New RF Biosensor based on Planar *LC* Resonant Circuit for Human Cancer Cells Characterization

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***Abstract* –In this paper, the new RF biosensor using planar *LC* (parallel inductor and capacitor) resonant circuit for human cancer cells (HepaG2 cells) characterization is proposed. The RF biosensor is designed on stopband resonator structure by using an interdigital electrodes (to be a capacitor) and two spiral transmission lines (to be an inductor) and operated frequency at around 23 GHz. The principle of proposed RF biosensor deals the different resonant energy coupling of cancer cells located on the interdigital electrodes. By evaluating the changes of resonant peak, the number of cancer cells can be effectively determined. The on-chip *LC* circuit allows an accurate and sensitive detection at cell-scale in this design. Experimental results show the good cancer cells detection capability with 50, 60 and 70 cells.** [1]

Keywords: RF biosensor, cancer cells, *LC* resonant circuit, on-chip

I. INTRODUCTION

In recent ten years, the biomedical applications have more interest as there is an important need for biomedical electronics and sensors that can quickly and accurately analyze biological molecules, DNA and/or cells.

For cells detection, different analyze methods have been developed based on mechanical [1], optical [2], electrical [3] and microwave techniques [4]. In order to achieve good detection sensitivity, labeling cells with a specific chemical and antigen or molecule are strongly changed the cell properties and seriously damage them. Microwave detection methods are very interesting as they can be label free and then allow a non-invasive analysis. After analysis, cells can be kept in original state and reused for further investigations in its primeval environment. In [4], an original approach for biological cell discrimination using impedance spectroscopy analysis at microwave frequency. This method allows a label free analysis really done at the cell scale using high frequency electromagnetic waves as a non-invasive tool to probe the intracellular medium. In [5], the biosensor chip allowing determination of intrinsic relative permittivity of biological cells at microwave frequencies. This sensor permits non-invasive cell identification and discrimination using an RF signal to probe intracellular medium of biological samples. However, the equivalent parameters of resistance and capacitance should be extracted and calculated from the measurement for further analyzing the properties of biological cells. These works are good and inspired us to further study this issue.

In this paper, the RF biosensor using planar *LC* (parallel inductor and capacitor) resonant circuit for human cancer cells (HepaG2 cells) characterization is proposed. The RF biosensor is performed by using an interdigital electrodes (to be a capacitor in detection area) and two spiral transmission lines (to be an inductor) at around 23 GHz. The principle of RF biosensor deals the different resonant energy coupling of

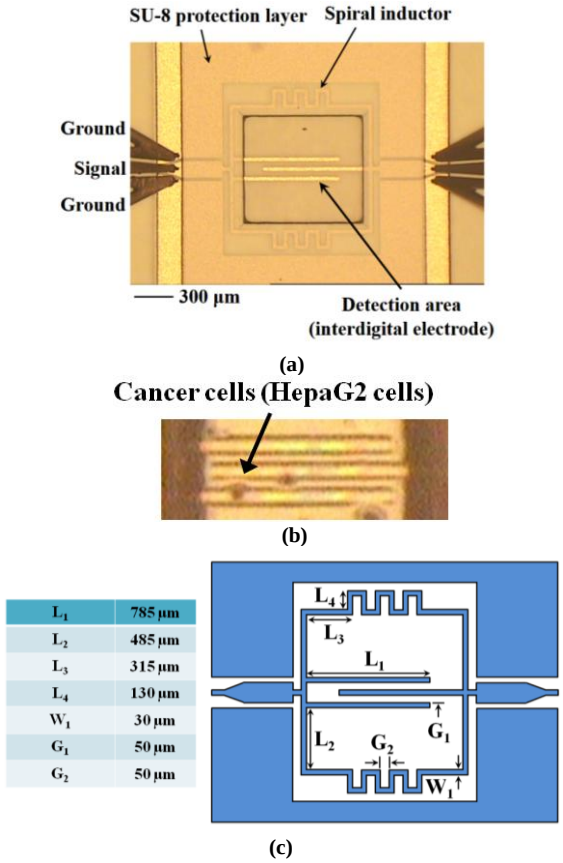

Fig 1. Configuration of (a) unloaded and (b) loaded and (c) structure dimension of the RF biosensor.

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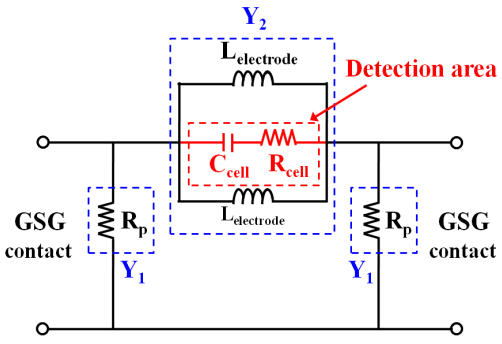


Fig 2. Equivalent circuit model of the RF biosensor.

cancer cells located on the interdigital electrodes for rapid cell counting. By evaluating the changes of resonant peak, the number of cancer cells can be effectively determined. The on-chip *LC* circuit allows an accurate and sensitive detection at cell-scale in this design. The extracted and calculated equivalent parameters of the RF biosensor are well demonstrated. Experimental results show the good cancer cells detection capability with 50, 60 and 70 cells.

II. Circuit Design

A. Planar LC Circuit Design

Fig. 1 shows configuration and dimension of RF biosensor. The proposed RF biosensor is based on a coplanar *LC* transmission lines that can be designed to operate at around 23 GHz with significant sensitivity detection to a very low concentration of cells. SU-8 protection layer is deposited on the chip to ensure the biological cells are all filled with the interdigital capacitor in microwave detection. The resonant circuit is worked by nature much more sensitive to small parameter changes. If the available analysis spectrum is limited to a narrow band around the resonant frequency, it will be easy to detect tiny interactions to very small number of biological elements. The RF biosensor is designed on a *LC* resonant circuit with bandstop response. Two spiral transmission lines and an interdigital electrode is associated by a parallel lumped stopband resonator so as to suit the RF biological detection better than conventional distribution-based half-wavelength transmission lines. Fig. 2 shows equivalent circuit model of the RF biosensor. The interdigital capacitor will cause a resonant frequency shifts in contact of tiny biological cells. The frequency shift can be easily linked to a change of capacitance (C_{cell}) and resistance (R_{cell}) using a simple equivalent circuit model. In Fig. 2, two inductor ($L_{electrode}$) are associated with spiral transmission lines. An interdigital capacitor (C_{cell}) is associated with microwave characteristics of biological cells. A resistance (R_{cell}) is associated with the impedance of biological cells. The parameter of R_p is associated with the resistance of the substrate. The structure and microwave characteristic of RF biosensor is simulated using ANSOFT HFSS [6]. The interdigital capacitor is formed by 50 μm gaps. The resonant frequency is adjusted changing the inductor meander number. In this work, *RLC* equivalent circuit parameters are

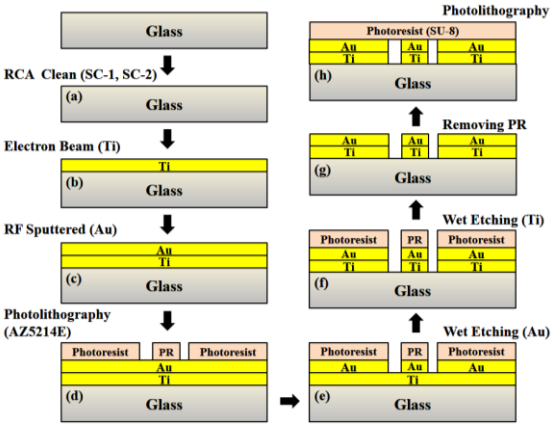


Fig 3. Process flow of the RF biosensor.

evaluated using AGILENT ADS circuit simulations [7]. The equivalent circuit is divided into three sections shown in Fig. 2. The equivalent circuit parameters can be extracted from π-network by using the relation between the measured S-parameters and the ABCD matrix as following

$$\begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix} \Leftrightarrow \begin{bmatrix} A & B \\ C & D \end{bmatrix}$$

where

$$A = \frac{(1+S_{11})(1-S_{22})+S_{12}S_{21}}{2S_{21}} = 1+\frac{Y_1}{Y_2}$$

$$B = Z_0\,\frac{(1+S_{11})(1+S_{22})-S_{12}S_{21}}{2S_{21}} = \frac{1}{Y_2}$$

$$C = \frac{1}{Z_0}\,\frac{(1-S_{11})(1-S_{22})-S_{12}S_{21}}{2S_{21}} = 2Y_1+\frac{Y_1^2}{Y_2}$$

$$D = \frac{(1-S_{11})(1+S_{22})+S_{12}S_{21}}{2S_{21}} = 1+\frac{Y_1}{Y_2}$$

A, B, C and D are the elements corresponded with the ABCD matrix [8], Z_0 is the characteristic impedance of the standard 50 Ω GSG contact. Therefore, the equivalent circuit parameters of the RF biosensor are related to the equivalent circuit parameters of the π-network, therefore

$$Y_1 = \frac{A-1}{B} = \frac{-1\pm\sqrt{1+BC}}{B} = \frac{D-1}{B} = \frac{1}{R_p}+jB_p \quad (1)$$

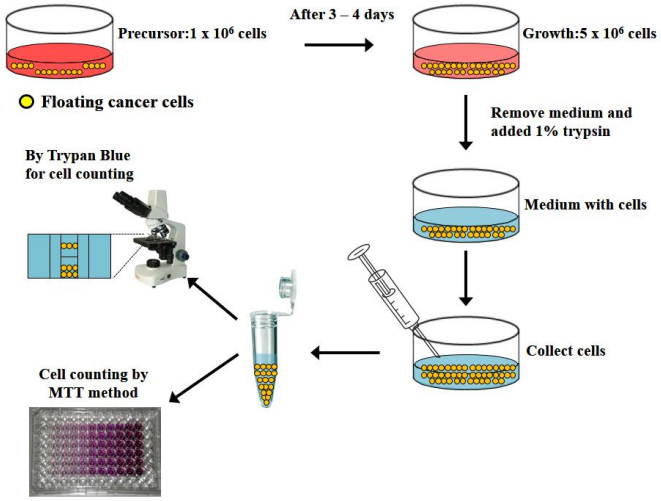
$$Y_2 = \frac{1}{B} = \frac{(1-\omega^2LC)+j\,\omega RC}{-\omega^2RLC+j\,\omega L} \quad (2)$$

$$R_{cell} = \frac{1}{\mathrm{Re}[Y_2]} \quad (3)$$

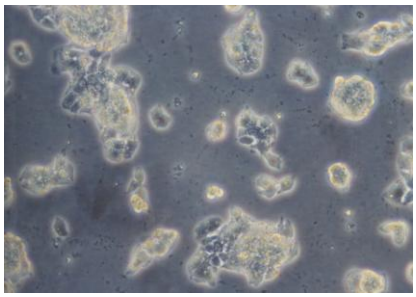
$$C_{cell} = \frac{\mathrm{Im}[Y_2]}{\frac{1}{2Z_0\omega_0}\left(\frac{\omega_0}{\omega}-\frac{\omega}{\omega_0}\right)} \quad (4)$$

$$L_{electrode} = \frac{1}{4\pi^2 f_0^2 C} \quad (5)$$

$$R_p = \frac{1}{\mathrm{Re}[Y_1]} \quad (6)$$



(a)



(b)

Fig 4. (a) Experimental protocol process flow used for on-chip cell culture and (b) photograph of HepaG2 cells (hepatic cancer cells)

B. Experimental Process

Fig. 3 shows process flow of the RF biosensor. Detailed process flow is described as follows. The glass substrate is cleaned by the universal standard RCA cleaning. A 1.5-µm-thick titanium (Ti) layer as the metal conductor of the device is achieved through the electron beam deposition. The electron beam power and pressure rate are set to 1000 W and 2×10-6 torr, respectively. Third, use RF sputtered deposition a 0.5-µm-thick gold (Au) layer. The RF power 500 W and Argon (Ar) flow 100 sccm, respectively. A photolithographically patterned with AZ5214E-photoresist mask to define the RF biosensor chip circuit structure, their thickness being 2 µm. Wet etch process is used to remove the exposed 1.5µm Ti and Au, respectively. After photoresist stripping by acetone, the *LC* structure with a high-resolution pattern is obtained. A 55µm thick SU-8 resist layer to define the effective detection area and can be effective prevent media to contact conductor lines, increase the accuracy of detection and reduce the possibility of short circuit. The microwave characteristics of the RF biosensor were measured by using an Agilent N5247 on wafer VNA to analysis, the change of the frequencies during experiment. Before microwave measurement short-open-load-through (SOLT) calibration technique has been applied using standard calibration substrate that came with the probe station.

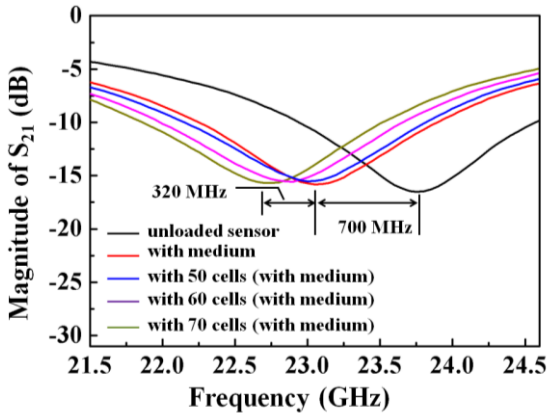


Fig 5. Measured magnitude of S_{21} under different conditions on the RF biosensor.

C. Human Cancer Cell Growth Protocol

Fig. 4(a) shows experimental protocol process flow used for on-chip cell culture. Using MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide) to detect cell living rate. The cell number is adjusted to 1 x 10^5 cells/ml and then putting cells to 96 micron-hole plate by using pippet. The density of cells in each micron-hole plate is 1 x 10^4 ~ 8 x 10^4 cells/100 µl. Due to cultured cells are adherent, the cells are adherent in 3 hours under 37 °C and 5% CO_2 concentration. When cells are already adherent on the bottom, the medium with 55 µL/micron-hole and 0.5 mg/mL MTT (DMEM medium) is added. After 3 hours, 0.04N HCl/100 µL added and oscillate within 30 minutes in oscillator for dissolving the ingrain agent. Using enzyme linked immunosorbent assay, ELISA reader to count the cell number at 540 nm. This method can confirm the cancer cell number and living rate and standard curve with relation of cell number and absorption simultaneously. The photograph of HepaG2 cells (hepatic cancer cells) is shown in Fig. 4(b).

TABLE I
EXTRACTED AND CALCULATED PARAMETERS OF THE RF BIOSENSOR

	f_0 (GHz)	$L_{electrode}$ (nH)	C_{cell} (pF)	R_{cell} (Ω)
unloaded sensor	23.74	0.156	0.288	0.949
with medium	23.07	0.152	0.313	0.938
50 cells	22.96	0.147	0.327	0.896
60 cells	22.89	0.141	0.343	0.805
70 cells	22.75	0.136	0.36	0.648

III. RESULTS

Fig. 5 shows measured magnitude of S_{21} under different conditions (unloaded sensor, loaded sensor with medium only, loaded sensor with medium and 50, 60 and 70 cells) on the RF biosensor. It can be observed that the sensitivity allows the detections of unloaded, with medium only, 50 cells, 60 cells and 70 cells on the sensor with accuracy limited by the width of the resonance peak. The resonant

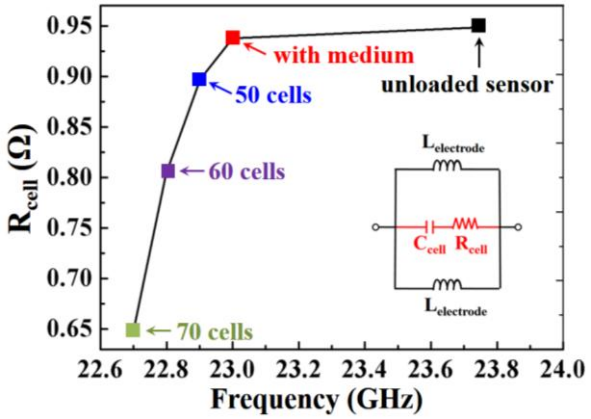


Fig 6. Extracted equivalent parameters of R_{cell} by different number of cells.

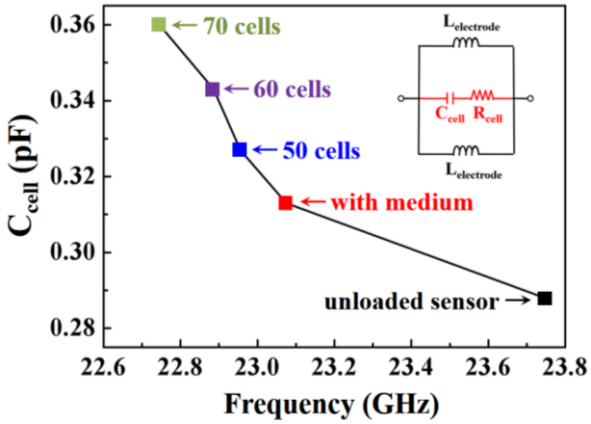


Fig 7. Extracted equivalent parameters of C_{cell} by different number of cells.

peaks shift has a major influence on the RF biosensor. The extracted and calculated equivalent parameters of $L_{electrode}$, R_{cell} and C_{cell} are summarized in Table I by following the equations in Section 2. Fig. 6 shows extracted equivalent parameters of R_{cell} by different number of cells. As HepaG2 cells are increased, the resonant peaks are shifted lower. The equivalent parameter of R_{cell} is shifted to lower from 0.9 Ω as 50 cells to 0.65 Ω as 70 cells. The resistance of dead cancer cells is limited to zero [9]. If the resistance of biological cells can be controlled by artificial, we might have a challenge to kill or damage the cancer cells by high frequency and high transmitted power electromagnetic waves. Fig. 7 shows extracted equivalent parameters of C_{cell} by different number of cells. The resonant peaks shift are induced by different conditions within 22 to 24 GHz for demonstrating the intrinsic dielectric properties of cells. As HepaG2 cells are increased, the resonant peaks are shifted lower. This is show HepaG2 cells are became more polarity to enhance the equivalent parameter of C_{cell}. Based on the information of RF detection, small cell amount can be quickly detected and analyzed for future cell counting.

IV. Conclusion

In this paper, the RF biosensor using planar *LC* resonant circuit for human cancer cells characterization has been proposed. The advantage of RF biosensor is to be well adapted on very low concentration conditions of cancer cells with 50, 60 and 70. By evaluating the changes of resonant peak, the number of cancer cells can be effectively determined. The equivalent lumped elements of *L* and *C* are well extracted and calculated by following the different number of cancer cells. The RF biosensor can be effectively applied on the applications of prior label-free cancer cells number detection for curing as early as possible.

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