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High radio frequency biosensor for a nano-concentration detection of the label free Prostate Specific Antigen cancerous cells

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Abstract—**This research presents an original and novel biosensor based on the transmission line geometry of a high radio frequency microstrip filter which aims to achieve rapid diagnostics of cancer. The analytical design of the biosensor has been computationally verified. The biosensor was fabricated by patterning a 10 µm nickel/gold metal layer on a 1.6 mm FR-4 dielectric base with a continuous metal back plane. Subsequently, the microstrip was integrated with a microfluidic channel. Characterization of scattering parameters was performed by a vector network analyzer (VNA), with results showing good agreement with the modelling. To assess functionality, sensitivity to prostate specific antigens (PSA) was quantified. Initially, the biosensor was functionalized by coating it with antibody receptors (Ab) to capture PSA. Thereafter, sensitivity and repeatability towards the detection of captured PSA was assessed by immobilization of PSA onto the treated golden surface. Measurements were taken directly after each stage of coating and capturing. A nano-concentrations (2000-20ng/ml) of PSA were sensed. The characteristic dips in the reflection signal parameter (S_{11}) all showed both an amplitude and frequency shift, most notably at 0.6, 1.08 and 1.32 GHz respectively. This therefore revealed the ability of this biosensor to immobilize and detect nano-concentrations of PSA analyte by measuring the signal changes due to the presence of PSA, with variability in results being attributed to the surface quality of the biosensor. Mostly, electrical biosensor research is focusing on low frequency (<1MHz), on the other hand, the work reported here proves the achievability of this new high frequency approach.**

Keywords—**High frequency biosensor, microstrip filter, electrical characterization, label free detection, PSA**

I. Introduction

Providing prompt treatment is significant to early and rapid prognosis and diagnosis of cancer, since it eliminates further risks and reduces cost. Nevertheless, early detection of abnormal levels of PSA indicates diseases in their earliest stage [1], and can therefore enhance treatment in the initial period. Due to advances in microfabrication technologies, development in sensors and sensing fields has reached an exceptional level. Consequently, development of biosensing technology has seen a range of approaches being explored, either electrical, mechanical or optical. Optical approaches offer very high sensitivity, although instruments tend to be lab based. Mechanical approaches do offer high sensitivity in a small form factor, even though, microfluidics and signal recovery add complexity to the system. Low frequency electrical biosensing offers the simplest and cheapest technology platform at the expense of reduced sensitivity. In other words, for point of care diagnostics, the technology should be simple to use, cheap to manufacture and provide clinically appropriate ranges and sensitivity levels, as well as increasing the possibility of sensing minute analytic sizes with relatively low cost. High radio frequency electrical biosensing offers a good compromise between these competing factors. Interest is growing towards the use of the high radio frequency (RF) biosensors in bio-sensing applications in which rapid and label free detection can be achieved, as well as miniaturization and portability [2]. Research is covering several potential fields. For example, observing, Y. Hong et al introduced a radio frequency biosensor for revealing label free biocells [3]. For point of care diagnostics, electromagnetic waves offer an investigation tool to analyse the variance of the electrical properties between normal and abnormal cells, C. Dalmay et al [4-6]. H. Lee et al developed an RF biosensor based on an SRR resonator and a microstrip line, a devise which exhibited a frequency shift for PSA of around 5MHz [7].

The purpose of this research is to design and fabricate a novel PSA radio frequency label free biosensor based on an Electrical Passive Stepped Low Pass Microstrip Filter and microfluidics. This paper will outline the basics, design and modelling of this biosensor and, finally, the medical application of the biosensor. Vector network analysis was used to measure the electrical characterization of the biosensor. Thenceforward, its performance against a clinical PSA-analyte was quantified. The described research demonstrates the possibility of this original high frequency attitude, revealing the ability of this radio frequency biosensor to detect a nano-concentration of PSA analyte by measuring the signal changes resultant from PSA existence.

II. Biosensor design

The proposed biosensor consists of two main parts; a microstrip filter and microfluidic. The design of the Passive Stepped Low Pass Microstrip Filter is characterised by a type

I Chebyshev response, having a steep pass/reject band transition with 1.0 dB ripple in the region of the pass band. Design parameters are: 1.4 GHz cut off frequency; fixed parts length with variable impedances and 50 Ω terminal impedances.

A. Prototype of the Low Pass Filter

The filter was built up from a prototype Normalized Low Pass Filter. Electrical values of the prototype Normalized Low Pass Chebyshev Filter elements are shown in table 1: g-values represent the filter components of capacitors, inductors and resistances [8].

Table 1 Elements numerical values for 1.0 dB ripple normalised low filter

g_0	g_1	g_2	g_3	g_4	g_5	g_6	g_7	g_8	g_9
1	2.204	1.131	3.147	1.128	1.194	3.147	1.131	2.204	1

Subsequently, the prototype pattern was scaled to a feasible filter according to a scaling scheme [9];

for capacitive parts: $C = \frac{C_n}{2\pi f_c R}$ (1)

and for inductive parts: $L = \frac{RL_n}{2\pi f_c}$ (2)

B. Microstrip filter

The microstrip filter was built from the previous electrical scaled filter. Transformation equations are required to estimate dimensions of the microstrip filter and realize the physical form, such as the following [10]:

Mainly inductive elements;

$$l_L = \frac{\lambda_{gL}}{2\pi} sin^{-1}\left(\frac{\omega L}{Z_{0L}}\right)$$

for short length: $l_L = \frac{f\lambda_{gL}L}{Z_{0L}}$ (3)

Mainly capacitive elements;

$$l_C = \frac{\lambda_{gC}}{2\pi} 2\pi sin^{-1}(\omega C Z_{0C})$$

for short length: $l_C = f\lambda_{gC}C$ (4)

The dimensions of the physical microstrip filter were realised by transformation schemes, in addition, according to substrate type (FR-4) and thickness (1.6mm), amended as necessary, as shown in table 2.

Table 2 Dimensions of the physical microstrip low pass filter,

Part	C1	L1	C2	L2	C3	L3	C4
width	31.15	1.728	31.15	1.728	31.15	1.728	31.15
length	8.356	6.553	12.47	7.549	12.47	6.553	8.356

Units: millimetre.

III. Modelling

Sonnet Lite and Ansys HFSS electromagnetic simulators completed geometric modelling. The proposed microstrip's metal was gold and the metal layer thickness was 10μm plated on 1.6mm FR-4 dielectric substrate and copper ground backplane. Wave scattering parameters S_{11} were characterized for the pass band frequencies. The results were in very good agreement with the design requirements; responses as shown in figure1.

IV. Fabrication

Designs were sent to Faraday Printed Circuits Company for fabrication. A 10 μm nickel/gold metal layer was patterned on the front surface to construct the device geometry with a continuous metal back plane on the rear of the 1.6 mm FR-4 dielectric. A solder resist layer was placed on the backside ground plane. However, the gold on the front surface was left exposed to allow for microfluidics and functionalization. Once fabricated, SMA connectors were soldered onto the terminal ports and laser-made acrylic microfluidic channel were integrated with the device, positioned to part cover the gold and FR-4, with double sided tape glue in between: figure 2.

V. Methodology

Biosensor characteristic scattering parameters were measured using a Rhode-Schwarz ZVL3 vector network analyzer VNA. VNA was calibrated up to the SMA connectors of the biosensor to de-embed effects of feeding lines, ports and cables. Microfluidic channels were integrated with the microstrip. VNA signal responses were used to characterize the bio functionality of the biosensor. The biosensor was implemented bio-medically via functionalizing it to detect nano-concentrations of PSA-analyte. Concentrations used were 2000-20 ng/ml. Experimental configuration is shown in figure 2.

The biosensor was initially prepared by coating the gold surface with DSP cross linkers to connect the antibodies Ab to those cross linkers. Thereafter, an antibody was added and bonded through DSP cross linkers to the gold's surface. Following Ab addition, a layer of BSA blocking agent was added to cover non-functional regions to enhance the sensitivity. Finally, PSA immobilization and data collection.

VI. Results and disscussion

A. Verification

Measured responses were in good agreement with the simulation results. An example of the S_{11} response is shown in figure 1, with the variation attributed to manufacturing tolerances in the device's fabrication.

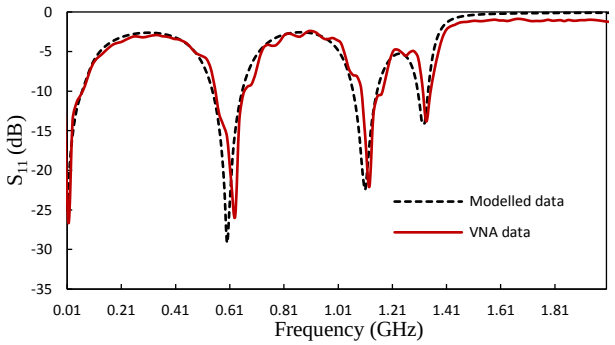

Fig. 1, Scattering parameters S_{11} from modelled design and VNA data of the fabricated microstrip filter

B. *Medical Implementation and Functionality*

The characteristic dips in the S_{11} parameters all demonstrated both an amplitude and frequency shift due to the coating process. After initial preparation, Biosensor sensitivity towards PSA was assessed. A 2000ng/ml concentration of PSA was immobilized on the functionalized surface, and was captured by the antibodies within 5 minutes. The presence of PSA led to a change in the surface geometry, therefore, leading to a signal change. VNA measurements indicated a significant signal adjustment, particularly the reflected wave of the incident scattering parameter S_{11} because of PSA capturing. Results are shown in figure 3 of the curve fitting. A fifth order polynomial was fitted to each curve to remove noises from the signal and to determine changes due to each step towards PSA capturing. Blocking of the surface demonstrated 0.6 dB, 0.75 MHz shift from the base point in the feature whilst immobilization of the PSA then showed 8.3 dB and 0.75MHz changes from the primary measurement of the blocking treatment. These results approved the biosensor detection functionality.

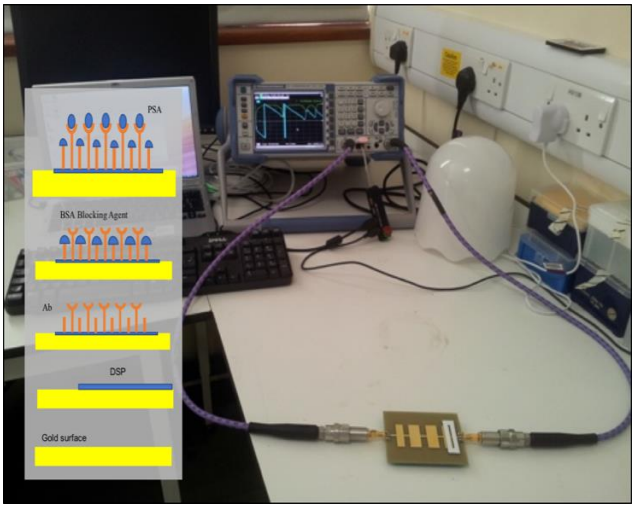

Fig. 2, Illustration diagram for the Prostate Specific Antigen capture procedure and experimental configuration.

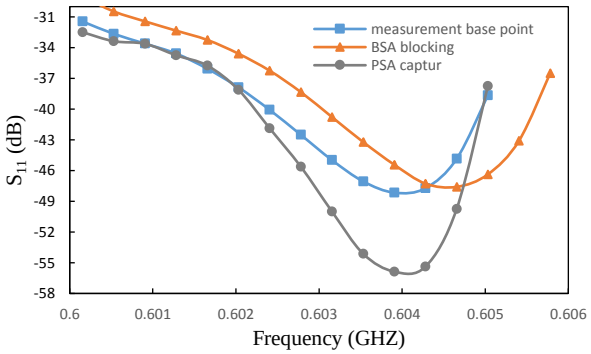

Fig. 3, Curve fitting of the resultant S_{11} scattering parameter for PSA immobilization, blocking and measurement base point at 0.6 GHz.

VII. Conclusions

A proposed novel biosensor based on the radio frequency Stepped Microstrip Low Pass Filter was fabricated and successfully tested. The microstrip device was designed from a Normalized Low Pass Prototype Filter and transformed into a physical microstrip filter. This microstrip biosensor was fabricated by standard PCB fabrication techniques and electrically characterized by a vector network analyser, thereafter, medically tested. The functionality of the biosensor towards PSA detection was quantified experimentally according to a biomedical protocol in a bio laboratory via the detection of PSA nano-concentrations. A change of 8.3 dB attenuation and 0.75 MHz frequency shift from the initial stage occurred due to the immobilization of the PSA. These original results demonstrated the feasibility of such radio frequency biosensors towards bio-medical applications. Work is currently progressing on miniaturization of this biosensor, as well as applications of higher frequencies to boost sensitivity for Nano volumes and Pico concentrations of the material under test, thereby determining sensitivity limits from such an approach.

Conflict of Interest

The authors declare that they have no conflict of interest.

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