verification of Dose Report for Microbiological Dose Record of Amendment

Date Issued: 9-Mar-13  
Plant Batch Number: S21303T037  
Customer: TCM Associates  
Product Description: GRN 0558999 Glucose Sensors 016AT

<table>
<thead>
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<th>Amendment Details</th>
<th>Date: 09-Mar-13</th>
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Amendment Justification

New Report

Signatures

Approved:  
Quality Manager/QA Officer  
[Signature] 18-Mar-2013
Verification of Dose Report for Microbiological Dose Setting Exercises

Customer: TCM Associates
Plant Batch Number: S21303T037
Product Description: GRN 0558999 Glucose Sensors 016AT

Valid From: 09-Mar-13

Introduction

Microbiological dose setting procedures, in accordance with recognised standards (BS EN ISO 11137) require exposure of product to low, predetermined irradiation doses. This report outlines the distribution of absorbed dose across the product detailed above, to provide objective evidence that the verification dose has been applied within +/- 10% of the specification.

Objective

The objective of this report is to obtain relevant data by placing the appropriate Perspex dosimeters in a predetermined pattern across an R&D container loaded with the customer's samples.

Methodology

Samples are processed in the R&D container with the key parameters of both sample and the total exposure time being recorded. After irradiation the dosimeters are recovered and the absorbed dose at each position on the samples is calculated and recorded.

Conclusion

The delivered dose in the product presentation illustrated on page 2 achieves requested dose specification of 5.0 kGy minimum dose and 6.1 kGy maximum dose.

Authorisation

<table>
<thead>
<tr>
<th>Position</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Manager</td>
<td></td>
<td>14-3-13</td>
</tr>
<tr>
<td>Quality Manager/QA Officer</td>
<td></td>
<td>18-Mar-13</td>
</tr>
</tbody>
</table>

Note:

It is the responsibility of the customer to routinely provide product in the presentation and orientation outlined in this report. Validation of the sterilisation effect and radiation induced material effects, if any, are not addressed by this qualification.
Verification of Dose Report for Microbiological Dose Setting Exercises

Plant Batch No: S21303T037

Product Detail
Customer Name: TCM Associates
Product Description: GRN 0558999 Glucose Sensors 016AT

Irradiation Container

400
120

5+6 I/S
7+8 I/S

3
4
Dosimeter Results

<table>
<thead>
<tr>
<th>Position</th>
<th>Dose (kGy)</th>
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<tbody>
<tr>
<td>1</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>8</td>
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</table>
Verification of Dose Report for Microbiological Dose Setting Exercises

Product Detail
Customer Name: TCM Associates
A/C No: ISO001
Issue Date: 09-Mar-13
Product Description: GRN 0558999 Glucose Sensors 016AT

Type of package: Bag
Total Number of Packages 2
Lot Number
Dimensions of Package: 380 x 280 x 60
Weight: 0.15 Density (g/cm³): various
No. of Packages/ Load 2 (10) Units
No. Of Turntable Loads
Plant Turntable
Plant Batch No: S21303T037
Dwell Time (mins): 145
Turntable Dose Rate (kGy/hr) 2.1
Dose Range Specification (kGy): 5.0 Min. 6.1 Max.

Dosimetry Results

<table>
<thead>
<tr>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>5.3</td>
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</table>

Comments
N/A
Appendix III

Peer reviewed publications and conference presentations during thesis

PUBLICATIONS:


INTERNATIONAL PRESENTATIONS:


