# Chapter 18

# Detection of protein adsorption on hydroxyapatite using electromagnetic sensors

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Hydroxyapatite (HA) is the main inorganic component of bone and possesses electrical properties. Thick films of HA have shown pressure induced capacitance changes, the dielectric behavior of HA thick films over the GHz range has been investigated. This chapter discusses microwave properties describing different type of microwave resonators. The interaction of proteins and HA over the GHz range will be discussed.

## 18.1 Introduction

The development of electromagnetic (EM) wave sensors has been extensively studied since they have a number of advantages which include real time sample monitoring, rapid analysis, and high sensitivity[1]. Microwave sensing can be used for the detection of materials and to analyze their interaction with EM waves. Based on the characteristics of the material analyzed i.e. its permittivity a unique microwave spectra is obtained. HA is a bio-ceramic widely used in bone and teeth regeneration as it is highly biocompatible[2]. The analysis of protein adsorption onto HA is of great interest since the adsorption of protein to materials has an effect on the cell attachment, migration, and proliferation. Protein adsorption depends on the chemical and physical properties of a surface such as topography[3], surface charge[4] and chemical composition[5]. The challenge of a better understanding of protein adsorption mechanisms resides in a better knowledge of the material's properties. This chapter focuses on the principles of microwave sensing and common EM wave sensor used. We also describe the use of an IDE microwave sensor for the detection of protein binding to a HA surface.

## 18.2 Microwave sensing

Microwave can be used to analyze the dielectric properties of materials as it measures the response of a material in the GHz frequency range. Microwave spectra arises from the molecular rotation when the sample is in contact with electromagnetic waves [6]. It measures the response to materials to an external electromagnetic excitation. Microwave spectra originate as a result of the dielectric constant of the material and from the interaction of the EM waves. Through measuring the material's response over a range of frequencies signature spectra can be obtained and changes can be monitored. Depending on the nature and the permittivity of the sample different spectra is obtained[7]. Permittivity (1) relates to how an electric field affects a dielectric medium.

$$\varepsilon_r = \varepsilon' + j\varepsilon'' \tag{1}$$

Therefore microwave spectra result from (i) the dielectric constant (ε') and (ii) the dielectric loss (ε''). The first arises from the reduction in velocity of microwaves due to the ability of the material to store energy as it is irradiated by EM waves[6]. The stored energy is slowly released back causing a reduction in the velocity of the wave. The second one is a reduction in the magnitude of the wave as molecules orientate in the electric field. Microwave sensing can measure changes in transmission and the magnitude of reflection. Changes in the composition and concentration of the material under test will result in a change in the dielectric properties. Microwave sensing is often accomplished by using designed microwave sensing structures i.e. an antenna or a cavity (to operate in a specific frequency) that allows measurement of the dielectric response of an analyte within the GHz frequency range[8].

### 18.2.1. *Microwave resonators*

Current dielectric sensing techniques are typically classified reflection or transmission technique and can use resonant or non-resonant systems that include open or closed structures. Resonant methods are highly accurate[9] thus are used to characterise materials using either a single frequency or a set of frequencies. This section focuses on microwave resonators and on current methods used i.e. cavity resonators, resonant lines and microstrip resonators.

A resonant cavity is an electromagnetic resonator (used at microwave frequencies) that allows the transmission of electromagnetic waves confined between two points e.g. a generator and an antenna[10]. A resonant cavity can be used to measure dielectric properties of materials and liquid samples. Moreover cavities are usually designed to operate at a desired frequency. Depending on the application the propagation of EM waves in resonant cavities can be in the transverse magnetic (TM ) mode or transverse electric (TE) mode. The sample under test is placed in the center where the electric field is at its highest. In here the material will interact with the EM waves and cause a perturbation on the cavity which is related to the dielectric properties of the material. While the use of cavities is the most common method for microwave measurements the material under examination is not fully in contact with the EM waves. Another type of cavity resonators is the coaxial cavity which allows measurements over a broad bandwidth.

Coaxial cavities are coaxial transmission lines with one or both en shorted. Similar to resonant cavities, the principal of operation of coaxial cavities is based in the perturbation theory. Electromagnetic fields excited in the resonator are reflected from the ends [11].

Line resonators enable the measurement of complex permittivity of materials by bringing the sensor in contact to the material under test. The opened ended sensors compromise a coaxial line with an input feed line located at a known distance ($L_1$) away from the shorted end ($L_2$) and from the open end section [12]. The sensor resonates at frequencies corresponding to the line lengths and is tuned prior to any measurements. When the sensor is placed in contact with the material, it detunes the response. This detuning is attributed to the permittivity of the material and is directly proportional to the change in length in the response.

Microstrip resonators (for the analysis of dielectric properties) were first described by Troughton[13]. The work describes a ring used as a resonator that can operate five wavelengths long at the frequency of interest. Ring resonators have been used in different applications such as the evaluation of dielectric permittivity of solvents [14] and the detection of biomolecules[15]. Ring resonators operate on the same principle as perturbation cavities. When a material is brought in contact with a ring resonator, depending on the dielectric properties of the analyte a shift in the resonant frequency is observed[16].

For our studies interdigitated electrodes were fabricated to act as a microwave resonator. Inderdigitated electrode (IDE) gold pattern printed on a woven glass and ceramic reinforced substrate acted as a microwave sensor. The sensor is connected to the vector network analyzed via an SMA connector. The SMA is choses as it excites the IDE sensor in a manner that the available signal is maximized[17]. Gold is used to maintain chemical neutrality and as a ground plane of the IDE sensor. The sensitivity of this type of IDE microwave sensor can change close to the sensor surface[18]. Precise control of the sensitivity is required. Finite element modeling was carried out to demonstrate this feature and can be seen in Fig. 1. Due to its features the use of IDE planar sensors eliminates some of the disadvantages found in cavities resonators.

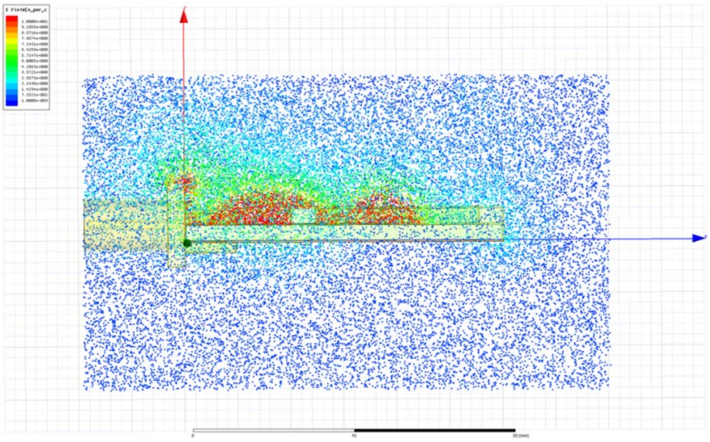

Fig. 1 An electromagnetic field distribution in the proximity of a sensor surface as modelled: side view.

## 18.3 Investigating material properties using electromagnetic waves

Microwave sensing has been applied to understand materials' properties by analysing its interaction with the EM waves. Material's dielectric properties are determined by its molecular structure[19]. As described in the previous section, changes in the molecular structure of a material lead to changes in their permittivity. Thus permittivity can determine how a material interacts with an EM field. Microwaves can propagate through a material in a non-invasive way thus microwave sensing has been previously used to investigate and detect components. The residual life of an activated carbon has been evaluated using microwave resonant cavity [20]. Changes in the response when carbon material was placed inside the resonator cavity was used to determine its residual life. Calibration curves were obtained which can be used to predict the residual life of activated carbon materials. In a similar study an IDE microwave sensor was used to analyse vegetable oil[21]. This method is of particular interest since measurements were carried out using low power microwaves and different response was obtained for each type of oil analyzed. Measurements were performed by bringing the IDE antenna in contact with the sample thus the material was in full contact with the EM waves. This type of IDE microwave sensor has also been previously used to monitor nitrate concentration in solutions[22]. These results show the potential of using microwave sensors for estimation of pollutants present in solution.

We are particularly interested in studying the interaction of biomaterials such as hydroxyapatite and their interaction with proteins. Our studies have focused on the microwave analysis of glucose oxidase as a model protein which will be discussed in the next sections.

## 18.4 Dielectric properties of hydroxyapatite

Hydroxyapatite (HA) is the main mineral component of bone and dentine. HA has been extensively used as scaffolds and as coating material for bone. It is important to note that HA presents electrical properties i.e. piezo-[23] and pyro-electricity[24]. Moreover HA thick films have shown pressure induced capacitance changes [25]. The electrical properties of HA and its interface with bone proteins and cells will be further discussed in chapter 27.

The response of HA thick films in the GHz frequency has been previously reported using a microwave cavity [26]. To study the dielectric properties of HA thick films an IDE sensor was placed in contact with the sample and measurements were taken in the 0.01-15 GHz frequency range (60,000 points for each measurement). It is important to note that all measurements were carried out in air. Figure 2 illustrates the spectra recorded for HA and air using and IDE microwave sensor in the 0.01-15 GHz frequency region. Different spectra are observed for air and HA indicating that HA responds to microwaves and thus can be detected. These results underline the dielectric properties of HA which are important to consider for the understanding of the bioactive behaviour of HA.

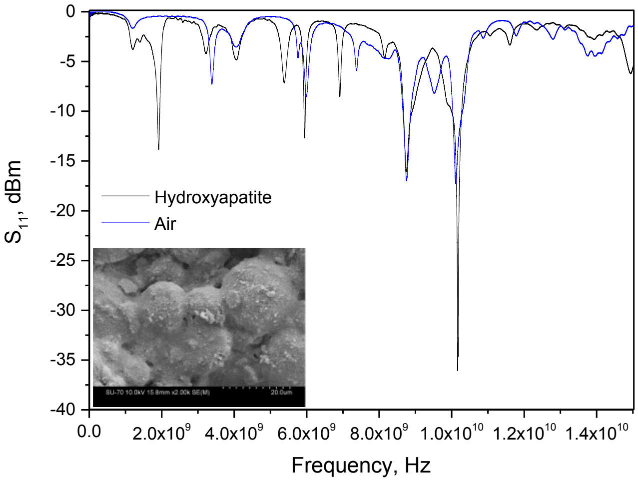

Fig. 2 Microwave response of IDE sensor in 0.01-15 GHz range for HA. Inset: SEM micrograph of HA thick film.

## 18.5 Analysis of protein adsorption on hydroxyapatite using microwaves

Changes in the electromagnetic wave signal in the microwave frequency range were used to detect protein adsorption. HA thick films were modified with glucose oxidase to analyse its response in the GHz range. Glucose oxidase(GOx) is a well-known dimeric glycoprotein which catalyses the oxidation of D-glucose [27] . The concentration of the adsorbed Gox to HA films was determined by using a molar coefficient of 14,100 $M^{-1}$ $cm^{-1}$ at 450 nm [28]. The calculated protein loading was of 1.6 nmol $cm^{-2}$. Measurements in the GHz range were recorded as detailed in the previous section. Fig. 3 illustrates the spectra of the modified HA films and HA and air focusing on the 6-10 GHz frequency range. While the obtained spectra show different resonant peaks for each sample; the difference observed is too small. The slight difference in signal observed could be due to the fact that the thickness of the protein layer is small in comparison with that of HA thick film (65 um). The approach described here shows the potential of microwave sensing for the detection of protein adsorption. To further analyse protein-ligand interactions a biological recognition element is required to attain selectivity.

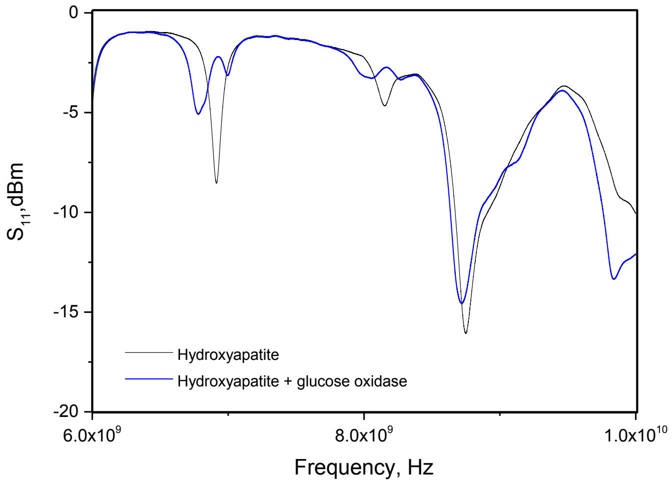

Fig. 3 Reflected signal of the IDE antenna for HA (—) and for HA samples modified with glucose oxidase (—), 6.0-10.0 GHz range.

## 18.6 Conclusions

The use of EM wave sensor for the analysis of materials and for the detection of protein adsorption has been discussed in this chapter. IDE microwave sensors can be fabricated to operate with high sensitivity in the GHz region. The dielectric properties of HA thick films were evaluated and the response in the GHz were measured. In addition the system described here was used to detect the binding of GOx to HA films. The results obtained confirm the performance of IDE sensor for microwave measurements and the potential as a sensing technique for biomaterials.

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