OPERATING FREQUENCY RANGES OF ULTRASOUND-BASED IMPLANTABLE GLUCOSE-SENSITIVE RESONATORS FOR IMPROVED SENSITIVITY AND LINEARITY

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ABSTRACT

Commercially available continuous glucose monitoring (CGM) devices with transdermic needles or connections can help to manage diabetes but face limitations due to foreign body responses impacting their lifespan. Recently, it was shown that using ultrasound to excite and read out an implantable stimulus-responsive hydrogel structure acting as an acoustic resonator can monitor glucose concentrations *in vitro* and *in vivo* and could help address these challenges. However, until now, a method to determine an appropriate excitation frequency for this application remained an open question. The presented study, therefore, describes a measurement setup and corresponding experiments to conduct a spectral analysis of glucose-sensitive hydrogel resonators to identify operating frequency ranges. The study explores frequencies from 2.5 to 4.5 MHz, and the results identify specific frequency bands (3.86 to 3.91 MHz and 4.02 to 4.08 MHz) near one dip (3.9 MHz) featuring high linearity and sensitivity within physiologically relevant glucose concentrations (0-12 mM). The findings demonstrate that careful ultrasound frequency selection with the described method can help to achieve a linear and sensitive glucose sensing system.

KEYWORDS

Ultrasound, Glucose Monitoring, Appropriate Frequency Selection, Sensitivity, Linearity, Acoustic Resonator, Hydrogel.

INTRODUCTION

Diabetes presents a significant global health challenge, impacting approximately 537 million individuals worldwide [1]. It represents a chronic metabolic disorder characterized by dysregulation of blood glucose levels due to inadequate insulin production [2]. In order to prevent severe complications associated with diabetes, such as hypertension, infections, neuropathy, and organ failure [3], early diagnosis and effective management are essential. Successful diabetes management often entails maintaining optimal blood glucose levels through regular monitoring as well as appropriate intervention [4]. Developments in continuous glucose monitoring (CGM) technology have enhanced the quality of life for diabetic patients [5] by offering real-time monitoring, thereby improving blood glucose control. However, existing CGM devices often utilize transdermal needles or connections that often cause foreign body responses [5], limiting their lifespan as implanted sensors.

Recently, the integration of a glucose-sensitive hydrogel-based sensing platform with an ultrasound (US) readout method for blood glucose monitoring has been reported [6], [7], [8]. This innovative approach effectively mitigates limitations encountered with other CGM devices, such as issues related to biocompatibility, mechanical compliance, and transdermal electronics. Hydrogels are three-dimensional cross-linked hydrophilic polymer networks [9] that offer excellent biocompatibility due to their high-water content and reduced mechanical mismatch at the hydrogel-tissue interface [10]. Glucose-sensitive hydrogels are designed to undergo volume-phase transitions (VPT) in response to changes in glucose concentration in the environment. Hydrogels, in general, show promise for reducing foreign body responses when implanted as they can be highly biocompatible, implantable, and biodegradable [11]. Using ultrasound to read this volume phase transition reduces the dependence on transdermic connections. Ultrasound, a non-invasive medical examination technique [12] with a proven safety record, is well-suited for reading out the volume change of hydrogel purpose due to its excellent visualization properties, affordability, portability, and real-time capabilities [13], [14].

The sensing principle of the hydrogel and ultrasound-based sensing platform relies on the ultrasound-induced oscillation of the hydrogel structure. Now, the shrinking of the glucose-responsive hydrogel structure in response to the presence of glucose in its environment affects its oscillation behavior under ultrasonic excitation. This change in oscillation behavior causes changes in the magnitude of reflected ultrasound waves (Fig. 1). If excited with a fixed frequency of ultrasound, the change in the reflected wave's intensity could be used to track the glucose concentration.

Determining the ultrasound operating frequency for inducing optimum oscillation is a critical factor in the operating principle for the sensor's functionality. In principle, this operating frequency should be close to the resonance frequency of the hydrogel structure to achieve a higher change in reflected wave's intensity due to greater energy absorption of the hydrogel structure to sustain the higher amplitude of vibration near its resonance frequency [15]. In previous work, due to experimental limitations, the operating frequency was chosen from a limited set of available frequencies without prior spectral characterization [6], [7], [8]. Here, expanding beyond previous results [16], a method is introduced to determine suitable operation frequencies for improved sensing performance using spectral characterization conducted in a more application-oriented reflection mode.

MATERIALS AND METHODS

Spectral Response Measurement

Spectral response measurements of the hydrogel sheet were conducted within a custom-made water tank setup (Fig. 2). To analyze the hydrogel assembly, a tunable ultrasound transducer (V380-SU, Olympus NDT Inc., USA) was used in the tank filled with degassed deionized water. A sinusoidal burst signal (peak-to-peak voltage: 10 V, repetition 3) spanning frequencies ranging from 2.5 MHz to 4.5 MHz was applied to the transducer gradually using a function generator (33521B, Keysight Technologies Inc., USA) to excite ultrasonic waves of corresponding frequencies. The hydrogel sheet, along with its acrylic solution container, was positioned opposite the transducer. Reflected ultrasonic waves from the hydrogel assembly were captured using a hydrophone (HGL-1000, ONDA Co., USA). The hydrophone's output was connected to a preamplifier (AH-1100, ONDA Co., USA), and the received signals were recorded using an oscilloscope (DSOX3024T, Keysight Technologies Inc., USA).

The data was evaluated using FFT after DC offset correction followed by filtering to stop the spectral leakage in the frequency domain. Then, the signal amplitude (I_S) for each frequency was extracted and referenced to the corresponding background signal without hydrogel (I_b) and plotted as the relative change in signal = $20 * \log (I_S / I_b)$.

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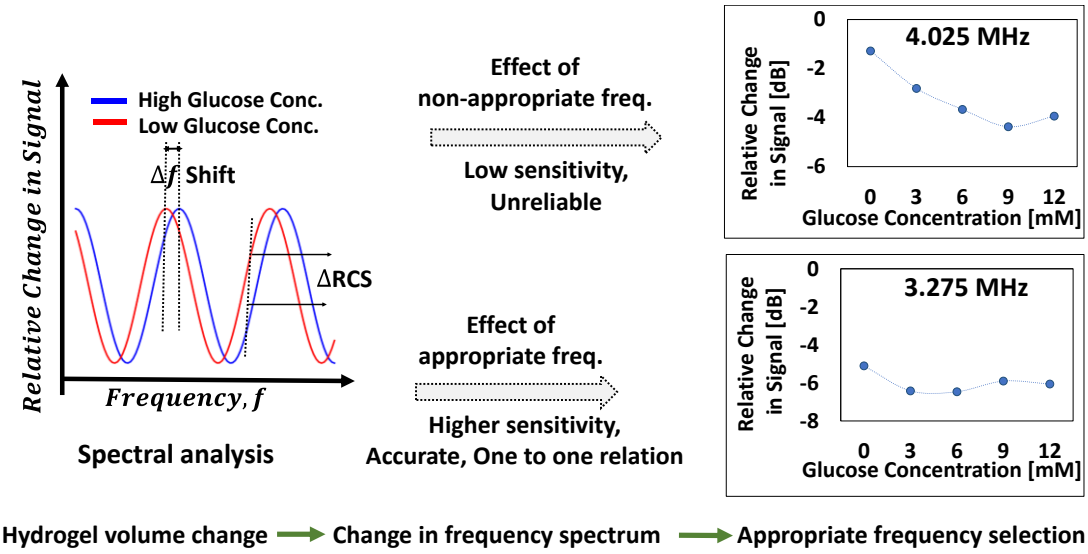

Figure 1: Effect of appropriate frequency selection for ultrasound readout of analyte-sensitive hydrogel structures based on resonance absorption of the ultrasound waves.

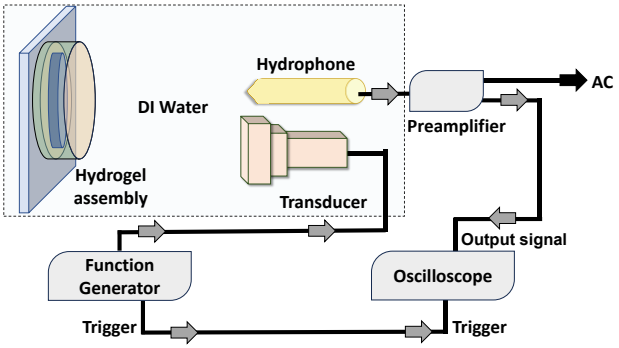

Figure 2: Schematic illustration of the measurement setup for the acoustic spectral characterization of stimulus-responsive hydrogel structures in reflection mode.

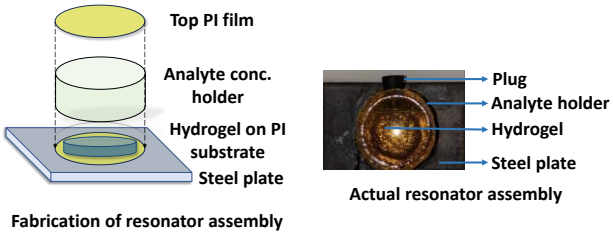

Figure 3: Fabrication of the resonator assembly showing the top PI film, hollow acrylic analyte holder, and hydrogel on surface-modified PI film being assembled on a steel plate and an actual fabricated assembly.

Sample Fabrication

The hydrogel sheets used in the study consist of a hydrogel layer, a polyimide substrate, and a solid backing. The preparation process involved following established protocols for the pre-polymer monomer solution as described in previous studies [7], [8]. Polyimide (PI) polymer films served as the substrate and underwent surface modification, as described in previous studies [17]. After modification, hydrogel sheets of 1000 µm thickness with a diameter of 1.5 inches were fabricated using PTFE spacers under a collimated UV source. The substrate, along with the hydrogel sheet, was attached to a steel plate using a cyanoacrylate-based adhesive to increase ultrasonic reflection. A hollow acrylic tube was affixed to the hydrogel side, and the assembly was sealed to ensure the analyte concentration introduced to the hydrogel did not get mixed with the DI water in the tank. Conditioning procedures were applied to each sample to improve measurement reproducibility measurements [18].

Glucose Solution Preparation

A 500 mM stock solution was first created by dissolving 18.1 g of D-(+)-Glucose (Sigma-Aldrich, USA) in 170 mL of 1X (10 mM phosphate concentration) phosphate-buffered saline (PBS) solution, and then increasing the volume to 200 mL with PBS. Then, an appropriate amount of 1X PBS phosphate buffer saline solution was added to the stock solution to prepare the required glucose solutions for this study.

RESULTS

Initially, the frequency spectrum of the hydrogel sheet was recorded in a glucose-free environment (0 mM) in 1X PBS solution. This baseline measurement was followed by the introduction of a 3 mM glucose solution (also in 1X PBS), after which the hydrogel was allowed a period of 7 hours to stabilize. The frequency response of the hydrogel to this glucose concentration was then logged. The procedure was repeated with a 6 mM glucose solution, with the hydrogel left to reach equilibrium overnight. The resulting frequency spectrum for the 6 mM concentration was recorded on the subsequent day. This method was consistently applied to obtain the spectra for 9 mM and 12 mM glucose concentrations, as illustrated in Fig. 4.

Following the acquisition of these spectra, the study further explored how the hydrogel structure responded to ultrasound excitation across these frequencies, focusing on the sensitivity and linearity of the hydrogel structure's output response (relative change in signal). Sensitivity, denoted as S(f), was determined for each frequency by comparing the hydrogel's response to the highest

(9 mM) and lowest (0 mM) glucose concentrations, utilizing the following formula for calculation.

Sensitivity, $\boldsymbol{S(f)} = \frac{|\boldsymbol{RCS}\,(f)_s^{9\,mM} - \boldsymbol{RCS}\,(f)_s^{0\,mM}|}{\boldsymbol{Highest\ Conc.} - \boldsymbol{Lowest\ Conc.}}$ where

$\mathrm{RCS}\,(f)_s^{9\,\mathrm{mM}}$ = Relative change in signal for 9 mM glucose and $\mathrm{RCS}\,(f)_s^{0\,\mathrm{mM}}$ = Relative Change in signal for 0 mM glucose. The findings of this sensitivity analysis are presented in Fig. 5.

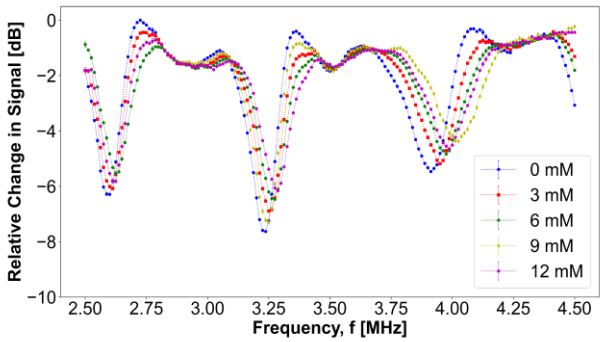

Figure 4: The effect of the change of hydrogel swelling state on the frequency spectrum induced by different glucose concentrations. Error bars represent one standard deviation from three repetitions while acquiring each data point

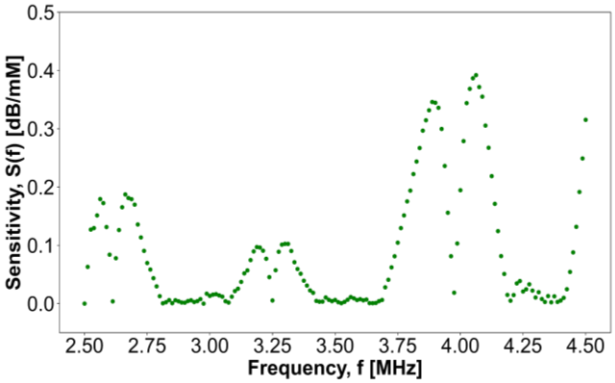

Figure 5: Sensitivity calculated at each frequency from the relative change in signal based on the 9 mM and 0 mM spectra since 9 mM represents the highest glucose response and 0 mM represents the lowest glucose response for this hydrogel structure.

The evaluation of the hydrogel structure's output response to varying glucose concentrations was conducted by fitting a linear regression model to its response curve across glucose concentrations ranging from 0 mM to 9 mM at each frequency. This leads to the calculation of the coefficient of determination, R^2, which reflects the fit's accuracy and is plotted against frequency. It is important to note that the analysis excluded the 12 mM glucose concentration due to the hydrogel's known tendency to reach saturation beyond a 9 mM glucose concentration, as documented in previous research [8]. outcomes of this linearity assessment are depicted in Fig. 6.

DISCUSSION

Figure 4 displays ultrasound resonance spectra for five glucose concentrations, suggesting potential operating frequencies near three absorption dips. Sensitivity and deviation from a linear line are then considered for refining the selection since good linearity and sensitivity are crucial for establishing a one-to-one relationship

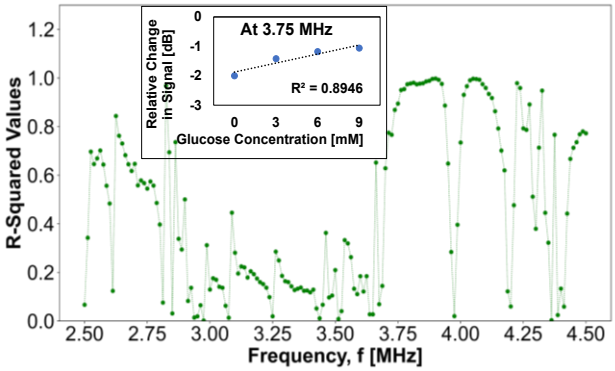

Figure 6: R-squared values representing the deviation from a linear fit for each frequency based on the spectra shown in Fig. 4. The inset shows an example of linear fit for the data obtained at 3.75 MHz. Since the 12 mM concentration response is due to the saturated hydrogel response for glucose [8]*, up to 9 mM is used for calculation.*

between output response and the corresponding analyte in implantable sensors. Results displayed in Fig. 5 and Fig. 6 then underline a narrow frequency band (3.86 to 3.91 MHz and 4.02 to 4.08 MHz) near the 3.9 MHz dip, exhibiting the least deviation from the fitted curve ($R^2 > 0.9$) as well as good sensitivity ($S(f) > 0.3$ dB / mM) to glucose concentrations. The least deviation from a linear fit means that at those frequencies, the hydrogel structure's output measured in the relative change in signal is able to show a one-to-one relationship with the corresponding glucose concentration (an example of output at these appropriate frequencies is shown in inset of Fig. 6). Similarly, an example of an inappropriate excitation frequency is shown in Fig. 1, where the characteristic curve at 3.275 MHz shows that reliable glucose measurement can be hindered at this frequency of excitation due to multiple glucose concentrations exhibiting relatively close output signals. While the hydrogel's glucose-induced response may inherently exhibit nonlinearity, achieving linear detection within specific frequencies can be attributed to the potential for nonlinear compensation within the detection methodology.

CONCLUSION

The research outlined in this paper provides insights into the appropriate selection of ultrasound excitation frequencies for the operation of hydrogel-based implantable resonators with ultrasound excitation and readout for continuous glucose monitoring. Here, we have demonstrated that while several frequency bands near the observed absorption dips in the hydrogel spectrum could serve as potential candidates to excite the hydrogel-based acoustic resonators using ultrasound, the careful evaluation to ensure one-to-one relationship between output response and glucose concentration is imperative for pinpointing an appropriate operating frequency. Our findings revealed that a narrow frequency range, between 3.86 to 3.91 MHz and 4.02 to 4.08 MHz, adjacent to only one of the absorption dips at 3.9 MHz, exhibited good linearity as well as good sensitivity, making it the most suitable for accurate and reliable glucose detection for the investigated hydrogel resonator. These results suggest the value of the assessment in determining the hydrogel ultrasound resonators' operating frequency. Understanding the optimal operating frequency range will facilitate the development of compact ultrasound units tailored to the hydrogel's resonance properties, ensuring improved sensor performance for continuous glucose monitoring platforms.

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CONFLICT OF INTEREST

The authors declare the following competing financial interests managed through the University of Utah conflict of interest management: Florian Solzbacher declares a financial interest in Blackrock Neurotech and Sentiomed, Inc.

The fundamental intellectual property of the sensing mechanism is protected by the following patent application: 'Implantable and biodegradable smart hydrogel micromechanical resonators with ultrasound readout for biomedical sensing,' US20210267573A1 with Christopher F. Reiche, Navid Farhoudi, Florian Solzbacher, and Prattay Deepta Kairy listed as inventors and also by the following patent: 'Ultrasound imaging of biomarker sensitive hydrogels', US11445997B2 with Christopher F. Reiche, Navid Farhoudi, and Florian Solzbacher listed as inventors.

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