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ScienceDirect

Procedia Engineering 120 (2015) 163 – 166

Procedia Engineering

www.elsevier.com/locate/procedia

EUROSENSORS 2015

Microfluidic-based Split-Ring-Resonator Sensor for Real-time and Label-free Biosensing

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Abstract

In this report, a split ring resonator (SRR), the most important building block of metamaterial, is fabricated and integrated with a microfluidic chamber for biosensing. The SRR is produced on a microwave printed circuit board while the microfluidic chamber is fabricated by casting of polydimethylsiloxane (PDMS). SRR was immobilized with Anti- Immunoglobulin G (IgG) for IgG detection by a standard covalent immobilization using Cystamine. The PDMS chamber was aligned and clamped on the circuit board and the electromagnetic response of the SRR sensor was continuously monitored when IgG analytes was flowed through the chamber. The reaction of Immunoglobulin G (IgG) and Anti-IgG results in a shift of resonance frequency. It was found that the response of the resonance frequency is sensitive to the IgG concentrations. Therefore, the SRR microfluidic scheme can be effectively used as an advanced bio-sensing device.

Peer-review under responsibility of the organizing committee of EUROSENSORS 2015

Keywords: Split ring resonator (SRR), metamatherial; microfluidic; biosensing

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Peer-review under responsibility of the organizing committee of EUROSENSORS 2015
doi:10.1016/j.proeng.2015.08.595

1. Introduction

Metamaterials are artificial materials with electromagnetic properties unfound in nature such as negative permittivity and negative permeability. They usually comprise periodic structures with geometric features smaller than the wavelength of interacting electromagnetic wave. [1-5]. A split ring resonator (SRR), a metallic open loop with a dielectric gap, is among the most common structures used in matamaterial [4-6]. It exhibits a strong electromagnetic resonance with its frequency and Q factor depending on the geometry and permittivity of the dielectric contained in the vicinity of the subwavelength resonator gap. Depending on their geometry and orientation, the inclusions can couple at a wavelength much longer than the periodicity. At resonance, the structures couple with free-space electric or magnetic waves, resulting in localized electric field in the gap and a strong current oscillation in the metallic loop. This local resonant field is very sensitive to a change in the dielectric properties of material in the gap region. This peculiar feature is thus promising for sensing applications.

Recently, various sensors based on SRR for determination of chemical [5-10] and biological analytes have been developed [11-13]. The basic structure of the SRR consists of a metallic open loop with a dielectric gap. The electromagnetic wave that transport through the loop induces the current inside the loop and those current will become strongest at its resonance frequency. The resonance frequency is depend on structure and dimension of the loop and dielectric gap. Since the structure of metallic loop is fixed then the resonance frequency will only depend on the analyte material in the gap. This kind of sensor has many key advantages such as high-sensitivity, real-time and label-free detection ability. However, there has been very few reports that demonstrate its full functionality in bio-sensing applications.

The detection of bio-molecules in real-time sensing requires the measurement in liquid-phase, which can cause significant loading effect to oscillation current inside the loop of SRR because the buffer for bio-analyte is normally highly conductive medium. Thus, most reports on SRR biosensors employ gas-phase detection method despite its limited sensitivity, poor repeatability as well as real-time detection inability. In this study, we minimize the effect of conductive liquid medium with microfluidic technology. The analyte presented to the gap is effectively controlled in both shape and volume to attain the stability in the sensor response and the real-time detection ability. The sensor is demonstrated as a label-free quantitative biosensor for determination of IgG after immobilization of the SRR gap with anti-IgG.

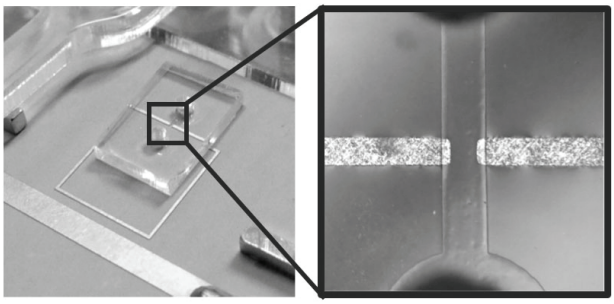

Fig. 1. The photographs of microstrip-coupled SRR integrated with PDMS microchamber.

2. Experimental

The SRR based sensor in this study was designed based on a microstrip-coupled SRR structure. The pattern was fabricated on the gold plated RT/duroid 6010.2LM high-frequency laminate PCB using the standard photolithography technique [3]. Polydimethylsiloxane (PDMS) micro fluidic chamber was fabricated by casting on a patterned silicon mold. Next, the PDMS chamber was aligned on the SRR gap and attached with inlet/outlet tubes as illustrated in Figure 1. The inlet tube was connected to the peristaltic pump for control of the sample flow while the outlet tube was drawn into a waste tank. The other end of the pump tube was immersed into the analyte solution.

Cystamine solution (25 mM), a bio-functional building block with two functional end groups (thiol (SH) and amine (NH_2)) for antibody immobilization [13], was then flowed into the chamber and incubated for 12 hours. The

sulfur atoms of thiol group were bound to gold surface through weak acid-base interaction while the amine group would be left for subsequent reaction. After that, PBS solution was flowed into the chamber while the sensor response was continuously monitored using a network analyzer (Agilent E5071C). The 20 mM Glutaradehyde (GA) was then fed into the chamber with 6.8 ul/min flow rate for 30 min. Next, the PBS solution then flowed into the chamber again for determination of resonance frequency change caused by GA. The Anti-IgG, an antibody that specifically binds with IgG, was then prepared at 1:100 v/v concentration and flowed into the chamber at the same flow rate for 45 min to form the sensing layer specific to IgG. Next, the excess Anti-IgG was removed by flowing of PBS buffer and the signal was recorded to determine the response due to Anti-IgG immobilization. The immobilized SRR-based sensor is then ready to be used for IgG bio-sensing as schematically demonstrated in Figure. 2. IgG solution in phosphate buffer saline (PBS) buffer will be injected into the flow channel. As IgG molecules pass through the SRR electrode, they will bind to anti-IgG on the electrode via immunological reaction, resulting a change of dielectric in the gap and its resonance frequency, which is continuously monitored by the network analyzer.

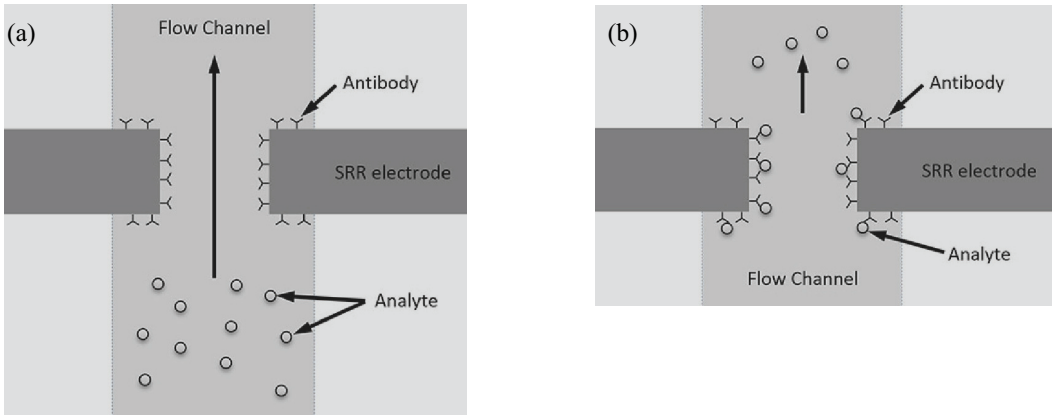

Fig. 2. The diagram illustration of SRR electrode with immobilized antibody in microfluidic flow channel (a) before and (b) after binding under analyte flow through a channel.

3. Results and discussion

Figure 3 (a) show the sensor transmission response (S_{21}) under different loading conditions. In the air, the maximum attenuation is occurred at the highest resonance frequency of around 2 GHz. When 1 μl of PBS buffer solution was dropped over the gap, the resonance frequency is shifted down to 1.55 GHz and the attenuation amplitude is reduced by more than 90%. In addition, the transmission response becomes quite unstable due to droplet vibration. To overcome the problems, the sensor will be operated in a closed chamber under flow through conditions. After attaching the PDMS chamber and flowing deionized (DI) water into the chamber, the attenuation response signal becomes higher with a sharper peak at a higher resonance frequency of 1.8 GHz. After adding PBS into DI water, the resonance frequency is slightly reduced but the attenuation amplitude is substantially reduced as much as 50% compared with that of DI water.

The measured frequency response shift due to the immobilization of Anti-IgG is shown in Figure 3 (b). It can be seen that the resonance frequency is decreased when the GA, Anti-IgG and 100 μg/ml IgG is flowed onto the SRR electrode and the frequency becomes increasing when the PBS buffer resumes. The net the frequency shift after 30 min GA, after 45 min Anti-IgG and after 45 min IgG flow in the PBS medium are around 10.5 MHz, 12 MHz and 9 MHz, respectively. The decrease of resonance frequency is due to the binding of GA, Anti-IgG and IgG on the gap, resulting in the increase of dielectric constant in the gap. In addition, the highest resonance frequency shift occurs after Anti-IgG immobilization, indicating that Anti-IgG causes the largest change in dielectric constant at the SRR gap.

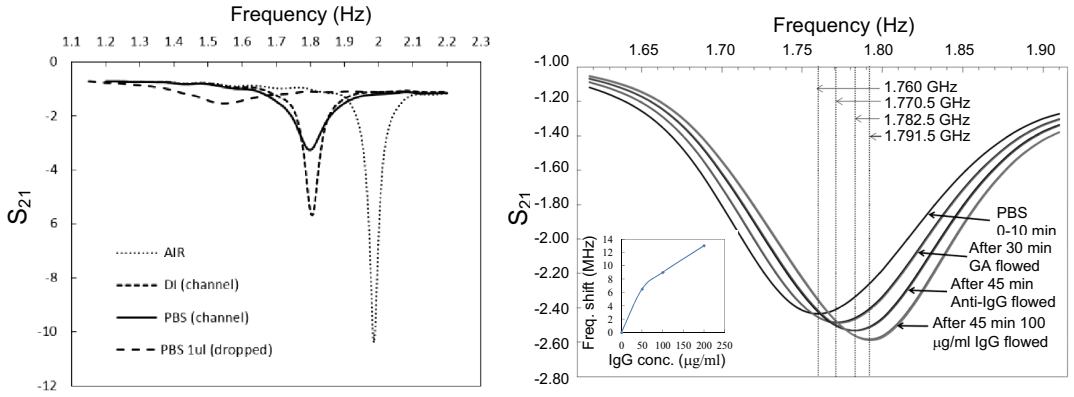

Fig. 3. Measured real-time frequency response of a SRR sensor under (a) different loading conditions and (b) due to various stages of Anti-IgG immobilization and 100 µg/ml IgG detection.

The immobilization process was repeated at other IgG concentrations to obtain the response characteristic curve for IgG detection as demonstrated in the inset of Figure 3 (b). It is seen that the resonance frequency shift increases monotonically and approximately linearly from 6.5 MHz to 13MHz as the IgG concentration increases from 50 to 200 µg/ml. The results demonstrate that the microstrip-coupled SRR integrated with PDMS chamber can effectively be used for real-time, label-free and quantitative detection of IgG and can be extended for use with other antibody-antigen biological analytes.

Acknowledgements

This research is funded by NECTEC under graphene based supercapacitor project (Grant no. P1201107) and Thailand's National research council (NRC) under 3D graphene project (Grant no. P1401107). A. Wisitsoraat would like to express his gratitude for Research Grant from NRC.

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