Gene-sensor on the basis of bioimpedance measurements assisted with nanotechnology: an instrumentation proposal

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Abstract. In this study, we propose basic instrumentation for a new gene-sensor based on Polymerase Chain Reaction (PCR) product and its multifrequency bioimpedance relative measurements assisted with nanotechnology. The instrumentation proposal is split into four basic blocks: A) Control. - The fundament is a microcontroller (µC) for wide bandwidth current excitation, as well as data acquisition and registration. B) Digital Synthesizer. - A wide bandwidth digital synthesizer will provide a sinusoidal excitation current. C) Bioimpedance measurement.- this section will detect relative multifrequency bioimpedance by comparison of a reference signal with the signal of the Analyte Under Test (AUT). Comparison is on the basis of magnitude ratio and phase shift. D) Electrical-Ionic Interphase. – This is based on gold electrodes in contact with the AUT for current injection and relative bioimpedance measurements. Deoxyribonucleic Acid (DNA) fragments as PCR products will be functionalized by magnetic nanoparticles-DNA biding and its magnetic DNA insulation. The specific electronic components for every section are out of the range of this study, and the general instrumentation structure proposed is intended to show tentative low cost technology for gene detection and obtain academic discussion and feedback.

1. Introduction

The ability to detect specific DNA sequences in the human genome has been of great interest in recent years as this provides important information in different disciplines of science such as criminology, the food industry and medicine. The detection of genetic diseases is of enormous value for medicine and the practice has improved in the last few decades thanks to available analytical methods [1]. Identifying specific DNA sequences requires a set of sophisticated and complex techniques, as well as highly trained personnel [2].

Several approaches for the simple, rapid and highly sensitive detection of DNA have been developed. Currently, there are several sensitive parameters on which the devices are based. These are: the hybridization of complementary DNA with specific chains of specific DNA anchored to the surface of the sensor changes its properties [1], measuring these changes allows us to detect the presence of the gene in question. In this paper we propose using the behavior of the electrical impedance of the system as a sensitive parameter.
It has been reported that electrical impedance measurements are sensitive to the concentration of DNA in a sample, to 2 mismatched base pairs and to the length of the DNA sequence [3-4]. One of the disadvantages of studies reported in current literature is that they require different independent and modular equipment to perform the measurements and they look for very high sensitivity, so the technology becomes more sophisticated [4]. Our group proposes to develop a biosensor device whose design integrates all the necessary systems to make the measurements and detect a specific DNA sequence from PCR product.

The sensitivity problem is overcome by using PCR product, where the amplification selectivity falls on the design of the primers. It has been reported that it is possible to carry out PCR processes with a very high specificity that even allows the detection of genetic contamination in bacteria [5-6]. Thus, we aim to satisfy the need for high specificity in the detection of DNA sequences of clinical interest. If the PCR product is the amplification of the specific gene, the sensitivity of the system will no longer be a problem due to the increase in the concentration of the specific gene in the sample.

It is sought that the DNA chains are immobilized in the test electrode or electrical-ionic interface to be able to perform the measurements of electrical impedance, where it will be possible to measure the changes in the electrical properties of the medium provoked by the hybridization of the complementary DNA with the sequence of immobilized single chain DNA. [1-3, 7, 8]. Although it is possible to carry out the measurements without any type of anchorage or immobilization [4], in our study we propose using magnetic nanoparticles as functionalization of the double strand of DNA and the application of a magnetic field in the test electrode or electrical-ionic interface for the immobilization of them.

2. Materials and Methods

2.1. General Instrumentation Design

In previous studies we used commercial equipment to test the operating principle. In this study, we propose using a specialized device that integrates the functions of every piece of equipment used to do the measurements. Our new design will use some commercial circuits, when possible, and will also have circuits of our own design. The specific electronic components for every section are out of the range of this study, and the general instrumentation structure proposed is intended to show tentative, low cost technology for gene detection and obtain academic discussion and feedback. The general design of the instrumentation of the proposed gene-sensor device consists of four modules: Control, Digital Signal Synthesizer, Relative Bioimpedance Estimator and Electrical-ionic interface. Figure 1 shows the block diagram of the general design of the instrumentation. A general description of each module is given below.

2.1.1. Control. The control block consists of the use of a microcontroller for the selection of the excitation current which will be a signal in the frequency range of 100Hz to 10 MHz. The microcontroller will also be used for the acquisition of the data resulting from the relative impedance measurement module, so it must have an integrated analog/digital converter. This will also carry out the processing and interpretation of the results, which will be displayed on an LCD monitor. The microcontroller will have two digital communication protocols configured: one serial (USB) and one wireless (Bluetooth), so that we can transfer and record the data to a personal computer for ease of use later.
2.1.2. **Digital Signal Synthesizer.** A multi-frequency signal synthesizer will be used to perform the injection of electric current to the sample; the signal will be a sinusoidal wave with an amplitude of 10 mV. The frequency of the signal will go from 100 Hz to 10 MHz with logarithmic increments to obtain measurements in the spectrum. An operational amplifier in a buffer configuration will be used to prevent the signal from falling.

2.1.3. **Relative Bioimpedance Estimator.** We will refer to **Relative Bioimpedance** as the measurements taken by this module since the measurements are relative to the signal across a constant resistance used as reference ($Z_{ref}$); also, the module measures the relation in amplitude and phase shift between two signals. This is an indirect form of measuring the electrical properties of the electric-ionic interphase. For the measurements, we will use a commercial, fully integrated system for measuring gain/loss and a phase module that compares two signals: the reference signal, which is the voltage that consumes $Z_{ref}$ due to the passage of the excitation current, and the signal of the AUT that will be the potential drop in the electrical-ionic interface. A voltage divider will be configured where we will have $Z_{ref}$ for the reference signal and the AUT, in series, as the variable impedance of interest. Thus, only two electrode terminals will be used for the measurements in the AUT. The module will compare the reference signal and the AUT signal and deliver a direct current (DC) voltage proportional to the magnitude relationship between the signals of both impedances. For the phase shift, the module will deliver another DC signal proportional to the phase angle between the signals. Hence, the system will be able to detect changes in the impedance of the AUT, which will correspond to the presence of the gene in question.

2.1.4. **Electrical-ionic interface.** The electrical-ionic interface consists of a gold surface, such as an electrode, in contact with the AUT for the injection of current and the measurement of relative impedance. For immobilization of the DNA, a magnetic field will be applied to the AUT-electrode complex that will attract the DNA strands with the attached magnetic nanoparticle, isolating them and immobilizing them on the electrode. This functionalization is explained in the following subsection.
2.1.5. Functionalization and Immobilization of DNA. For our gene-sensor proposal, the PCR product will be functionalized, to which magnetic nanoparticles will be attached to the 5'-phosphate group of the DNA sequences, which will give magnetic properties to the functionalized chains. The immobilization will be carried out by applying a magnetic field to the electrical-ionic interface so that the functionalized DNA strands will adhere to the surface of the electrode. The magnetic field may affect the electric field applied to the Analyte Under Test but, since the measurements will be relative to a reference and the magnetic field is common in all the measurements, the effect of it may be negligible; in any case, analyzes should be carried out in order to offer more reasonable guaranties. The magnetic field will be applied to the lower part of the AUT-electrode complex using a magnet. The principle of functionalization and immobilization at the electrical-ionic interface is shown in figure 2.

![Diagram of Functionalization and Immobilization](image)

**Figure 2.** - A) PCR product binding magnetic nanoparticles. B) Functionalized DNA immobilized on the electrode for current injection and relative impedance measurement.

As the DNA sample will be immobilized by magnetic adhesion, the test electrode will be reusable which reduces the cost of the analysis, considering that this device will reach the clinical laboratories of health centers.

2.2. Experimental Proposal

To verify the operating principle, a previous experimental proof of concept was developed with individual and commercial equipment. The system consists of four blocks similar to the ones we propose here: A) Microfluidic pump for sample transportation. B) Electric-ionic interphase which consists of a gold electrode in contact with the analyte under test. C) SciospecTM module (ScioSpec, ISX-3, Germany) for the bioimpedance measurements. And D) Personal Computer in which the programming of the ScioSpec module and the storage of the data were carried out. The experimental protocol, which will also be used for testing the device proposed in this study, is described below.

The experimental proof of concept consisted of three groups with the following conditions: A) Control: without anchoring DNA, only Mili-Q water was passed through. In this case we did not create any structural changes to the electric-ionic interphase. B) Positive Case: *Helicobacter pylori* DNA plus specific primers for the region of the universal bacterial gene (rDNA16S) were added to the PCR and passed the functionalized PCR product through the electric-ionic interphase. In this case we intend to create structural changes to the surface due to DNA + magnetic nanoparticle adhesion, and C) Negative control: *Helicobacter pylori* DNA plus specific primers for a non-bacterial gene (human beta globin). The final non-amplified PCR product and magnetic nanoparticles should create minimal structural changes in the electric-ionic interphase, only due to the effect of magnetic nanoparticles. Each assay...
was performed in triplicate. The comparison of the measurements made will allow us to identify the electrical differences between the samples that contain the specific gene and those that do not, and thus, define a parameter for the classification of the results. Figure 3 shows a graphical representation of the experimental design of the proof of concept.

**Figure 3.** Experimental design of previous proof of concept. - Quartz crystals in the electrical-ion interface module and the corresponding analytes were infused. Specific gene (rDNA16S): Injection of the specific gene and functionalized with magnetic nanoparticles. Negative control: Injection of anchoring PCR product for the missing gene for bacteria (human beta globin).

### 3. Results and Discussion

Figure 4 shows previous proof of concept results of the experiments made to test the operating principle with commercial and independent devices.

**Figure 4.** Electrical bioimpedance magnitude (A) and phase (B) results of the three tests of the proof of concept: Control: without DNA; Gene Positive: with specific DNA sequence; and Gene Negative: with unspecific DNA material.
The results were as expected and the selectivity of the PCR helped us to differentiate between the characteristics of the controls since, in the absence of the specific gene, there will be no amplification which will result in a lower impedance as opposed to when there is amplification (in the sample with the specific gene). As expected, the bioimpedance of the control sample was greater than that of the positive and negative controls and, in turn, the positive and negative control bioimpedances present noticeable differences between each other. This is based on what is reported in [4] where they describe that the DNA molecules have a negative charge and that the charge is proportional to the number of base pairs so that the greater amount or concentration of genetic material, the impedance decreases due to the free charges of the DNA molecules.

The experimental validation design will offer us reasonable assurances to believe that, in any DNA sample, if the specific gene in question exists, the PCR process will amplify this gene, so there will be a higher concentration of DNA in the measurement of electrical impedance. This will result in comparable changes in the results that will enable the presence of the gene to be identified by our device.

The lengths of the DNA strands and the specific gene should also be considered to offer even more reasonable guarantees. However, it is expected that this will not influence the classification of results because we expect the differences in the impedance to be significant among the samples with the gene in question present as opposed to the samples without it.

To validate the amplification of the gene in the PCR product, we used standard techniques such as agar gel electrophoresis to corroborate that the amplification had an effect on the impedance changes and thus rule out other possible variables that may affect the measurements. Figure 5 shows the agar gel that we ran where we can see the amplification of a specific gene of 500 base pairs in the positive group and the absence of it in the negative control.

![Figure 5](image)

**Figure 5.** 2.5% agarose gel showing in 1) 250 bp molecular size marker. 2) Positive Control, with specific DNA sequence. And 3) Negative Control, with unspecific DNA material.

### 4. Conclusion

In this study, we propose to design a gene-sensor based on relative electrical impedance measurements. The interaction of the different modules described in this study will allow us to detect the presence of a specific DNA sequence in PCR products. We also propose an experimental basis for validating the operation of the equipment where we can rule out the influence of extraneous factors in the measurements. Thus, it will be possible to attribute the possible changes presented in the relative bioimpedance between the controls to the presence of the specific gene in question. The development of the system and the implementation of the validation will be carried out in the next two years by Gerardo Ames as a thesis project to obtain his Master of Science degree. Once the device is finished and it is proved to be functioning correctly it is intended to obtain the necessary certification required to make this product available on the market.
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Conflict of Interest
The authors declare that there is no conflict of interest.

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