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CONTROVERSIES in
Continuous Glucose Monitoring

Recent Advances in Continuous Glucose Monitoring: Biocompatibility of Glucose Sensors for Implantation in Subcutis

Peter H. Kvist, Ph.D.,[1] and Henrik E. Jensen, DVSci.[2]

Abstract

Tight glycemic control slows or prevents the development of short- and long-term complications of diabetes mellitus. Continuous glucose measurements provide improved glycemic control and potentially prevent these diabetic complications. Glucose sensors, especially implantable devices, offer an alternative to classical self-monitored blood glucose levels and have shown promising glucose-sensing properties. However, the ultimate goal of implementing the glucose sensor as the glucose-sensing part of a closed loop system (artificial pancreas) is still years ahead because of malfunctions of the implanted sensor. The malfunction is partly a consequence of the subcutaneous inflammatory reaction caused by the implanted sensor. In order to improve sensor measurements and thereby close the loop, it is crucial to understand what happens at the tissue-sensor interface.

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Introduction

The prevalence of diabetes is rapidly increasing, almost reaching epidemic proportions (WHO, Fact sheet No. 236). The main target in the treatment of diabetes mellitus is to control the plasma glucose levels, as persistent or frequent episodes of hyperglycemia lead to damage of various organs (long-term effect). However, intensive medical treatment to control plasma glucose levels might lead to increased hypoglycemic events (short-term effect). Therefore, one of the central themes in controlling the short- and long-term effects of diabetes mellitus is the monitoring of plasma glucose levels. Human trials have convincingly demonstrated that strict control of hyperglycemia will delay the onset and progression of long-term effects of diabetes mellitus.[1–3] The most common approach to control hyperglycemia, including medical treatment, involves effective and regular monitoring of the blood glucose concentration. The preferred method has been the self-monitoring of glucose levels (SMBG) by puncture of capillaries in the finger tip and withdrawal of blood for analysis.[4,5] However, this method only provides a snapshot of the glucose concentration, and nocturnal hypoglycemia will remain undetected by SMBG. In contrast, continuous glucose measurements (CGM) potentially deliver the

Author Affiliations: Departments of [1]Pharmacology, LEO Pharma A/S, Ballerup, Copenhagen, Denmark and [2]Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark

Abbreviations: (CGM) continuous glucose measurements, (HE) hematoxylin and eosin, (ISF) interstitial fluid, (PC) phosphorylcholine, (PLGA) poly(lactide-co-glycolide), (SMBG) self-monitoring of glucose levels

Keywords: animal model, biocompatibility, continuous glucose monitoring, glucose sensor, *in vivo* model, inflammation, subcutis

Corresponding Author: Peter Helding Kvist, Ph.D., Department of Pharmacology, LEO Pharma A/S, Industriparken 55, DK-2750 Ballerup, Denmark; email address peter.kvist@leo-pharma.com

information needed for optimal control of blood glucose levels. The benefit of CGM vs SMBG is shown in **Figure 1**.

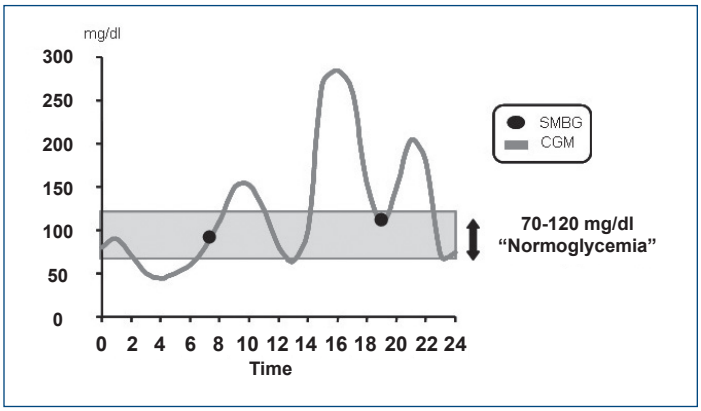

Figure 1. Comparison of information from standard SMBG and CGM from a person with diabetes mellitus. According to blood glucose measurements, the diabetic person controls glucose levels in the normal glycemic range and seems to be controlling the blood glucose levels well. However, CGM reveals that there is undetected hypoglycemia at 04.00 and hyperglycemia at 10.00, 16.00, and 21.00.

Different invasive and noninvasive techniques have been developed to obtain continuous glucose measurements.[6–11] In general, invasive techniques seem to be superior to noninvasive techniques because of better accuracy and reduced lag time of glucose measurements.[6,10] One of the most promising invasive techniques involves the implantation of glucose sensors in the subcutaneous tissue, but the sensors may malfunction, causing unreliable glucose measurements.[12–15] Suggested causes of the observed instability of glucose measurements are protein/cellular biofouling at the membrane surface, tissue interferents affecting the electrode, enzymatic dysfunction, and unstable levels of oxygen.[12,16–19] In order to elucidate these problems, it is important to focus on the inflammation caused by implanted sensors. Thus, it is crucial to understand what happens at the tissue–sensor interface in the future development and improvement of glucose sensors for implantation in the subcutis.

Continuous Glucose Measurements in Subcutis

The subcutis is easily accessible and suitable for implantation of glucose sensors. However, needle-type glucose sensors implanted in the subcutis do not measure the blood glucose concentration per se, but rather the glucose concentration in the subcutaneous interstitial fluid (ISF). Obviously, the measurements must correlate to blood glucose levels if this method is to be used as a clinical tool for the intervention of glucose levels in diabetic patients.

A milestone in glucose sensor development was demonstration of a correlation of glucose levels in the blood and ISF as shown by Fischer et al.[20] Their results opened the gate for the development and application of subcutaneous glucose sensors. Since then, several studies in animal models, e.g., cynomolgus monkeys,[21] pigs,[22] dogs,[15,23–25] rats,[26–29], cats,[23,30] and horses,[23] have shown a correlation between glucose concentrations in the blood and the ISF in subcutaneous tissue. Furthermore, there are numerous human reports on this subject and, as in animal studies, there is a correlation between blood and ISF glucose levels in healthy volunteers,[31–33] nondiabetic and insulin-dependent diabetes mellitus volunteers,[34] type 1 diabetics,[35–37] and pediatric type 1 diabetic patients.[38]

However, despite these encouraging reports of subcutaneously implanted glucose sensors, the method has not resulted in widespread use, partly because of several problems in relation to implantation. Differences between *in vivo* and *in vitro* performance of sensors in dogs have been demonstrated,[39-40] highlighting the need for an *in vivo* calibration procedure. However, the calibration drift can be overcome with multiple calibrations against a reference. An example is that measurements from the MiniMed Continuous Glucose Monitoring System® (CGMS, MiniMed Medtronic, Northridge, CA) are based on four daily calibrations.

After implantation of sensors in the subcutis, several studies have revealed that the *in vivo* sensor characteristics, i.e., zero (=background) current, linearity, and sensitivity, are different between sensors and, more importantly, also fluctuate for the same sensor over time.[12–15,22] This is partly because of the lack of reproducibility in the production of sensors, but most likely also because of physiological differences at the implantation site. This is further emphasized by the demonstration that the sensitivity of glucose sensors changed significantly after explantation from the subcutis and following placement in an *in vitro* environment.[7,40,41] This indicates the role of a reversible biological factor affecting the sensor measurements.

Furthermore, there is a delay in ISF glucose equilibrium after changes in the plasma glucose concentration, as the glucose sensor inevitably has an intrinsic delay due to glucose diffusion over membranes. The delay is reported to vary from 3 to 15 minutes[15,20,36,42,43] depending on species, sensor design and size,[44] and the applied stimulus (meal, intravenous, subcutaneous, or oral glucose or insulin administration).

In conclusion, the major drawback of subcutaneously implanted electrochemical sensors is the bioinstability with unpredictable drift and reproducibility of sensor measurements.[13,20,43,45,46] The bioinstability is partly explained by the sensor design, but obviously is also affected by the subcutaneous inflammatory reaction to implanted sensors. Suggested causes of the observed bioinstability have been attributed to protein/cellular biofouling in or on the membrane, tissue interferents affecting the electrode, enzymatic dysfunction, or unstable levels of oxygen.[12,16–18] Therefore, there is a need for a more detailed evaluation of the sensor-related subcutaneous inflammatory reaction.[12,47,48]

Inflammation and Biocompatibility of Implanted Materials

Understanding the interaction between tissue/cells and the implanted glucose sensor is indispensable for the optimization of continuous glucose measurements in the subcutis. Immediately after implantation of a biomaterial, e.g., a glucose sensor, an inflammatory process is initiated.[11,49] This is followed by different characteristic phases of the inflammatory response, optimally leading to total resolution after the biomaterial/sensor has been removed.

Initiation and Development of Inflammation

The manifestations of acute inflammation are related to vascular and cellular changes.

Once implanted, the polymeric material, i.e., the sensor membrane, is covered rapidly with plasma proteins as a result of increased vascular permeability and/or disrupted vessels, where fibrinogen especially seems to play an important role in the subsequent development of inflammation.[49,50–52] In parallel with these initial events, there is a release of cell-mediated factors, causing inflammatory cells to leave blood vessels and migrate to the implant where they are activated. In chronic stages, the implant is walled off by granulation tissue and eventually a fibrous capsule is formed.[53] Here the continued presence of an implant prevents the tissue from returning to normal, but it is possible to achieve a steady state where no progressive changes occur.[54]

Inflammatory Reaction to Subcutaneously Implanted Glucose Sensors

The aforementioned description of inflammatory responses to biomaterials is based primarily on functional studies to characterize the mechanisms behind the inflammatory reaction to biomaterials in general. Few reports have been published on the subcutaneous inflammatory reaction caused by implanted glucose sensors. Publications related to the evaluation of the *in vivo* biocompatibility of implanted glucose sensors are summarized in **Table 1**. The infiltration of neutrophils and macrophages in the tissue surrounding the implanted sensor is a common observation. The study of Mang and colleagures[55] contributed to future decisions in material selection for sensor membranes, and Klueh and Kreutzer[56] established the first murine model for the *in vivo* evaluation of implantable glucose sensors. However, other animal studies probably have little relevance in comparison with modern sensors for short-term implantation. This is mostly because of suboptimal experimental design,[12,26] uncontrolled sensor production, i.e., ill-defined material selection[57] or nonsterile sensors,[58] size of sensors,[58] and duration of implantation.[26,55,57] Moreover, evaluation of the inflammation has in general been restricted to a histomorphological examination performed on hematoxylin and eosin (HE)-stained sections. Selective staining (Masson's trichrome stain) was used in only one study to evaluate the extent of fibrosis.[56] In conclusion, so far only a few studies have focused on *in situ* detection of specific immune cells, proteins, or genes in evaluation of the inflammation caused by implanted glucose sensors.

Future Perspectives

Sensor characteristics such as sensitivity, zero current, and linearity have been found to differ between *in vitro* and *in vivo* environments.[39,40] Furthermore, it has also been shown that *in vivo* sensor characteristics change over time, i.e., the local tissue reaction affected sensor measurements. It has also been found that the biocompatibility of glucose sensors should be considered from a perspective linked to the events of inflammation. The technique used to describe the inflammatory reaction, i.e., the biocompatibility of sensors, is the detection of specific immune cells (cell surface markers) or cytokines at the protein and the gene levels.

Today, certain proteins, genes, and immune cells have now been identified as key players in the development of lesions caused by an implanted glucose sensor over time, i.e., knowledge that will be beneficial in future attempts to control inflammation, thereby improving the sensing properties.[22,49,59,60] However, the specific events of inflammation around the sensor need to be linked to the quality of sensor measurements before a targeted approach to dampen components of the immune response can be performed successfully.

Table 1.

***In Vivo* Studies in Evaluation of the Inflammatory Reaction (Biocompatibility) to Subcutaneously Implanted Glucose Sensors[a]**

Aim of study	Technique/enzyme	Outer membrane	Duration of implantation/ animal species	Comment on histopathology	Reference
Sensing properties and biocompatibility of sensor	Electrodes covered by membranes/GOD	MPC	24 h, 72 h, 28 and 56 days/rats	Fibrin deposition and increased numbers of infiltrated neutrophils, eosinophils, and lymphocytes were present at 24 and 72 h. Fibrous capsule formation at 4 and 8 weeks postimplantation.	Henninger *et al.*, 2007[60]
Sensing properties and biocompatibility of sensor	Electrodes covered by membranes/GOD	Polyurethane/PDMS	1, 2, 24, 48, 72 h and 7 days/pigs	Fibrin deposition from 1 h to 7 days, and various extents of infiltration of neutrophils, eosinophils, macrophages, B and T lymphocytes. The proinflammatory cytokines tumor necrosis factor-α and interleukin-1 are involved in inflammation.	Kvist *et al.*, 2006[22,49,59]
Sensing properties and biocompatibility of sensor	Electrodes connected with wires and covered by membranes/GOD	Nafion® (electrodes) and Teflon (connecting wire)	1, 3, 7, 14, and 30 days/mice	The inflammatory reaction ranged from edema, necrosis, infiltration of neutrophils and macrophages to giant cell formation and collagen deposition. Moreover, there was a significant decrease in inflammation in the tissue around the wire and reference electrode compared with the working electrode.	Klueh and Kreutzer, 2005[56]
Biocompatibility of glucose sensor	Membranes on foil and/or porous hollow fiber (polyamide)/GOD	MPC and/or polyamide	10 days/rats	Inflammatory reaction signifcantly increased around electrode. Significant decrease of inflammation when using MPC, porous membrane, and a combination.	Mang *et al.*, 2005[55]
Biocompatibility of glucose sensor	Electrodes covered by membranes/GOD	Regenerated cellulose	50 h/dogs	There is considerable infiltration of inflammatory cells at the tissue–sensor interface and a diffusion barrier of exudative fluid around the sensor.	Fischer *et al.*, 1994[58]
Sensing properties and biocompatibility of sensor	Electrodes covered by membranes/GOD	Cellulose acetate or polyurethane	14–96 h/dogs	Characteristic acute inflammation together with exudative fluid (<0.5 ml) in tissue surrounding the sensor. Bacteria were cultivated from the exudative fluid.	Rebrin *et al.*, 1992[12]
Sensing properties and biocompatibility of sensor	Electrodes covered by membranes/GOD	Polyurethane (working electrode) and Teflon	3, 4, and 7 days/rats	Histological examination showed a fibrovascular tissue reaction with infiltration of mainly macrophages, plasma cells, and few neutrophils.	Moatti-Sirat *et al.*, 1992[26]
Sensing properties and biocompatibility of sensor	Electrodes covered by silicone rubber tube/GOD	Silicone rubber and enzyme; enzyme is cross linked to serum albumin with glutaraldehyde	10 days/rats	The border is sharply defined at the tissue–silicone rubber interface and is characterized by capillary-rich connective tissue and few inflammatory cells. In contrast, tissue adjacent to the enzyme layer is infiltrated by many inflammatory cells.	Ertefai and Gough, 1989[57]

[a]GOD, glucose oxidase; MPC, 2-methacryloyloxyethyl phosphorylcholine; PDMS, polydimethylsiloxane.

An obvious way to eliminate or reduce the inflammation caused by short-term implanted glucose sensors, thereby potentially reducing the bioinstability of measurements, is the release of drugs from the device. A dexamethasone/poly(lactide-co-glycolide) (PLGA) microsphere system was used to reduce the inflammation around subcutaneously implanted materials (cotton threads), and this method showed a significant reduction in tissue reaction in rats.[61] However, it did not improve the long-term effect on foreign body reaction.[62] The dexamethasone/PLGA system has been tested with glucose sensors.[63] However, that study did not focus on showing that a reduction in inflammation around the sensors could be correlated with improved sensor measurements. A glucose sensor releasing the anti-inflammatory factor nitrite oxide from the outer membrane has also been tested.[64] It was shown that the inflammatory response at the implantation site was reduced and that the sensing properties seemed to improve. Furthermore, a novel technique of coating devices with a DNA-based structure demonstrated the devices to be fully histocompatible after subcutaneous implantation.[65] In combination with functionalization of DNA-based coatings with angiogenic factors, this technique showed an increase in the vascularity around the implant.[65] The future may show if the technique is compatible with the production of glucose sensors for implantation and if the increased vascularity will improve measurements in *in vivo* settings.

As the local release of drugs might have systemic effects, different procedures to reduce local inflammation might be preferred. Therefore, modification of the outer membrane to improve biocompatibility and reduce inflammation is a major field of interest and has been excellently reviewed elsewhere.[66,67] A simple strategy to reduce local inflammation is to inhibit the protein adsorption to the sensor surface by coating the outer membrane with phosphorylcholine (PC). PC mimics red blood cell surface, thereby transferring the nonthrombocytogenic properties to the sensor surface. This method has been applied to glucose sensors with success.[55] Despite the question asked and the strategy chosen for reduction of the local subcutaneous inflammation and improvement of sensor measurements, the newly established *in vivo* models will be useful in achieving proper answers (**Figures 2 and 3**).[22,49,56,60]

The ultimate goal, creating an artificial pancreas, can be realized with an automated insulin dosage system, but it requires a reliable glucose measurement system. In the last decade, glucose sensors have been introduced to the market, but the systems are still not ready for implementation as the glucose-sensing part of the artificial pancreas. With a better understanding of problems related to the biology, chemistry, and physics of sensors, the goal of developing an artificial pancreas will come closer. Meanwhile, diabetic patients can improve their glycemic control with implantable glucose sensors.

Figure 2. The pig is a good model for evaluating the performance and biocompatibility of implantable glucose sensors due to practical, anatomical (e.g., subcutis), physiological, and immunological reasons. Here seven sensors were implanted in the subcutis, and performance of the sensors was tested with a setup of a glucose pump connected to a venous (implanted in the jugular vein) catheter and a catheter for blood sampling. After termination of the experiment, tissue around sensors were sampled (see Figure 3).

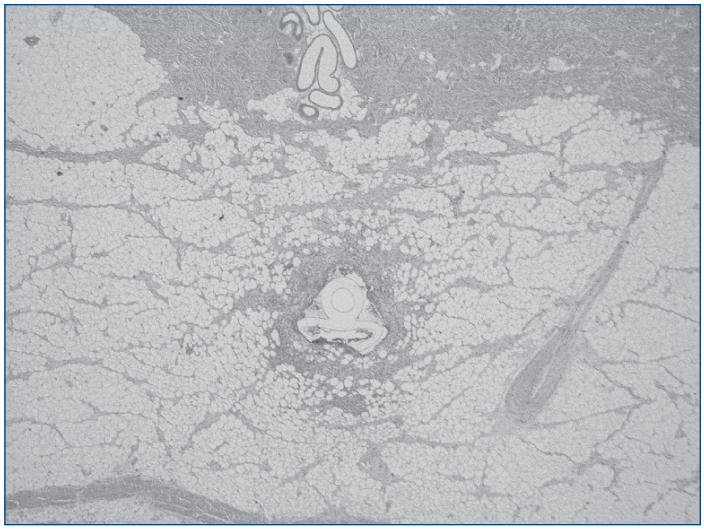

Figure 3. Skin 3 days after implantation of a MiniMed Continuous Glucose Monitoring System® sensor. Inflammatory cells have infiltrated the tissue in a limited area around the sensor; HE ×40.

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