Fundamental monomeric biomaterial diagnostics by radio frequency signal analysis

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A B S T R A C T

We present a new diagnostic technique of fundamental monomeric biomaterials that do not rely on any enzyme or chemical reaction. Instead, it only uses radio frequency (RF) signal analysis. The detection and classification of basic biomaterials, such as glucose and albumin, were demonstrated. The device was designed to generate a strong resonance response with glucose solution and fabricated by simple photolithography with PDMS (Polydimethylsiloxane) well. It even was used to detect the level of glucose in mixtures of glucose and albumin and in human serum, and it operated properly and identified the glucose concentration precisely. It has a detection limit about 100 μM (1.8 mg/dl), and a sensitivity about 58 MHz per 1 mM of glucose and exhibited a good linearity in human blood glucose level. In addition, the intrinsic electrical properties of biomaterials can be investigated by a de-embedding technique and an equivalent circuit analysis. The capacitance of glucose containing samples exhibited bell-shaped Gaussian dispersion spectra around 2.4 GHz. The Albumin solution did not represent a clear dispersion spectra compared to glucose, and the magnitude of resistance and inductance of albumin was higher than that of other samples. Other parameters also represented distinguishable patterns to classify those biomaterials. It leads us to expect future usage of our technique as a pattern-recognizing biosensor.

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1. Introduction

Molecular diagnostics have been applied in various fields and applications, such as molecular biology, biosensors, analytical chemistry, and others by using numerous techniques, e.g., electrochemical, optical, and magnetic resonance methods, and so on. Recently, radio frequency (RF, GHz range) electrical signal-based sensors (RESs) have been considered as novel diagnostic tools for biological sensors (Lee et al., 2012, Lee and Yook, 2014; Park et al., 2014a, 2014b; Grenier et al., 2009; Kim et al., 2013; Kim et al., 2015; Yang et al., 2010); chemical sensors (Rauch et al., 2014; Barochi et al., 2011; Rossignol et al., 2013; Tanguy et al., 2015; Potyrailo and Morris, 2007; Zhixin Li et al., 2014); and humidity sensors (Kim et al., 2006; Bernou et al., 2000). This is due to their unique features, such as low cost, low power consumption, and their ability to provide non-invasive and real-time sensing. Another remarkable potential of RESs is their direct sensing capability, which depends on the interaction between the microwaves

and materials. According to the dielectric theory, it is widely known that biomolecules exhibit different behaviors of dielectric dispersion at specific frequencies. This has been demonstrated with glucose in the category of monosaccharides (Moran et al., 2000; Fuchs and Kaatze, 2001; Chan et al., 1986; Noel et al., 1996) and albumin, it has been demonstrated in the category of monomer proteins (Grant et al., 1968; Eden et al., 1980; Oncley, 1942). In aqueous solution, albumin represents two distinct dielectric dispersions, i.e., in the moderate frequency region (10^5–10^7 Hz) and in the high frequency region (10^{10}–10^{12} Hz). However, glucose exhibits strong dielectric dispersion in the low-GHz range. Thus, by observing the electrical property, especially capacitance related to dielectric constant, of glucose and albumin subjected to the electrical signal in the low-GHz range, we could utilize this characteristic of glucose and albumin for the detection and classification of glucose and albumin. In the low-GHz range, In the past few decades, various kinds of glucose sensing techniques were developed, including electrochemical (Harper and Anderson, 2010; Oliver et al., 2009), optical (Bahshi et al., 2009), Raman spectroscopy (Olga Lyandres et al., 2008), reverse iontophoresis (Potts et al., 2002), electro-thermal (Park et al., 2014a, 2014b) and field

effect transistor-based (Miyahara and Moriizumi, 1985) methods. Each of these methods has its particular advantages and successes to date. However, such methods are not possible without some bulky enzymes, such as glucose oxidase, hexokinase, or glucose-6-phosphate dehydrogenase for more accurate results (Oliver et al., 2009). This is the intrinsic and inevitable prerequisite in these methods. The use of enzymes or even simple chemicals also increases cost and creates production problems. Thus, we expect that the RES, which is a direct detection method for glucose, can slightly ameliorate these drawbacks. Currently, the number of people who have diabetes mellitus caused by imbalances in their glucose levels has increased due to changes in lifestyle and diet (Cowie et al., 2006). Thus, glucose research, especially for the detection and surveillance of glucose, is considered more important now than it was earlier.

Albumin, a non-glycosylated, multifunctional, negatively-charged plasma protein, is a fundamental protein in humans (He and Carter, 1992). It comprises 50% of the total protein in the plasma, making it the most abundant protein and monomeric proteins in human plasma. One of its main functions in the human body is to regulate the osmotic pressure of blood. It has hydrophobic binding sites in its center and hydrophilic binding ligands in its outer parts (Farrugia et al., 2010). Due to its abundance in mono-protein of albumin, it has been studied extensively in protein diagnostics, immunology, and other areas of research in which antigen-antibody reactions are used (Theodore, 1985; Doumas et al., 1971).

Even albumin and glucose are fundamental biomaterials, and it is worthwhile to investigate them due to their importance to human life, it is not easy to examine them at once. In human bloods or any other real samples, it is inevitable that glucose and albumin exist as a mixture, and we must purify the samples to obtain the respective materials. Thus, it is extremely difficult to examine the properties of target molecules in the mixed condition without any supporting specific reaction for detecting them. In this regard, as mentioned previously, the distinct dielectric behavior of the glucose and albumin merits attention for direct sensing. However, since the glucose and albumin exhibited their own unique properties in response to RF signals, simply investigating the dielectric properties of various bio-samples with a device, such as a dielectric spectrometer, is not sufficient for use as a biosensor. The amount and concentration of the samples required for sensing are much too high to be used as a sensor application, and the work required in using a dielectric spectrometer is quite cumbersome. To solve these problems, we constructed a device that operated as a kind of reflection notch filter in the RF range and exhibited clear resonance of the inductance from the electrode and the capacitance from the sample (Matthaei et al., 1980). The electrode was designed to generate electrical resonance with glucose in a few milli-molar range, which corresponds to the concentration range in human blood. Compared to the previous report of Wang's group (Kim et al., 2015) and our previous work (Park et al., 2014b), this work has some advantages. First, this work has an improvement about detecting limit. Limit of detections (LODs) of the report of Wang's group and our previous work are 8.01 mg/dl (0.45 mM), 18 mg/dl (1 mM), respectively. Additionally, the electrical properties of the samples were extracted from the de-embedding technique and the analysis of equivalent circuit.

RF signals were measured easily as a scattering S-parameter that is a power signal. In doing so, we are able to observe the resonance condition very quickly. To gather more a comprehensive understanding of the electrical properties of biomaterials under RF electrical signal and more reliable discrimination between biomaterials, we tried to de-embed the RF signals from the multi-dimensional electrical parameters by using suitable equivalent circuit analysis (Jun et al., 2007). An R-L-C (resistance-inductance-capacitance) circuit model was constructed that could be analyzed

freely from the contact resistance and the contact capacitance between the target sample and the electrode to extract the properties of the targets. The various parameters that were obtained by processing the analytical signal yielded more reliable results for instant in-situ sensing, with each parameter exhibiting distinct patterns depending on the contents of the sample, even with mixtures of glucose and albumin. The capacitance of each sample that were well-matched to dielectric theory. Thus, we also expect that the analysis of the various parameters de-embedded by our model has a potential for use recognizing direct patterns using RF signals.

2. Materials and methods

Detailed descriptions of the preparation methods of the samples are provided in the Supporting information S1. A highly resistive (> 4000 Ω/cm) silicon (Si)/silicon oxide (SiO$_2$, 500 nm oxide layer) substrate was selected in order to minimize the signal loss to the substrate, and then a ground-signal-ground (GSG) electrode pattern was fabricated on the substrate by photolithography. The electrode material consisted of Ti/Au (10/150 nm) that was deposited using e-beam evaporation. In a GSG electrode, two ground electrodes were used as the ground base around the signal (Fig. 1d), and the signal electrodes were two transmission electrodes (with a 3-μm gap that became more narrow (2 μm) at the end of the tip). These electrodes were designed to generate electrical resonance with certain bio-samples (glucose) (Fig. 1e). In addition, to eliminate other unexpected influences on the experiment, a polydimethylsiloxane (PDMS) elastomer well (1.5 mm well in diameter and 3 mm thick) was also deposited on the electrode to facilitate dropping the target solution (6 μl) in an exact position (in the middle of the signal electrode) and creating uniformly-shaped droplets between the signal electrodes. The schematic diagram in Fig. 1b and the photograph of the device in Fig. 1e show the completed device. Fig. 1f shows the equivalent circuit between the two ports of our RES. The RF signals were obtained as S-parameter forms that were measured using a network analyzer (E5071 C, ENA Series Network Analyzer by Agilent Technologies) at −20 dBm. The S-parameter is the relative value of the voltage of the two ports. Generally, a notation such as S$_{ab}$ is used to indicate the S-parameter, where "b" represents the applied incident (input) voltage of the port "b", and "a" signifies the measured voltage of the port "a". Thus, S-parameter is composed of 4 parameters: S$_{11}$, S$_{21}$, S$_{12}$ and S$_{22}$. S$_{11}$ and S$_{22}$ demonstrate reflected signals of the device and S$_{21}$ and S$_{12}$ exhibit signals transmitted through the solution and the electrode. Before observing the S-parameters of the samples, the signal to the network analyzer was calibrated using the short-open-load-through standards. After dropping a target solution in the well, the S-parameters were measured and used to determine the contents of the sample and the concentration of the solute. Contingent upon the kind and the concentration of the sample, the samples had diverse effects on the electric field in the RF range. The S-parameter that was obtained was decomposed to interrogate the electrical properties of the sample itself, i.e., the resistance (R), the inductance (L), the capacitance (C), and the contact properties of the sample with electrodes, namely, the contact resistance (R$_c$), and the contact capacitance (C$_c$), and the parasitic capacitance (C$_{ps}$). The decomposition of the S-parameter into electrical parameters was conducted by the de-embedding technique and numerical analysis with an equivalent circuit. Detailed descriptions of the techniques are provided in the next section. Furthermore, the relative signal loss resulting from the absorption by the target solution was also calculated by the following loss equation.