Label-Free cDNA Detection Based on Radiofrequency Scattering Parameters: A New Approach for an Inexpensive Gene Sensor

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A new gene detection technique that is fast, inexpensive, and easy-to-use is urgently needed in hospitals, clinics, and laboratories without access to expensive equipments. The lack of a practical, minimally invasive, and economical method constitutes the main impediment to the promotion of genetic medicine in developing countries. Radiofrequency scattering parameters are an inexpensive gene sensor potentially capable of noninvasively identifying biological materials. They represent a quantitative value for the electromagnetic reflection/transmission characteristics of certain molecular markers in a given frequency domain. The S_{21} parameter is the difference between the signal received and that transmitted. The aim of this study is to evaluate the S_{21} transmittance parameters (magnitude and phase) as an indirect impedance measurement for detecting the label-free complementary deoxyribonucleic acid (cDNA) amplification of the 16S ribosomal subunit gene. S_{21} values showed differences associated with distinct cDNA concentrations. Hence, this technique could possibly facilitate the design of an inexpensive, label-free, and easy-to-use gene sensor. [DOI: 10.1115/1.4045909]

Keywords: DNA, gene sensor, impedance, S *parameter, PCR*

Introduction

The focus of genetic medicine is the diagnosis of a patient through the detection of deoxyribonucleic acid (DNA) fragments or genes that are associated with specific pathologies. This approach has important advantages, including a fast and precise diagnosis, the identification of specific therapies according to the particular metabolic gene profile, and the monitoring of the effectiveness of a given therapy in real-time (thus preventing relapses). As one example of a genetic technique, the examination of self-assembled monolayer (SAM)-based electrochemical biosensors has sparked great interest in the point-of-care diagnostics community [1–5].

Due to the advantages of diagnostic methodologies based on genetic medicine, intense efforts have been made to develop gene sensors over the past 10 years, resulting in a large number of DNA biosensors. The technological advances have allowed for the development of simple devices and fast procedures showing the potential for lower costs [6]. However, available technology is still too expensive for molecular analysis to be widely used in developing countries due to the need for expensive reagents and/or highly trained health personnel. Hence, it is important to develop an economical and easy-to-use gene sensor for small hospitals, clinics, and laboratories without access to expensive equipment.

The common techniques of molecular analysis consist of multiple steps, and therefore are time consuming and labor intensive. They also require expensive and sophisticated devices and costly reagents [7–9]. Polymerase chain reaction (PCR), a procedure that normally involves agarose gel electrophoresis, must be carried out by highly trained personnel [9,10]. Although the methodology itself is relatively simple, it lacks sensitivity [8]. Improved sensitivity is obtained with quantitative real-time PCR (qPCR) and dot blot hybridization, which unfortunately rely on complex labeling procedures [8–10]. Consequently, there is interest in developing

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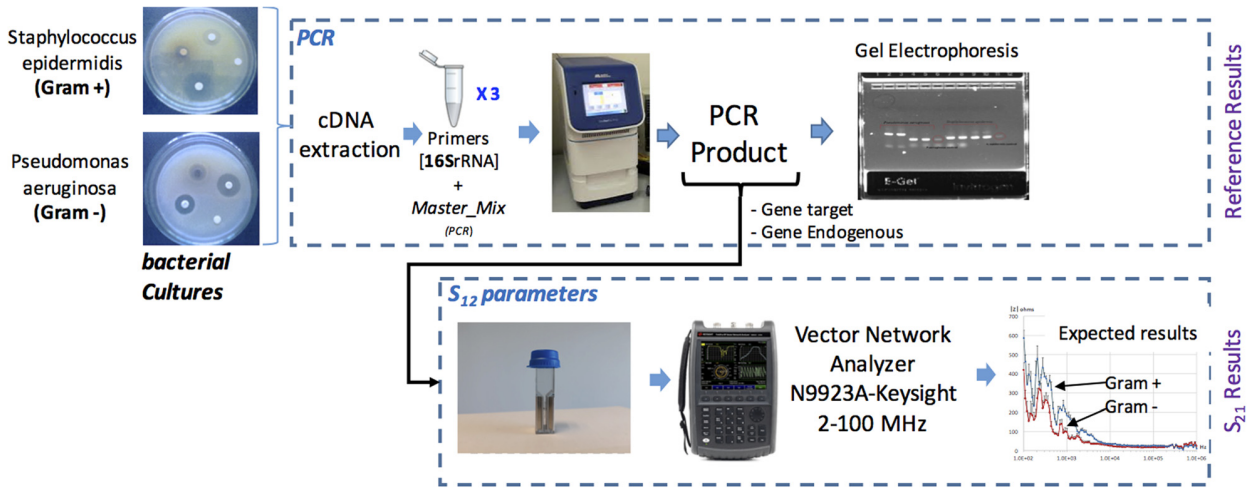

Fig. 1 The experimental design

strategies for detecting PCR products that do not require labeling [10]. As one such alternative, nucleic acid biosensors have the potential of providing relevant information about specific sequences with faster, simpler, and less-expensive procedures than those used in current methodologies [7,11,12].

Among the various PCR techniques is second-step asymmetric PCR, utilized in one study to amplify a gene in the *Salmonella* genome [8]. Complementary sequences of the target gene were immobilized by means of the biotinylation of a test chip. Subsequently, denatured PCR products were passed through the chip to detect amplification. Evaluation of the hybridization of the gene and its immobilized complementary sequence was achieved by surface plasmon resonance, and the results were compared to real-time PCR [8]. In another study, complementary ssDNA was immobilized on a gold electrode and then PCR products were passed through the target gene, which was amplified and denatured to analyze hybridization by differential pulse voltammetry and electrochemical impedance spectroscopy (EIS) [9]. Additionally, tagged PCR products have been assessed with magnetoresistance to detect amplification [6]. Other approaches involve electrical microarrays [12] or cyclic voltammetry [11] to detect the amplicons of PCR products.

Although real-time detection of DNA amplification is of particular interest, it is currently accomplished with optical biomarkers integrated into qPCR, the use of which implies complex and expensive technology. The one in charge of the procedure must master the technique as well as acquire advanced knowledge of its theoretical basis. PCR amplification has also been examined cycle by cycle through EIS measurements. For this purpose, PCR is performed on the test interface by first immobilizing single-strand complementary DNA on the electrode and then employing these strands as primers [13]. Different strategies have been explored to find more accessible alternatives to qPCR for developing countries, including optical (e.g., fluorescence) and other techniques [14,15].

Scattering parameters (*S*-parameters) show electromagnetic reflection/transmission characteristics in a given frequency domain. For a device based on microwave frequencies, the scattering parameter S_{21} is the forward voltage gain, being the

Table 1 The PCR mixture

Component	Quantity (μL) for one reaction
PCR mixture	12.5
Fwd primer	2.5
Rev primer	2.5
cDNA	7.5
Water	0
Total volume	25

Table 2 The cycling parameters for PCR

Step	Temperature (°C)	Time
Initial denaturation	95	30 s
Denaturation	95	5 s
Primer annealing	55	10 s
Extension	72	30 s
Final extension	72	5 min

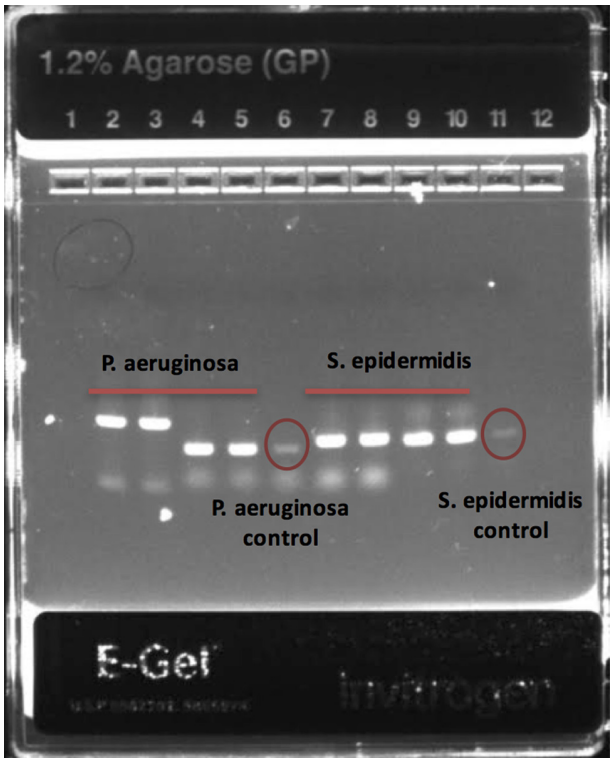

Fig. 2 Image of the PCR products of 16S rRNA from 1.2% agarose gel electrophoresis

Table 3 The concentration of the 16S gene and the S_{21} (10 MHz) values for magnitude in different samples

Sample type	cDNA concentration (ng/μL)	S_{21} magnitude at 10 MHz (dB)	Spearman correlation coefficient ($p < 0.05$)
Pseudomonas	1859.6	0.355	0.960
Pseudomonas diluted 50%	876.1	0.256	
Control	368	0.189	
Epidermidis	1383.4	0.379	0.997
Epidermidis diluted 50%	730	0.276	
Control	282.5	0.259	

relationship between the power of the output port (port 2) and the input port (port 1). S_{21} transmission parameters have not yet been reported, to our knowledge, for the rapid detection of label-free DNA from PCR products. The aim of this study was to analyze the possible use of S_{21} to detect label-free complementary deoxyribonucleic acid (cDNA) amplification of the 16S ribosomal subunit gene (16S rRNA). The latter gene, which exists in all types of bacteria, can be instrumental in the classification of bacterial species and in molecular diagnostics for the identification of infectious diseases [16–19]. It consists of highly conserved nucleotide sequences interspersed with variable genus- or species-specific regions [20]. The current experimental design is a proof-of-concept approach to the evaluation of a new, inexpensive, label-free, and easy-to-use gene sensor that is based on the measurement of the multifrequency bioelectrical impedance of PCR products. The project is a binational effort by the Tel Aviv University in Israel and the National Polytechnical Institute in Mexico.

Methodology

Experimental Design. Standard techniques were used to culture one gram-positive bacterium (*Staphylococcus epidermidis*)

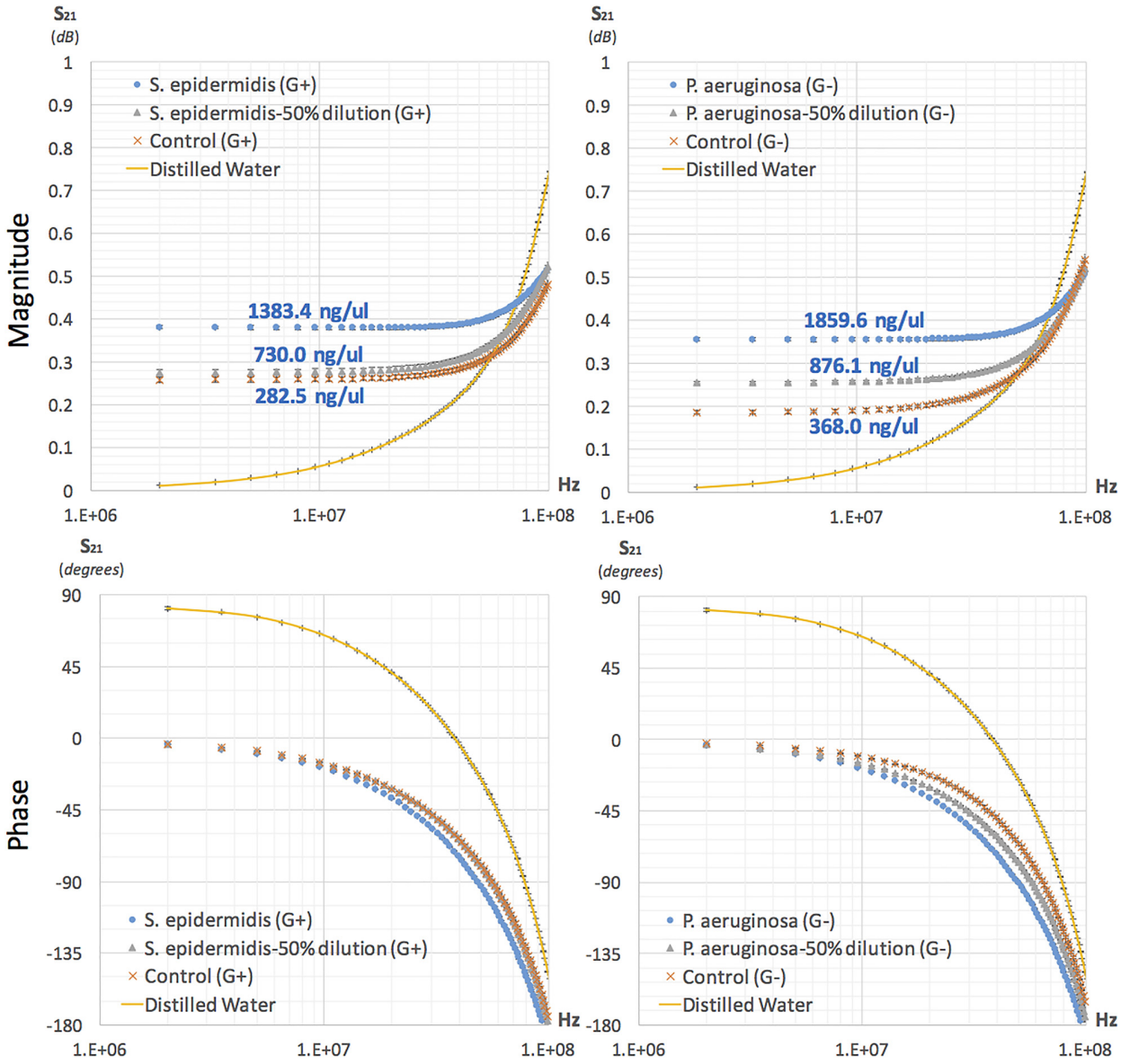

Fig. 3 The ordinate is S_{21} (magnitude or phase) for the final PCR products of the 16S ribosomal subunit (including 50% dilutions of the same). The values are taken from a gram-positive bacterium (*Staphylococcus epidermidis*), a gram-negative bacterium (*Pseudomonas aeruginosa*), the corresponding endogenous products (positive control), and distilled water (the vehicle, negative control). The abscissa is the measured frequency.

and one gram-negative bacterium (*Pseudomonas aeruginosa*). After extracting ribonucleic acid (RNA) from each bacterium, the reverse transcription process was carried out to obtain cDNA. This complementary DNA was amplified by retro transcriptase-quantitative PCR (RT-qPCR). The final PCR products and their 50% dilutions (in 200 μL of distilled water) served as samples for determining S_{21} values. Endogenous products were used as the control, and all assays were performed in triplicate. Products were evaluated by molecular weight and DNA concentration. The experimental design is depicted in Fig. 1.

cDNA Amplification of the 16S Ribosomal Subunit. For each organism and with at least 2 mL cell suspension, total mRNA was isolated from bacterial samples with the EZ-RNA and total RNA isolation kits (Biological Industries, Inc., Israel). The extracted mRNA was then converted to cDNA with the enzyme reverse transcriptase and PCR mixture (Table 1).

PCR amplification was performed with DNA polymerase, according to the five steps noted in Table 2. The primers were purchased from IDT, Inc. (Israel). The final PCR products were examined by electrophoresis with agarose gel (RHENIUM, Inc., Israel) to determine their molecular weight and on a Nano-Drop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA) to quantify the concentration of DNA.

Measurement of S_{21} Parameters. The evaluation of the scattering parameters for PCR products was conducted with a 2 mm gap electroporation disposable cuvette (model 610, BTX/Harvard Apparatus, Inc., Holliston, MA) connected to a vector network analyzer (model N9923A, Keysight, Inc., Santa Rosa, CA). S_{21} parameters (magnitude and phase) were measured in the frequency range of 2–100 MHz, utilizing 300 Hz bandwidth steps and a power level of 5 dBm. The analysis of PCR products, their corresponding dilutions and the controls was performed in triplicate and values are expressed the mean.

Results

The PCR products of 16S rRNA resulting from 1.2% agarose gel electrophoresis are shown in Fig. 2. It was found that bands 2–5 are related to amplicons from *Pseudomonas aeruginosa*, bands 7–10 to amplicons from *Staphylococcus epidermidis*, and bands 6 and 11 to amplicons from the controls. The amplicon size was determined and expressed in kilobase pairs (kbp), findings 2 and 3 have 530 kbp, 4 and 5 have 336 kbp, 7 and 8 have 387 kbp, and 9 and 10 have 365 kbp (Fig. 2). The concentrations of the PCR products are listed in Table 3.

Discussion and Conclusion

Many biosensors have been developed for examining diverse analytes, the size of which includes individual ions, small molecules, nucleic acids, proteins, and whole viruses or bacteria [21]. For instance, real-time detection of *E. coli* 16S rRNA carried out under mild conditions with a DNA sensor resulted in high specificity, evidenced by its ability to differentiate *Bacillus subtilis* from *Escherichia coli* 16S rRNA sequences. Moreover, the sensor responded to the presence of the analyte within seconds [22].

Similarly, the S_{21} values of this study (graphed according to magnitude and phase; Fig. 3) show clear differences between the final PCR products, their corresponding dilutions and the control. The differences seem to be associated with the concentration of cDNA. Sensitivity is evident in the 20–50 MHz bandwidth for both magnitude and phase. The γ dispersion arose at this frequency, perhaps related to the polarization caused by the reorientation of water molecules and their interaction with the vibration of DNA molecules. Hence, the extent of the polymerization of nucleotides should be reflected in the energy transmittance of the sample, which is expressed in a variation of S_{21} values in function of the concentration of hybridized DNA.

The current contribution was designed as a preliminary investigation to assess the feasibility of using S_{21} parameters to distinguish between types of bacteria. Since the DNA concentration was determined separately for each of the two types of bacteria included herein, the robustness of the technique for evaluating unknown samples or concentrations is unknown. However, data were processed to identify the most sensitive points on the S_{21} parameter curves for differentiating one cDNA concentration from another.

Data on the S_{21} parameters were analyzed by the Mann–Whitney U test at every frequency of the whole bandwidth. Significant differences between groups and conditions were found in the bandwidths of 2–50 and 20–50 MHz for magnitude and phase, respectively. As a first approach, the S_{21} magnitude was measured at 10 MHz, resulting in a significant correlation between the S_{21} values and the concentration of cDNA, as indicated by the Spearman correlation coefficient (>0.97, Table 3). The present findings justify further research to explore the potential of the technique to examine unknown samples and concentrations, which would imply different trials with the same bacteria at a wide range of concentrations.

As the sensitivity of S_{21} was herein associated with the concentration of cDNA, the proposed method could possibly be instrumental in the design of an inexpensive and easy-to-use gene sensor for small hospitals, clinics, and laboratories in areas of developing countries without access to expensive equipment. An easy-to-use system could be controlled by a personal computer interface or cell phone application. Future investigation is warranted to establish an accurate and practical system.

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Nomenclature

cDNA = complementary deoxyribonucleic acid
DNA = deoxyribonucleic acid
EIS = = electrochemical impedance spectroscopy
mRNA = messenger ribonucleic acid
PCR = polymerase chain reaction
16S rRNA = 16S ribosomal subunit gene

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