Pacifier Biosensor: Toward Noninvasive Saliva Biomarker Monitoring

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Supporting Information

ABSTRACT: Wearable sensors for noninvasive monitoring of physiological parameters is a growing technology in the clinical field. Especially in neonates, the development of portable and nonharmful monitoring devices is urgently needed because they cannot provide any feedback about discomfort or health complaints. However, in infant monitoring, only wearable sensors measuring physical parameters for vital signs have been developed. Here, we describe the first chemical wearable sensor for newborn monitoring. This fully integrated pacifier operates as a portable wireless device toward noninvasive chemical monitoring in the infant’s saliva. The infant’s mouth movements on the pacifier result in efficient saliva pumping and promote unidirectional flow from the mouth to the electrochemical chamber. The integrated electrochemical detection chamber, containing the enzymatic biosensor, is located outside of the oral cavity. The capabilities of the platform were studied for glucose detection in diabetic adults and compared to their blood levels with good correlation, demonstrating the sensor’s good performance. This baby-friendly device integrates saliva sampling with electrochemical sensing, along with miniaturized wireless electronics on a single pacifier platform. Such integration simplifies the infant’s health monitoring in a real-time and selective fashion, representing the first wearable sensor focusing on chemical saliva sensing in newborns. This initial demonstration of glucose monitoring introduces new possibilities for metabolites monitoring in infants and neonates using saliva as a noninvasive sample.

Wearables sensors for noninvasive monitoring of physiological parameters is a growing technology in the clinical field; however, similar prototypes for babies have been less explored. The current sensors for infants still present some limitations such as (1) not being fully portable since they need a hardwired net to connect the device to the main base unit and (2) uncomfortable for the wearer since they are usually placed on the cloths or contain multiple rigid interfaces, not providing an intimate attachment to the skin. Furthermore, all these platforms may involve risks for the newborn, since the skin is not fully developed and the infant may be prone to injuries and scarring. These limitations complicate the care of these babies and also the rigid devices and wire net can hinder breastfeeding, frustrate basic clinical tasks, and/or create physical barriers between parent and neonate, which is important for helping in the baby recovery.

Recently, Rogers’ group developed a binodal module for noninvasive epidermal monitoring in newborns. This platform consists of one device placed on the chest to record electrocardiograms for heart activity and another placed on the base of the foot to measure blood oxygen levels. This system has also been applied for continuous monitoring of skin temperature and respiration rate being successfully tested to be unharmful in neonate’s skin. Despite the recent advances in wearable babies monitoring, all devices developed so far are strictly limited to the monitoring physical parameters but any chemical biomarker. However, monitoring chemical biomarkers in newborns is essential for rapid control of low birthweight babies or metabolic diseases such as diabetes.

In this sense, glucose monitoring is especially important for the babies (bio)-chemical monitoring due to the harmful effects that glucose deregulation can cause in babies.
including a significant risk of perinatal morbidity or long-term health problems in the case of very low birth-weight compromising their neurological outcome. Current approaches for monitoring glucose in newborns are not portable and involve invasive and costly methods exceptionally used in neonatal intensive care units. Additionally, these approaches usually require piercing the skin to reach the interstitial fluid, putting the baby’s health at risk. These advances in continuous glucose monitoring are available only in major clinical facilities, while nearly half of all newborns have no access to these hospitals. Thus, early detection and monitoring of metabolic diseases is essential and demands low-cost, user-friendly platforms, for rapid, portable, and noninvasive monitoring of newborns. Recently, sweat glucose colorimetric sensors and external optical glucose measurements through the skin, i.e., finger and ear lobe, have been explored as noninvasive approaches. However, these promising emerging technologies still face several limitations, such as nonspecific absorption by biological chromophores and thermal noise for the external optical sensors along with low sensitivity for the colorimetric ones. Therefore, the use of specific and sensitive electrochemical biosensors, combined with noninvasive biofluids, seems as an extremely promising approach.

The already existing wearable sensors developed for glucose monitoring have been demonstrated through various noninvasive human fluids including sweat, saliva, urine, and interstitial fluid. Therefore, saliva represents an ideal biofluid for noninvasive sampling which contains clinically significant biomarkers, making it extremely suitable for monitoring physiological and metabolic states. Especially in newborns and babies, saliva is a meaningful biofluid because of (a) its easy accessibility, (b) large availability, and (c) good correlation with blood glucose levels. The field of wearable salivary sensing has experienced considerable recent progress, in the integration of sensing devices using saliva as the noninvasive sample in mouthguards, intraoral dental accessories, or even foodstuffs such as lollipops. However, these platforms still present certain challenges for glucose on-body applications including (a) the necessity of long-term exposure of some devices inside the mouth, which make them uncomfortable and nonuser friendly for easy translation to real applicability, (b) the direct contact of sensor materials such as the potentially harmful protective (antifouling) layers, e.g., poly(o-phenylenediamine) used to maintain high electrode stability, and (c) the limitation of their use just for adults or children and not for newborns. One of the major challenges in the development of wearable devices for infants is the safeguard of materials and chemicals. Safety is particularly important for devices which are in direct contact with the baby’s mouth. Hence, to improve the practical usage of wearable salivary sensors forthcoming efforts would require user-friendly platforms, based on biocompatible nontoxic materials with external electrodes and circuitry as well as the prospect to expand these platforms to newborns and infants. Thus, leveraging these features for designing new noninvasive sampling in comfortable platforms may expand the current technologies in the field of babies’ health monitoring.

Here, we demonstrate a proof-of-concept of a fully integrated wearable pacifier-based platform toward wireless noninvasive biomarker monitoring in newborns’ saliva. While other pacifiers have been already designed to sense physical parameters such as suction pressure and temperature, to the best of our knowledge, this wearable pacifier for saliva monitoring represents the first example of baby’s wearable device for chemical biomarker sensing. The feasibility of this newborn and infant wearable platform has been demonstrated for real-time amperometric monitoring of glucose. The biosensor relies on glucose-oxidase (GOx)-based enzymatic detection on a Prussian Blue (PB) electrode transducer. The pacifier sensing platform integrates (a) a compatible wireless amperometric circuitry, coupled with a Bluetooth communication system for miniaturization and low-power operation, (b) an isolated electrochemical detector for preventing leakage of materials to the mouth, and (c) a nontoxic polymeric nipple containing a safety rectifying channel for saliva sampling. This rectifying channel consists of a silicone tube with asymmetrical conical constrictions, which enables noninvasive saliva collection and unidirectional saliva flow from out of the mouth. The external electrochemical detection during pacifier usage constitutes a safe and wireless system for the baby. Thus, the combination of a pump-free system, along with the unidirectional flow to an external collection outlet, facilitates a baby-friendly safe system that can be fully integrated into a baby’s daily life. Furthermore, we showed on-body performance through glucose determination in saliva from type I diabetes patients under fasting and meal intake states, demonstrating the applicability for real-time monitoring of diabetes in saliva. Thus, we demonstrated the first example of a pacifier-based electrochemical device for chemical sensing, and particularly, for monitoring salivary biomarkers and metabolic diseases in newborns. The new platform holds a considerable promise for saliva-chemical monitoring in infants and neonates, since it could be used in Neonatal Intensive Care Units (NICUs) as a supplementary tool of the developed skin devices for physical parameters monitoring, providing full information about all of the newborn’s parameters.

**EXPERIMENTAL SECTION**

**Screen Printing Process and Electrode Modification.** The electrodes were fabricated via a screen-printing process. A semiautomatic MMP-SPM screen printer (Speedline Technologies, Franklin, MA, USA) and customized stencils were used. These were developed using AutoCAD software (Autodesk, San Rafael, CA, USA) and produced by Metal Etch Services (San Marcos, CA, USA) using square stainless-steel stencils (12 ×12 in²). The reference electrode and the conductive contacts were printed on a flexible polyethylene terephthalate (PET) sheet using an Ag/AgCl ink (E2414, Gwent Inc., Torfaen, UK). The printed traces were cured at 85 °C for 15 min. Then, carbon-Prussian blue (PB) ink (C2070424P2, Gwent Inc., Torfaen, UK) was used to print the working and counter electrodes using the same curing procedure. Finally, an insulator layer was printed to define the electrode area (Aleené’s, Inc, Fresno, CA) and was cured in a similar manner.

The biosensor modification consisted of an initial 0.5 μL of chitosan (Sigma-Aldrich, St Louis, MO, USA) 0.5% w/v prepared in 0.1 M acetic acid, which was first drop-casted into the working electrode and dried at room temperature. Subsequently, 0.5 μL of glucose oxidase (GOx) from Aspergillus niger type X-S (EC 1.1.3.4) (Sigma-Aldrich, St Louis, MO, USA) in a 34 mg/mL concentration prepared in 0.1 M phosphate buffer, pH 7.4 (Panreac, Spain), was drop cast into the working electrode and dried overnight at 4 °C.

**Device Fabrication.** The pacifier biosensor was fabricated using different parts of commercially available NUK pacifiers.
such as the nipple (Orthodontic pacifier 0–3 months, silicone, NUK, USA) and the core containing the chamber (Juicy Orthodontic Pacifier, 0–6 months, NUK, USA). The nipples used for saliva flow studies were silicone nipples (Gerber First essentials, USA). A 4 mm diameter inlet hole was created in the nipple using a biopsy punch (Biopunch, Redding, US).

The cap of the pacifier containing the electrochemical cell was customized using a 3D printer. Acrylonitrile butadiene styrene (ABS)-based 3D printed piece (2.4 × 1.8 × 0.7 cm³) included a chamber (4 × 4 × 4 mm³) for performing the electrochemical measurements and a 0.5 × 0.5 mm² hole at the bottom right corner, which connected the measurement chamber with the outside of the pacifier by a hydrophilic cellulose-based thread (Figure 1). Additionally, an external 3D sealed case can be further incorporated for the full integration of both potentiostat and the correspondent battery for future applicability.

Figure 1. Glucose pacifier sensing concept. (A) Glucose pacifier working-principle. (a) Real picture and use of pacifier-biosensor. (b) On-body saliva monitoring for a healthy individual. Signal interpretation: a dry device (I), saliva reaches and starts to fill the electrochemical chamber (II), stabilization of the signal (III), glucose signal (IV), and saliva elimination from the pacifier (V). (B) Schematic of the assembled wireless pacifier-biosensor. (C) Schematic of the glucose enzymatic biosensing approach on the PB electrode. (D) Parts of the pacifier: (a) pictures of the three different parts: nipple, electrochemical cell, and electronics. (b) Detail of the electrochemical cell. (E) Scheme of the assembling of the pacifier pieces: nipple of the pacifier (1), inlet for collecting saliva (2), electrochemical chamber (3), PB-GOx electrode (4), central piece (5), outlet (6), insulator pacifier cap (7), integrated wireless potentiostat (8), and back cap of the pacifier (9).
The saliva sampling was performed by fabricating a customized dropper using lab-accessible materials. The dropper was based on a PVC tube channel with 3.6 cm length and 0.15 cm diameter including polystyrene constrictors with 6 mm length with shape and size specified in the Supporting Information. A 4 mm hole was cut on the tip of the nipple using a biopsy punch (Biopunch, Redding, US) where the dropper channel was tidily placed avoiding any possible swelling of the parts from the user. The dropper channel conduces the saliva to the electrochemical chamber, allowing complete soaking of the electrode surface. Finally, the outlet was fabricated by using a 7 cm long hydrophilic cellulose-based thread, along with a 1 cm diameter hydrophobic filter (protection filter 10 mL, Eppendorf, Hamburg, Germany), placed at the back of the chamber fixed with adhesive blue tack (Bostik, Unipessoal, Portugal) to the pacifier.

**In Vitro Studies.** All electrochemical measurements were carried out using a portable multi potentiostat µSTAT 8000P (Dropens, Oviedo, Spain). Electrochemical detection of the enzymatic product (H$_2$O$_2$) was performed by amperometry at fixed potential $-0.20$ V vs Ag/AgCl, in room temperature.

All solutions for the in vitro measurements were prepared in artificial saliva. The artificial saliva was prepared by dissolving 5 mM of NaCl (Panreac, Spain), 15 mM of KCl (Sharlab, Spain), 1 mM of citric acid (Fluka, Switzerland), 1 mM of CaCl$_2$, 1.1 mM of KSCN (Merk, Germany), and 4 mM of NH$_4$Cl (Sigma-Aldrich, St Louis, MO, USA) in distilled water. The pH of artificial saliva was adjusted to 6.7, which is an average pH of human saliva. The artificial saliva was adjusted to 6.7, which is an average pH of human saliva. 

Additionally, a blue candy and gel food color (Dr. Oetker), purchased in a local supermarket, was used as a blue ink to dye real and artificial saliva, respectively, in order to facilitate the saliva flow visualization.

**On-Body Studies and Sample Collection.** The on-body experiments were performed using saliva from one healthy and four type-1 diabetic volunteers in the age group between 25–60 years old. The salivary studies were performed in strict compliance with the protocol that was approved by the institutional review board (IRB) at the University of California, San Diego, protocol 151879.

The on-body studies for the healthy person were carried out using the pacifier with the PB-GOx sensor 30 min after a meal, as detailed in the scheme shown in Figure 1A and in Video S1. In the case of the diabetic patients, the volunteers were asked to follow a protocol adapted from a previously published procedure to collect the saliva. The protocol consisted in

1. brushing the teeth using toothpaste and carefully rinsing the mouth with plenty of water, avoiding toothpaste residue. This step is done before the saliva testing, to avoid possible glucose contamination.
2. (Minimize swallowing and hold saliva in the mouth (typically < 1 min).
3. Collect raw saliva in a clean 2 mL Eppendorf. After that, saliva and blood glucose levels were measured before and after the food intake by diabetic patients.

For on-body measurements, a commercial glucometer Glucocard G Meter (Arkay, MN, USA) was used to assess the corresponding glucose blood concentrations. In this case, saliva samples were placed in the beginning of the pacifier inlet and the movement of the mouth was simulated by manually pressing the pacifier nipple, simulating the mouth suction movement during the pacifier use for the effective flow of saliva along the tube. A new GOx modified electrode was used for each experiment. Nipples and PVC tubes were sterilized and/or discarded after use, between experiments. For sterilization, it is recommended to clean the nipple and sample holder under boiling water for a few minutes to prevent the growth of germs and bacteria inside the pacifier followed by rinsing with ethanol and purified water.

A previously reported time-correlation for the highest values of glucose in saliva and blood was used for the sampling measurement. According to this, the salivary glucose value reaches its peak value 15–40 min after food intake while the blood glucose increases to its highest value around 30–60 min in healthy subjects and 1–2 h for diabetic patients after food intake. Thus, in all subjects, saliva and blood glucose were measured 30 and 60 min, after food intake, in saliva and blood, respectively, for a good data correlation.

**RESULTS AND DISCUSSION**

**Glucose Pacifier Concept.** A pacifier-based wearable biosensor has been designed to address the challenge of real-time monitoring of salivary metabolites, such as glucose, in newborns and infants (Figure 1A). Considering the safety in operation and measurement, the prototype integrates an electrical circuitry, and a disposable electrode placed in an external sensing chamber, fully sealed by the pacifier nipple that was minimally modified for easy acceptance by the infant.

The electrodes are easily replaceable via insertion through a slot (Figure S1) which fits in the 4 x 4 mm$^2$ electrochemical chamber, where the saliva is accumulated to carry out the amperometric measurement. The fluidic system is closed with a thread which connects the inside of the pacifier with the atmosphere, soaking the fluid, acting as the outlet (see the Supporting Information).

The electrochemical measurement was carried out with our customized electrodes inserted on the electrochemical chamber (Figure 1B). The electrodes were fabricated using low-cost scalable screen-printing technology on a PET layer, including the three electrodes system containing working and counter PB electrodes and the Ag/AgCl reference electrode. As a proof of principle toward noninvasive saliva biomarker monitoring, we have selectively detected glucose in saliva by using GOx. To this aim, the enzyme was immobilized on the working electrode surface using chitosan, a nontoxic polysaccharide, to attach the enzyme. The GOx-based biocatalytic oxidation of glucose is followed by low-potential (−0.20 V vs Ag/AgCl) amperometric detection of the H$_2$O$_2$ product at the PB transducer, which leads to highly selective glucose monitoring (Figure 1C). Figure 1A shows the continuous on-body amperometric monitoring for a healthy individual using the pacifier biosensor. Initially, the device is dry, and the observed current is zero (I). This signal increases when saliva reaches the electrochemical chamber due to capacitive current (II). Next, it leads to a stable signal when the electrochemical cell is filled with saliva (III). Stability of this signal may be considerable affected by the amount and viscosity of saliva. Then, when the enzymatic reaction has been completed, the glucose signal is obtained, (IV) and it is obtained from the difference of the current before (III) and after (IV) the enzymatic reaction of GOx. Finally, the signal decreases slowly to return to the baseline when the saliva is removed from the chamber by the outlet (V).

The pacifier biosensor prototype demonstrates the simplicity of the device. This fully integrated design of the pacifier toward
the initial proof-of-concept prototype includes three main assembled parts (Figure 1D): the integrated portable potentiostat connected to the electrode for a wireless connection, the electrode chamber where the electrochemical measurement takes place, and the nipple for the saliva collection from the user.

In summary, the schematic of the pacifier biosensor assembly is shown in Figure 1E. Thus, the pacifier biosensor comprises the nipple of the pacifier as the inlet for the saliva collection, which is driven through a unidirectional constricted channel to the electrochemical detection chamber, where our customized disposable electrode is inserted.

Characterization of the Continuous Saliva Sampling in the Pacifier. Since the final application of the device is the monitoring of saliva glucose in infants, the location of the inlet and nipple shape was optimized accordingly with the body position while using the device for effective saliva collection in several body positions. To this aim, an edible blue candy was used to stain the saliva and visually monitor the volume collected. Cylindrical and orthodontic nipples were drilled with 4 mm holes along their whole surface and filled with absorbent white paper. Upon eating the blue-candy, the saliva volunteer stained blue and the pacifier was used for 30 s. After use, the pacifier was examined to determine, according to the level of dye on the absorbent paper, the different amount of saliva collected on the nipple. Different nipples (cylindrical and orthodontic) and modalities (sitting, lying on the right and left sides, and facing up and down) were explored, with the orthodontic format with a single inlet on the tip of the nipple being the best inlet location for all body positions (Figure S2).

Thus, the design of the pacifier has taken into account the body position to obtain the maximum saliva amount in a regular use by a baby. The final assembling of the pacifier is shown in Figure 2A–C, as well as the dropper channel inlet used to sample saliva (Figure 2D). The dropper channel consists of a 3.4 cm long semiflexible Polyvinyl chloride (PVC) tube located in the tip of the nipple. This channel goes through the nipple, connecting the inlet (facing the mouth) with the electrochemical chamber where the electrode is placed. To ensure the safety of the pacifier biosensor, the channel has been designed to ensure only forward direction saliva flow (Figure 2E). The saliva flows from the inlet toward a 4 × 4 mm² electrochemical chamber with a small volume for fast saliva monitoring and easy integration of our customized electrode. This chamber also contains a 0.5 × 0.5 mm² opening at the right bottom corner containing a hydrophilic outlet thread, connecting the inside part of the cell with the outside of the pacifier in order to remove the saliva from the chamber (Figure 1D).

To ensure the safe use of the pacifier by the infant, a rectifier system was incorporated to enable a forward saliva flow with no backflow to the baby’s mouth. The working principle of the rectifier system is similar to the mechanism of a dropper. The saliva transport happens in a small PVC tube placed inside the nipple (Figure 2F) connecting the pacifier tip (in contact with the mouth) to the back of the nipple, the electrochemical cell (where the sensor is located). The flow happens without any backflow to the infant’s mouth because of the reduction of the internal volume and increasing of the stiffness on the sensor end using three polystyrene constrictors inserted in the PVC tube reducing the cross section (Figure 2D).

The final plastic valve is cut to produce a droplet with a volume less than or equal to half of the volume of the fluid displaced from the compression of the nipple by the baby to

Figure 2. Characterization of the continuous saliva sampling. (A) Photograph of the full integrated device. (B) Schematic of the pacifier. (C) Side (a) top and (b) bottom views of the pacifier. (D) Working principle of the inlet for saliva sampling. (E) Schematic of the nipple and flow direction. (F) Photograph of (a) the nipple containing the channel for saliva collection in contact with the mouth, (b) the outlet of the channel that drops the saliva into the electrochemical chamber, and (c) schematic of the configuration of the inlet through the pacifier.
ensure the unidirectional flow (see more details in the Supporting Information). Thus, when the tube is compressed by the infant’s mouth (indicated by the arrows in Figure 2D), the fluid inside the bulb is displaced moving outward and reaches the end of the tube allowing the droplet formation. This droplet grows to a maximum volume after which, it breaks and leaves the channel running into the electrochemical cell. Once the droplet releasing occurs, this amount of fluid does not return to the infant’s mouth. Afterward, when the nipple is relaxed, the bulb returns to its original volume decreasing the

Figure 3. Biosensor and pacifier performance. (A) Electrochemical performance in artificial saliva. (a) Amperograms obtained for increasing glucose concentration (event indicated with an arrow) and resulting calibration curve (linear range 0.1−1.4 mM) where the current value is calculated as the difference in the response (Δcurrent) before and after induction (n = 5), right side. (b) Selectivity test: response to 0.6 mM glucose in the presence of common electroactive physiological interferents in saliva (200 μM UA and 20 μM AA). (B) Correlation between current obtained in saliva using the pacifier sensor (black) and glucose concentration in blood (red) in 5 different persons (healthy and diabetic) before and after a meal. (C) In vitro raw saliva test for two type I diabetes subjects (a,b) before and after a meal. (D) Scheme of the procedure used for on-body glucose sensing experiments: saliva is collected ∼30 min after a meal to measure the maximum glucose levels using the GOx-modified PB detector by chronoamperometry. When the saliva reaches the biosensor, a decrease in the current change is observed until the sensor is fully covered with saliva and then the signal becomes stable and the enzymatic reaction takes place reaching a stable current due to the enzymatic reaction for ∼200 s due to the chitosan layer. (E) Amperograms for on-body glucose monitoring saliva using the pacifier biosensor. On body measurements in the same patient for (b,e) before meal and (c,f) after a meal and (a,d) after a meal without enzyme in two different individuals.
pressure and pulling fluid inside initiating a new cycle. Once the saliva reaches the sensing chamber, the electrochemical measurement starts. To allow a continuous and completely unidirectional flow, a second safety accessory was included to remove the measured saliva, connecting the electrochemical chamber to the atmosphere. The full saliva replenishment in the electrochemical chamber is necessary to avoid saliva carryover during the measurements, replacing the saliva already used and avoiding possible overflow of saliva to the electrical contacts or back-flowing to the mouth. To this aim, a small opening (0.5 × 0.5 mm²) at the right bottom corner connects the inside part of the cell with the outside of the pacifier through a hydrophilic thread. Once the thread outlet is soaked inside the electrochemical chamber, the end part, exposed to the air, is constantly drying by evaporation allowing a continuous flow. In addition, the outlet was optimized for fast removal of saliva. Therefore, to increase the fluid absorption rate and to reduce drying time, a filter was placed in the back part of the electrochemical cell in contact with the thread, providing the fastest drying time (800 s) compared to other studied hydrophilic outlets (Table S1 and Figure S3). One important consideration to the design was the concerning location of the filter which was placed on the back part of the chamber piece facing directly the electronic board with great risk of wetting it. To avoid the contact of saliva with the electronics, a plastic cap was placed between the sensing chamber and the miniaturized potentiostat, enclosing the outlet and isolating the system. The drying process was not compromised, once the thread, connected with the filter paper, was long enough to be exposed to the open air, thus allowing saliva evaporation.

**Analytical Performance and On-Body Glucose Monitoring.** Once the device actively and continuously collects and eliminates saliva autonomously, optimization of the glucose detection was required for the efficient applicability of the sensor. Glucose in vitro analysis using amperometry at −0.20 V in artificial saliva was performed using an integrated disposable screen printed PB electrode modified by chitosan and GOx. Figure 3A shows the calibration curve for glucose with the linear range between 0.1 and 1.4 mM, consistent with glucose concentration ranges in diabetic saliva.28−30 Using this approach in artificial saliva offers an excellent linearity (R² = 0.994), with an intercept of 0.04 ± 0.03 nA, and good sensitivity (0.69 ± 0.04 nA mM⁻¹), reflecting the good reliability of the sensor. The limits of detection (LOD) and quantification (LOQ) obtained were 0.04 mM and 0.1 mM, respectively, which were enough to quantify the regular levels of glucose in diabetic patients, even before eating.28−30 The LOD has been calculated according to the 3 σb/m criteria (m is the slope of the linear portion in the calibration graph, and σb was estimated as the standard deviation (n = 10) of the amperometric signals measured for glucose at the lowest concentration level of the calibration graph). Additionally, highly favorable intersensor reproducibility was also obtained for 0.6 mM glucose, which is a regular glucose concentration in diabetic patients, with RSDs ≤ 10% (n = 9). A deviation of 6 and 9% was found for high and low glucose concentrations, respectively. Thus, this technology offers satisfactory reproducibility, low fabrication costs, and convenient replacement for the proposed application.

Uric acid (UA) and ascorbic acid (AA) were also examined as potential saliva interfering compounds toward selective determination of glucose at −0.20 V. As illustrated in Figure 3A(b), 0.6 mM glucose produces the expected response whereas uric acid and ascorbic acid does not show any response at the basal concentration of these molecules in saliva (200 and 20 μM, respectively).30 Note also that a defined glucose response is obtained after the UA and AA measurement. Furthermore, little or no interference from milk’s sugars should be found due to milk left-over or reflex in the baby’s mouth30 and the negligible affinity of GOx to fructose.41

A continuous on-body monitoring of glucose in saliva was carried out using healthy and diabetic human subjects (Figure 3B). After about 200 s of using the pacifier (see the Experimental Section, device operation), the saliva reaches the electrode which provokes an increment in the amperometric signal. This signal stabilizes within a few seconds and increases (negatively) 100 s later, once the reaction with the GOx takes place, due to the presence of glucose in the saliva. The working operation of the pacifier sensors is shown in Video S1 in which the pacifier is used by a healthy subject after a meal at the same time that the amperometric signal is monitored. Thus, the concept of the pacifier-sensor has been proved in monitoring differences between glucose concentration before and after a meal in diabetic patients, which would have further applications for newborns and infants.

In this sense, to prove the suitability of the sensor to monitor glucose concentration in saliva, raw saliva from two different patients diagnosed with type I diabetes has been tested in vitro using the PB-GOx electrode before and after they had a meal (see the Experimental Section for detailed information about sample collection). As can be seen in Figure 3C, a good correlation between the current obtained with the PB-GOx sensor and the commonly used glomerulator-fingertip blood signal for glucose in saliva was achieved with RSDs of 8% and 9% in the two sample subjects analyzed, respectively. In this sense, the glucose concentration in saliva before and after a meal in 5 different individuals (healthy and diabetic) using the pacifier was correlated with blood glucose concentration as well, obtaining a very good correlation between these two approaches measuring glucose in two different fluids (Figure 3C). The measurements of the diabetic patient’s saliva using the pacifier-sensor have been carried out 30 min after a meal as is detailed in the schematic shown in Figure 3D. Using this approach, different diabetic patients were asked to have a meal in order to monitor their glucose content in saliva before and after the food intake. As shown in Figure 3E, the signal obtained in a fasting state (b,e) was lower than the one obtained after a meal (c,f). To be sure of the good operation of the PB-GOx sensor, after meal saliva was also tested in the pacifier-sensor without including GOx in the configuration, showing a huge decrease in the signal due to the absence of the enzyme-based glucose recognition (a,d). This proof of concept clearly demonstrates the suitability of the sensor to monitor changes in glucose concentration using saliva as a noninvasive sample. The good results obtained in terms of selectivity, sensitivity, and reproducibility between different sensors establish the basis for the potential clinical applicability of the pacifier in the diabetes monitoring of newborns and children. This platform provides a new alternative to the current issues related to sample collection involving invasive samples such as blood, making necessary the development of new chemical wearable sensors using non-invasive samples like saliva, especially in newborns’ diseases. Additionally, the capability of the sensor to be fully integrated with wireless transduction makes it even more convenient for
saliva monitoring in a pacifier, considering the encouraging results obtained using the active saliva monitoring.

**CONCLUSIONS**

We have demonstrated, for the first time, a fully integrated baby’s wearable device prototype for chemical biomarker sensing using a pacifier. As a proof of concept, we have tested the device for glucose monitoring, integrating sampling, and measurement onto the same platform. We combined a pump-free system allowing unidirectional flow to an external collection outlet, in a baby-friendly platform, to facilitate an intimate and safe system to the neonate. The new device is capable of integrating saliva sampling with electrochemical sensing, along with miniaturized wireless electronics on a single pacifier platform. Such integration simplifies the infant’s health monitoring in a real-time and selective manner. The attractive performance can rapidly alert the wearers and parents about abnormal glucose patterns being possible to be implemented for adults in another accessory. The pacifier platform could be readily reconfigured and expanded to the monitoring of other diseases and saliva biomarkers in newborns or for multianalyte monitoring in connection to different bioreceptors and printed transducers.

With this platform, we attempt to access the infant world with an accessory toward direct and real-time saliva sampling and detection. In addition, we are adopting the advantages of the electrochemical wearable field platforms, including user-friendly and fast diagnostics into an unnoticed group of patients with rare diseases, who usually face delayed diagnostics and monitoring, and also into the premature babies group with very low birthweight, who requires strict glucose monitoring for parenteral nutrition in neonatal intensive care units.

However, several challenges would need to be overcome for clinical translation. The duration of the sensing operation is currently limited by safety reasons and by the stability of the chitosan layer which provides the enzyme modification into the electrode, in addition to the biofouling effects from the complex saliva matrix. Here, biofouling and lifetime challenges have largely been avoided because of the single-use of the sensors which is also an advantage considering point of care applications. However, alternative sensing electrolyte layers should be investigated toward higher stability and extended operations, such as proposed polymeric coatings for avoiding biofouling. In order to improve the safety aspects of the device, a complete single silicone-based nipple piece and dropper channel could be created for eliminating the risk of losing the internal parts. Further improvements in the detection limits could be realized through the use of other ink materials, and improvements in the saliva collection would provide better diagnostics.

Future efforts aim to expand the pacifier sensing platform toward the monitoring of additional chemical markers for achieving comprehensive timely information about the health status of neonates. We envision a future pacifier sensor that can provide simultaneous real-time monitoring of multiple saliva biomarkers, obtaining a useful assessment of the health status of neonates who cannot provide any feedback about discomfort or health complaints. Thus, the necessity to control these parameters would be addressed by using the wearable pacifier since it constitutes a comfortable tool which can be fully integrated for health monitoring into newborn daily life.

**REFERENCES**