Reduction of measurement noise in a continuous glucose monitor by coating the sensor with a zwitterionic polymer

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Continuous glucose monitors (CGMs), used by patients with diabetes mellitus, can autonomously track fluctuations in blood glucose over time. However, the signal produced by CGMs during the initial recording period following sensor implantation contains substantial noise, requiring frequent recalibration via finger-prick tests. Here, we show that coating the sensor with a zwitterionic polymer, found via a combinatorial chemistry approach, significantly reduces signal noise and improves CGM performance. We evaluated the polymer-coated sensors in mice as well as in healthy and diabetic non-human primates, and show that the sensors accurately record glucose levels without the need for recalibration. We also show that the coated sensors significantly abrogated immune responses, as indicated by histology, fluorescent whole-body imaging of inflammation-associated protease activity and gene expression of inflammation markers. The polymer coating may allow CGMs to become standalone measuring devices.

Diabetes mellitus is a metabolic disorder where blood glucose (BG) regulation is hampered, and affects hundreds of millions of people worldwide with many still undiagnosed1–4. The current gold standard clinical treatment for diabetic patients is to practice self-monitoring of blood glucose (SMBG) by measuring their BG levels (BGs) from finger-prick-drawn blood several times a day, followed by injecting insulin as necessary to bring BG back into a normal range5–6. However, SMBG is not capable of capturing BG fluctuation over time. These deficiencies together with the pain associated with repetitive finger-prick tests render SMBG an unfavourable practice for both patients and doctors7,8.

In the past several decades, more sophisticated implantable devices for glycemic tracking such as continuous glucose monitors (CGMs) have been developed9–12. Of note, three companies, Medtronic/MiniMed13, Dexcom14 and Abbott, have competing technologies that allow continuous recording of BG fluctuations in interstitial fluid of the subcutaneous space15. In contrast to SMBG, traditional CGMs can capture BG fluctuations continuously and therefore enable complete tracking of blood glucose trends over time16. However, issues with reliability and short-term noise, as well as requirements for daily calibrations, have limited their potential, and the Food and Drug Administration (FDA) has not, until recently, approved the use of traditional CGMs for up to 6 days post-implantation, but only alongside constant finger-prick blood recalibrations (that is, four on the first day of use and one every 12h thereafter)16–19,22,23. The noise period ends up complicating ~30 to 50% of approved CGM duration, and the requirement for such frequent recalibration, which is both wearisome and painful for users, can lead to inaccurate BG measurements22,24–26. While the exact mechanisms behind CGM noise and fluctuation remain unclear, measurement of glucose with commercial sensors is oxygen based (glucose oxidase)27,28, and has been shown to be influenced by the presence of various pharmacological agents or cells that result in significant oxygen or glucose fluctuation29,30. We hypothesized that the inflammatory response to the materials that the CGM is composed of, as also implicated by others31,32, might be the driving force behind signal fluctuation following implantation.

Foreign-body response including inflammatory events and fibrosis due to wound-healing processes are a major hindrance to implanted biomaterial sensors33–34. To combat such responses, which can interfere with sensing and lead to device failure, surface modifications35 or drug delivery systems36,37 have been developed to enhance their biocompatibility. However, limited success has been achieved on alleviating host response to CGMs to fully restore their performance.

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functional reliability. Among the many natural and synthetic materials used as coatings for implantable devices, zwitterionic polymers have received considerable attention due to their ultralow fouling properties and hindering of non-specific protein adsorption, leading to reduced capsular formation46–49.

Beyond the initial phase of noise, CGMs face additional challenges to long-term use including (1) fibrinotic capsule formation around the sensor which can limit analyte transport50–54, (2) long-term, onboard data recording and battery life55, (3) adhesive glue durability and safety56, and (4) inactivation of glucose oxidase and BG sensitivity57. All of these issues, however, are not as significant until the end of the 6 to 7-day range (now 10-day range with Libre) of the current FDA-approved CGM lifespan55–59,60. In addition, the recently FDA-approved Libre platform from Abbott utilizes an internal factory calibration to largely address sensor to sensor variability; however, issues of inaccuracy, noise and host response remain (Supplementary Section 1.1)60,61.

Zwitterionic library screen for identifying chemistries

In this work, a coating based on zwitterionic polymers was developed using combinatorial chemical approaches, and applied to Medtronic CGMs, with the goal to mitigate the acute, interfering host inflammation and potential effects on signal noise (Fig. 1a). Uncoated sensors show significant noise within the first day after implantation and required BG calibration (at least four times on the first day of use and at least two times every day thereafter) every day to correct signal trends. In contrast, the zwitterionic polymer-coated sensors showed significant improvement on eliminating sensing noise and were able to record glucose levels accurately without recalibration beyond the first glucose blood measurement, necessary to convert raw sensor signals to real-time BG values57. These results were observed in both SKH1 mice and non-human primate diabetic and healthy non-diabetic models. Inflammation profiles following sensor implantation were measured using a number of orthogonal methods including in vivo imaging using ProSense fluorescence imaging, histological studies and gene profiling. Here we show that while inflammation is a primary cause of sensor noise, the critical variation seen early following implantation is not due to fibrous capsule formation58. The zwitterionic polymer coating reduced inflammation and CGM noise compared with naive sensors. This technology is significant for subcutaneously embedded glucose monitors as it overcomes the most significant issue of sensor noise, deviation from actual measured BGs, while also improving user experience by eliminating the need for recalibration, traditionally often occurring multiple times throughout the first week of sensor use.

To assess the sensor coating and functionality we used the Medtronic MiniMed CGM as a model sensor for experimentation, as it is a typical CGM, commercially available, and the data can be exported for research purposes56,61. The Medtronic CGM consists of a recorder and a glucose sensor that detects glucose through a conductive gold electrode coated by a thin membrane consisting of a conductive gold electrode coated by a thin membrane embedded with the glucose specific enzyme, glucose oxidase (GOx)62. This layer selectively immobilizes GOx but also allows small molecules such as glucose, O2 and H2O2 to diffuse through. The GOx catalyses the oxidation of glucose to gluconolactone, and produces H2O2 that changes the electrical current on the electrode surface53,54,55 (Fig. 1c and Supplementary Section 2). The electrical signal (ISIG signal) is recorded in the CGM and can be later exported for analysis. To improve CGM biocompatibility and reduce associated inflammation, a zwitterionic polymer was developed to coat the sensor electrode. Using a combinatorial approach, 64 various zwitterionic polymer hydrogels (Fig. 1d and Supplementary Section 3) were synthesized using four-arm polyethylene glycol (PEG) polymers (2 kDa or 5 kDa) as crosslinkers, and screened for their immuno-nostimulatory properties. PEG is desirable as its use allowed for a wide range of chemical-structure library synthesis. While other zwitterionic hydrogels have been used on CGMs, to our knowledge they have not been tested in vivo63,64. For in vivo characterization, zwitterionic polymer hydrogels were injected in SKH1 mice subcutaneously, as this site had the lowest potential for PEG hydrogel degradation kinetics of all those tested55. Inflammation to the hydrogels was measured using a ProSense substrate for inflammation-associated protease activity (Fig. 1e) at day 7, a time point that is more optimal for hydrogels that are less immunogenic than polymer plastics65. This is due to the fact that the biomaterial class of hydrogels is less immunogenic than hard polymers and metals, which are both part of the multicomponent CGM devices. SKH1 mice, while they do have a lower background than C57BL/6 mice, due to not having any fur, do mount robust foreign-body or fibrosis responses in the subcutaneous implant space (Supplementary Fig. 3.2)55. While it is difficult to fully attribute inflammatory profiles of different PEG chemistries to specifically higher molecular weight or to degradation, we saw a generally lower inflammatory profile for zwitterionic chemistries utilizing shorter PEG crosslinkers (Fig. 1e), but did not macroscopically observe degradation on histological assessment (Supplementary Fig. 3.2).

Effects of modifications on CGM function and signal noise

In addition to issues with mechanical stability and shearing off CGM surfaces following insertion into the body, zwitterionic hydrogels can absorb water, resulting in swelling and subsequent inflammation inside body (Supplementary Fig. 3.3). As such, hydrogel coatings were not used; instead, the lead zwitterionic polymer, methacryloyloxyethyl phosphorylcholine polymer (poly(MPC)), was identified from our library screen (* in Fig. 1e) and used to coat the CGM sensor electrode. Poly(MPC) (number-averaged molecular mass, Mn: 10 kDa, polydispersity index, PDI: 1.1) containing free pendant thiol groups along the backbone was yielded from a reversible addition–fragmentation chain-transfer polymerization of MPC and lipic acid methacrylate monomers, followed by disulfide reduction (Fig. 2a). Dopamine-mediated conjugation56–58 was used to couple the poly(MPC) to the sensor electrode (Fig. 2b). By immersion of the sensor electrode in a 3 mg ml−1 dopamine solution (pH 8.5) for 24 h, polydopamine films were coated to the electrode surface by oxidative self-polymerization of dopamine. After rinsing, poly(MPC)–SH was conjugated to the polydopamine-coated electrodes by treating the electrode with poly(MPC)–SH solution (pH 8.0) at 37°C for 24 h. The coating of the electrode with the zwitterionic polymer was confirmed using X-ray photoelectron spectroscopy (XPS) analysis (Fig. 2c,d and Supplementary Section 4). The characteristic peak of phosphorus groups of the poly(MPC) at 188 eV (P 2p peak) and at 131 eV (P 2s peak) indicates the zwitterionic polymer was successfully coated on the polymer shell of the electrode (Fig. 2d). Before coating, phosphorus groups were not observed on the electrode (Fig. 2c).

The coated sensors were examined using an in vitro glucose sensing assay, to evaluate whether sensing performance and sensitivity were compromised by the polymer coating or the coating process. In the assay, the initial glucose concentration was 100 mg dl−1 and it was successively increased to 200, 300 and 400 mg dl−1 every 30 min, allowing the sensors (two non-coated control sensors and two coated sensors) to track the change in glucose concentration (Supplementary Section 4). The recorded signal was plotted as signal versus time and normalized (Fig. 2e). For both coated and control sensors, as the glucose concentration increased, correspondingly the signal immediately increased, indicating sensitive response to the glucose concentration. The rising edge of the signal is not completely straight, likely due to time required for equilibration of the
The sensing curves of the coated and non-coated sensors overlapped well and the signal responses of the sensors were linearly correlated to the glucose concentration. Overall, these in vitro results indicate that the zwitterionic poly(MPC) coating did not disrupt the performance and response of the sensors.

**Fig. 1 | Illustration of CGM sensing in vivo.**

- **a**, Non-coated sensor induces inflammatory immune cascade, and the host response causes sensor noise and inaccuracy requiring frequent BG calibrations. The zwitterionic polymer-coated sensor overcomes the hostile in vivo environment, eliminating sensor noise and the requirement for BG recalibrations.

- **b**, Components of the CGM, including bright-field and scanning electron microscopy images of the CGM electrode.

- **c**, Illustration of the enzymatic mechanism of glucose detection by the electrode.

- **d**, Examples of different zwitterionic copolymer units utilized for constructing biomaterial combinatorial library. MPC stems from phosphorylcholine, CB1–CB3 stem from carboxybetaine with different spacer lengths, and SB1, SB2 represent slightly different sulfobetaine zwitterionic structures.

- **e**, Biocompatibility (inflammation profile) results from the zwitterionic biomaterial library screen. The combinatorial library contained 64 various zwitterionic polymer hydrogels using four-arm PEG polymers (2 kDa or 5 kDa) as crosslinkers. Note: inversion of monomers (that is, CB1–SB1 and SB1–CB1) indicates that they have the same polymeric structure with different mole ratios of the monomers (see the Supplementary Information for further elaboration and a list of full chemical names). Experiments were repeated at least two or three times.
In vivo sensing of zwitterionic polymer-coated sensors and control sensors (without coating) was performed in SKH1 mice. The electrode was inserted subcutaneously with a guide needle, and the sensor base and recorder were immobilized on the back of mice with an adhesive tape harness (Supplementary Section 5). Following insertion, the mice recovered from anaesthesia and were able to move freely (Fig. 3a). The electrode interacted with interstitial fluid containing glucose, and the electrical signal was recorded at 5 min intervals for three continuous days. Glucose challenges of 250 mg per mouse were performed to induce glucose-level spikes and BG fluctuations on days 1, 2 and 3 of recording. BG values were measured with glucose test strips (8 times each day) on days 1–3 covering the period of glucose challenges. At the end of the three-day recording period, recorders were retrieved from the mice and the stored electrical signals were exported. The results of two representative control sensors and two coated sensors are shown in Fig. 3b–e, while the results with more sensors (six control sensors and six coated sensors in total) are shown in Supplementary Section 6.

Fig. 2 | Zwitterionic polymer coating of Medtronic CGMs. a, Synthesis scheme for the identified hit polymer, poly(MPC). CTA, 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (RAFT chain transfer agent); ACVA, 4,4’-azobis(4-cyanovaleric acid) initiator (azo initiator); DMAc, dimethylacetamide (solvent). b, Illustration of the modification of the sensor electrode surface with zwitterionic poly(MPC) through dopamine-mediated conjugation. c,d, Examination of the electrode surface coating using XPS analysis. The characteristic peaks of phosphorus groups of the poly(MPC) at 131 eV (P 2p peak) and at 188 eV (P 2s peak) were examined. e, The control and coated sensors were examined using an in vitro glucose sensing assay. The black line indicates the initial glucose level and the black arrows the time point when the glucose concentration was successively increased. Experiments were repeated at least three times.

In vivo sensing of zwitterionic polymer-coated sensors and control sensors (without coating) was performed in SKH1 mice. The electrode was inserted subcutaneously with a guide needle, and the sensor base and recorder were immobilized on the back of mice with an adhesive tape harness (Supplementary Section 5). Following insertion, the mice recovered from anaesthesia and were able to move freely (Fig. 3a). The electrode interacted with interstitial fluid containing glucose, and the electrical signal was recorded at 5 min intervals for three continuous days. Glucose challenges of 250 mg per mouse were performed to induce glucose-level spikes and BG fluctuations on days 1, 2 and 3 of recording. BG values were measured with glucose test strips (8 times each day) on days 1–3 covering the period of glucose challenges. At the end of the three-day recording period, recorders were retrieved from the mice and the stored electrical signals were exported. The results of two representative control sensors and two coated sensors are shown in Fig. 3b–e, while the results with more sensors (six control sensors and six coated sensors in total) are shown in Supplementary Section 6.
Medtronic CareLink iPro Therapy Management software was used to convert electrical signals to glucose levels by correlating the electrical signal to a corresponding measured BG value at that same time point. At the time point when BG measurement was performed, the electrical signal was plotted on the x axis, and the measured BG was plotted on the y axis. As shown in Fig. 3b, these data points were plotted as open circles, with different colors indicating different days. Within a short-time period (<12 h) the signal versus BG value followed a linear trend. However, for longer periods (>24 h), this mathematical algorithm to describe the relationship became more complex due to possible changes of the physiologic environment induced by host response including inflammation. The CareLink software fitted the relation between the electrical signal and the BG value for each day using a single linear equation. For the control sensors, the linear relationships of signal calibration versus BG value continued to deviate over all three days indicating sensor performance was disturbed during that timeframe (Fig. 3b, control #1 and #2). In contrast, the coated sensors all showed linear relationships of signal versus BG values that overlapped well across each measurement day, suggesting sensor performance was not disturbed (Fig. 3b, coated #1 and #2). In both cases, however, open circle BG values with higher deviation from linear regression lines occurred during glucose challenges (Supplementary Fig. 6.3), which may possibly be due to calibration algorithms dampening high BG fluctuations due to Kaman filtering, utilized to reduce signal-to-noise patient-to-patient variability.

Generated linear regression equations can also be used to fit signal values to BG values, allowing for glucose levels to be calculated based on the recorded electrical signal at the corresponding time point (Fig. 3b, solid dots). By using all the measured BG values from different days to calibrate the signal (with ‘recalibration’), the calculated glucose level versus time was plotted as the...
sensor function and the ability for CGMs to accurately and reliably suggest that an early host inflammation response is interfering with space. The inflammation profiles decreased with time from day 1 within the first 1–3 days following insertion into the subcutaneous tissue. Each mouse was implanted with either a control or coated electrode with an in vivo fluorescence imaging system (IVIS) using a ProSense libration. These results suggest that the zwitterionic polymer coat-15 recording period, are statistically shown in Fig. 3e (N = 6 for each group). Control (uncoated) sensors showed 73 ± 36% inaccuracy of non-recalibrated glucose levels compared with actual BG values; however, recalibration significantly decreased this inaccuracy to 15 ± 17%. Conversely, coated sensors showed only an inaccuracy of 17 ± 11% without recalibration and 11 ± 9% inaccuracy with recalibration. These results suggest that the zwitterionic polymer coating significantly improves the performance and accuracy of CGMs in mice and, equally important, eliminates the (industry norm and manufacturer directed) requirement of recalibration by painful, repetitive finger-prick BG normalization, often required of users multiple times each day.

Improved biocompatibility and reduced signal noise
The inflammation profiles after sensor insertion were evaluated with an in vivo fluorescence imaging system (IVIS) using a ProSense substrate indicating inflammation-associated protease activity. Each mouse was implanted with either a control or coated electrode and monitored over multiple days post-implantation (Fig. 4a). The IVIS inflammation profiles were quantified and shown in Fig. 4b. Correlating with the timeline of observed CGM sensor noise, host inflammation responses are largely acute and most prominent within the first 1–3 days following insertion into the subcutaneous space. The inflammation profiles decreased with time from day 1 to day 8, and, on average, at all time points measured (days 1, 3 and 8 post-insertion), skin around zwitterionic coated sensors had reduced inflammation profiles compared with the control sensors (Fig. 4a). The similar kinetics of this dissipating response to those of decreasing signal noise over time following CGM implantation suggest that an early host inflammation response is interfering with sensor function and the ability for CGMs to accurately and reliably measure glucose levels. Furthermore, the observed inflammation is also foreign-body induced and independent of wound injury, as insertion and immediate removal of the delivery-assisting guide needle does not result in significant inflammation (Fig. 4a, bottom left, mock mice). Therefore, the observed interfering host inflammatory response requires a material implant to be present. In addition, four electrodes, including two control electrodes, one control polyurethane tubing and one coated electrode, were also inserted into the same mice to compare their inflammation profiles directly (Fig. 4c). Similar to the results observed in Fig. 4a, the inflammation profiles on the skin around each inserted material decreased with time from day 1 to day 8. The inflammation on the skin around the coated electrode was also significantly reduced compared with the other three control samples.

To better understand this inflammation, sensor-embedded tissues were dissected and prepared for histological examination. Cellular infiltration (haematoxylin and eosin (H&E)) and fibrotic tissue overgrowth (Masson’s trichrome) surrounding both implanted electrodes increased with time from day 1 to day 8 in SKH1 mice (Fig. 4d). Overall fibrosis for the zwitterionic coated sensors appeared reduced (Fig. 4d, top versus bottom). While fibrosis increases over time, sensor noise is limited to early immune inflammation throughout the first 3 days following insertion (Fig. 3). As CGM noise also continuously decreases over this time in an inversely proportional relationship, we believe that these data are consistent with the hypothesis that capsule formation is not the primary driver in early sensor signal noise. Furthermore, while the possibility that additional hydrogen peroxide species production, which might result as a consequence of onboard glucose oxidase, it is not clear that the amount of hydrogen peroxide produced by these FDA-approved sensors is a key factor driving noise and inflammatory profile. Of note, when we measured reactive oxygen species (ROS) levels with two different substrate systems (Supplementary Fig. 7.3), we found no difference between measured ROS for uncoated control and coated sensors. As ROS levels do not correlate with the presence or lack of observed sensor noise, we therefore do not feel they are responsible. By comparison, as there was a clear reduction in IVIS-measured inflammatory signature, we hypothesized that other components of the inflammatory response must be involved and that the zwitterionic polymer is acting by reducing this particular type of biomaterial implant-induced inflammation.

At this point, multiplexed NanoString gene-expression analysis was performed to compare tissues from control and coated implants with mock saline injected background (Fig. 4e). The results indicate many inflammatory markers, cyto/chemokines Cxcl15, Tfnsf18, Cxcl17, IL17b and Ccl20, as well as B-cell marker Cd19, were increased in tissues with control electrodes, especially one day following implantation (Fig. 4e, control day 1 versus mock). Importantly, these factors were not increased, and in some cases were suppressed below mock background levels in tissues surrounding zwitterionic coated CGMs (Fig. 4e, coated day 1 versus control day 1 and mock). In addition, coated sensors also had suppressed (lower than mock background) levels of macrophage markers C6d8 and Emr1 (F4/80), eosinophil marker Prg2, immune marker Kit, as well as numerous other cytokines and cytokine receptors. Corroborating the earlier Masson’s trichrome histology, tissue surrounding coated sensors also had lower levels of fibrosis-associated genes such as myofibroblast marker alpha Smooth muscle actin (αSMActin) and collagen 1a1 (Col1a1; Fig. 4e). These results suggest that the zwitterionic coating eliminates numerous inflammatory responses that may play a role in or be responsible for sensor-associated noise.

Elimination of signal noise in non-human primate models
Zwitterionic polymer-coated sensors were also tested in non-human primates (NHPs). For each testing run, four coated and four control sensors were subcutaneously inserted into the back of non-diabetic or diabetic NHPs (Fig. 5a). The recorders were also sutured into blue curve (Fig. 3c). Actual BG values were plotted as black dots for comparison. Yellow banded regions in Fig. 3c were used to visually indicate the time period of day 2. To understand manufacturer-mandated requirement of calibrating the control sensor every day, we generated glucose-level trends from signal data using only a single, initial measured BG value on the first day, without further calibration (‘non-recalibration’) for the next two days (Fig. 3c, red curve). Spiking glucose levels are due to glucose challenges. Based on these results, it was found that the recalibrated glucose levels (blue curve) recorded from control sensors did not overlap well with actual BG values (black dots). Even with frequent recalibration there remained imprecision throughout the first day of recording indicating that calibration of the control sensors cannot completely get rid of inaccuracy and noise. Furthermore, the non-recalibrated glucose trend (red curve) from control sensors deviated more significantly from both the actual BG value and the recalibrated glucose trend, showing that control sensors failed to depict accurate glucose levels without frequent calibration. Conversely, for coated sensors, the recalibrated and non-recalibrated glucose trends (blue versus red curve) overlapped with each other as well as with measured BG. Thus, coated sensors are able to record glucose levels accurately even without the need to recalibrate every day. These results were observed consistently on all six control and six coated sensors (Supplementary Section 6).

For all sensors in Fig. 3c, the recalibrated and non-recalibrated glucose trends were compared with measured BG values at the appropriate corresponding time points, and their deviation (% difference) from BGs is shown in Fig. 3d. Control sensor-recorded glucose levels showed a significant difference compared with measured BGs after day 2 when no recalibrations were performed, while the coated sensors showed no significant deviation. For coated and control sensors, comparisons between non-recalibrated versus (re) calibrated glucose trends, non-recalibrated versus measured BGs, and recalibrated versus measured BG values, during the entire recording period, are statistically shown in Fig. 3e (N = 6 for each group). Control (uncoated) sensors showed 73 ± 36% inaccuracy of non-recalibrated glucose levels compared with actual BG values; however, recalibration significantly decreased this inaccuracy to 15 ± 17%. Conversely, coated sensors showed only an inaccuracy of 17 ± 11% without recalibration and 11 ± 9% inaccuracy with recalibration. These results suggest that the zwitterionic polymer coating significantly improves the performance and accuracy of CGMs in mice and, equally important, eliminates the (industry norm and manufacturer directed) requirement of recalibration by painful, repetitive finger-prick BG normalization, often required of users multiple times each day.
the skin, and a zipped jacket was placed over the sensors to prevent them from falling out or being pulled out by the NHPs, which have social grooming behaviour. Glucose levels were then recorded for five continuous days. Non-diabetic and diabetic NHPs were glucose-challenged with 0.5 g glucose kg\(^{-1}\) NHP body weight by intravenous infusion on each of the first three or four days, respectively, to induce dynamic signal spikes and fluctuations in their glucose levels. This was done to test control (non-coated) and zwitterionic polymer-coated sensors not only over an extended period of time but also across BG extremes, for accuracy assessment over a larger dynamic range of glucose measurements. In non-diabetic NHPs, resting or steady-state glucose levels were stable except during glucose challenges, whereas in the diabetic NHPs, as expected, glucose levels in between intravenous glucose tolerance tests (IVGTTs) showed wide fluctuations. The glucose levels of diabetic NHPs fluctuated significantly throughout the day, allowing us to evaluate the performance of the sensors with variable glucose levels such as can be experienced by diabetic patients. After recording, the recorders

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**Fig. 4 | CGM biocompatibility in SKH1 mouse model is improved with the coating.** a, Left: schematic of subcutaneous CGM sensor implantation, and the results of mock (guide needle only and subsequent removal following) insertion into SKH1 mice for biocompatibility testing. Right: IVIS inflammation monitoring of uncoated control (top) versus coated CGMs (bottom) after 1, 3 and 8 days post-insertion. b, Quantification of IVIS inflammation signals from a and statistical analysis showed our zwitterionic coating resulted in significantly reduced inflammation at all measured time points. Data are presented as mean ± s.d. * indicates statistically significant compared with the group ‘control’ at the level of \(P < 0.05\) using two-way analysis of variance. \(N = 5\) mice per group. c, Additional IVIS imaging was performed to examine inflammation of coated CGMs versus both uncoated control CGMs and polyurethane tubing implanted in the same mice. d, While fibrosis was not eliminated completely, zwitterionic coated CGMs reduced overgrowth at 1, 3 and 8 days post-insertion, as indicated by histological analysis (H&E, cellular infiltration; Masson’s trichrome, collagen deposition) of retrieved tissues with embedded CGMs. Scale bar, 400 \(\mu m\). e, NanoString expression analysis showing inflammation (cytokine, chemokine and immune) markers significantly increased following 1 day of control sensor implantation and suppressed/inhibited in tissues surrounding zwitterionic coated sensors, analysed from tissue RNA extracts: fold changes presented on a base 2 logarithmic scale. Experiments repeated at least two or three times. NanoString performed once.
were retrieved from both diabetic and non-diabetic NHPs, and the recorded data were exported.

As done previously in the SKH1 model, the electrical signal exported to the iPro2 Medtronic CareLink software was plotted as time on the x-axis, and with measured BG values on the y-axis (Fig. 5b). Linear regression of signal versus BG values showed decreased slopes over time, highest on day 1 and significantly reduced into days 2 and 3 of continual sensing. This trend, similar to what was seen for the SKH1 mouse model, was also observed for control sensors in both diabetic and non-diabetic NHPs, indicating sensor performance was disturbed to a greater extent by an early period response consistent with when we observe significant host inflammation to foreign objects (that is, CGM insertion and presence).

In contrast, coated sensors showed near-identical (not statistically different) slopes of signal versus BG values over days 1, 2 and 3 of continuous measurement, indicating sensor performance was left undisturbed.

Electrical signals were converted to glucose levels by using either a single BG value on the first day without additional recalibration or recalibration (using all measured BG) data for both non-diabetic and diabetic models. Non-recalibrated versus recalibration with BG comparisons for both non-diabetic and diabetic NHPs. In c and d, the yellow regions indicate day 2. e, f. Significance of various comparison methods of non-diabetic (e) and diabetic (f) models (N = 3 sensor for each group, and each sensor recording >1,000 data points). Data are presented as mean ± s.d. Significance was calculated by one-way analysis of variance. *P < 0.05; NS, not significant. g. Representative histology images (H&E and trichrome staining) of tissue with subcutaneously inserted coated and non-coated electrodes in non-diabetic and diabetic NHPs (N = 2 or 3 animals per health state; from each of which 24 sensors total (3 rounds of 4 controls and 4 coated) were collected). Scale bars, 400 μm.
measured BG values were plotted for comparison (Fig. 5c, black dots). We note that the in-study use of 4–6 finger-prick BG measurements per day on the first 3 days is higher than vendor instructions to patients to carry out only 2 each day beyond the first day. The results of representative control and coated sensors are shown in Fig. 5c, while additional sensors (totally 3 control and 3 coated) are shown in Supplementary Section 8. Furthermore, recalibrated versus non-recalibrated glucose-level trend differences were quantified throughout the entire recording period (Fig. 5d). While trends shown here reflect the current approved CGM timeframe of 6–7 days, we did attempt longer-term (15 day) recording on diabetic NHPs and note that coated sensors do not show a later delayed period of noise (Supplementary Section 9). While signal trends from coated CGMs showed deviation from the BG-calibrated data at later stages of recording (days 11–15) (Supplementary Fig. 9b–e, rightmost column), this error occurred beyond the FDA-approved range of use of 7 days. More relevant, coated CGM error was not significant over the first 10 days of use (Supplementary Fig. 9b–e, middle two columns). These data indicate that the zwitterionic coated CGMs performed efficiently with little signal error disagreement between non-recalibration and recalibration trends over the approved range of use. In contrast, glucose-level trend recording by the control sensors on both non-diabetic and diabetic NHPs showed a significant difference compared with actual, measured BGs. As was the case with testing in mice, coated sensors showed no significant difference compared with recorded glucose levels. In the non-diabetic model, the control sensors showed a 48 ± 26% inaccuracy of the non-recalibrated glucose level compared with measured BGs, while recalibration significantly decreased this inaccuracy to 21 ± 17% (Fig. 5e). The non-recalibrated versus recalibrated trends were also significantly different from each other. For the coated sensors, they only showed an inaccuracy of 18 ± 17% for non-recalibration and 16 ± 13% inaccuracy for recalibration every day. These findings were also observed in diabetic NHPs, where control sensors showed 32 ± 30% inaccuracy non-recalibrated, or 23 ± 14% with recalibration (Fig. 5f). Coated sensors only showed an inaccuracy of 22 ± 14% for non-recalibration and 24 ± 14% inaccuracy for recalibration every day. These data indicate the zwitterionic coating still has a functional and significant effect on the functional readout of CGMs and in reducing overall noise in higher-order non-human primates, regardless of diabetic state. The only diabetic model-specific phenomenon was a slight trend decrease in BG values into week 2, which we attribute to NHPs becoming more comfortable with (1) breathable jackets zipper over to protect the CGMs and (2) dedicated vet technicians, resulting in improved dispensation of daily insulin, control of normoglycemia and reduced diabetic state over time (Supplementary Fig. 9). Lastly, both coated and non-coated sensors were implanted in both non-diabetic and diabetic monkeys, and the tissue with embedded sensors was dissected and stained for histological examination. Similar to the results in SKH1 mice, tissue overgrowth for both coated and uncoated sensors increased with time from day 1 to day 8 (Fig. 5g).

Current CGMs attempt to reduce the impact of signal noise, including variability among patients, using software algorithms4–6. Since calibration using software dampening algorithms does not eliminate interfering host inflammation nor completely address signal noise, recalibration using finger-prick test is still required by the vendor16–19,22,23. The zwitterionic polymer coating developed here significantly improves the performance and accuracy of CGMs in our higher-order NHP model. Furthermore, and of equal significance, the coating allows for just a single standalone BG calibration taken immediately after insertion, for the entire recording period. In doing so, this coating-based technology eliminates the requirement for constant BG calibration multiple times (4–6 times by manufacturer guidelines) throughout the first day of use and at least twice every day thereafter, a tedious and stressful process for patients to do with finger-prick tests4. The result of the zwitterionic polymer coating improving sensor accuracy is repeatable and holds true across two animal models (SKH1 mice and NHPs) as well as in both healthy and diabetic animals. The CareLink iPro2 software is used only in the post-data collection phase and does not affect glucose measurements during the experiment. The CareLink iPro2 software is reported to be compatible with CGMs from other vendors, and is not specified only for human use20. Recent studies reported incorporation of Medtronic iPro2 CGM and associated MiniMed-disseminated (CareLink Transmitter Utility) software for use in rodents (rat)24–26,29. In addition, one recent 2018 study also reported that CGM iPro2 has good clinical accuracy when used in cats26. Our study obtained data on iPro2 usage with NHPs, and while we haven’t seen any species-specific effects in alteration of iPro-based data calibration, we can report that basal ISIG values are different between mice and NHPs, corresponding to known BG range differences for what is considered in each species to be normoglycemic.

In summary, we have shown that a zwitterionic polymer coating, developed using combinatorial chemical approaches, eliminates sensor noise and the requirement of frequent BG recalibration. Coated and naive sensors were tested across two animal models, SKH1 mice and non-diabetic and diabetic NHPs. Across all animal models tested the coated sensors were able to record BG levels accurately without recalibration and showed significant improvement in reducing sensing noise, whereas non-coated sensors began to show significant noise even at the beginning of the first day following implantation. Inflammation-profile studies indicate that the zwitterionic coating significantly reduced inflammation during the early stages after sensor implantation. Gene-expression profiling also established that the zwitterionic coating completely inhibited or suppressed (below background) levels of numerous cytokines and immune markers that may be associated with noise for control CGMs. No differences in ROS were detected between tissues implanted with control and coated CGMs, at least suggesting another mechanism must be at play. Improving function via improving the biocompatibility of implantable glucose monitors should in turn help CGMs gain independence from the requirement of constant concomitant BG finger-prick testing, which constitutes a major hurdle towards FDA approval for standalone monitoring devices for diabetic patients.

Methods

Synthesis of zwitterionic polymers for the combinatorial library. All commercially available reagents and lab supplies were purchased from Sigma-Aldrich, other than the exceptions below.

1. (Methacryloyloxy)ethyl 2-(trimethylammonio)ethylphosphate and (R)-α-lipoic acid were purchased from TCI Chemicals. N-(8-Boc-aminopropyl) methacrylamide was purchased from Polysciences. tert-Butyl 4-bromobutyrate was purchased from Oakwood Chemical. Regenerated cellulose ester dialysis tubing was purchased from Spectrum Labs. Four-arm PEG maleimide polymers (2kDa, 5kDa) were purchased from Creative PEGWorks.

1H NMR and 13C NMR spectra were recorded on Varian Inova 500 MHz NMR spectrometer, using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). High-resolution mass spectral (HRMS) data were obtained on 7 Tsk Bruker Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer. Molecular weight and PDI values of the water soluble polymers were estimated by gel permeation chromatography (GPC) in aqueous buffer containing 0.05 M sodium nitrate. One guard column and three Tosoh GMPWxL columns were calibrated with poly(ethylene oxide) standards. Flow rate was set to 1.0 ml min⁻¹ and Viscomet refractive index detector was used for conventional calibration. Molecular weight and PDI values of tetracydrofurin (THF) soluble polymers were estimated by GPC in THF mobile phase calibrated with polystyrene standards, operating at 1.0 ml min⁻¹ with a Viscomet refractive index detector, and three Viscotec LT6000L columns at 35°C.

Flash chromatography was performed on a Teledyne Isco CombiFlash RF-200 chromatography equipped with ultraviolet-visible and evaporative light scattering detectors.

Synthesis of lipoic acid monomer. A suspension of 2-aminoethyl methacrylate hydrochloride (5.0 g, 27.17 mmol) in dichloromethane (100 ml) was treated with triethylamine (4.54 ml, 32.60 mmol) at room temperature and stirred for a few
in a preheated oil bath at 70 °C. The reaction was terminated at 20 h by rapid cooling and exposure to air. The polymer was purified by dialysis in water. After lyophilization, a pinkish solid (2.09 g) was obtained.

The resultant polymer was dissolved in mixed solvents of methanol (5 ml) and water (2 ml). The solution was cooled to 0 °C under N2 atmosphere. A freshly prepared solution of NaBH4 (200 mg) in water (2 ml) was slowly added. The reaction was stirred at 0 °C for 1.5 h, and then terminated by adding 2 N HCl to adjust the pH 3. Water was added up to 40 ml. The product solution was dialysed in water at 4 °C in a dark room for 2 d. After lyophilization, a white solid (1.96 g) was obtained (Mw: 9 kDa, PDI: 1.2, aq. GPC).

General procedure for the polymerization of sulfobetaine containing polymers (SB2 as an example). 2-(Methacryloyloxy)ethyl dimethyl ammonium hydroxide monomer (SB2) (1.5 g, 5.38 mmol), lipoid acid monomer (90 mg, 0.75 mmol), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (14.5 mg, 0.057 mmol) and 4,4′-azobis(4-cyanovaleric acid (3.2 mg, 0.011 mmol) were weighed in a weighing boat and transferred into a 10 ml Schlenk flask. Methanol (5.0 ml) was added. The flask was sealed with a rubber septum. The mixture was vortexed to get a homogeneous solution, which was purified with nitrogen for 20 min. The flask was immersed in a preheated oil bath at 70 °C. It was noticed that the solution turned yellow so the reaction was terminated. The polymer was dissolved in water (~40 ml), which was purified by dialysis in water. After lyophilization, a pinkish solid (0.87 g) was obtained.

The resultant polymer was dissolved in water (25 ml). The solution was cooled to 0 °C under N2 atmosphere. A freshly prepared solution of NaBH4 (100 mg) in water (1 ml) was slowly added. The reaction was stirred at 0 °C for 1.5 h, and then terminated by adding 2 N HCl to adjust the pH 3. Water was added up to 40 ml. The product solution was dialysed in water at 4 °C in a dark room for 2 d. After lyophilization, a white solid (0.7 g) was obtained (Mw: 10.5 kDa, PDI: 1.4, aq. GPC).

General procedure for the polymerization of control polymer (PEG). Poly(ethylene glycol) methyl ether methacrylate (PEG475) (2.0 g, 4.21 mmol), lipid acid monomer (333 mg, 1.05 mmol), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (14.8 mg, 0.053 mmol) and 4,4′-azobis(4-cyanovaleric acid (2.94 mg, 0.011 mmol) were weighed in a 10 ml Schlenk flask. DMF (1.9 ml) was added. The flask was sealed with a rubber septum. The mixture was vortexed to get a homogeneous solution, which was purified with nitrogen for 20 min. The flask was immersed in a preheated oil bath at 70 °C. The reaction was terminated at 20 h by rapid cooling and exposure to air. The polymer was purified by dialysis in water. After lyophilization, a pinkish solid (2.17 g) was obtained.

The resultant polymer was dissolved in mixed solvents of methanol (5 ml) and water (2 ml), and then lyophilized to dryness at 0 °C under N2. A freshly prepared solution of NaBH4 (200 mg) in water (2 ml) was slowly added. The reaction was stirred at 0 °C for 1.5 h, and then terminated by adding 2 N HCl to adjust the pH 3. Water was added up to 40 ml. The product solution was dialysed in water at 4 °C in a dark room for 2 d. After lyophilization, a colourless oil (1.74 g) was obtained (Mw: 11 kDa, PDI: 1.3, THF GPC).

General procedure for the polymerization of amine polymer (amine polymer). N-(t-Boc-aminopropyl)methacrylamine (1.5 g, 6.86 mmol), lipid acid monomer (244 mg, 0.77 mmol), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (21.5 mg, 0.077 mmol) and 4,4′-azobis(4-cyanovaleric acid (4.5 mg, 0.015 mmol) were weighed in a weighing boat and transferred into a 10 ml Schlenk flask. Methanol (3 ml) and N,N-dimethylacetamide (2 ml) were added. The flask was sealed with a rubber septum. The mixture was vortexed to get a homogeneous solution, which was purified with nitrogen for 20 min. The flask was immersed in a preheated oil bath at 70 °C. The reaction was terminated at 24 h by rapid cooling and exposure to air. The polymer was precipitated from diethyl ether (300 ml), filtered, washed with diethyl ether (100 ml) and then dried to get 1.18 g pinkish solid (Mw: 8.6 kDa, PDI: 1.2, THF GPC).

To remove the Boc-protecting group, the resultant polymer was dissolved in dichloromethane (15 ml). TFA (30 ml) was added. The reaction was stirred at room temperature overnight. TFA was removed on rotovap to leave viscous oil, which was dissolved in distilled water (40 ml), dialysed in water, and then lyophilized to dryness. The protected polymer was dissolved in mixed solvents of methanol (8 ml) and distilled water (10 ml), dialysed in water, and then lyophilized to dryness. The deprotected polymer was dissolved in mixed solvents of methanol (5 ml) and water (10 ml). Using 1 N NaOH pH was adjusted to 7–8. The solution was cooled to 0 °C under N2 atmosphere. A freshly prepared solution of NaBH4 (150 mg) in water (1.5 ml) was added. The reaction was stirred at 0 °C for 1.5 h, and then terminated by adding 2 N HCl to pH 3. Water was added up to 40 ml.
The product solution was dialysed in water at 4°C in a dark room for 2 d. After lyophilization, a white solid (1.36 g) was obtained.

General procedure for the polymerization of control polymer (carboxylate polymer). tert-Butyl methacrylate (2.0 g, 14.06 mmol), lipid acid monomer (495 mg, 1.56 mmol), 4-cyan-4-((phenylcarbonyloxy)thio)pentanoic acid (43.5 g, 0.156 mmol) and 4'-azobis(4-cyanoveric acid (8.7 mg, 0.031 mmol) were weighed in a weighing boat and transferred into a 10 ml Schlenk flask. Methanol (3.6 ml) and N,N-dimethylacetamide (2.4 ml) were added. The flask was sealed with a rubber septum. The mixture was vortexed to get a homogenous solution, which was purged with nitrogen for 20 min. The flask was immersed in a preheated oil bath at 70°C. The reaction was terminated at 5.1 h by rapid cooling and exposure to air. The reaction solution was transferred into a 100 ml round-bottom flask. The solvents were removed on a rotovap to leave viscous oil (M_{r}: 11.6 kDa). FTIR: 1,273 cm⁻¹ (C-O); 1,541 cm⁻¹ (C=N).

Above viscous oil was dissolved in dichloromethane (15 ml). TFA (30 ml) was added. The reaction solution was stirred at room temperature overnight. TFA was removed on rotovap to leave viscous oil, which was dissolved in nano pure water (40 ml) by adding 2 N NaOH to adjust pH to basic, dialysed in water, and then lyophilized to partial dryness. The deprotected polymer was dissolved in mixed solvents of methanol (5 ml) and water (30 ml). The solution was cooled to 0°C under N₂ atmosphere. A freshly prepared solution of NaBH₄ (200 mg) in water (2 ml) was added. The reaction was stirred at 0°C for 1.5 h, terminated by adding 2 N HCl to pH 3. Water was added up to 50 ml. The product solution was dialysed in water at 4°C in a dark room for 2 d. After lyophilization, a white solid (1.36 g) was obtained.

Gelation protocol. Hydrogels are prepared via thiol-maleimide Michael addition reaction with four-arm PEG maleimide as a cross-linker. The copolymer was dissolved in 1 x PBS to make 25 w/v% stock solution. Appropriate volumes of polymer and cross-linker stock solutions resulted in an equimolar stoichiometric ratio of thiol and maleimide functional groups which were combined to yield hydrogels within a 30 s.

Subcutaneous inflammation/cathespin (IVIS) measurements of bulk hydrogels. Following pre-sterilization and washing of cross-linked zwitterionic library hydrogels with ethanol, materials were then washed 3 times with 10 ml PBS, and incubated in PBS at 4°C overnight. They were then broken up using pipetting action through a 5 ml serological pipette (pipet up and down repeatedly, until they are crushed so that the small pieces can go through the pipette smoothly). All hydrogels were subjected to mechanical disruption/crushing to make sure not only that they were able to be injected subcutaneously but also that they were of similar, relatively monodispersed size and shape distribution (pieces ~100 by 200 µm, as determined by microscopy and histology, see Supplementary Fig. 3.2).

Subcutaneous evaluation of the zwitterionic library was then performed with multiple implantations per mouse as described in Bratlie et al. Each hydrogel solution was loaded separately into a 1 ml syringe (100 µl gel + 200 µl PBS = 300 µl in total) and subcutaneously injected into bilateral sites on the dorsal surface of female SKH1 mice, in appropriate format (single-spot injection or six-array format, depending on the experimental design), 0.8 cm paramedian to the midline and 1 cm between adjacent sites. After subcutaneous injection, mouse diet was changed to special alfalfa-free (low fluorescence background) food pellets. Six days later (and 16–24 h before the imaging time), 100 µl of ProSense 750 was intravenously injected through the tail vein. The next day (day 7), IVIS imaging of the mice was performed. Inflammation heatmap data fold changes are shown as ratios of signal for each hydrogel compared with non-zwitterionic control (n = 5 in all cases).

Coating of CGM sensors with zwitterionic polymer. The poly(MPC) polymer was coupled to the Medtronic MiniMed Sof-Sensor electrode with dopamine-mediated conjugation. Briefly the sensors were immersed in a 3 mg dopamine ml⁻¹ Tris buffer solution (pH 8.5) for 24 h such that the electrode surface was coated with polycyanoacrylate films by oxidative self-polymerization of dopamine. The sensors were rinsed with endotoxin-free water and placed in a 5 mg/poly(MPC) ml⁻¹ Tris buffer (pH 8.5) and degassed with argon gas for 3 min and covered from light. The sensors in polymeric solutions were placed on an orbital shaker at 37°C for 24 h then rinsed with endotoxin-free water and stored at room temperature before use.

XPS analysis. XPS analysis was performed with Physical Electronics Model PHI 5000 VersaProbe II instrument with a monochromatic Al K-alpha X-ray source (1486.6 eV), operating at a base pressure of 3.7×10⁻¹⁰ torr.

Glucose sensing assay in vitro. The in vitro glucose sensing assay was performed with glucose solutions at different concentrations. A 12-well plate was filled with 100 mg/ml glucose solution and covered with paraffin to overcome evaporation and prevent changes in glucose concentration of the solution. Sof-Sensors were inserted through the paraffin and immersed in the glucose solution. After a 20 min hydration period, the iPro Recorder was attached to the sensor. The glucose solution was spiked every half hour increment with a concentrated amount of glucose to bring the final solution correspondingly higher to 200, 300 and 400 mg d⁻¹. The signal from the two non-coated and the two coated sensors was plotted as signal versus time, normalized and graphed.

Experimental animals. Female, 8–12-week-old SKH1 mice (Jackson Laboratory), male 8–12-week-old C57BL/6 mice (Jackson Laboratory) and male, non-diabetic and diabetic, Macaque non-human primates, 4–6 kg (University of Illinois-Chicago) were used. Mice were housed in a climate-controlled room under 12/12 h light/dark cycle with food and water ad libitum. The Animal Care and Use Committees of Massachusetts Institute of Technology (MIT) and University of Illinois-Chicago approved all testing procedures. Deep anaesthesia was maintained with 3% isoflurane during sensor insertions for the mice.

Sensor functional testing in mice. All MIT committee on animal care (CAC) guidelines for the care and use of laboratory animals were observed. SKH1 mice were anaesthetised using 3% isoflurane. Control and coated Medtronic MiniMed Sof-Sensor glucose sensors were implanted subcutaneously in the mice. Adhesive harness was placed on mice to ensure the sensors stayed in place. After a 15 to 20 min sensor hydration period, the iPro2 recording unit was plugged into the sensor base, after which a green blinking light on the unit indicating the device-initiated data recording. Note: All reported CGM sensor trends were obtained on individual mice (one CGM per mouse).

Blood glucose testing in mice. After 1–2 h of sensor insertion in the mice, the first glucose reading was taken. BGs were taken frequently during this period (10–13 times a day) for the first three days. However, as required by iPro2 and Medtronic manuals, BG calibration was minimally performed at least four times on the first day of use and at least two times every day thereafter. BGs were measured using the Clarity hand-held monitor and test strips (Clarity Diagnostics). For each measurement one drop of blood was collected from the tail vein using a lancing device (Leepine). Values were calculated using the Clarity IVIS technical manual and tested on a YSI 2900 biochemical analyser, per manufacturer instructions (YSI/Xylem).

Sensor functional testing in NHPs. The NHPs were anaesthetised and four coated and four non-coated Medtronic MiniMed Sof-Sensor glucose sensors were inserted subcutaneously on the back. Recorders were attached and a breathable jacket was placed over the sensors so the animals would not grab/pull off the sensors due to their social grooming behaviours. Four glucose readings were taken on day 1 and standard IVGGT (50% dextrose solution) was performed. On day 6, the recorders were taken off the sensors and the data was uploaded to the online Medtronic CareLink iPro Therapy Management software portal.

CGM recording data analysis. The key information recorded by the Sof-Sensor was 'time' and 'ISIG signal'. For the recalibration method, all the manually measured BG reads during the entire recording period (3–5 d) were used for calibration. The Medtronic CareLink iPro Therapy Management software autonomously calculated a 'glucose level' at each time point based on the average of the ISIG signal. By fitting the linear relation, the 'glucose level' for the rest of the days was calculated. To compare ISIG values, the difference of ISIG values was calculated using the Clarity IVIS software tool (Difference(t)) = (Glucose level(t) – BG(t))/BG(t), where Difference(t), Glucose level(t) and BG(t) were the 'difference', 'glucose level' and 'BG' at a specific time point (t). For the statistical analysis of the comparison method differences, the Difference(t) value of each sensor was averaged.

IVIS imaging and ProSense inflammation. For CGM biocompatibility testing: 18–24 h before imaging, a dose of 100 µl ProSense 750 Fast Fluorescent Imaging agent (PerkinElmer) was injected intraperitoneally into SKH1 mice with previously inserted Sof-Sensors. In vivo fluorescence imaging was performed using the IVIS Spectrum measurement system (Xenogen). The mice were maintained under 3% isoflurane. All imaging were performed using the manufacturer's Living Image acquisition and analysis software (Caliper Life Sciences).

For hydrogel zwitterionic library screen: following pre-sterilization and washing with ethanol, materials were then washed 3 times with 10 ml PBS, and incubated in PBS at 4°C overnight. They were then broken up using pipetting action through a 5 ml serological pipette (pipet up and down repeatedly, until they are crushed so that the small pieces can go through the pipette smoothly). Subcutaneous evaluation of the zwitterionic library was then performed with multiple implantations per mouse as described in Bratlie et al.. Each hydrogel solution was loaded separately into a 1 ml syringe (100 µl gel + 200 µl PBS = 300 µl in total) and subcutaneously injected into bilateral sites on the dorsal surface of female SKH1 mice, in appropriate format (single-spot injection or six-array format, depending on the experimental design), 0.8 cm paramedian to the midline and 1 cm between adjacent sites. After subcutaneous injection, mouse diet was changed to special alfalfa-free (low fluorescence background) food pellets. Six days later (and 18–24 h before the imaging time), 100 µl of ProSense 750 was injected. The next day (day 7), IVIS imaging of the mice was performed. Inflammation heatmap...
data fold changes are shown as ratios of signal for each hydrogel compared to non-zwitterionic control (n=5 in all cases).

**Histology.** Subcutaneous tissue sections were fixed in 4% paraformaldehyde and sent to the Swanson Biotech Histology Core at the Koch Institute. Sections (5 μm) processed with both H&E and Mason’s trichrome stains were then imaged using an EVOS microscope (Thermo Fisher Scientific) at various magnifications, as indicated.

**Gene-expression profile.** RNA was extracted from frozen tissues containing control and coated sensors using the Trizol Reagent protocol. The RNA was quantified, diluted and used with a NanoString machine/kit for gene-expression analysis.

**ROS activity assessment.** Protein lysates were prepared from retrieved CGMs with neighbouring subcutaneous tissues retrieved 1 and 3 days after SQ implantation into SKH1 mice. 50 μg of each lysate were aliquoted twice and incubated for 30 min at 37°C with two different ROS substrate solutions: 10 μM APF (Cat# A36003, Invitrogen) and 5 μM CellROX (Cat# C10422, Invitrogen) diluted in PBS. Following appropriate incubation times, samples were read in a black-well 96-well plate with a Tecan fluorescent-capable infinite M1000 plate reader.

**Induction of diabetes in NHPs.** Streptozotocin (STZ) diabetes induction was performed as follows: before STZ administration, animals were anesthetized with ketamine 10 mg kg⁻¹ intramuscularly. An intravenous catheter was inserted and a perfusion fluids (30 ml kg⁻¹) before and after peritoneal injection of STZ to decrease the risk of kidney damage (60 ml kg⁻¹ total). Glucose levels were then measured frequently daily until the NHP was documented as euglycemic or hyperglycemic after STZ injection followed by twice daily glucose monitoring. Glargine (0.5 U kg⁻¹) insulin was also administered twice daily subcutaneously once NHPs became hyperglycemic. After 2 weeks of insulin treatment, fructosamine levels were checked and insulin dosages altered accordingly. Animals that exhibited hyperglycemia with fasting blood glucose ≥250 mg dl⁻¹ were used in the study. If the monkey did not become hyperglycemic after STZ, another 1/3 of the original dose was given to induce diabetes. Excluding the original injection, the maximum additional STZ injection can occur three times.

**Reporting Summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information.

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**References**

1. Yach, D., Stuckler, D. & Brownell, K. D. Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. Nat. Med. 12, 62–66 (2006).
2. Zimet, P., Alberti, K. G. M. M. & Shaw, J. Global and societal implications of the diabetes epidemic. Nature 414, 782–787 (2001).
3. Gabir, M. M. et al. The1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care 23, 1108–1112 (2000).
4. Zhao, X. et al. The lifetime cost of diabetes and its implications for diabetes prevention. Diabetes Care 37, 2557–2564 (2014).
5. Clar, C., Barnard, K., Cummins, E., Boyle, P. & Waugh, N. Self-monitoring of blood glucose in type 2 diabetes: systematic review. Health Technol. Assess. (Roxchi) 14, 1–140 (2010).
6. Kovatchev, B., Bette, M. & Clarke, W. Analytical methods for the retrieval and interpretation of continuous glucose monitoring data in diabetes. Methods Enzymol. 454, 69–86 (2016).
7. Boland, E. et al. Limitations of conventional methods of self-monitoring of blood glucose. Diabetes Care 24, 1858–1862 (2001).
8. Newman, J. D. & Turner, A. P. F. Home blood glucose biosensors: a commercial perspective. Biosens. Bioelectron. 20, 2435–2453 (2005).
9. Hovorka, R. Continuous glucose monitoring and closed-loop systems. Diabet. Med. 23, 1–12 (2006).
10. Shichiri, M., Yamasaki, Y., Kawamori, R., Hakui, N. & Abe, H. Wearable artificial endocrine pancreas with needle-type glucose sensor. Lancet 360, 1129–1131 (1996).
11. Hovorka, R. Closed-loop insulin delivery: from bench to clinical practice. Nat. Rev. Endocrinol. 7, 385–395 (2011).
12. Veiseh, O., Tang, B. C., Whitehead, K. A., Anderson, D. G. & Langer, R. Managing diabetes with nanomedicine: challenges and opportunities. Nat. Rev. Drug Discov. 14, 45–57 (2014).
42. Englert, K. et al. Skin and adhesive issues with continuous glucose monitors: a sticky situation. J. Diabetes Sci. Technol. 8, 745–751 (2014).

43. Abbott Laboratories. Freestyle Libre, flash glucose monitoring system. Abbott Libre https://www.freestylelibre.us (2018).

44. Bailey, T., Bode, B. W., Christiansen, M. P., Klaff, L. J. & Alva, S. The performance and usability of a factory-calibrated flash glucose monitoring system. Diabetes Technol. Ther. 17, 787–794 (2015).

45. Hoss, U. & Budiman, E. S. Factory-calibrated continuous glucose sensors: the science behind the technology. Diabetes Technol. Ther. 19, S44–S50 (2017).

46. Bequette, B. W. Continuous glucose monitoring: real-time algorithms for calibration, filtering, and alarms. J. Diabetes Sci. Technol. 4, 404–18 (2010).

47. Doloff, J. C. et al. Colony stimulating factor-1 receptor is a central component of the foreign body response to biomaterial implants in rodents and non-human primates. Nat. Mater. 16, 671–680 (2017).

48. Prichard, H. L., Schroeder, T., Reichert, W. M. & Klitzman, B. Bioluminescence imaging of glucose in tissue surrounding polyurethane and glucose sensor implants. J. Diabetes Sci. Technol. 4, 1035–1062 (2010).

49. Yang, W., Xue, H., Carr, L. R., Wang, J. & Jiang, S. Zwitterionic poly(carboxybetaine) hydrogels for glucose biosensors in complex media. Biosens. Bioelectron. 26, 2454–2459 (2011).

50. Yang, W. et al. The effect of lightly crosslinked poly(carboxybetaine) hydrogel coating on the performance of sensors in whole blood. Biomaterials 33, 7945–7951 (2012).

51. Reid, B. et al. PEG hydrogel degradation and the role of the surrounding tissue environment. J. Tissue Eng. Regen. Med. 9, 315–318 (2015).

52. Bratlie, K. M. et al. Rapid biocompatibility analysis of materials via in vivo fluorescence imaging of mouse models. PLoS ONE 5, e10032 (2010).

53. Vegas, A. J. et al. Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates. Nat. Biotechnol. 34, 345–352 (2016).

54. Yesilyurt, V. et al. A facile and versatile method to endow biomaterial devices with zwitterionic surface coatings. Adv. Healthc. Mater. 6, 1601091 (2017).

55. Lee, H., Dellatore, S. M., Miller, W. M. & Messersmith, P. B. Mussel-inspired surface chemistry for multifunctional coatings. Science 318, 426–430 (2007).

56. Lee, H., Rho, J. & Messersmith, P. B. Facile conjugation of biomolecules onto surfaces via mussel adhesive protein inspired coatings. Adv. Mater. 21, 431–434 (2009).

57. Kang, S. M. et al. One-step multipurpose surface functionalization by adhesive catecholamine. Adv. Funct. Mater. 22, 2949–2955 (2012).

58. Facchinetti, A., Sparacino, G. & Cobelli, C. Signal processing algorithms implementing the ‘smart sensor’ concept to improve continuous glucose monitoring in diabetes. J. Diabetes Sci. Technol. 7, 1308–1318 (2013).

59. Vallejo-Heligon, S. G., Klitzman, B. & Reichert, W. M. Characterization of porous, dexamethasone-releasing polyurethane coatings for glucose sensors. Acta Biomater. 10, 4629–4638 (2014).

60. Salesov, E., Zini, E., Riederer, A., Lutz, T. A. & Reusch, C. E. Comparison of the pharmacodynamics of protamine zinc insulin and insulin degludec and validation of the continuous glucose monitoring system iPro2 in healthy cats. Res. Vet. Sci. 118, 79–85 (2018).

61. Chen, X., Lawrence, J., Parellkar, S. & Emrick, T. Novel zwitterionic copolymers with dihydrolipoic acid: synthesis and preparation of nonfouling nanorods. Macromolecules 46, 119–127 (2013).

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Author contributions
X.X., J.C.D., V.Y., A.S. and D.G.A. designed experiments, analysed data and wrote the manuscript. X.X., J.C.D., E.Y., A.S., J.J.M., M.O., S.F., D.I., S.G., I.J., J.L., W.W., A.B. and K.A.W. performed experiments. X.X., J.C.D., V.Y., A.S., H.H.T., J.T., H.-J.C. and B.Y. performed statistical analyses of datasets and aided in the preparation of communicating datasets. R.L. and D.G.A. provided conceptual advice. R.L. and D.G.A. supervised the study. All authors discussed the results and assisted in the preparation of the manuscript.

Competing interests
The authors declare no competing interests.

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- □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No sample size calculation was performed. Instead, we relied on journal guidelines for a minimum of n = 5 animals/treatment group, except with the non-human primate study, where animals were limited. Numbers in this case are also clearly reported in the relevant figure legends. In addition, we adhered to sample-size requirements necessary for determining statistical significance.

Data exclusions

No data were excluded from the analyses.

Replication

All attempts at replication were successful. Experimental repeat numbers are also reported in the figure legends.

Randomization

Animal groups were randomized by body weight and/or the level of their diabetic state (that is, blood-glucose status following fasting and prior to transplantation).

Blinding

Blinding was not possible for part of the data analysis, especially in vitro, due to visual observations of chemical modification (that is, having different shading) identifying the treatment groups. However, with that said, analyses on the biology/immunology side were generally blinded from the mainstream chemistry analyses, and vice versa.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study

☑ ☒ Unique biological materials
☐ ☐ Antibodies
☐ ☐ Eukaryotic cell lines
☐ ☐ Palaeontology
☐ ☒ Animals and other organisms
☐ ☐ Human research participants

Methods

n/a Involved in the study

☑ ☐ ChIP-seq
☑ ☐ Flow cytometry
☑ ☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All unique biological materials are readily available from standard commercial sources.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Animals used were 8–12 week-old female SKH1 or male C57BL/6 mice (healthy and STZ-induced diabetic), and non-human primate male cynomolgus monkeys (body weights: 4–6 kg).

Wild animals

The study did not involve wild animals.

Field-collected samples

The samples did not involve samples collected from the field.