Journal of Diabetes Science and Technology
Volume 1, Issue 6, November 2007

ORIGINAL ARTICLES

Blood-Induced Interference of Glucose Sensor Function *in Vitro*: Implications for *in Vivo* Sensor Function

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Abstract

Background:
Although tissue hemorrhages, with resulting blood clots, are associated with glucose sensor implantation, virtually nothing known is about the impact of red blood cells and red blood cell clots on sensor function *in vitro* or *in vivo*. In these studies, we tested the hypothesis that blood can directly interfere with glucose sensor function *in vitro*.

Methods:
To test this hypothesis, heparinized human whole blood (HWB) and nonheparinized human whole blood (WB) were obtained from normal individuals. Aliquots of HWB and WB samples were also fractionated into plasma, serum, and total leukocyte (TL) components. Resulting HWB, WB, and WB components were incubated *in vitro* with an amperometric glucose sensor for 24 hours at 37°C. During incubation, blood glucose levels were determined periodically using a glucose monitor, and glucose sensor function (GSF) was monitored continuously as nanoampere output.

Results:
Heparinized human whole blood had no significant effect on GSF *in vitro*, nor did TL, serum, or plasma-derived clots from WB. Sensors incubated with WB displayed a rapid signal loss associated with clot formation at 37°C. The half-life was 0.8 ± 0.2 hours (n = 16) for sensors incubated with WB compared to 3.2 ± 0.5 (n = 12) for sensors incubated with HWB with a blood glucose level of approximately 100 mg/dl.

Conclusion:
These studies demonstrated that human whole blood interfered with GSF *in vitro*. These studies further demonstrated that this interference was related to blood clot formation, as HWB, serum, plasma-derived clots, or TL did not interfere with GSF *in vitro* in the same way that WB did. These *in vitro* studies supported the concept that the formation of blood clots at sites of glucose sensor implantation could have a negative impact on GSF *in vivo*.

J Diabetes Sci Technol 2007;1(6):842-849

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Abbreviations: (CGM) continuous glucose monitoring, (GSF) glucose sensor function, (HBSS) Hanks' balanced salt solution, (HEPES) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, (HWB) heparinized whole blood, (PBS) phosphate-buffered saline, (PPC) platelet-poor plasma clots, (PPP) platelet-poor plasma, (PRC) platelet-rich plasma clots, (PRP) platelet-rich plasma, (RBC) red blood cells, (RSL) rapid sensor loss, (STC) silastic tubing chamber, (TL) total leukocyte, (TRT) tissue response triad, (WB) whole blood

Keywords: blood, blood clots, diabetes, implantable glucose sensor, red blood cells, sensor function *in vitro*, tissue hemorrhages

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Introduction

Significant effort has been invested in developing subcutaneously implantable glucose sensors. Although most of these glucose sensors show excellent to satisfactory sensor performance upon implantation,[1–6] a significant number of sensors also experience a delay of sensor functionality postimplantation. This delay of sensor functionality, which is also referred to as the run-in time of implantable biosensors and is defined as the time from implantation of the biosensor to the actual stabilization of the sensor baseline signal,[7] can last a few hours to several days. The exact mechanisms or substances involved in this delay of functionality are still unknown, but it is generally believed that this initial delay in sensor functionality is thought to be the consequence of biofouling of sensors by tissue response to sensor implantation. Biofouling of glucose sensors is induced by influxing leukocytes, which are known to accumulate in high numbers at sites of sensor implantation and bind to protein-coated sensors.[7,8]

Currently, virtually all methods of sensor implantation cause tissue trauma and frequently tissue hemorrhage. Although hemorrhage, with associated blood clot formation, is frequently associated with glucose sensor implantation and movement of sensors in the tissue, virtually nothing is known about the impact of hemorrhage and blood clots on sensor functionality. Therefore, for the present study we tested the hypothesis that blood and blood clots can directly interfere with glucose sensor function *in vitro*. To test this hypothesis, heparinized whole blood (HWB) and nonheparinized whole blood (WB) were obtained from normal nondiabetic individuals. Aliquots of HWB and WB samples were also fractionated into plasma, serum, and total leukocyte (TL) components. Next, resulting HWB and WB components were incubated *in vitro* with an amperometric glucose sensor for 24 hours at 37°C. During incubation, blood glucose levels were determined periodically using a standard FreeStyle® blood glucose monitor (Abbott Diabetes Care, Alameda, CA), and glucose sensor function (GSF) was monitored continuously. These studies demonstrated that human blood clots interfere with GSF *in vitro*. These studies further demonstrated that this interference was related to blood clot formation, as HWB, serum, plasma-derived clots, or TL did not interfere with GSF *in vitro* in the same way that WB did. These *in vitro* studies supported the concept that the formation of blood clots at sites of glucose sensor implantation could have a major impact on GSF *in vivo*.

Experimental Section

Blood and Blood Components

Heparinized whole blood and nonheparinized WB were obtained from normal, nondiabetic individuals. In addition, HWB and WB were also fractionated into platelet-rich plasma (PRP) and platelet-poor plasma (PPP). PRP was prepared by centrifuging blood (HWB or WB) at 150 *g* for 5 minutes at 4°C. Using a plastic pipette, the resulting cell-free fluid (PRP) was transferred to a plastic container and placed on ice until used. PPP was prepared by centrifuging blood (HWB or WB) at 1500 *g* for 15 minutes, and the resulting cell-free fluid (PPP) was transferred to a container and placed on ice until used. Because no anticoagulant is present in WB, WB-derived plasma clotted when warmed to 37°C. Plasma-derived clots from WB were designated as platelet-rich plasma clots (PRC) and platelet-poor plasma clots (PPC). In order to investigate the effect of increasing blood glucose levels on sensor functionality, WB was spiked with an increasing amount of glucose prior to the start of the experiment. University of Connecticut Health Center institutional review boards (Farmington, CT) approved all human blood studies.

Isolation of Total Blood Leukocytes

For studies of the effect of human leukocytes on sensor function *in vitro*, human leukocytes were isolated from 4 milliliters of HWB as described previously.[9] Briefly, HWB was centrifuged and PRP was removed and discarded. The remaining solution was placed on ice for 10 minutes to lyse red blood cells (RBC). Total leukocytes were recovered (5 minutes at 500 *g*) and washed three times with Hanks' balanced salt solution (HBSS)/4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (1× HBSS, 20 m*M* HEPES, pH 7.2) minus calcium and magnesium. Leukocytes were reconstituted at their original concentration of 5×10^6/ml in PRC or PPC and tested for their effect on sensor function *in vitro*. The viability of the leukocyte preparation was greater than 98% as observed by Trypan blue staining. PRC + leukocytes and PPC + leukocytes were incubated *in vitro* with an amperometric glucose sensor for about 24 hours at 37°C. In addition, WB with a leukocyte count of 5×10^6/ml (same donor as HWB) was also incubated *in vitro* with a glucose sensor.

Evaluation of Sensor Function

The continuous glucose monitoring (CGM) system used for these studies is presented in **Figure 1**. Abbott Diabetes Care provided all glucose sensors used in these studies.[10,11] In order to protect the sensor "wiring" from

shorting out, sensors were covered in electrical moisture sealant as described previously.[11] Sensor performance *in vitro* was conducted for up to 24 hours using the CGM system described previously with modifications.[3,11] Briefly, sensors were inserted into a silastic tubing chamber (STC) by making a small opening using a 23-gauge needle on 3-centimeter-long silastic tubing (Nalgene 50 silicone tubing, size 0.25-inch inside diameter × 0.375-inch outside diameter, Nalgene Company, Rochester, NY). Prior to sensor insertion, the STC was sealed at both ends with microcentrifuge tube caps (Fisher Scientific, Pittsburgh, PA). Blood or blood components were injected directly into STC using a 1-milliliter tuberculin syringe with a 26-gauge needle. For CGM, glucose sensor leads were connected to a potentiostat and data acquisition system (Abbott Diabetes Care) as described previously[11] **(Figures 1B** and **1C)**. Data were acquired at a frequency of 20–60 points per minute. The performance of the sensor *in vitro* was tested by placing STC with a sensor into a heated sand box, which was maintained at 37°C. Once blood or blood components were added to the container holding the sensor, the containers were immediately submerged into the sand in order to avoid sensor signal drifting as a consequence of temperature shifts **(Figures 1B, 1C, and 1D)**, and the CGS system was initiated. During incubation, blood or plasma glucose levels were determined periodically by withdrawing blood or blood components from STC using a tuberculin syringe and measuring blood glucose levels using a standard FreeStyle blood glucose monitor. Blood glucose results were logged into the continuous data system. All sensors were tested in phosphate-buffered saline (PBS) with the addition of glucose (around 100 mg/dl) prior to the start of the experiment and after exposure to blood or plasma in order to evaluate any loss of sensor functionality.

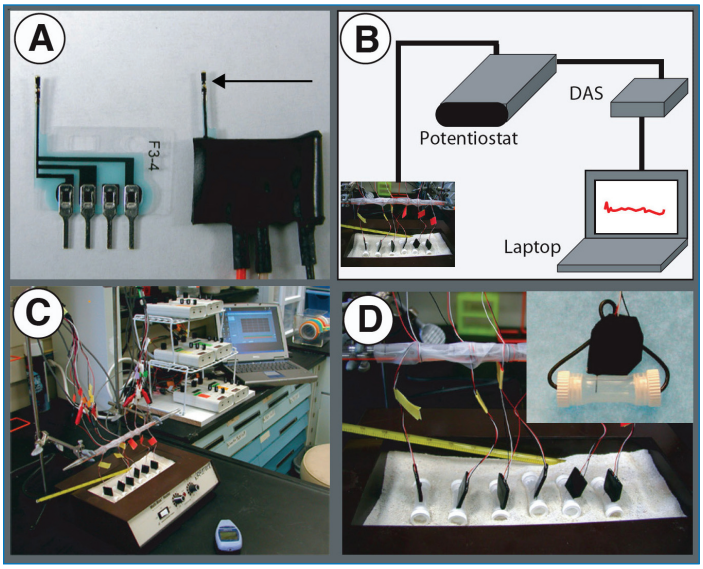

Figure 1. Continuous glucose monitoring system used for the study of blood and blood components *in vitro*. **(A)** The CGM system used for *in vitro* blood studies included an Abbott Diabetes Care glucose sensor with (right) and without (left) protective electronic coating, with the arrow indicating the sensing tip. **(B)** Schematic diagram of CGM system. **(C)** CGM experimental setup reaction vessels in a sand bath to maintain constant temperature (37°C). **(D)** Close-up of reaction vessels used for the *in vitro* blood study.

Data Analysis

The half-life of sensors as defined by the time for sensor current to decay to half of its initial value was calculated for each sensor incubated with WB and for each sensor incubated with HWB. Average half-life and standard deviation were calculated for these sensors incubated with blood. All sensor blood tests were done with blood glucose levels of approximately 100 mg/dl if not stated otherwise for specific experiments.

Results

Effect of HWB and WB on Glucose Sensor Function

To investigate the effect of blood and blood clots on sensor function, we initially evaluated the *in vitro* impact of HWB and WB on sensor functionality. To achieve this, we incubated sensors together with freshly drawn HWB and WB from nondiabetic individuals in the STC, as shown in **Figures 2B** and **2C**, respectively. Because of density difference, HWB quickly separated into plasma and RBC layers **(Figure 2B)**. RBC normally make up about 50% of the total blood volume so the plasma and RBC layers occupy approximately equal volumes. As can be seen in **Figure 2B**, the sensor tip was embedded in the RBC layer with the rest of the sensor exposed to the plasma. It should be noted that glucose sensing only occurred at the very tip of the sensor[3] where RBC were located. As can be seen in **Figure 2A**, HWB (nonclotted) did not interfere with sensor functionality *in vitro*, i.e., the fall in blood glucose levels paralleled the drop in sensor output. The decline in sensor response over the 6-hour testing period appeared to be due to the decrease in glucose levels in the blood from glucose metabolism by cells. Because RBC are the majority of cells in the blood of healthy individuals (i.e., 1,000 RBC for every white blood cell), they are responsible for the majority of glucose metabolism in the blood. As can be seen **(Figure 2A,** red diamonds**)**, the glucose level fell from 115 mg/dl to below 20 mg/dl over the time of the experiment. The sensor response tracked the fall in glucose consumption accordingly. The half-life for sensors incubated with HWB was 3.2 ± 0.5 hours (n = 12). In the case of WB, it is interesting to note that the blood clot (i.e., RBC + fibrin) was attached to the sensor tip **(Figure 2C)**. It appears

that fibrin is the likely "glue" that adheres the blood clot to the sensor. The sensor response in this case declined more rapidly than in HWB and reached baseline levels within 1.5 to 2 hours after the start of the experiment. Interestingly, the actual drop in blood glucose levels in withdrawn samples of clotted blood did not match the rapid decline of sensor output, i.e., actual blood glucose levels were higher than apparent glucose levels indicated by sensor output. For example, in **Figure 2A**, the WB glucose level was measured to be 74 mg/dl about 3 hours into the experiment, whereas the sensor response was only at a background level (blood glucose levels for WB not shown). The half-life was 0.8 ± 0.2 hours (n = 16) for sensors incubated with WB. It should be noted that, in both cases, normal sensor function was restored after washing the sensor with PBS and testing with glucose (data not shown). Experiments were repeated at least twice with different blood donors each time and with 6 sensors per experiment for a total of 12 sensors. These experiments clearly indicate that heparinized whole blood does not interfere with sensor functionality *in vitro* but that blood clots do interfere with sensor functionality *in vitro*.

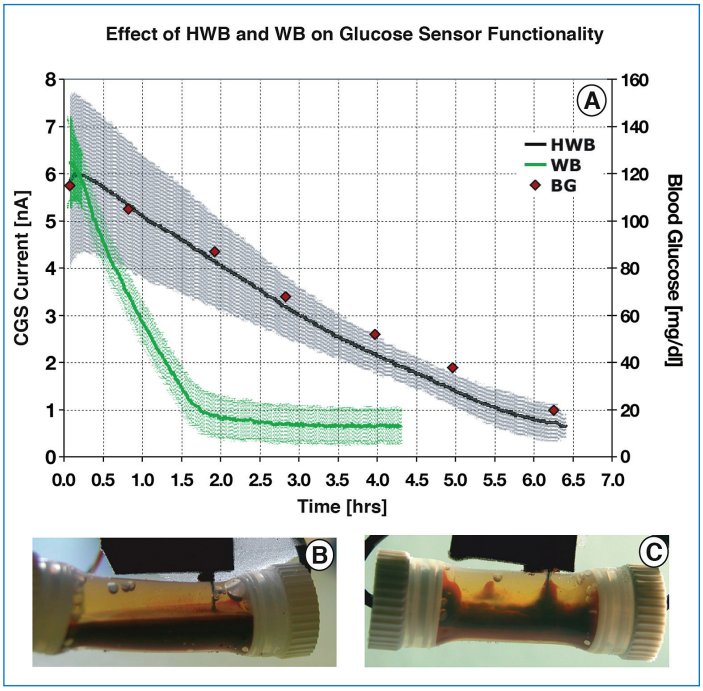

Figure 2. Effect of HWB and WB on glucose sensor functionality. To determine the impact of HWB or WB on sensor function *in vitro*, approximately 1 milliliter of HWB or WB was added to the reaction vessel containing a glucose sensor and incubated at 37°C with continuous glucose sensing **(A)**. Reaction vessels were also sampled periodically for blood glucose levels by utilizing an external glucose monitor. These studies were repeated at least twice with a total of six sensors used per series. **(A)** Results of one series for HWB and one series of WB. Data presented as the range of sensor response (± 1 standard deviation) are shown for both HWB and WB. The appearance of HWB **(B)** and WB **(C)** reaction vessels demonstrated that although WB incubation with the sensors resulted in adhesion of the blood clots to the sensors **(C)**, this did not occur in the HWB samples.

Effect of PPC and PRC on Glucose Sensor Function

Previous studies have suggested that the protein coating of glucose sensors can affect sensor function.[7,8,12] Here we found that clot formation of blood had a tremendous impact on sensor functionality *in vitro*. This observation raised the question of the role of plasma proteins and fibrin clots on sensor function *in vitro*. Therefore, we next investigated whether PPC or PRC would have any effects on sensor functionality, as occurred with WB. The effect of PPC on glucose sensor function is presented in **Figure 3A**, which shows that clot formation around the sensor did not interfere with glucose sensor function *in vitro*. Additionally, we noted that the plasma glucose level in PPC only changed insignificantly over the 5-hour time frame of the experiment, likely as a consequence of the limited amount of platelets in PPC (see **Figure 3A**). Because no drop in sensor functionality was apparent in the first few hours, the experiment was terminated after about 5 hours. This experiment was repeated twice with a total of six sensors in each experiment (n = 12).

In order to investigate if platelets had an impact on sensor functionality, we next incubated sensors with PRC. As was the case with PPC, PRC did not interfere with sensor functionality *in vitro* **(Figure 3B)**. Because a higher number of platelets were present in the PRC solution, the glucose consumption was slightly higher when compared to the PPC solution. For completeness we also investigated the effect of PRP and PPP (i.e., heparin addition; no clot formation) on glucose sensor function *in vitro*. We found that PRP and PPP did not affect sensor function or glucose levels (data not shown), similar to what was shown with PPC or PRC. These studies demonstrated that neither plasma clots nor plasma proteins contributed to WB interference in sensor function *in vitro*.

Effect of RBC Concentration on Glucose Sensor Function

Initial studies with blood and plasma indicated that sensor functionality was lost rapidly only if the sensor was present in WB. Because this rapid sensor function loss only occurred in the presence of both clot formation and whole blood, we hypothesized that RBC played a key role in the loss of sensor function seen in WB-treated sensors. To evaluate the role of RBC in sensor interference, we varied the RBC concentration by mixing WB and plasma (both platelet rich and poor) at different ratios. As shown in **Figure 4**, the result demonstrated that decreasing the number of RBC in clots decreased the interference proportionally. This study emphasized that RBC are key elements in WB interference in sensor function *in vitro*.

Contribution of Leukocytes to WB-Induced Interference in Glucose Sensor Function

Previous studies suggested that inflammatory leukocytes can have negative effects on sensor function *in vitro* and *in vivo*.[8,12] To establish whether leukocytes submerged in plasma clots interfere with glucose sensor function, we isolated total blood leukocytes and resuspended them in nonheparinized plasma (e.g., PRC and PPC). WB and plasma were placed on ice while total leukocytes were processed. As can be seen in **Figure 5**, the decrease in sensor response was greatest in WB. Alternatively, leukocytes suspended in PRC or PPC induced only a slight fall in sensor response *in vitro*. The sensor response declined faster in leukocytes in PRC when compared to PPC, likely because of the higher glucose consumption of

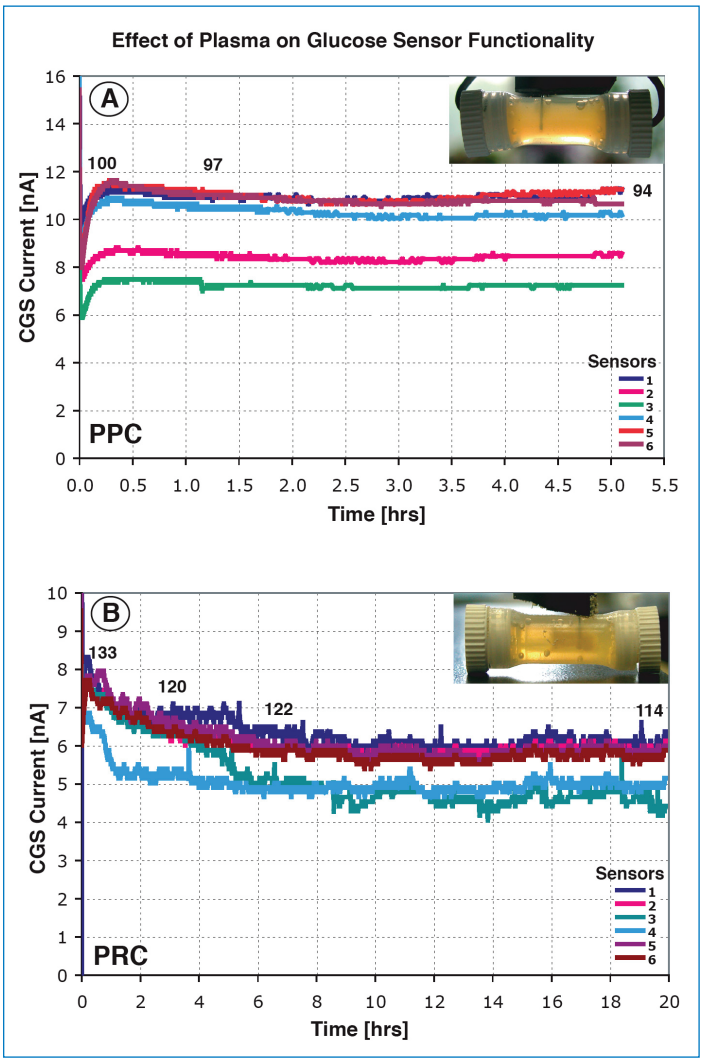

Figure 3. Effect of plasma on glucose sensor functionality. To determine the impact of PPC and PRC on sensor function *in vitro*, approximately 1 milliliter of PPC or PRC was added to the reaction vessel containing a glucose sensor and incubated at 37°C with continuous glucose sensing. The reaction vessels were also sampled periodically, and blood glucose levels were determined by a glucose monitor. These studies were repeated at least once with six sensors used per series. Results of one series for PPC and one series of PRC are presented. Data are presented as sensor responses for PPC **(A)** and for PRC **(B)**. (Insets) PPC and PRC reaction vessels are presented in A and B, respectively.

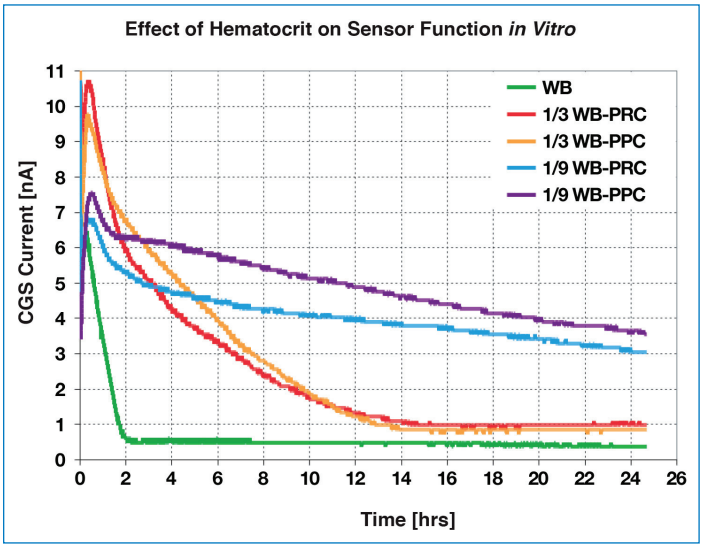

Figure 4. Effect of hematocrit on sensor function *in vitro*. To determine the impact of hematocrit on sensor function *in vitro*, WB was diluted in PPP and PRP at dilutions of 0, 1/3, and 1/9. The resulting WB or WB dilutions were added to the reaction vessel containing a glucose sensor and incubated at 37°C with continuous glucose sensing. Reaction vessels were also sampled periodically for blood glucose levels utilizing a glucose monitor. These studies were repeated once with 6 sensors used per series, for a total of 12 sensors each. Results from one series are presented.

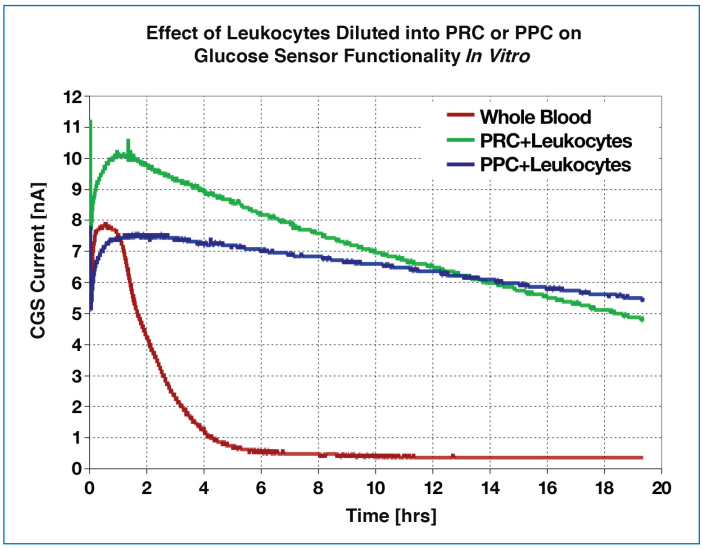

Figure 5. Effect of leukocytes diluted into PRC or PPC on glucose sensor functionality in vitro. To determine the impact of leukocytes suspended in PPC or PRC on sensor function in vitro, approximately 1 milliliter of 2 × 10^6 leukocytes was added to PPC or PRC, and the resulting suspension was added to the reaction vessel containing a glucose sensor and incubated at 37°C with continuous glucose sensing. The reaction vessels were also sampled periodically, and blood glucose levels were determined by external monitoring. These studies were repeated once with six sensors used per series. Results of one series for leukocytes suspended in PPC and PRC are presented, as well as WB as a reference point for the leukocyte studies.

PRP. It was also apparent that sensor output decline in WB was not at the expected 1.5- to 2-hour time frame but rather around 4 hours. We believe that this initial delay of WB was a consequence of the initial low temperature of around 4°C of the WB. WB was placed on ice for approximately 1 hour until leukocytes had been isolated from HWB and the experiment was initiated. In these conditions, blood was unable to clot until the temperature increased. In order for blood and plasma to reach equilibrium with the temperature in the incubator (37°C), a significant amount of time was needed. Therefore, the delay, which was also noticeable by the plateau of the whole blood clot, was due to the low temperature at the beginning of the experiment. In any case, it was shown that leukocytes appear *not* to contribute significantly to WB interference in sensor function *in vitro*. The decrease in sensor response was due to the decrease in plasma glucose level. As expected, the decrease in the blood glucose level was higher in PRC when compared to PPC because of the glucose consumption of platelets present at higher numbers in PRC than in PPC. For these studies, each experimental condition utilized six sensors.

Effect of Glucose Levels in WB

Because WB was the only medium having a significant effect on glucose sensor function, we investigated if loss of sensor function was different for various levels of blood glucose. For that, we spiked normal human blood with increasing amounts of glucose, ranging from 111 to 560 mg/dl. Results shown in **Figure 6** indicated that the sensor half-life increased with glucose levels. It was also interesting to note that all samples, regardless of starting blood glucose levels, had approximately the same rate of decrease in sensor response within the first 4 hours. However, the rates began to diverge after 4 hours based on starting blood glucose levels. We currently do not know what may be the cause of this divergence in rates that occur at higher blood glucose levels.

Discussion

Loss of sensor function associated with the tissue response triad (TRT) of inflammation, fibrosis, and vessel regression generally resulted in a slow and progressive loss of sensor function over several hours/days, without any "rebound" or return of sensor function once lost.[13] Alternatively, an unexpected rapid loss of sensor function, i.e., within minutes/hours, was also seen in a significant number of implantable sensors.[14,15] This rapid apparent loss of sensor function [i.e., rapid sensor loss (RSL)] usually occurred within the first 24 hours postsensor implantation. Although significant efforts had been undertaken to understand the factors and mechanisms involving the loss of sensor function associated with sensor-induced TRT,[13,16–18] little is known about the cause of RSL *in vivo*. In an effort to begin to fill this gap in our knowledge, we developed an *in vitro* model to determine the impact of blood, blood clots, and various blood components on sensor function *in vitro*. Using this model, we tested the hypothesis that blood clots can induce an "apparent" loss of sensor function. Results of these studies demonstrated that (1) as expected, WB and related blood components clotted rapidly when incubated at 37°C, whereas HWB and related components did not; (2) HWB or heparinized plasma had no significant effect on GSF *in vitro*, nor did serum or plasma clots derived from WB; (3) TL preparations, at concentrations equivalent to normal blood levels, did not interfere with sensor function *in vitro*; but (4) sensors incubated with WB displayed a rapid signal loss associated with clot formation at 37°C. The half-life was 0.8 ± 0.2 hours (n = 16) for sensors incubated with WB compared to 3.2 ± 0.5 (n = 12) for sensors incubated with HWB for a blood glucose level of approximately 100 mg/dl. (5) Furthermore, removal of blood clots from the sensor reversed blood clot interferences (e.g., testing sensors in PBS with the addition of glucose). These data clearly demonstrated that

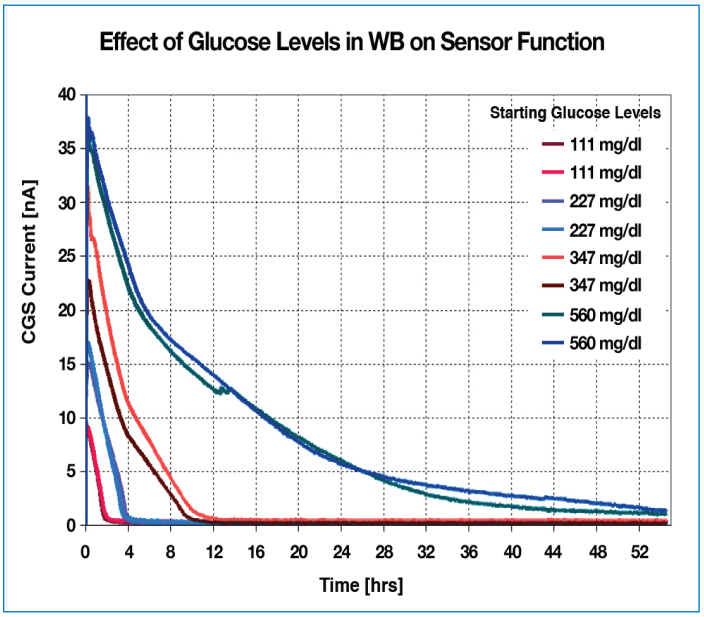

Figure 6. Effects of glucose levels in whole blood on sensor function. To determine the impact of blood glucose levels in whole blood effects on sensor function *in vitro*, WB (blood glucose 111 mg/dl) was spiked with glucose to final concentrations of 227, 347, and 560 mg/dl as determined by external monitoring. The resulting samples were added to reaction vessels containing a glucose sensor and incubated at 37°C with continuous glucose sensing. Two sensors were used for each glucose level.

although RBC (heparinized blood) can slowly metabolize glucose in the blood, blood clot formation around the sensor can decrease the sensor response dramatically (i.e., output). We hypothesized that this "apparent" loss of sensor function *in vitro* was the result of "metabolism and membranes" of RBC. A decrease of glucose levels within the microenvironment of the sensor was the result of glucose metabolism by RBC **(Figure 7B)**. In addition, this "apparent" loss of sensor function *in vitro* may also be a consequence of the deformability of RBC; sensor membranes were blocked mechanically, diminishing glucose diffusion to the sensor **(Figure 7C)**.

Usually, serum/plasma and tissue-derived proteins and peptides, as well as inflammatory cells, are thought to be major contributors to the loss of sensor function associated with *in vivo* biofouling. Interestingly, although tissue hemorrhage occurs during sensor implantation, as well as postsensor implantation, likely because of movement of the sensor, the role of hemorrhage with associated clot formation in RSL and the progressive loss of sensor functionality have not been addressed. Hemorrhage with associated blood clot formation in close proximity of sensor location in the tissue would lead to an immediate accumulation of RBC around the sensor **(Figure 7B)**. RBC and plasma-derived fibrin(ogen) released in the hemorrhage would congeal into a "blood clot" rapidly, which would contract/retract and draw RBC even closer to the glucose sensor **(Figure 7B)**. Alternatively, in the case of heparinized blood, RBC are not congealed around the sensor, allowing diffusion of glucose to the sensor membrane **(Figure 7A)**. Because RBC are metabolically active cells, this accumulation of RBC at the site of sensor implantation can act as a metabolic barrier to glucose for the sensor **(Figures 7A and 7B)**. This "metabolic barrier" is the result of RBC consumption of the bulk of the glucose diffusing in from the vasculature, thus preventing glucose from reaching the sensor **(Figure 7C)**. As a result of this RBC-based "metabolic barrier," the microenvironment surrounding the sensor is depleted of glucose. This glucose depletion is reflected by the low sensor output (i.e., low nanoamperes). This "apparent" loss of sensor function, relative to circulating blood glucose levels, actually reflects the glucose levels that are occurring within the microenvironment of the sensor-blood clot. Vice versa, this glucose level in the microenvironment of the sensor does not reflect glucose level distant from the sensor. Clearly, future *in vivo* studies to investigate these possibilities need to be undertaken to dissect the role as well as the mediators and mechanisms of blood clot-induced sensor interference.

Conclusion

The present studies demonstrated that blood clot interference of the Abbott Navigator sensor is not related

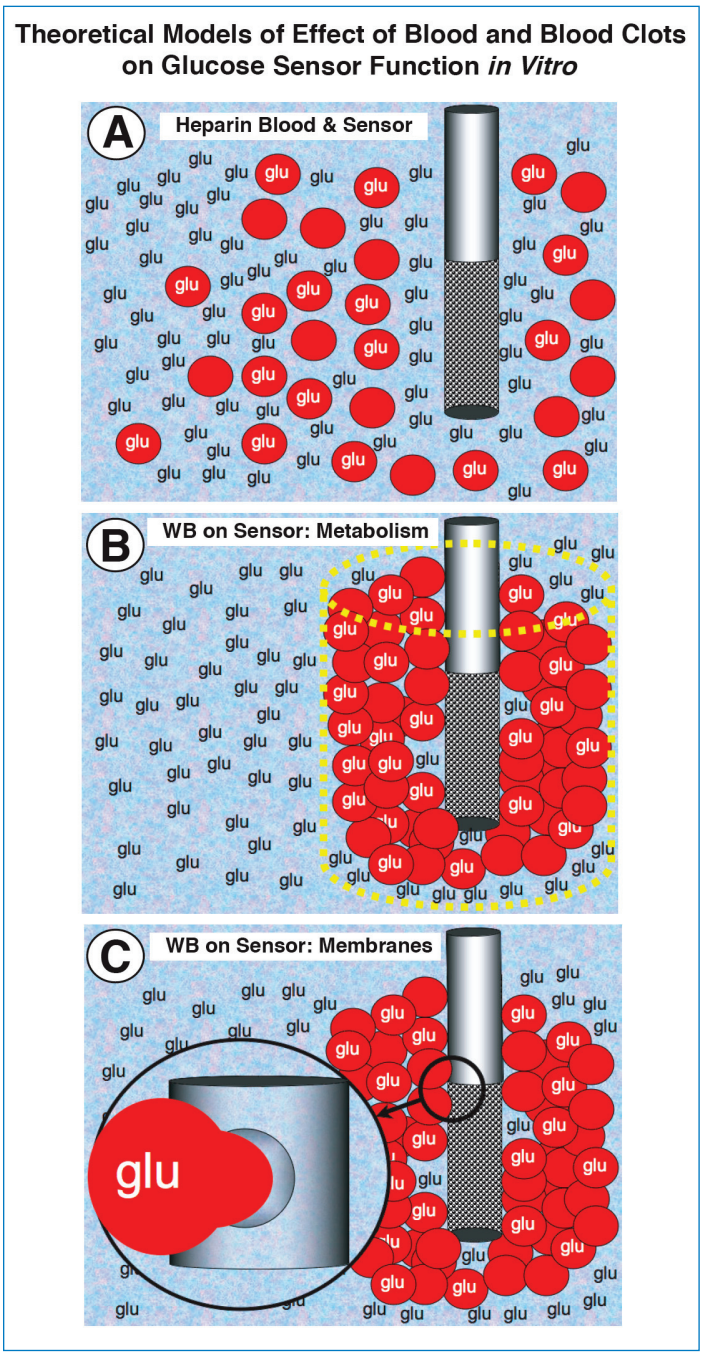

Figure 7. Theoretical models of the effect of blood and blood clots on glucose sensor function *in vitro*. This theoretical model presents possible mechanisms involved in blood **(A)** and blood clot **(B** and **C)** effects on sensor function *in vitro*. **(A)** A model of slow glucose metabolism with a parallel fall in sensor output. **(B)** RSL as a result of "coating" of the blood clot (RBCs plus fibrin) around the sensor, resulting in a metabolic barrier to glucose diffusion into the sensor. **(C)** A model in which the fibrin blood clot causes membrane blockage of sensor pores, thus blocking glucose diffusion into the sensor. It is likely that the mechanisms presented in **B** and **C** are both involved in RSL in vitro and act similarly to induce loss of sensor output *in vivo*.

to biofouling by proteins, including clot proteins, as HWB, serum, plasma-derived clots, or TL did not interfere with GSF *in vitro*. Additionally, the apparent rapid decrease in sensor function for sensors incubated in WB is not the result of biofouling of the sensor membrane but rather reflects mainly RBC metabolism in the microenvironment of the sensor. These studies demonstrated that human blood clots interfere with GSF *in vitro*, likely by increasing RBC density around the sensor. This high local density of RBC around the sensor increases glucose metabolism and hence a rapid depletion in local glucose levels around the sensor (metabolism). Additionally, deformability of RBC allows possible mechanical blocking of sensing area (membranes). "Metabolism and membranes" both cause a sharp decrease in sensor functionality. These studies support the hypothesis that hemorrhages, with associated formation of blood clots at sites of glucose sensor implantation, have an impact on GSF *in vivo*. Minimizing hemorrhage and blood clot formation at sites of sensor implantation could enhance the sensor performance and life span of sensors *in vivo*. Furthermore, any tissue injury with associated hemorrhage will also present a more significant problem to long-term glucose sensing, as they will induce additional inflammation and associated fibrosis, thereby decreasing the sensor life span. Ongoing *in vivo* experiments in the mouse support our initial hypothesis that hemorrhages at the site of glucose sensor function cause a drop in sensor functionality. This loss of sensor function is usually of a temporary nature. We anticipate that these *in vivo* mice studies will be submitted for publication in the near future and will provide additional insights into the role of blood and blood clots in the acute loss of sensor function *in vivo*. Nonetheless, these studies should help in designing strategies and approaches in overcoming the initial delay in sensor functionality, as well as long-term effects on sensor function *in vivo*.

Acknowledgment:

This work was supported by Abbott Diabetes Care, Alameda, California.

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