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*COMMENTARY*

# Glucose Sensing in the Subcutaneous Tissue: Attempting to Correlate the Immune Response with Continuous Glucose Monitoring Accuracy

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Safe and effective blood glucose (BG) control using a closed-loop artificial pancreas system requires a continuous glucose monitoring (CGM) system that is reliable, accurate, timely, and easy to use.[1–5] The real-world performance of commercial needle-type subcutaneous tissue glucose sensors has significantly improved over the past 20 years, due to optimization of their mechanical design, porous membranes, enzyme electrochemistry, signal processing, automated introducer, and method of manufacture.[6–8]

Despite these technological advances, the accuracy and longevity of current CGM are limited due to the subcutaneous tissue's cellular and humoral immune response to glucose sensor insertion and maintenance.[9–14] The tissue's reaction to short-term sensor implantation (1–7 days), medium-term implantation (7–14 days), and long-term implantation (>14 days) includes tissue injury, blood–biomaterial interaction, provisional matrix formation, acute inflammation, chronic inflammation, granulation tissue development, foreign body reaction, and fibrosis/fibrous capsule formation.[15] The duration of each sequence and degree of immune reaction depend upon the tissue type, the amount of initial tissue trauma, the biomaterial's size, shape, composition, and surface texture, additional tissue trauma due to macro-/micromotion, chemical leaching, electric current leaching, and the age and immune competency of the individual patient.[11,14–22]

Insertion of a glucose sensor's needle and electrode through the epidermis and dermis into the subcutaneous tissue damages cells, connective tissue, and extracellular matrix.[9,11,12] The sensor's thin and flexible electrode remains within this region of subcutaneous tissue after removal of the introducer needle. Injured arterioles, capillaries, and venuoles release plasma, red blood cells, white blood cells, and activated platelets into the surrounding tissue.

Arterioles may initially constrict to limit blood loss but will eventually dilate to increase local blood flow for nutrient delivery and waste product removal. Activation of the coagulation cascade and the release of cytokines cause capillaries to leak protein-rich plasma into the wound that coats the electrode with a layer of proteins and thrombus.[15] A chemical gradient guides the migration of acute inflammatory cells from adjacent vascular tissue to the local environment surrounding the implanted electrode.[24]

Neutrophils and monocytes/macrophages infiltrate the surrounding tissue and actively phagocytize bacteria, injured cells, and debris, and release protease enzymes and reactive oxygen species. Fibroblasts, lymphocytes, eosinophils, and mast cells also migrate from adjacent capillaries into the wound and secrete a wide variety of cytokines, chemokines, and growth factors that modulate the acute inflammatory and wound healing process.[11,14,15,17,22–24]

Glucose sensor performance (accuracy, time lag, and signal stability) can be highly variable for 1–12 h after insertion of the electrode into the subcutaneous tissue. The reasons for this variability are multifactorial, complex, and poorly understood.[25,26] Loss of functional capillary vessels and local vasoconstriction can decrease the delivery of glucose, oxygen, and other nutrients, and decrease the removal of cellular waste products. Injured lymphatic vessels can limit the removal of edema fluid, cellular waste products, and phagocytized debris. The tissue surrounding the glucose sensor electrode may become acidotic due to the accumulation of carbon dioxide, lactic acid, and protons, an environment that may cause variable enzyme function (glucose oxidase/dehydrogenase) or function of the electrochemistry.[11,27] The large number and high metabolic activity of the neutrophils and macrophages that surround the implanted electrode may further decrease the local concentration of oxygen and increase interstitial fluid acidosis.

A layer of acute inflammatory tissue (containing damaged cells, connective tissue, edema fluid, and immune cells) may surround the working electrode to become a mechanical or physical barrier that significantly inhibits/slows the inward diffusion of glucose and oxygen. Metabolically active cells adjacent to the electrode (red blood cells, macrophages, and

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neutrophils) actively consume glucose, significantly decreasing the number of glucose molecules reaching the working electrode. Macrophages have been identified as the major cell type producing a ''Cell-Based Metabolic Barrier'' that limits the diffusion of glucose from the adjacent interstitial fluid to the sensor's electrodes, causing an artificially low sensor output signal.[10,12,28] Thus, performance of an enzyme-based electrochemical glucose sensor may be significantly affected by the dynamically changing local tissue environment immediately adjacent to the working and reference electrodes.[16,25]

The cellular, humoral, and chemical environment surrounding a CGM electrode will start to stabilize within several hours and become more stable within 12 h of implantation, depending upon the amount of initial tissue trauma, ongoing tissue trauma due to body movement, and the degree of immune response produced by the individual patient. Thrombus will undergo fibrinolysis, neutrophils and macrophages will continue to phagocytize debris, and capillary vessels will regain their vasomotor control and no longer release protein-rich fluid into the wound.[15,27]

Neutrophil numbers will decrease after a few days if the wound does not become contaminated/infected with bacteria. Macrophage numbers will increase over time to further phagocytize debris and modulate the immune response. Fibroblasts will transform into myofibroblasts in 2–5 days and synthesize extracellular matrix and collagen with fibrils that may parallel the surface of the electrode in an attempt to confine or wall off the foreign body.[19–21] A dense layer of macrophages may surround the electrode and combine to produce foreign body giant cells.[15,22] A layer of inflammatory cells and fibrous tissue that becomes thick, dense, and continuous may significantly affect sensor performance and limit longevity.[10,17,22] The formation of granulation tissue with new normally functioning capillary and lymphatic vessels may take several weeks to develop and probably will not form in the environment immediately adjacent to the CGM's electrodes.[29,30]

Early generation subcutaneous tissue glucose sensors were Food and Drug Administration (FDA) approved for only 3 days of use and required frequent recalibration multiple times per day to maintain sufficient accuracy for real-time monitoring, but not good enough for dosing insulin without a confirmatory BG measurement. Improvements in electrode insertion, size, softness, flexibility, membrane biomaterial, and electrochemistry have decreased the degree of initial and ongoing tissue damage, leading to enhance sensor stability/performance.

These enhancements along with improvements in signal averaging and filtering have improved accuracy enough for the FDA to approve the use of real-time CGM data to dose insulin without requiring a confirmatory BG measurement.[4–8,31] Recent clinical trials using current CGM in closed-loop and hybrid closed-loop artificial pancreas systems have been extremely promising.[1–3] Despite these advances, the accuracy, lag time, reliability, longevity, and overall clinical performance of current commercial CGM systems are limited by the variable subcutaneous tissue's immune response to needle/electrode insertion and maintenance.

In a recent issue of the journal *Diabetes Technology & Therapeutics*, Rigla et al. describe a human clinical trial that correlated the subcutaneous tissue's immune response with a metric of CGM accuracy.[32] The authors should be commended for completing a systemic study in ambulatory humans that included a qualitative/quantitative analysis of tissue histology surrounding implanted glucose sensors for 1 and 7 days. These data are important because the number of CD68 positive macrophages/0.01 $mm^2$ surrounding the electrodes was significantly higher in those CGM that had a mean absolute relative difference (MARD) >10%, whereas the CGM electrodes surrounded by significantly fewer macrophages had a lower MARD% (better accuracy). This association between macrophage location/density on CGM performance and other histology findings is consistent with prior in vitro and animal study results.

Unfortunately, the current human study methods have many limitations that may seriously affect the conclusions. The iPro®2 CGM system was used with a second-generation Enlite® glucose sensor and CareLink iPro software (Medtronic MiniMed, Northridge, CA) to determine MARD for the 7-day study.[33,34] This system records sensor signals for 6 days and requires a retrospective calibration using two reference BG measurements at start up and at least three reference BG measurements per 24 h, no longer than 12 h apart.[35] The number of BG measurements used to calculate MARD was limited to one to four per day because the 12 study subjects self-monitored their blood glucose (SMBG) using a commercial glucose meter and test strips only $5.7 \pm 1.6$ times per day.

In addition, the methods do not describe which SMBG measurements were used for CGM calibration or used for calculating MARD. SMBG measurements used for calibration should be obtained during a period of glucose stability to minimize calibration error.[34,36] Since five of the study subjects were not diabetic and seven of the subjects had type 2 diabetes mellitus (T2DM), the overall mean BG level was $113 \pm 17$ mg/dL with only an 87.6–158 mg/dL range. Diabetes is known to affect the immune response to tissue injury and wound repair. The results do not report whether diabetes status affected per day MARD or histology results, and whether BG range or rate of change during SMBG measurements used for calibration affected MARD. A quality CGM accuracy study requires a much larger number of BG measurements per day using a reference analyzer over a wider range of BG values and rates of change.[4,5,8,34,37–39]

Of note, the number of macrophages and tissue histology results obtained on day 7 were correlated with MARD results from CGM data recorded for the entire 7-day period. This correlation should have been limited to CGM data recorded from day 7 only or perhaps days 6 and 7 due to the dynamically changing environment surrounding the CGM electrodes. There may have been few macrophages located adjacent to the electrode for several days after implantation of some sensors. In addition, the histology analysis should have quantified the thickness, density, and continuity of the surrounding layer of inflammatory/fibrous tissue to determine whether these mechanical parameters correlated with MARD.

In conclusion, Rigla et al. successfully demonstrated a significant correlation between the number of macrophages surrounding the CGM electrodes implanted in humans for 7 days and overall glucose sensor accuracy. These data support the importance of the local tissue environment immediately adjacent to the CGM electrodes on glucose sensor performance, especially the location and number of

metabolically active macrophages. The authors are encouraged to continue this important area of research in patients with type 1 diabetes mellitus (T1DM) and T2DM using real-time CGM systems over a wide range of glucose values, understanding the difficulty in recruiting patients scheduled to undergo abdominoplasty surgery. Study of the subcutaneous tissue's acute inflammatory reaction to CGM implantation over a 14-day period would facilitate the development of next-generation glucose sensors with enhanced accuracy and longevity.

### Author Disclosure Statement

J.IJ. is a cofounder and equity owner of Capillary Biomedical, Inc., a company dedicated to developing glucose sensor and insulin delivery systems for people with diabetes mellitus. He is also the Chairman of Capillary Biomedical's Scientific and Clinical Advisory Boards. J.IJ. is also an equity owner and member of the Scientific Advisory Board of Thermalin Diabetes, Inc., a company dedicated to developing novel insulin formulations for people with diabetes. M.C.T. is an equity owner and member of the Scientific Advisory Board of Capillary Biomedical. Research at the Jefferson Artificial Pancreas Center related to insulin delivery has been funded by NIH-NIDDK, The Juvenile Diabetes Research Foundation (JDRF), Frederick Banting Foundation, Capillary Biomedical, and Thermalin Diabetes. G.E., D.D., A.K., and C.L. have nothing to disclose.

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