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A percutaneous device to study glucose kinetics in subcutaneous tissue fluid

M. GERRITSEN[1], J. A. LUTTERMAN[2], J. A. JANSEN[1]*
[1]*Department of Biomaterials, University of Nijmegen, 6500 HB Nijmegen, The Netherlands*
[2]*Department of Internal Medicine, University Hospital St. Radboud, Nijmegen, The Netherlands*
E-mail: J.Jansen@dent.kun.nl

In the current study subcutaneous glucose kinetics were investigated in tissue fluid collected with a percutaneous device (PD). PDs containing a subcutaneous tissue chamber were implanted subcutaneously in New Zealand white rabbits. Sintered titanium fiber mesh sheets were used for subcutaneous anchorage of the PD. The bottom of the subcutaneous tissue chamber was either covered with a titanium fiber mesh sheet, a cellulose acetate membrane, or left uncovered. Subcutaneous glucose kinetics were determined after injection of octreotide and glucagon. The tissue reaction to the implants was evaluated histologically. No dynamic relationship was observed between glycaemia and subcutaneous tissue fluid glucose for all membrane covered devices. Histological evaluation showed that the presence of a seroma cavity in combination with obstruction of the membrane prevented adjustment of the subcutaneous glucose concentration in response to changes in glycaemia. In the uncovered devices, on the other hand, changes in glycaemia were reflected in subcutaneous tissue fluid. Our results prove that it is possible to measure changes in the glucose concentration in subcutaneous tissue fluid collected with a percutaneous device. Therefore, we conclude that a percutaneous device has an application as model to study the *in vivo* performance of implantable glucose sensors. The use of porous membranes in such devices has to be avoided.

1. Introduction

It is generally accepted that subcutaneously implantable glucose sensors can be of great importance for the clinical management of diabetes mellitus [1, 2]. Establishing the relationship between the output of such a sensor and the blood glucose concentration is a key factor in the development of a clinically applicable concept for continuous glucose monitoring. Subcutaneously implanted glucose sensors, in general, show a significant drift in sensor signal over the implantation period [1–3]. At present, it is not clear whether their response can be used at all for long-term glucose monitoring. With different sampling procedures like microdialysis, the wick technique, filtrate collection, ultrafiltration and suction effusion, it has been shown that the subcutaneous interstitial glucose concentration is in equilibrium with glycaemia under steady-state conditions, during oral glucose loads, as well as glucose or insulin infusions [4–7]. The reason for the long-term failure of subcutaneously implanted glucose sensors, however, has not been elucidated. Suggested failure modes have been related to sensor properties, protein deposition, actions of inflammatory cells, or tissue response to the implanted sensor [8–10].

To investigate the mechanisms involved in the poor performance of implanted sensors, a model is needed in which the sensor can easily be changed. Ideally, this should occur without influencing the environment surrounding the sensor and without inducing a trauma upon re-insertion. A percutaneous device (PD), which provides a permanent connection between the exterior and interior of the body, can perhaps solve the above mentioned problem [11]. Such a device can be used as carrier of an implantable glucose sensor, which allows easy replacement without surgery. First, however, it has to be demonstrated that it is possible to follow changes in blood glucose concentration with a PD.

The PD used in this experiment contains a subcutaneous chamber for the collection of tissue fluid. This idea of a subcutaneous chamber is derived from earlier studies in which implanted tissue cages have been used to study the subcutaneous interstitial compartiment [12–16]. Tissue fluid was collected from these devices at various intervals for analysis. It was shown to be a representative

*Author to whom all correspondence should be addressed.

of the subcutaneous interstitial fluid and in equilibrium with the interstitial fluid surrounding the implants. A major disadvantage of completely implanted subcutaneous chambers is that frequent sampling is not feasible. Because a PD provides permanent access to the subcutaneous tissue, it allows more frequent sampling of tissue fluid. Consequently, we suppose that monitoring of changes in the subcutaneous glucose concentration in response to changes in the blood glucose concentration should be possible.

The subcutaneous chamber in this experiment is covered with either a titanium fiber mesh sheet, a cellulose acetate membrane, or is left uncovered. With the titanium fiber mesh sheet a tissue cage-like construction is created. Titanium is used as an alternative to stainless steel, a material frequently employed in the construction of such cages. This fiber mesh allows the ingrowth of vascularized connective tissue. Sharkawy *et al*. [17–19] showed that implant porosity has a positive influence on the fibrosity and vascularity of the tissue encapsulating the implant. As a consequence, the diffusion properties of the tissue for small analytes such as glucose are also improved. We hypothesize that, through the use of a porous membrane, the diffusion of glucose into the tissue fluid collected in the subcutaneous chamber is also positively influenced. As a comparison, implants covered with a cellulose acetate membrane with a pore-size that prevents the ingrowth of tissue were used [13, 20]. Uncovered devices were included in this study as controls. The objective of this study, therefore, is to investigate glucose kinetics in subcutaneous tissue fluid collected with a percutaneous device as a possible model to investigate the long-term failure of implanted glucose sensors. Besides, the effect of different methods to cover the subcutaneous chamber on glucose diffusion is examined.

2. Materials and methods

2.1. Implants

Implantable percutaneous devices as shown in Fig. 1 were used. The devices consisted of a KELF (polychloride-trifluoro-ethylene) body in which a subcutaneous chamber was formed with a volume of 500 µl, in connection with a percutaneous part that penetrated the skin. The access to the subcutaneous chamber was closed with a stainless steel screw. A sintered titanium fiber mesh sheet (volumetric porosity 80%, fiber mesh weight 600 g/m^2, fiber diameter 50 µm, Bekaert Fiber Technologies, Belgium) was used for subcutaneous anchorage of the device. In previous experiments this material showed good biocompatibility and anchorage in soft tissue [21–23]. The bottom of the device was either covered with titanium fiber mesh ($n = 6$), cellulose acetate (Millipore®, pore size 0.2 µm) ($n = 6$), or left uncovered ($n = 12$).

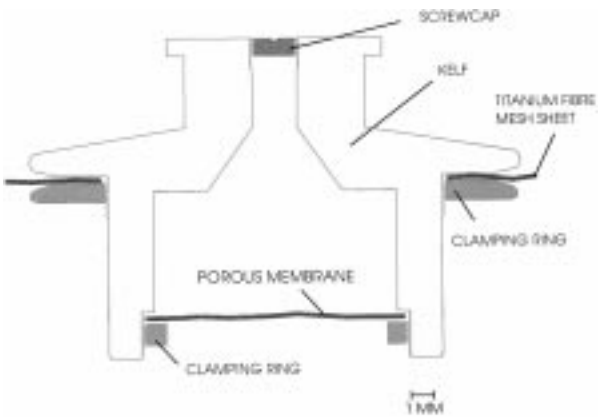

Figure 1 Graphical cross-section of a membrane-covered percutaneous device as used in the experiment.

2.2. Animals and surgical procedure

The percutaneous devices were inserted in the dorsum of female New Zealand white rabbits ($n = 24$), age 3 months, weight approximately 2.5–3 kg. One percutaneous device was implanted in each rabbit. Before insertion, the implants were sterilized in an autoclave. Surgery was performed under general anaesthesia by intravenous injection of Hypnorm® (0.5 ml/kg) and atropine (0.5 mg).

The backs of the rabbits were shaved, depilated, washed and disinfected with iodine on both sides of the spinal column. The percutaneous devices were placed using a one-stage implantation procedure, i.e. placement of the subcutaneous part and creation of the percutaneous exit site in the same session. Therefore, paravertebrally a longitudinal incision was made through the full thickness of the skin. Subsequently, lateral to the incision, a subcutaneous pocket was created by blunt dissection with scissors. This was followed by the creation of an exit site lateral of the first longitudinal incision. After placement of the implant, the wound was carefully closed with sutures. To reduce the perioperative infection risk, an antibiotic (Terramicine®) was administered postoperatively by a subcutaneous injection. The animals were separately housed in cages. After surgery, the animals were left three weeks to allow for wound healing. Guidelines for the care and use of laboratory animals were observed.

2.3. Tissue fluid analysis

To facilitate glucose diffusion into the subcutaneous chamber, the devices were filled with 0.9% NaCl at least 24 h before each experiment. After removal of the stainless steel screw, the chamber was accessed through the percutaneous part of the device. Sampling was performed by using blood collection capillary tubes (Ciba Corning Diagnostics Limited, UK). Only clear samples, free of haemoglobin, were used for analysis. Glucose kinetics in the subcutaneous interstitial fluid were investigated. Before the experiment, basal glucose concentrations in blood and tissue fluid were determined. The animals were sedated by intramuscular injection of 0.5 ml Hypnorm®. Blood glucose levels were elevated by subcutaneous injection of 6 µg octreotide and intramuscular injection of 0.15 mg glucagon. At regular intervals up to 3 h after injection, subcutaneous glucose concentrations were measured and related to blood glucose concentrations. Duplicate experiments were

performed. Obtained values were compared with the corresponding blood glucose concentrations. Therefore, blood samples were obtained from a cutaneous aural vein. Glucose analysis in tissue fluid was performed by the APEC glucose analyzer (Stam Instruments B.V., The Netherlands). Blood glucose measurements were performed by the Gluco-Touch portable blood glucose meter (Lifescan, Belgium).

2.4. Histologic procedure and evaluation

Four months after the start of the experiment the animals were sacrificed using an overdose of Nembutal®. The implants with their surrounding tissues were excised immediately and fixed in 4% buffered formalin. After dehydration, excess tissue was removed and the samples were embedded in methyl methacrylate (MMA). Histologic sections of approximately 10 μm thickness were prepared using a sawing microtome [24, 25], stained with methylene blue and basic fuchsin and investigated by light microscopy. The tissue reaction to the percutaneous device was evaluated histologically by examining epithelial attachment and quality of the tissue inside the titanium fiber mesh. The quality of the fibrous capsule covering the bottom of the device was also evaluated.

3. Results

3.1. Macroscopic clinical findings

3.1.1. Membrane covered implants

All twelve animals appeared in good health during the implantation. The implants showed good healing with the surrounding soft-tissue with various degrees of limited epithelial migration. Around one implant clinical signs of a mild inflammatory reaction were present. In this implant with clinically present inflammation, migration proceeded. Finally, this resulted in exposure of the KELF flange of the percutaneous part of the device.

3.1.2. Uncovered implants

One rabbit had to be sacrificed during the experiment because of the appearance of a Pasteurella abcess underneath the implant. The other eleven animals appeared in good health during the implantation period. All implants showed good healing with the surrounding soft-tissue with various degrees of limited epithelial migration. No clinical signs of inflammation were observed.

3.2. Tissue fluid analysis

3.2.1. Membrane covered devices

The basal glucose concentration in tissue fluid sampled from the percutaneous devices was shown to be lower than glycaemia, $= 1.58 \pm 1.32$ mM vs $= 5.62 \pm 0.58$ mM (paired t-test, $P < 0.001$). No differences were observed between cellulose acetate or titanium fiber mesh covered devices. Upon microbial examination, the tissue fluid was found to be sterile.

In all animals, injection of octreotide and glucagon resulted a prolonged increase in glycaemia. On the other hand, this increase in glycaemia was not followed by a significant increase in tissue fluid glucose. Fig. 2 shows a representative example. No differences were observed between cellulose acetate or titanium fiber mesh covered devices.

3.2.2. Uncovered devices

In uncovered implants, the basal glucose concentration was also lower than the blood glucose concentration, $= 2.15 \pm 1.63$ mM vs $= 6.10 \pm 0.87$ mM (paired t-test, $P < 0.001$). No statistical difference was observed with the basal glucose concentration in membrane covered devices (general t-test, $P > 0.05$). Upon microbial examination, the tissue fluid was found to be sterile.

In one animal, the glucose kinetics could not be investigated because of a persistent infection inside the subcutaneous chamber. In the remaining ten animals, a clear and prolonged increase in glycaemia was observed after injection of octreotide and glucagon. This time, the increase was followed by a significant increase in the tissue fluid glucose concentration in all cases. On the other hand, the subsequent decrease in blood glucose concentration was not always completely reflected in the tissue fluid in this particular experiment. In ten of the twenty experiments, the tissue fluid glucose concentration followed glycaemia during the increase as well as the decrease. Fig. 3 shows a representative example.

3.3. Histological evaluation

Light microscopic evaluation of the tissue surrounding the percutaneous devices revealed that the tissue reaction to all implants was relatively uniform. Only limited epithelial downgrowth was observed. In one case the downgrowth proceeded, resulting in exposure of the KELF flange of the percutaneous part of the device. The titanium fiber mesh sheets used for subcutaneous anchorage were filled with immature connective tissue, generally free of inflammation (Fig. 4).

Evaluation of the subcutaneous part of the device showed that all implants, the membrane-covered as well as the uncovered ones, were surrounded by a relatively

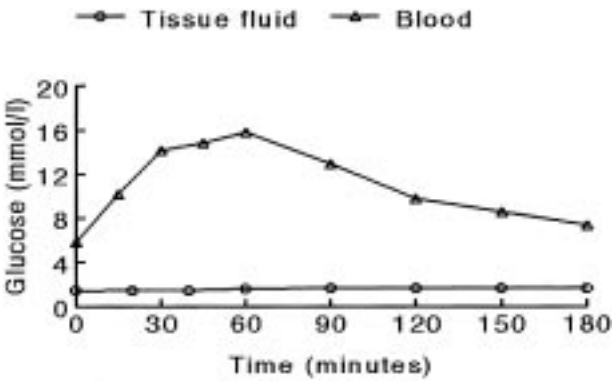

Figure 2 Blood glucose and subcutaneous tissue fluid glucose concentration after injection of octreotide and glucagon. Example of membrane-covered subcutaneous chamber. The changes in glycaemia were not reflected in the tissue fluid up to 3 h after injection.

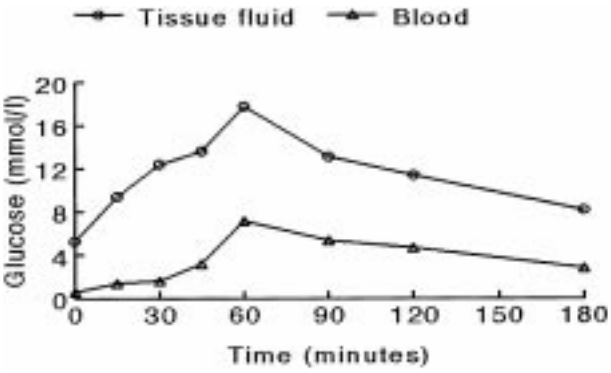

Figure 3 Blood glucose and subcutaneous tissue fluid glucose concentration after injection of octreotide and glucagon. Example of uncovered subcutaneous chamber in which the increase as well as the decrease in glycaemia were reflected in the subcutaneous tissue fluid.

Figure 5 Histologic section showing a thin fibrous capsule containing small blood vessels and few inflammatory cells covering the bottom of an uncovered subcutaneous chamber after four months of implantation. Original magnification × 133, bar 75 μm.

thin fibrous capsule containing small bloodvessels (Fig. 5). No differences were observed in thickness or vascularity of the fibrous capsule between the different devices. The capsule was generally free of inflammatory cells. Only occasionally accumulations of inflammatory cells were observed. In all membrane-covered devices, the formation of a seroma cavity between the fibrous capsule and the membrane was observed (Fig. 6). The fiber mesh sheets did not contain viable connective tissue and were filled with an amorphous deposition. The cellulose acetate membranes, as expected, never showed ingrowth of connective tissue.

4. Discussion and conclusions

We observed a good tissue reaction to the percutaneous part of the device and the titanium fiber mesh used for subcutaneous anchorage. This is in agreement with the findings of previous experiments with percutaneous devices [21–23]. Evidently, the most important finding of this study is that it is possible to measure changes in the glucose concentration in subcutaneous tissue fluid collected with a percutaneous device. However, a prerequisite is that the bottom of the created subcutaneous sampling chamber is left uncovered. Only then was tissue fluid glucose shown to respond to changes in glycaemia. This is in corroboration with the findings of other investigators, who were able to measure a rapid adjustment of the glucose concentration within the fibrous capsule surrounding their implanted sensors in response to blood glucose alterations [26–32]. On the other hand, in our experiment we observed a significantly larger delay in response. The reason for this is not clear. We suppose that the relatively large volume of the fluid reservoir in the subcutaneous chamber influenced the rate of glucose diffusion and prevented rapid equilibration between the two compartments. Also, specific tissue aspects like fibrous capsule thickness and vascularization can have influenced glucose diffusion to the subcutaneous chamber. This possible effect of fibrous capsule properties can also explain that the decrease in blood glucose concentration was only reflected in the subcutaneous tissue fluid of only half of the used animals. More *in vivo* data are needed to provide a definitive answer.

In contrast with the above mentioned, no dynamic relationship was observed between blood glucose and tissue fluid glucose concentration in the membrane-covered percutaneous devices. Histological evaluation of

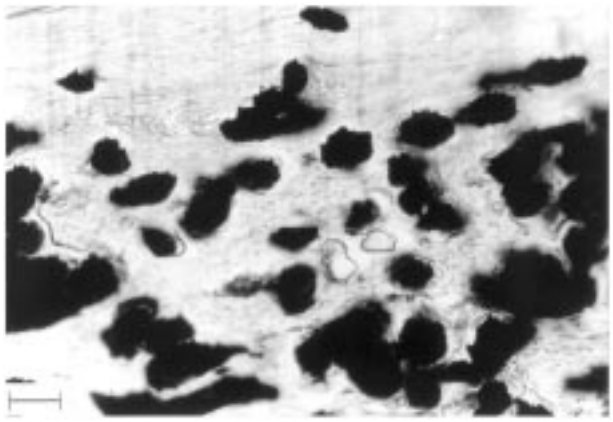

Figure 4 Histologic section showing the porosity of the titanium fiber mesh sheet used for subcutaneous anchorage of the percutaneous device after four months of implantation. The mesh porosity is filled with immature connective tissue containing fibrocytes, collagen and small blood vessels. Original magnification × 147, bar 68 μm.

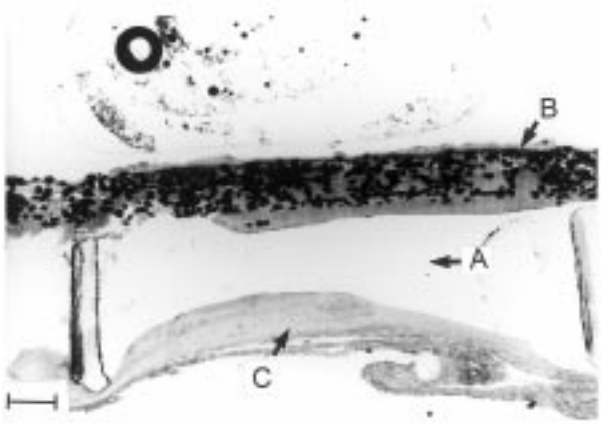

Figure 6 Histological cross-section after four months of implantation showing the formation of a seroma cavity (A) between the titanium fiber mesh membrane (B) and the fibrous capsule (C). Original magnification × 18.4, bar 544 μm.

the tissue surrounding the subcutaneous part of our implants revealed the formation of a seroma cavity between fibrous capsule and membrane. Apparently, the presence of the seroma cavity in combination with obstruction of both the fiber mesh and cellulose acetate membranes prevented a rapid adjustment of the subcutaneous glucose concentration within the fibrous capsule. In addition, we observed that the design of the percutaneous device hindered the ingrowth of connective tissue into the titanium fiber mesh. This is illustrated by the finding that in all implants the fiber mesh was filled with an amorphous deposition. We suppose that this absence of tissue ingrowth is due to a prolonged reduction of stress on the titanium fiber mesh. Similar to the situation with bone implants, this stress shielding effect influences the viability of the connective tissue inside the mesh [33].

Further, the results with the cellulose acetate-covered devices revealed that also the use of porous membranes that prevent the ingrowth of connective tissue should be avoided in carriers of implantable sensors. Deposition of surface-active components, like proteins, diminishes membrane permeability and prevents rapid equilibration of the glucose concentration in the measuring compartment.

In conclusion, this study was aimed at the direct collection of tissue fluid with a percutaneous device to investigate subcutaneous glucose kinetics. No dynamic relationship was observed between glycaemia and subcutaneous tissue fluid glucose for membrane covered devices. The presence of a seroma cavity in combination with obstruction of the porous membrane prevented adjustment of the subcutaneous glucose concentration in response to changes in glycaemia. In the uncovered devices, on the other hand, changes in glycaemia were reflected in subcutaneous tissue fluid. Our results show that it is possible to measure changes in the glucose concentration in subcutaneous tissue fluid collected with a percutaneous device. Therefore, we would like to propose a percutaneous device as a model to study the *in vivo* performance of implantable glucose sensors. The use of porous membranes in such devices has to be avoided.

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