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A Label-free, non-Intrusive, and Rapid Monitoring of Bacterial Growth on Solid Medium using Microwave Biosensor

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***Abstract*—Microwave resonator sensors are attractive for their contactless and label-free capability of monitoring bacterial growth in liquid media. This paper outlines a new label-free microwave biosensor based on a pair of planar split ring resonators for non-invasive monitoring of bacterial growth on a solid agar media. The sensor is comprised of two split ring resonators with slightly different resonant frequencies for differential operation. The transmission coefficient (S_{21}) of the sensor is considered as the sensor's response with a designed and measured quality factor above 200 to ensure a high-resolution operation of the biosensor. Two resonant frequencies of 1.95 and 2.11 GHz represent the sensing signal and the reference signal, respectively. The developed sensor demonstrates high performance in monitoring the growth dynamics of Escherichia coli (E. coli) on Luria-Bertani (LB) agar with 4 mm thickness. The sensor's resonant amplitude response demonstrated 0.5 dB variation corresponding to the bacterial growth over 48 hours when bacteria were spread on LB agar starting with initial OD_{600} = 1.5. Moreover, 0.6 dB change in the sensor's response was observed over 96 hours of bacterial growth starting with an initial OD_{600} = 1.17 spotted on LB agar. The measured results fit well to the curves created using Richards' bacterial growth model, showing the strength of the sensor as a potential candidate for use in predictive food microbiology systems.**

***Index Terms*— Bacterial growth monitoring, Differential microwave sensor, *E. coli* growth, LB agar, Ring resonator**

I. Introduction

AGAR based bioassays are indispensable for microbiological applications, especially in clinical, food, and environmental settings for pathogen detection and functional screening of metabolites, pollutants, and enzymes [1]–[3]. In Canada alone, food borne pathogens caused 1.6 million illnesses, 4000 hospitalizations, and 105 deaths per year [4]. In recent years there has been huge demand for microbial studies using structured solid systems in predictive food microbiology approaches to achieve a more realistic model relative to a liquid matrix[5]. Compared to liquid broth, agar based assays provide an opportunity for multiple screenings in a single agar plate which can significantly reduce the cost and analysis time, while also simplifying the procedure and providing high selectivity and sensitivity [6], [7]. The drawback of this technique is the time it takes to visually observe colonies growing on the agar medium. When attempting to detect infectious pathogens, the extended incubation period, usually 48-72 hours but sometimes even longer, delays the treatment and can lead to life threatening conditions [8]. Currently more advanced and expensive techniques such as high-throughput automated systems based on DNA amplification/sequencing are being utilized for pathogen detection [9], [10]. However, the cost and expertise required to operate these techniques limits their adoption on a global scale. Therefore, an improvement of agar-based assays facilitating bacterial growth monitoring in real time, enabling early detection of pathogens, can be a paradigm shift in clinical research and treatment.

Recently, microwave sensors have demonstrated significant performance as real time industrial sensors [11]–[14] and biosensors [15], [16]. They offer several advantages such as being non-invasive to cells and their environment, non-destructive, and label-free. They operate based on the interaction between the electromagnetic field and the surrounding environment, which is then interpreted as a variation of power transmission or impedance in a sensing circuit. As an electric field is applied, molecular dipoles and free charges are excited, while the net response is based on the material's dielectric properties (permittivity, conductivity, and loss tangent). The understanding and sensing of a cell's dielectric properties is particularly beneficial for cell characterization, separation, and identification [17]. In recent years, microwave sensors have been implemented by several groups for the detection of biological cells. Notably, Afshar *et al*. used dielectrophoresis (DEP) to investigate the dielectric properties of Chinese hamster ovary (CHO) cells subjected to nutrient deprived conditions [18] and heat shock [19] over a frequency range of 100 kHz to 300 MHz. Microwave sensors have also been successful in detecting cancerous cells [20]. Grenier *et al*. [21] used microwave dielectric spectroscopy to discriminate between sub-populations of B lymphoma cell lines with a frequency range of 40 MHz to 40 GHz and Chen *et al*.

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[22] used a coplanar waveguide (CPW) transmission line for the dielectric characterization of HepG2 cells with a frequency range of 1 to 40 GHz. Although these broadband devices have shown great potential for characterizing and monitoring biomatter, they ultimately have lower sensitivity, limiting practical use.

Planar microwave resonators have exhibited enhanced sensitivity and an improved quality factor compared to broadband techniques [23], [24]. Advantages of these devices include low-cost, design flexibility, and simple fabrication relative to other microwave sensing techniques. They are also compatible with microfluidic lab-on-a-chip systems, enabling testing of aqueous biological solutions [25]. Furthermore, they have been successfully implemented in wearable technology devices [26]. They operate by quantifying the interaction between electric field and material under test by measuring the resonant frequency, which is dependent on the dielectric properties of the material. In recent years, they have demonstrated substantial potential in biosensing and biomedical applications [25]. Narang *et al*. [27][28] demonstrated that a microwave-microfluidic biosensor can detect the concentration and growth of E. coli, which has applications related to antibiotic susceptibility testing (AST). A microwave sensor as a capacitive matrix for label-free detection of *Escherichia coli* in Luria-Bertani medium, with the detection limit of 10^3 CFU/ml, has been reported [29]. Microwave sensors monitor bacterial growth by virtue of variations in dielectric properties associated with growth [30]. Mondal *et al*. [31] successfully detected different concentrations of glucose in water which can benefit individuals with diabetes. To further improve monitoring, a double resonator array, where each resonator acts independently, can be implemented [24], [32]. This enables non-contact, real-time monitoring of a biological sample as well as allowing additional distinction between biological sample variation and unknown environmental conditions. Notably, Velez *et al.* [33] implemented two split ring resonators to monitor and measure electrolyte concentrations of NaCl, KCl, and $CaCl_2$ in diluted aqueous solutions. A double planar microwave resonator integrated with agar based bioassays can be an effective tool to better understand real-time bacterial growth in agar assays.

In this work, a new biosensor platform based on solid growth media with microwave sensor integration was investigated. The main goal was to provide a reliable, non-invasive, real-time method that can monitor bacterial growth in isolated areas without direct contact. The developed structure was theoretically and practically investigated using standard simulation software programs, which demonstrated good alignment between simulation and experimental results. The capability of label-free monitoring of bacterial growth by the developed method mitigates damage to analytes, operates with small volumes, and reduces implementation and testing complexities. This paper is organized as follows, section 2 discusses materials, methods, and the principal of the operation for the sensor; section 3 presents the measurement results and discussions, and section 4 concludes the paper's major results and findings.

II. Sensor Design and Simulations

Planar split ring resonator sensors have the advantages of non-contact sensing, low fabrication costs, and structural robustness owing to their microstrip structure. The sensor structure in this work comprised of two planar split ring resonators (SRRs) operating within a frequency span of 1.8 to 2.2 GHz. The planar sensor was designed and simulated in High Frequency Structure Simulator (HFSS) and presented in Fig. 1a. The operation frequency of the sensor was selected considering the fact that cytoplasm (material within a cell) is mainly composed of water, and water's loss factor increases beyond this frequency span, reducing sensitivity at higher frequencies [34].

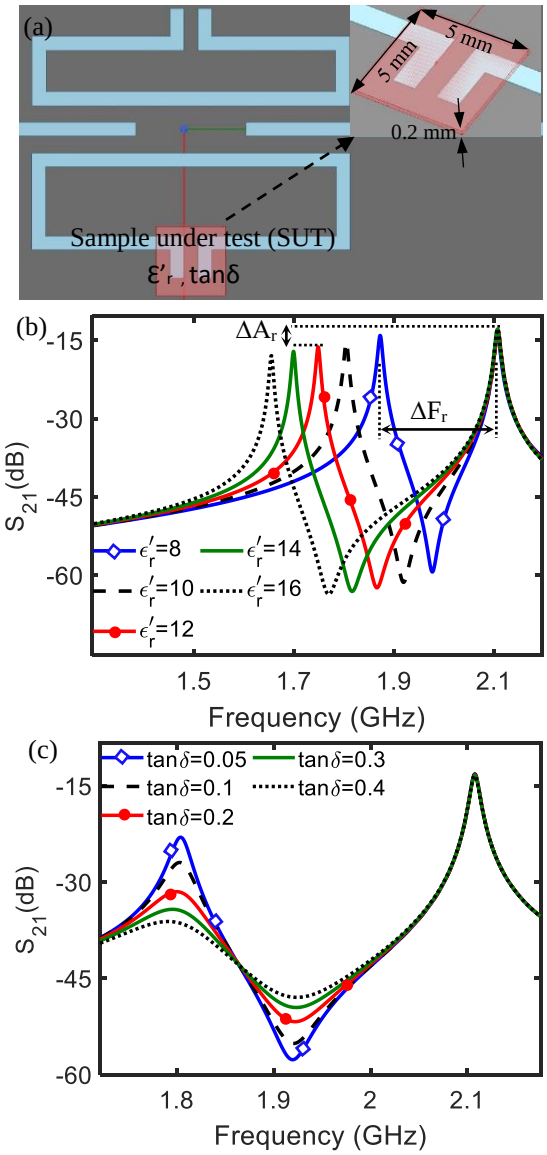

Fig.1 HFSS simulation results, (a) sensor structure with a sample (5×5×0.2 mm^3) over the ring gap of the sensing resonator, (b) transmission coefficient (S_{21}) response of the sensor in the presence of a sample with various permittivity at a loss tangent of 0, and (c) transmission coefficient (S_{21}) of the sensor to the sample's loss tangent variation (0.05 to 0.4) with a constant permittivity of 8.

The resonators were designed to achieve a high -3 dB quality factor, which corresponded to high resolution in detecting permittivity variation in the vicinity of the sensor.

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The motivation for using two resonators in this microwave sensor was to eliminate the common electronic noise and the impact of common dielectric property variation due to temperature and relative humidity changes local to the sensor [24]. This structure enabled the precise monitoring of changes in dielectric properties in an agar environment with a very high dielectric loss.

In order to guarantee an adequate electromagnetic isolation between the sensing and reference resonator, the layout of the sensor was designed to consider the maximum distance between the ring gaps of the resonators. As shown in Fig.1b,c, putting a dielectric slab with permittivity of 8 and loss tangent of 0 over the ring gap of the sensing resonator would mainly affect the response of the sensing resonator, while the reference resonator profile was remained unaltered, indicating acceptable decoupling between the two resonators. HFSS simulation results demonstrated the capability of the sensor to detect

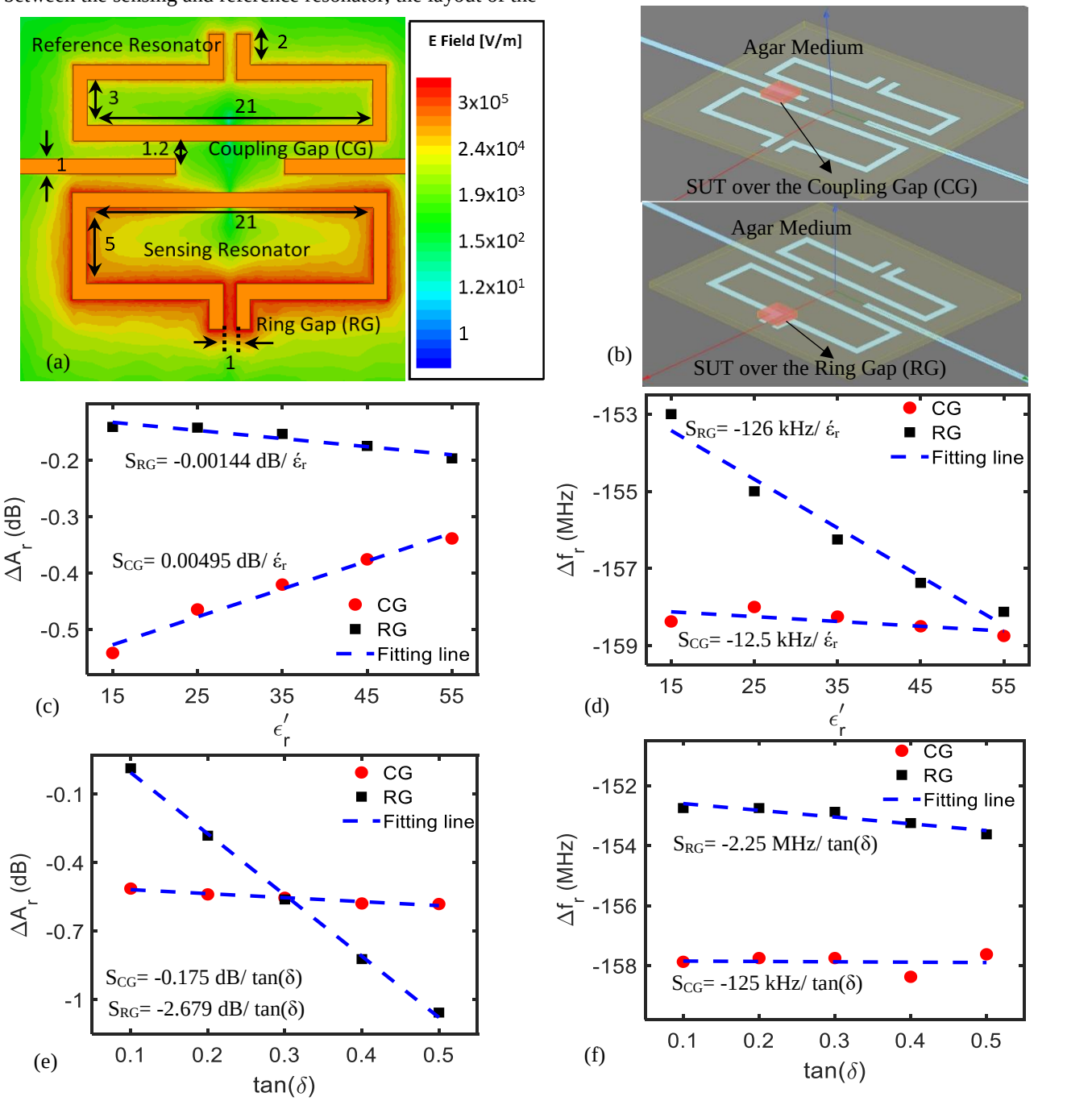

Fig. 2. HFSS simulation results: (a) E-field analysis at 1.94 GHz and the sensor's dimensions in mm corresponding to a half-wavelength (λ/2) resonance ; (b) two different configurations of the sample (3×3×1 mm^3) for sensitivity analysis by considering the common mode effects as a slab (30×26×1 mm^3); (c) the differential resonant amplitude as a function of the permittivity of the sample placed on the resonator gap and the coupling gap for a constant loss tangent of 0.15; (d) changes in resonant frequency difference with respect to the change of the permittivity of the sample placed on the resonator gap and the coupling gap for a constant loss tangent of 0.15; (e) changes in resonant amplitude difference with respect to the change of the loss tangent of the sample placed on the resonator gap and the coupling gap for a constant permittivity of 15 ; (f) changes in resonant frequency difference with respect to the change of the loss tangent of the sample placed on the resonator gap and the coupling gap a constant permittivity of 15.

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dielectric property variation of the sample material over the ring gap of the sensing resonator by tracking the simulated changes in the transmission coefficient (S_{21}) of the sensor.

A resonator with a slightly smaller physical size was designed to operate as the reference resonator, with reference resonant frequency (*RRF*) and reference resonant amplitude, (*RRA*). The resonator with the larger size operated as the sensing resonator, attributed with a sensing resonant frequency (*SRF*) and sensing resonant amplitude (*SRA*). Independent operation of the two resonators allowed a linear subtraction between *RRF* and *SRF* or *RRA* and *SRA* to eliminate the effect of temperature and moisture variation in the environment over the response of the sensor. The adjusted responses of the sensor were defined as ΔF_r and ΔA_r, which were the differences between RRF and SRF, and the *RRA* and *SRA*, respectively (Fig. 1b).

Fig. 2(a) represents the electric field distribution on the sensor when the input signal is 1.94 GHz, the *SRF*. A high sensitivity was confirmed via electric field analysis near the ring gaps of the sensing and reference resonators by demonstrating a high electric field intensity near these regions in comparison to other areas over the bare sensor at the resonant frequencies of 1.95 GHz and 2.11 GHz, respectively. A sensitivity analysis has been performed in the ring and coupling gap of the sensing resonator in order to have an accurate and complete comparison. The effect of the agar layer and container (common mode effects) were considered by covering both resonators with a material slab with a permittivity of 81 and loss tangent of 0.157 at a distance of 0.5 mm from the surface of the resonators. A sample under test (SUT) with initial permittivity of 15 and loss tangent of 0.15 was alternately placed over the agar material on the ring gap, then the coupling gap of the sensing resonator as shown in Fig. 2(b). The sensitivity of the sensor to permittivity and loss-tangent variation of the sample material on the coupling or the ring gap area of the resonators was studied in HFSS simulations. Fig. 2(c, d) presents ΔA_r and ΔF_r variation versus permittivity change of a test sample over the ring and coupling gaps with the associated sensitivity (the partial derivative of the output with respect to an input factor, i.e. $S_{CG} = \frac{\Delta(\Delta A_r)}{\Delta \varepsilon_r}$) to compare the sensitivity of the sensor's response for those regions. The results in Fig. 2 show that a higher sensitivity to variation in dielectric properties was achieved in the ring gap area in comparison to the coupling gap region.

III. Materials And Methods

A. Bacteria Sample Preparation

Bacterial culture was grown in LB agar medium. LB agar was prepared by adding LB broth (20 g/L) and agar (15 g/L) (Fisher Bioreagents) in RO water. The media was sterilized by autoclaving at 121 °C at 15 psi for 15 minutes and poured into Petri dishes (60 × 15 mm^2) to make agar plates. Plates were inoculated with an overnight culture of E. coli grown in LB broth. The plates were then incubated over a resonator at room temperature (22 ±1 °C) for 72 hours. Following incubation, the area in the agar plate covered by bacterial growth was carefully scraped off, suspended in sterile water, and measured for optical density at 600 nm wavelength (OD_{600}) using a spectrophotometer.

B. Split Ring Resonator Fabrication and Experimental Setup

A two-port resonator sensor with two decoupled split ring resonators was fabricated on a Rogers RT/duroid 5880 high frequency substrate with dielectric thickness of 0.79 mm, covered by a 35 µm copper cladding on both sides. The relative permittivity and loss-tangent of the substrate were 2.2 ±0.02 and 0.0009, respectively.

The Petri dish containing E. coli was placed over the microwave sensor covering both resonators. The microwave sensor was connected to a phase network analyzer (N5242A-PNA) from Keysight through phase-stabled cables to measure the S_{21} profile of the sensor. The output power of the PNA was 0 dBm and the bandwidth was set to 300 Hz for measurements. All microwave cables were mechanically secured against vibration and bending forces to avoid any drift in the sensor's response. The *S*-parameters of the microwave sensor were recorded using LabVIEW software every 2 minutes. Visible bacterial growth was observed and recorded using a Celestron 5 MP Handheld Digital Microscope Pro.

C. Functional Validation of the Sensor

The performance of the fabricated sensor was initially validated through a set of test measurements. The response of the sensor in the presence of a set of standard 5×5 mm^2 substrates with varying thickness, permittivity and loss tangent, listed in Table I, was recorded.

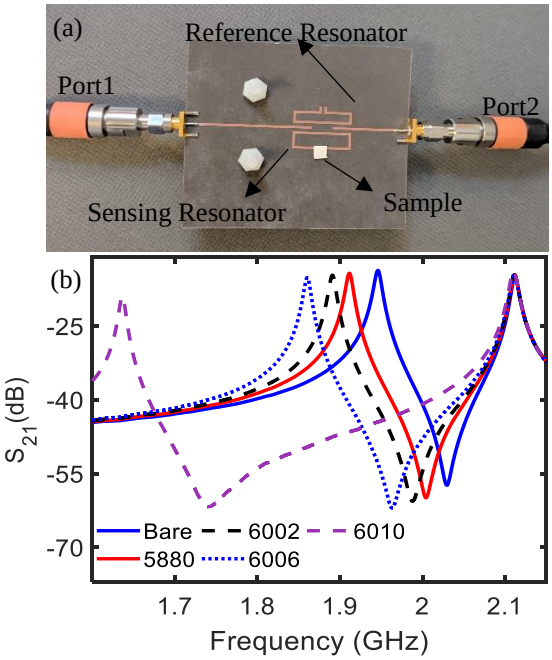

Fig. 3. (a) Test setup (fabricated sensor and sample) and (b) S_{21} response of the sensor to different standard substrates with different thickness, permittivity, and loss tangent for initial functionality validation of the sensor.

As shown in Fig. 3, the experimental results not only validated the functionality of the fabricated sensor for monitoring

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variation in dielectric properties, but also demonstrated low coupling between the sensing and reference resonators, leading to high resolution and accuracy.

TABLE I
DIELECTRIC PROPERTIES OF THE SUBSTRATES

Rogers Substrate	Permittivity	Loss tangent	Thickness (mm)
5880	2.2	0.0009	1.57
6002	2.92	0.0012	0.76
6006	6.15	0.0027	1.91
6010	10.2	0.0023	1.91

IV. MEASUREMENT RESULTS AND DISCUSSION

The S_{21} response of the fabricated bare sensor was measured using a PNA showing resonant frequencies at 1.95 and 2.11 GHz, resonant amplitudes of -13.17 dB and -15.36 dB, and -3 dB quality factors of 212 and 234 for the sensing and the reference resonators, respectively. To avoid mutual coupling between the resonators, individual resonators were separated by 0.2λ to achieve a low coupling factor. Fig. 4 compares the simulated and measured results of S_{21} profiles for the bare sensor and the sensor with an agar layer. The characteristic parameters of the responses are presented in Table II. A good agreement was observed between the simulated and measured S_{21} profiles at room temperature. The differences derived from the impact of soldering and SMA connectors as well as radiation loss. However, the *SRF* of the measured S_{21} profile for the sensing resonator with agar media deviated from simulation results due to the complexity of determining the dielectric properties of agar medium in the Petri dish.

In order to monitor bacterial growth, a sealed plastic Petri dish with 4 mm thickness of LB agar was inoculated by spreading 50 µL of E. coli (OD_{600} =1.5) over half of the plate (28.2 cm^2), the other half was not inoculated as a reference. The plate was placed over the sensor to align the inoculated portion over the sensing resonator and the reference agar over the reference resonator. The dielectric property changes in the growth media as a result of bacterial growth were continuously monitored and recorded by the sensor along with microscope images taken at regular intervals. Meanwhile, the reference resonator monitored the dielectric property changes in agar media solely due to temperature and humidity variations. Fig. 4(a) presents the experimental setup with the automated data acquisition system. The experiment was carried out inside an insulated

TABLE II
PRESENTS A COMPARISON BETWEEN THE MEASURED RESULTS AND THE HFSS SIMULATED RESULTS

Sensor Responses	Bare Sensor Simulated	Sensor-Agar Simulated	Bare Sensor Measured	Sensor-Agar Measured
$SRF(GHz)$	1.94	1.88	1.95	1.91
$RRF\ (GHz)$	2.10	2.07	2.11	2.07
$\Delta F_r\ (MHz)$	-160	-190	-160	-160
$SRA\ (dB)$	-12.65	-12.31	-13.17	-18.11
$RRA\ (dB)$	-14.26	-12.49	-15.36	-19.76
$\Delta A_r\ (dB)$	1.61	0.18	2.18	1.65
Q_S	212	188	212	118
Q_R	218	209	234	120

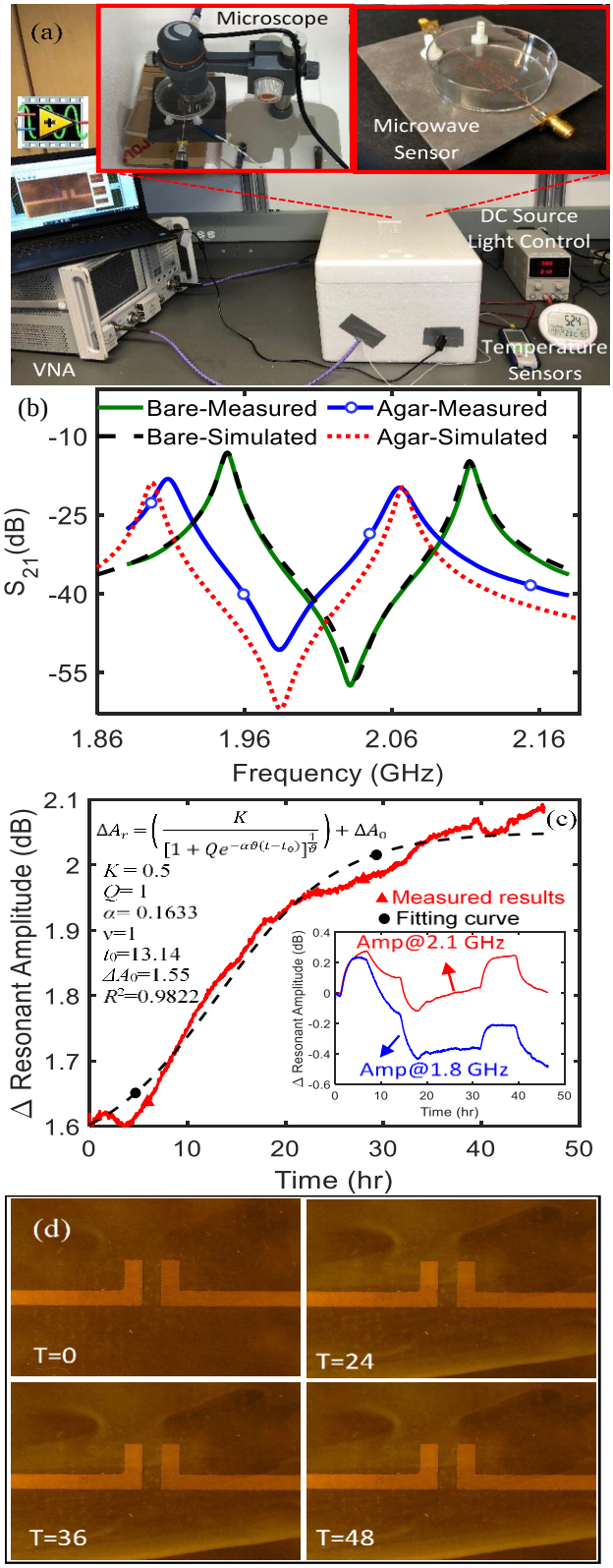

Fig. 4 (a) The experimental setup with a vector network analyzer, two temperature sensors to monitor the sensor and room temperature, (b) measured S_{21} profile for the bare resonator and the resonator with agar compared to the simulated results of the sensor in HFSS for bare and with agar; (c) transient response of ΔA_r variation in accordance to bacterial growth over 48 hrs of experiment on half of the agar surface; (d) time-lapsed microscope images of the medium with spread bacteria.

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Styrofoam box in order to stabilize the environment for measurements at 24℃. In addition, both temperature and humidity were constantly monitored and recorded. The variations in temperature inside the chamber during the experiments (96 hours) was minimal with 24.0 ±0.2℃, ensuring that the sensing parameters can only be considered as a function of bacteria growth. Because dielectric properties of bacteria are related to physiological characteristics such as cellular structure, biochemical components, and cellular activities [35]–[37]. It is well known that bacterial growth phases are associated with the changes in the physiological characteristics[30], which is reflected in the dielectric properties. The variations in dielectric properties in correlation with *E.coli* growth as evident from this work is further supported by several studies provided in the literature [38]. The relative permittivity (ε_r) of *E.coli* cell suspension was proved to be proportional to cell concentration by experimental evidence.

As shown in the inset of Fig.4 (c), both *RRA* and *SRA* responded to the dielectric property changes due to temperature and moisture variations in the agar medium. However, bacterial growth over the sensing resonator produced an additional impact on the transient response of the resonant amplitude of the sensing resonator (Fig. 4(c)). Bacterial growth on the agar affected the effective dielectric properties of that resonator. The change in the permittivity and loss tangent was reflected in the shifting resonant amplitude and resonant frequency of the sensing resonator. The change in the resonant amplitude over the experiment time was fitted to Richards' growth curve [39], which demonstrated a good agreement (R^2 >0.96) between the fitted curve and the measured microwave response. Equation (1) presents Richards' growth function.

$$Y(t) = \left(\frac{K}{[1+Qe^{-\alpha\vartheta(t-t_0)}]^{\frac{1}{\vartheta}}} \right) + Y_0 \quad (1)$$

where $Y(t)$ is the yield over time, Y_0 is initial concentration, K is the upper asymptote, α is the growth rate, and $Q = -1+(K/Y(t=t_0))^{\upsilon}$.

The microwave responses of the sensor were compared to the photos that were taken during the experiment using an optical microscope (Fig. 4d). As shown in Fig. 4d, negligible changes can be observed between T=36 hr. and T=48 hr. while the microwave sensor clearly demonstrated growth in the agar media above the sensing resonator.

In the second experiment, 1.5 µL of *E.coli* (OD_{600} = 1.17) grown for 24 hours and stored at 4 °C was spotted on an agar area surface (0.0314 mm^2). The inoculated region was placed over the ring gap of the sensing resonator and bacterial growth was monitored over a period of 96 hours. Both ΔF_r and ΔA_r demonstrated an increasing trend over the experiment period. However, ΔA_r variation started earlier (approximately 6 hrs.) than ΔF_r (approximately 40 hrs.), which can be attributed to the fact that the resonant amplitudes were influenced by conductivity, loss factor and permittivity variation, where the resonant frequencies were dominated by only the permittivity change in the agar medium. The response of the sensor was fitted to the Richards' model, which for both ΔF_r and ΔA_r demonstrated a good match between the predictive model and the sensor's response. Time-lapse images were taken and compared to the responses of the microwave sensor, which clearly indicated bacterial growth over the agar media. The first

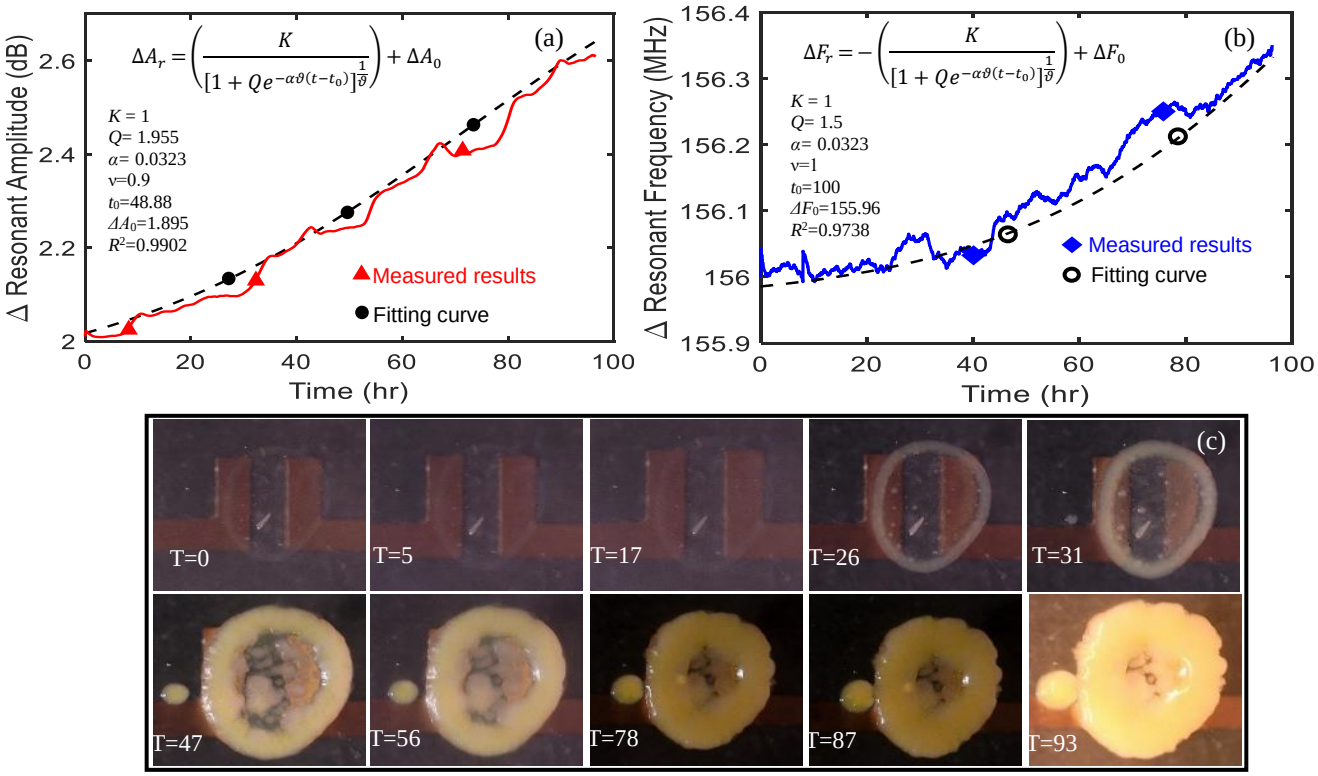

Fig.5. Measurement results for bacterial growth in the ring gap of the microwave sensor; (a) variation of resonant amplitude difference (ΔA_r), (b) variation of resonant frequency difference (ΔF_r), over time, (c) captured time-laps photos of bacterial growth associated with the experiment.

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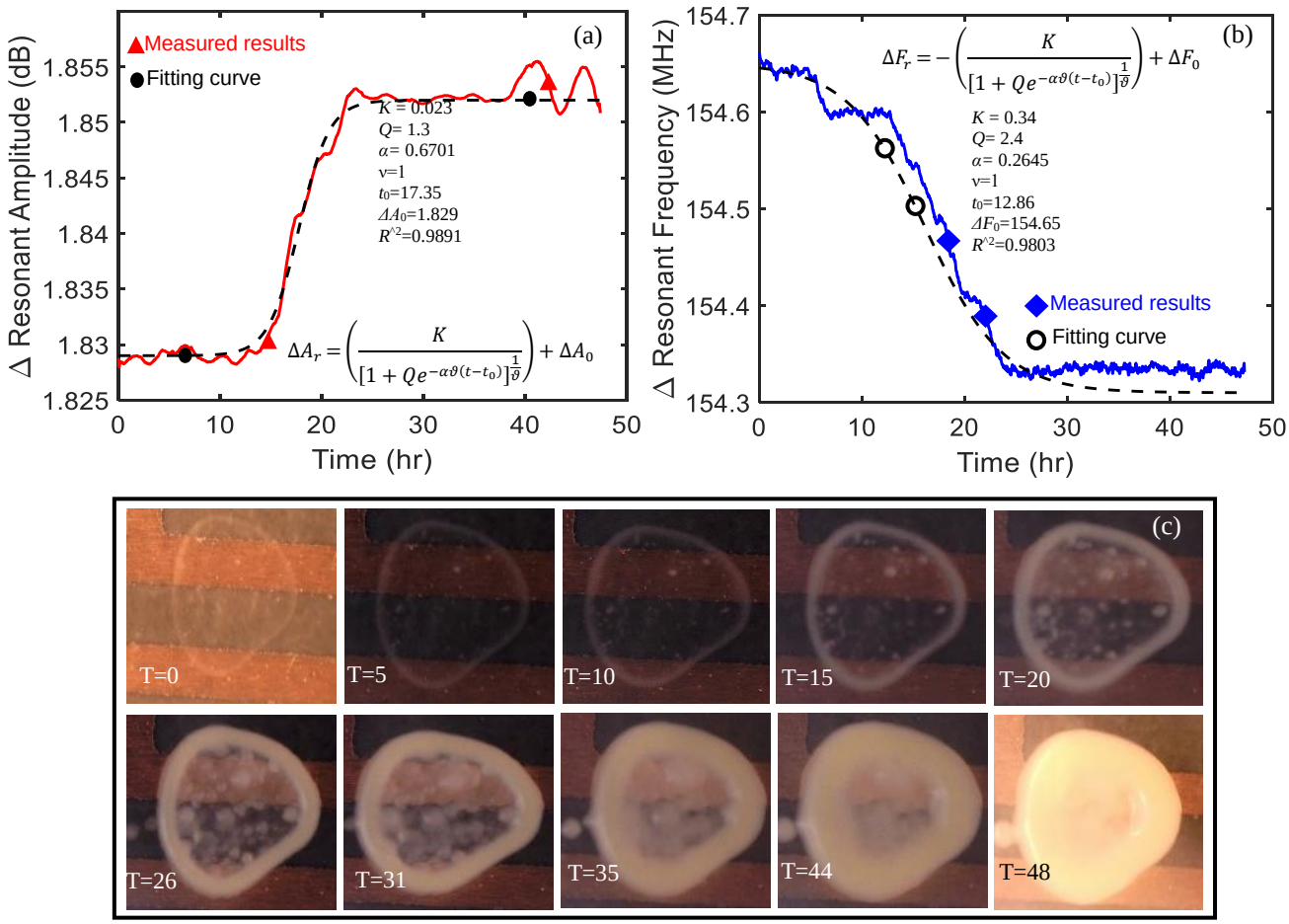

Fig.6. Measurement results for bacterial growth in the coupling gap of the microwave sensor; (a) variation of resonant amplitude difference (ΔA_r) over time, (b) variation of resonant frequency difference (ΔF_r) over time; (c) captured time-lapse photos of bacterial growth associated with the experiment.

noticeable change observed in the microscope images was 17 to 26 hours after the experiment was started, whereas the change was noticeable in approximately 6 hours for the microwave signal (Fig.5).

The sensitivity of the designed sensor for bacterial growth monitoring on LB agar medium was investigated by spotting 1.5 µl of a fresh E. coli culture (OD_{600} =1.5) as a single spot (0.0314 mm^2). The inoculated spot was placed over the coupling gap of the sensing resonator and bacterial growth was monitored over a period of 48 hours. ΔA_r of this experiment is presented in Fig. 5a and demonstrated 10 to 15 times less sensitivity relative to placing the bacteria sample over the ring gap over the same time period (Fig. 5a). This behavior was predicted from the HFSS simulation results, Fig. 2d.

It is worth mentioning that the sample size and volume of bacteria in the first and second experiment is different due to demand for the multiple microbiology plating techniques used. Both spread plating and spotting plating techniques have been employed to test the sensing ability of the device, these two microbiology plating techniques which are widely used for clinical studies [40].

However, ΔF_r comparison between the two experiments demonstrated a shorter lag-time in Fig. 5b, which is most likely due to the higher initial bacteria concentration (OD 1.5 vs OD 1.17). Time-lapse photos confirmed the bacteria growth over the experiment period (Fig. 6c). The first noticeable change was observed 5 to 15 hours after the experiment began. Comparing the microwave sensor's response (ΔF_r – Fig. 6b) to the recorded images concluded that the microwave response was able to detect bacteria growth earlier and at lower populations than the low-cost optical method. Besides, the sensing methodologies for detecting and measuring bacterial growth reported so far are exclusively on liquid medium (Table III). This work presents a microwave sensing platform to monitor bacterial growth by switching to solid medium (LB agar) from generally adopted liquid medium. The efficiency and performnace of the microwave sensor to accurately measure bacterial growth kinetics in the solid medium was validated through measurements. Compared to previous studies measuring bacterial growth on various liquid matrixes, the developed and fabricated sensor in this study has demonstrated the capability of monitoring bacterial growth kinetics in solid medium (Luria-Bertani Agar) with a low concentration of 1.8×10^7 cells/cm^2.

V. Conclusion

In this research we developed a novel, label-free, microwave-based biosensor that can monitor bacterial growth on a translucent solid medium. The sensor consisted of two split ring resonators with slightly different resonant frequencies operating at 1.95 and 2.11 GHz. Bacterial growth resulted in a measured resonant amplitude difference variation of 0.5 dB.

TABLE 3
STATE OF THE ART OF BACTERIA SENSORS

Biosensor Type and Sensing Technique	Bacterial Substrate and concentration	Potential Limitation
Metal-Oxide Semiconductor, Impedometric (Capacitive) sensor Integrated with microfluidic channel [41]	Luria-Bertani Broth, 10^6/mL, 10^7/mL	-expensive and unable to track different phases of bacteria growth over time, including lag, exponential, stationary and death.
Metal-Oxide Semiconductor, Conductometric system based on the resistance of the test sample between two electrodes [42]	Luria-Bertani Broth, 4×10^2 to 4×10^4 colony forming unit (CFU)/mL).	-In direct contact with sample under test
Microfluidic-Microwave Ring Resonator Biosensor, Interaction between electric field and sample (Dielectric properties) for detection of different concentration of bacteria [27],[28]	Mueller Hinton Broth, 2.8×10^6/mL	-Sensitive to changing environmental parameters (temperature, humidity and etc.)
Silicon nanowire field effect transistor, based on quantification of changes in the source–drain current caused by varying pH values [43]	Luria-Bertani and M9 Broth, 10^8/mL	-In direct contact with sample under test
Vision-based method, Image processing by using Fast Fourier transform for detection of different concentrations of bacteria [44]	Tryptic Soy Broth, 2×10^6 CFU/mL	-Require post-processing (Image processing)
Optical surface cytometer, Measure the fluorescence of bacteria labelled with DNA-intercalating dyes such as SYBR Green I. [45]	Luria-Bertani Broth, 10^4 cells/mm^2 as the limit of detection	-Require labeling (fluorescently-labelled bacteria)
Differential Microwave Sensor, Interaction between electric field and sample (Dielectric properties) for monitoring bacteria growth [this work]	Luria-Bertani Agar, 1.8×10^7 cells/cm^2.	- Sensitivity to the thickness of the solid growth medium

The developed method functioned by comparing the response of the sensing resonator to the reference resonator, which accounted for uncontrolled environmental parameter fluctuations such as temperature and humidity. The efficacy of the developed sensors was validated by determining the growth curve of E. coli. This sensor is an attractive candidate for monitoring bacterial growth on solid media, expanding the application of current microwave sensing methods, which are still largely depended on liquid samples. This opens a new avenue for promising new research in the detection of bacterial growth on solid media. Further work will be focused on the application of this biosensor in similar solid matrixes to expand its applications in the food, environmental, and pharmaceutical industries.

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