Gene-sensor on the Basis of Magnetic Immobilization and Bioimpedance Measurements: a proof of concept

D U Laguna¹, V Sanchez², G Ames³ and C A González*¹

¹ Instituto Politécnico Nacional, Escuela Superior de Medicina. CDMX, México.
² Instituto Politécnico Nacional, Escuela de Medicina y Homeopatía. CDMX, México.
³ Universidad Autónoma Metropolitana-Iztapalapa, Ci3M, CDMX, Mexico.

*cgonzalezd@ipn.mx

Abstract. Detection of molecular markers or specific DNA sequences (genes) represents the future in genomic medicine. Gene detection requires expensive equipment and specialized technicians, thus biosensors development that allows such detection quickly, economically and simply is fundamental in the first level of health care. In this study the development and evaluation of a genosensor based on magnetic anchoring of DNA sequences and electrical bioimpedance spectroscopy measurements is presented. As a first proof of concept, bioimpedance measurements were developed in PCR product anchored by magnetic nanoparticles and two conditions were compared; PCR product and absence of amplicons. The experimental evaluation indicates technical feasibility of developing a biosensor of molecular markers or specific genes through electric bioimpedance measurements assisted with magnetic nanoparticles. The observations show that the DNA biosensor proposal offers the possibility of detecting a PCR product and differentiating it from the absence of amplicons.

1. Introduction

The development of technology in the biomedical field has been influenced by the need to make timely diagnoses. The standardization of molecular markers and with them the development of biosensors, has enabled the implementation of new techniques to detect genes of clinical interest in a fast, economical and simple way. The detection of genetic diseases is of great importance for medicine and such practice has improved in the last few decades thanks to the analytical methods available [1]. Identifying specific DNA sequences requires a set of sophisticated and complex techniques, as well as highly trained personnel [2]. Currently, there are several sensitive parameters on which the devices are based and the hybridization of complementary DNA with specific chains of specific DNA anchored to the surface of the sensor changes its properties [1,3,4,5]. In 2011, a selective, sensitive and rapid electrochemical sensor was proposed to distinguish the union of coincident oligonucleotide sequences [6]. It has been reported that electrical impedance measurements are sensitive to the concentration of DNA in a sample and to the length of the DNA sequence [3-4]. Recently, the use of magnetic micro and nano particles in the development of biosensors has been described and the advantages offered are associated with low background interference, easy functionality and the best understanding of molecular interactions; thus detecting interactions such as DNA-DNA hybridization [7]. One of the disadvantages of the research reported in existing literature is that it requires different independent and modular equipment to perform the measurements and look for very high sensitivity, so that the technology becomes more sophisticated [4]. This paper presents the development and evaluation of a genosensor based on electrical bioimpedance spectroscopy measurements. The results indicate the technical possibility of developing a biosensor of molecular markers or specific genes from measurements of electric bioimpedance assisted with magnetic nanoparticles.
2. Materials and Methods

2.1. Description of the Technological Proposal

The development of the geno-sensor is based on two foundations: 1) Anchoring or immobilization of DNA amplicons (PCR product) on a gold surface embedded in a quartz crystal, in turn the amplicons have magnetic nanoparticles attached that enable the anchoring by the effect of a permanent magnet, and 2). The estimation of multifrequency electric bioimpedance changes as a function of the modification of molecular and structural components in the gold surface (see Figure 1).

![Diagram](image)

**Figure 1.** A) PCR product binding magnetic nanoparticles. B) Functionalized DNA immobilized on the electrode for current injection and relative impedance measurement.

2.2. Description of the DNA biosensor system

The biosensor system of DNA sequences consists of four modules: 1) Infusion pump, 2) Electrical-ionic interface, 3) ScioSpec bioimpedance meter and 4) Personal computer. The infusion pump has an adapted insulin syringe which performs the controlled infusion of the analytes towards the electrical-ionic interface by means of a capillary tube. The electrical-ionic interface houses the quartz crystal with a gold surface. In this module, the DNA anchoring process is performed by magnetic effect, as well as by the interconnection of the electrodes for bioimpedance measurement. The Sciospec™ module (ScioSpec, ISX-3, Germany) is the instrument that allows us to interact with the electrical-ion interface module, injects a potential difference and, at the same time, measures the current to estimate the impedance of the system. The PC is the last module, in which the programming of the ScioSpec module and storage of the data in manipulable format is carried out.

2.3. DNA amplification of the 16S ribosomal subunit (ADNr16S) and concentration adjustment.

For this test, the DNA of the bacterium Helicobacter pylori (ATCC 43504) was used as a positive control. The concentration and purity of the DNA was determined with the Epoch spectrophotometer (Biotek). The amplification of the rDNA16S was carried out using the universal primers for bacteria DG74: 5'AGGAGGTGATCCACCCGCA3' and RW01 5'AACCTGGAGGAAGGTGGGAT3' in a final volume of 25 uL containing 5 uL of DNA (approximately 100 ng / ul), 0.5 U of Taq polymerase (Invitrogen), 1 mM of each primer, 3.75 mM MgCl2, 0.2 mM of each dNTP and Taq polymerase buffer. The amplification was carried out in an Amp PCR System 9700 thermocycler (Applied Biosystem) using the following reaction
conditions: an initial denaturation of 5 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, alignment at 62 °C for 30 s and extension at 72 °C for 30 s. Finally, an extension was carried out at 72 °C for 5 min. As a negative control, PCR with DNA from the same bacterium was carried out, but using universal primers for a human beta globin gene region GH20 5'GAAGAGCCAAGGACAGGTAC-3' and PC045'CAACTTCATCCACGTTCACC-3' reaction which is standardized under the same reaction conditions. The PCR products were verified in electrophoretic assay, running on a 2.5% agarose gel and were quantified and diluted at a concentration of 50 ng/ul.

2.4. Bioimpedance spectroscopy measurements
The bioimpedance spectroscopy measurements were made through a ScioSpec ISX3 spectrometer controlled by a personal PC (HP mini 110-1100) which was programmed to perform measurements in a frequency range of 100Hz to 10MHz in 126 logarithmically spaced steps. Measurements of bioimpedance in triplicate were made and stored in its resistance and reactance components.

2.5. Experiment design
The experiment to evaluate the potential utility of the geno-sensor system in the detection of a specific gene is described as follows (see Figure 2): The analytes were passed in a microfluidic vehicle through the electrical-ionic interface in order to perform the anchoring of amplicons in three steps: 1) cleaning the system with purified water (Mili-Q water), 2) anchoring by magnetic effect of DNA on the gold surface, and 3) measurement of the multifrequency electrical bioimpedance on the resulting gold surface. Each step of analyte infusion was performed for 12 minutes followed by a rest time of 30 minutes. In order to evidence changes in the electrical bioimpedance as a function of the modification of biochemical and structural components in the resulting gold surface, the experiment was designed in three independent tests in order to generate the following conditions: A) Control.-without anchoring of DNA, only Mili-Q water was passed through, so there are no structural changes in the gold surface. B) Specific region of the universal gene for bacteria ADNr16S, got through PCR product of the specific and functionalized gene, so there are structural changes in the surface due to DNA + magnetic nanoparticles, and C) Negative control.- product anchoring of PCR for a region of the non-specific gene for bacteria (human beta globin), the final, non-amplified product and magnetic nanoparticles were got through, so that there are structural changes in the surface due only to the effect of magnetic nanoparticles. Each assay was performed in triplicate. When obtaining the measurements of both the resistive and the reactive parts, the magnitude and phase of each one was determined. Following this, an average of the three measurements was calculated for magnitude and phase.
3. Results

Figure 3 shows the PCR products in which, as expected, only amplification for a region of the 16S rDNA gene specific for bacteria (positive gene) can be observed, and there is an absence of amplification for a region of the human beta globin gene which is not present in bacteria (negative gene) (Figure 3). Figure 4 shows the bioimpedance spectrum measured in magnitude and phase. The control test shows substantially different behavior with respect to the positive and negative control tests. We observed that, for the positive test and the negative control, there is a notable difference in the results due to the existence of the PCR product in the first case (positive gene) and the absence of it in the second case (negative control). The graphs are in similar dynamic ranges resulting from the anchoring performed in both conditions. An ANOVA test indicates statistical differences between groups for: Control (without DNA), Positive Gene (anchoring of DNA amplicons) and Negative Gene (anchoring of PCR product without DNA amplicons) $p< 0.05$. 

**Figure 2.** 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).
4. Discussion

Some authors have proposed DNA chain immobilization to measure the changes in the electrical properties of the medium promoted by the hybridization of the complementary DNA, and important efforts have been developed to perform the measurement without any type of anchorage or immobilization [3-6]. In our work we propose the use of 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. Changes in the magnitude of bioimpedance of the three experimental conditions evaluated can be explained in terms of changes in electrical conductivity associated with differences in the effectiveness of the electrical-ion exchange. Such an effect is a function of the concentration of DNA molecules for each product of PCR evaluated. The characteristic changes in the differentiable phase in the three tests in turn indicate a change of the reactive component of the system and, therefore, of the capacitive effect at the electrical-ionic interface, which is explained by the change in the dielectric content associated with different concentrations of DNA. Such hypotheses are still preliminary and the observations and findings of the present study are still subject to analysis and study, so additional experiments are required to verify the observations.
5. Conclusion
The experimental trials show evidence of the technical feasibility of developing a geno-sensor of molecular markers or specific genes through the magnetic immobilization of DNA and its detection through measurements of electrical bioimpedance spectroscopy. The observations show that the developing biosensor DNA system offers the possibility of detecting a PCR product and differentiating it from the absence of amplicons.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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