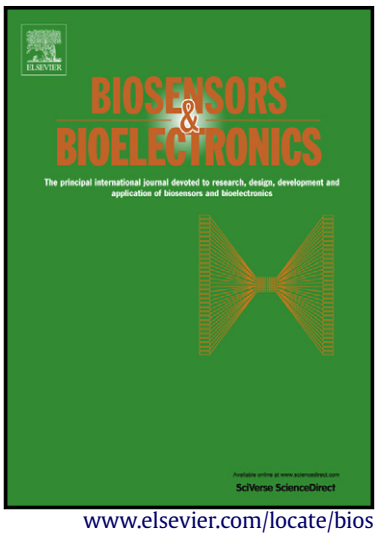

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40 GHz RF Biosensor Based on Microwave Coplanar Waveguide Transmission Line for Cancer Cells (HepG2) Dielectric Characterization

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Abstract – This paper presents a 40-GHz RF biosensor that involves using a microwave coplanar waveguide (CPW) transmission line for the dielectric characterization of cancer cells (Hepatoma G2, HepG2). In the past, conventional resonator-based biosensors were designed to operate at a specific resonant peak; however, the dielectric sensitivity of the cells was restricted to a narrow bandwidth. To provide a very wide bandwidth (1–40 GHz), biosensors were based on a microwave CPW transmission line. The proposed biosensor can rapidly measure two frequency-dependent cell-based dielectric parameters of HepG2 cells, microwave attenuation ($\alpha(f)_{cell}$) and the dielectric constant ($\varepsilon_r(f)_{cell}$), while removing the microwave parasitic effects (including the cultured medium and substrate materials). The proposed biosensor can be applied in postoperative cancer diagnosis.

Keywords: biosensor, microwave, dielectric characterization, coplanar waveguide (CPW), HepG2 cells

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1. Introduction

Cancer cells (and cancerous stem cells) have undifferentiated properties that are difficult to identify and characterize. Biologists are currently attempting to determine an efficient and specific labeling system for tagging antigens and molecules in their undifferentiated state (Bidlingmaier et al., 2008). Cell differentiation degree is nearly impossible to evaluate using a microscope without an efficient labeling system. Therefore, a label-free electronic detection method for noninvasive analysis is necessary, because the cells are maintained in their original state, and easy to reproduce during the measurement.

The dielectric characterization of cells is one of the most crucial tools for cellular biologists and can be used for rapid cell detection after surgery. Currently used methods include optical, electrochemical, piezoelectric, and microwave sensing techniques that involve using metal and/or semiconductor nanoparticles (Moerner and Orrit, 1999; Drummond et al., 2003; Kim et al., 2006; Chien et al., 2007; Perng et al., 2007; Campbella and Mutharasanb, 2008; Agasti et al., 2010; Nandakumar et al., 2011; Yang et al., 2012). However, the detection limit and sensitivity of these methods, including functional nanoparticles, is approximately 5 pM to 100 fM for the optical method, 10 pM to 500 fM for the electrochemical/electrical method, and 1 pM to 100 fM for the piezoelectric method.

Recently, using microwave methods for detecting dielectric properties of cells has yielded compelling results. Microwave methods have been developed to achieve rapid, reliable, accurate, and high-sensitivity biodetection (Anderson et al., 2005; Blacklock et al., 2009). Biosensors based on planar filtering devices have also been developed (Dalmay et al., 2010). The biosensors were demonstrated by conducting only a few biological cell analyses for detecting the dielectric properties of the cells. However, these biosensors involve using a resonator-based (semilumped elements as parallel of inductances and capacitances) structure at a specific resonant peak (Dalmay et al., 2009; Yang et al., 2010; Lee et al., 2012). Although these biosensors are measured under open-air conditions without any support media, living cells must be preserved using a paraformaldehyde solution (PFA 4%). Adding the PFA blocks cells in their physiological living state, allowing more complex measurements to be taken over a longer duration. Some literature proposes biosensors that involve using interdigital (IDT) electrodes (Radke and Aloculja, 2004; Zou et al., 2007;

Zhang et al., 2013). However, capacitive sensing by using IDT electrodes is more challenging because the capacitance shift is less than 5% of the overall coupling capacitor value (Li and Uttamchandani, 2009; Muley and Boldor, 2013). The dielectric sensitivity of these biosensors is restricted to a very narrow bandwidth and the microwave parasitic effects (including the cultured medium, functional nanoparticles, and substrate materials) are not completely removed. Other previous works have used an EM simulation tool to fit and optimize the electrical circuit model of the cells (Kim et al., 2008; Ferrier et al., 2009). However, these fitted electrical circuit parameters cannot accurately express the dielectric properties of the cells.

This study proposes a new label-free biosensor based on a microwave coplanar waveguide (CPW) transmission line for the dielectric characterization of HepG2 cells The biosensor provides a very wide bandwidth (1–40 GHz), and can effectively measure and calculate two frequency-dependent cell-based parameters (microwave attenuation $\alpha(f)_{cell}$ and dielectric constant $\varepsilon_r(f)_{cell}$) of HepG2 cells while removing all of the microwave parasitic effects. Three main objectives were considered in developing the proposed biosensor. The first objective was to implement a new biosensor based on a microwave CPW transmission line. In considering previous RF biosensors (Dalmay et al., 2009; Yang et al., 2010; Lee et al., 2012), this study is the first to investigate the dielectric properties of HepG2 cells at a very wide bandwidth. The second objective was to design the biosensor to operate at microwave frequencies for detecting HepG2 cells in biomedical diagnosis. Two frequency-dependent cell-based dielectric parameters (microwave attenuation $\alpha(f)_{cell}$ and dielectric constant $\varepsilon_r(f)_{cell}$) of the HepG2 cells are accurately measured and calculated. The scanned bandwidth of the biosensor is more intensive than the dc or lower frequency (below 100 MHz). The third objective was to create a new approach to removing microwave parasitic effects while the biosensor performs microwave on-chip measurement. Using this approach, the real dielectric properties of the cells can be obtained without including the microwave parasitic effects. The biosensor can be used for cancer diagnosis after surgery.

2. Materials and Methods

2.1. Configuration of the biosensor

Fig. 1a shows the configuration of the proposed biosensor. The biosensor was designed based on a microwave CPW transmission line. The biosensor was fabricated on a glass substrate with a relative dielectric constant (ε_r) of 5.27, a loss tangent (tan δ) of 0.003, and a thickness (h) of 700 μm (Corning EAGLE XG). The detection area was etched at the center of the conductor line by using a standard semiconductor fabrication process. A 55-μm-thick SU8 protection layer was deposited on the biosensor surface and patterned to create a microchamber and prevent unwanted microwave interaction with cells in the other areas of the biosensor. The detection area was designed to locate the HepG2 cells in the area where the electromagnetic (EM) field is strongly concentrated. The simulated frequency response of the biosensor was verified using a 3D full-wave EM simulator (HFSS, ANSOFT). In contrast to previous works (Dalmay et al., 2009; Yang et al., 2010; Lee et al., 2012), this biosensor is based on a microwave CPW transmission line and provides a very wide bandwidth. Different cell densities exhibit distinct microwave properties, allowing identification of cell numbers even when cell density is extremely low (≤ 20 cells/μL).

2.2. Biosensor fabrication

Fig. 1b shows the fabrication flow diagram of the biosensor. The biosensor was fabricated on a lossless dielectric substrate (Corning Eagle XG series). The glass substrate was cleaned using universal standard RCA cleaning. The Au/Ti films were deposited on the glass substrate by RF magnetron sputtering using a Ti metal target (99.9995% purity, 20 cm in diameter, 0.5 cm thick) and e-beam evaporation using an Au chip (99.999% purity). The glass substrate was ultrasonically cleaned in acetone, rinsed in deionized water and subsequently dried in flowing nitrogen gas before deposition. The dimensions of the glass substrate were 60 × 60 × 0.7 mm. The 1.5-μm-thick bottom Ti layer was sputtered on the glass substrate. The sputtering was performed in argon atmosphere (purity: 99.99%) with a target-to-substrate distance of 15 cm. The conditions of the RF sputtering process are depicted in Fig. 1c. After the sputtering of the bottom Ti layer, 0.5-μm-thick Au layers were deposited on the bottom Ti layer by using e-beam evaporation. The e-beam chamber was vacuumed to 5×10^{-4} torr prior to deposition. The substrate temperature was measured using a thermocouple gauge. The variation in substrate temperature during deposition was

maintained within a range of 25–35 °C, with a target substrate temperature of 30 °C. The conditions of the e-beam evaporation process are illustrated in Fig. 1d. Au was used as the material for designing the electrodes because of its excellent electrical properties and biocompatibility. A mask was photolithographically patterned with 2-μm-thick AZ5214E-photoresists to define the biosensor structure. A wet etching process was used to remove the exposed 1.5-μm-thick layer of Ti and 0.5-μm-thick layer of Au. After photoresist stripping using acetone, the CPW transmission line structure with a high-resolution pattern was obtained. A 55-μm-thick SU8 protection layer enabled concentrating cells in the detection area and reducing the possibility of a short circuit in the proposed biosensor architecture.

2.3. HepG2 cell growth experimental protocol

The HepG2 cells (Bioresource Collection and Research Center; Hsinchu, Taiwan) were cultured in DMEM containing 10% fetal bovine serum, 100 units/mL of penicillin, 100 μg/mL of streptomycin, 0.37% (w/v) $NaHCO_3$, 0.1 mM nonessential amino acid, and 1 mM sodium pyruvate, and stored in a humidified incubator at 37 °C in 5% CO_2. In our study, HepG2 cells were seeded on 6-cm dishes with a cell density of 1×10^6 cells/mL. After 3–4 days of incubation, the cells in the dish became confluent and were detached using trypsinization. The cells were harvested for counting by using Trypan Blue solution. The indicated cell number (20, 200, 1000, and 2000 cells/μL) was prepared for on-chip microwave measurement. Cell viability was confirmed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (Mosmann 1983). In brief, after the supernatant was removed, the cells were incubated at 37 °C in MTT (0.5 mg/mL in medium) for 4 h. The medium was aspirated and the cells were solubilized in DMSO for 3 h. The OD values were measured at 570 nm. A photograph of the cultured HepG2 cells on the biosensor is shown in Fig. 2. The HepG2 cells can be directly injected into the detection area of the biosensor because HepG2 cell adhesion occurs more frequently on a glass substrate than in gold lines and in the SU8 protection layer. After measurement, the biosensor can be washed again with deionized water and dried for a few minutes, then reused.

3. Results and Discussion

3.1. On-chip measurement

The dielectric properties of the HepG2 cells were measured using an Agilent N5247 on-wafer vector network analyzer. The scanning frequency ranged from 1 to 40 GHz. The short-open-load-through calibration technique was used to apply a standard calibration substrate to the probe tips using a de-embedding reference plane (A-A' and B-B'), as shown in Fig. 3. The temperature of the laboratory during measurement was set to 20 °C. To facilitate movement of the specimen into the detection area, the conductor line of the biosensor was used to provide the characteristic impedance (Z_0) of 30 Ω and to accommodate ground-signal-ground 150-μm pitch microwave probes.

3.2. New method for removing microwave parasitic effects

Fig. 4 shows S_{21}-magnitude measurements at different HepG2 cell densities. The microwave parasitic effects can be divided into the cultured medium and substrate materials. The unloaded biosensor is measured before the HepG2 cells are injected into the detection area, as indicated by the black line. The biosensor with only the cultured medium (without HepG2 cells, ≈ 1 μL) is then measured, as indicated by the red line. When the cell density changes from 2×10^1 cells/μL to 2×10^3 cells/μL, the EM waves penetrate the HepG2 cells, causing microwave attenuation and S_{21}-magnitude degradation. The HepG2 cells can be considered electric charges remaining within the homogeneous dielectric material system. Therefore, S_{21}-magnitude degradation at different cell densities is associated with polarization effects (including ion vibration and deformation losses) between cells at microwave frequencies. The lower detection limit of the proposed biosensor is approximately 20 cell/μL.

In this work, four calibrated scattering parameters (S-parameters) measured on the biosensor are obtained, and the frequency-dependent propagation constant ($\gamma(f) = \alpha(f) + j\beta(f)$) is derived from the eigenvalues of the transmission matrix (*ABCD* matrix), where $\alpha(f)$ is the microwave attenuation and $\beta(f)$ is related to the wave number of the eigenvalues (Collin 1992). To remove the microwave parasitic effects from the biosensor, the frequency-dependent cell-based S-parameters of the HepG2 cells must be modified as follows:

$$\begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix}_{cell} = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix}_{loaded} - \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix}_{medium} - \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix}_{unloaded} \quad (1)$$

where $[S]_{cell}$, $[S]_{loaded}$, $[S]_{medium}$, and $[S]_{unloaded}$ indicate the measured S-parameters of the HepG2 cells, the loaded biosensor, the biosensor with only the cultured medium, and the unloaded biosensor, respectively. Therefore, the frequency-dependent cell-based $\alpha(f)_{cell}$ and $\varepsilon_r(f)_{cell}$ of the HepG2 cells can be obtained as follows:

$$\alpha(f)_{cell} = 8.686 \cdot \left[-\frac{1}{L_1} \mathrm{Re}\left\{ \ln\left[\frac{1 - S_{11}^2 + S_{21}^2}{2S_{21}} \pm \left[\frac{(S_{11}^2 - S_{21}^2 + 1)^2 - (2S_{11})^2}{(2S_{21})^2} \right]^{1/2} \right]^{-1} \right\} \right] (\mathrm{dB/\mu m}) \quad (2)$$

$$\beta(f)_{cell} = -\frac{1}{L_1} \mathrm{Im}\left\{ \ln\left[\frac{1 - S_{11}^2 + S_{21}^2}{2S_{21}} \pm \left[\frac{(S_{11}^2 - S_{21}^2 + 1)^2 - (2S_{11})^2}{(2S_{21})^2} \right]^{1/2} \right]^{-1} \right\} = \frac{2\pi \cdot f \sqrt{\varepsilon_{eff}(f)_{cell}}}{c} (\mathrm{rad/\mu m}) \quad (3)$$

where c is the light speed (3×10^8 m/s) and L_1 is the length of the CPW line (L_1 = 6600 μm). The effective dielectric constant $\varepsilon_{eff}(f)_{cell}$ is extracted from Eq. (2), as follows (Simons 2001):

$$\varepsilon_{eff}(f)_{cell} = 1 + \frac{\varepsilon_r(f)_{cell} - 1}{2} \cdot \frac{K(k_1)}{K(k_1')} \cdot \frac{K(k_0')}{K(k_0)} \quad (4)$$

To achieve the characteristic impedance ($Z_0(f)_{cell}$), the dielectric constant $\varepsilon_r(f)_{cell}$ must first be obtained. The term $Z_0(f)_{cell}$ can be then calculated as follows:

$$Z_0(f)_{cell} = \frac{30\pi}{\sqrt{\varepsilon_{eff}(f)_{cell}}} \frac{K(k_0')}{K(k_0)} \ (\Omega) \quad (5)$$

where

$$k_0 = \frac{S}{S + 2G}, \quad k_1 = \frac{\sinh(\pi S / 4h)}{\sinh(\pi (S + 2G) / 4h)}, \text{ and } k_i' = \sqrt{1 - k_i^2} \quad (i = 0, 1)$$

K(k) is the elliptical integral of the first type, and h is the thickness of the glass substrate. The expressions for the ratio of $K(k_i)/K'(k_i)$ are given as

$$\frac{K(k_i)}{K(k_i')} = \pi / \ln\left[2\frac{1 + \sqrt{k_i'}}{1 - \sqrt{k_i'}} \right] \quad \text{for } 0 \le k_i \le \frac{1}{\sqrt{2}} \quad (i = 0, 1) \quad (6)$$

$$\frac{K(k_i)}{K(k_i')} = \frac{1}{\pi} \ln\left[2\frac{1 + \sqrt{k_i}}{1 - \sqrt{k_i}} \right] \quad \text{for } \frac{1}{\sqrt{2}} < k_i \le 1 \quad (i = 0, 1) \quad (7)$$

where $K'(k_i) = K(k_i')$. The CPW line-based biosensor is designed on a dielectric substrate of finite thickness. The method can be applied to any single-layer CPW line-based biosensor with quasi-TEM propagation.

3.3. Dielectric properties of HepG2 cells

Fig. 5 shows the measured and calculated frequency-dependent cell-based microwave attenuation $\alpha(f)_{cell}$ at different HepG2 cell densities. As the cell density changes from 2×10^{1} cells/μL to 2×10^{3} cells/μL, the microwave attenuation $\alpha(f)_{cell}$ surpasses that of the unloaded biosensor. The $\alpha(f)_{cell}$ indicates the microwave attenuation of the HepG2 cells without the microwave parasitic effects. After the cell density increases, the microwave attenuation $\alpha(f)_{cell}$ are 0.12×10^{-3} dB/μm for 2×10^{1} cells/ μL, 0.58×10^{-3} for 2×10^{2} cells/ μL, 0.81×10^{-3} for 1×10^{3} cells/ μL, and 1.26×10^{-3} dB/μm for 2×10^{3} cells/ μL at 40 GHz, respectively. The $\alpha(f)_{cell}$ variation by cell density is caused by the polarization current in the HepG2 cells. Additionally, the $\alpha(f)_{cell}$ is also related to the dielectric loss of the HepG2 cells, described by the loss tangent $\tan\delta(f)_{cell}$. The average values of the $\tan\delta(f)_{cell}$ at different cell densities are 0.021 (2×10^{1} cells/μL), 0.032 (2×10^{2} cells/μL), 0.056 (1×10^{3} cells/μL) and 0.102 (2×10^{3} cells/μL). Such results are useful because considering dielectric loss is crucial in biomedical diagnosis.

Fig. 6 shows the frequency-dependent cell-based dielectric constant $\varepsilon_r(f)_{cell}$ of the HepG2 cells. As the cell density increases, the $\varepsilon_r(f)_{cell}$ shows an average of 11.37 for 2×10^{1} cells/μL, 13.58 for 2×10^{2} cells/μL, 14.6 for 1×10^{3} cells/μL, and 16.4 for 2×10^{3} cells/μL from 15–40 GHz, respectively. The $\varepsilon_r(f)_{cell}$ is associated with the permittivity of the HepG2 cells by polarization effects within a range of 1–40 GHz. The $\varepsilon_r(f)_{cell}$ is highest at lower frequencies and decreases in distinct and consecutive plateaus as the frequency increases. The γ-dispersions ($\geq 10^{9}$ to10^{11} Hz) play a central role in polarization effects, which are caused by ion vibration, deformation, and the aqueous content of the HepG2 cells (Pethig and Kell, 1987). The polarization of the HepG2 cells can be expressed approximately as the relationship between the polarizability and the EM field of the cells as follows (Kittel 1996):

$$P = \sum_{j} N_j \alpha_j E(j) \tag{8}$$

where N_j is the cell density, α_j is the polarizability of the cells and E(j) is the EM field at cell site j. The $\alpha(f)_{cell}$ and $\varepsilon_r(f)_{cell}$ of the HepG2 cells are dominated by the cell density. According to the results, the dielectric detection of the HepG2 cells by using the microwave CPW line on the developed biosensor was successfully completed.

4. Conclusion

This paper presents a new and original biosensor based on a microwave CPW transmission line for the dielectric characterization of HepG2 cells. Compared with previous works, the proposed biosensor can provide a wider bandwidth for high-sensitivity detection. The biosensor is designed for label-free detection and effective measurement of frequency-dependent cell-based parameters ($\alpha(f)_{cell}$ and $\varepsilon_r(f)_{cell}$) of HepG2 cells at cell densities varying from 2×10^1 cells/μL to 2×10^3 cells/μL. The microwave parasitic effects were successfully removed using the proposed method. The sensitivity of the biosensor is associated with the cell density, making it an effective tool for detecting the cell density rapidly, even when cell density is extremely low. The findings of this study could be widely used for the dielectric characterization of any type of cell.

Acknowledgement

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Figure Caption

Fig. 1. (a) 3D illustration, (b) fabrication flow diagram of the biosensor, (c) table for the E-beam evaporation process conditions and (d) table for the RF sputtering process conditions. (L_1 = 6600 μm, L_2 = 3000 μm, d = 600 μm, W = 900 μm, G = 25 μm, S = 1160 μm, t_{Ti} = 1.5 μm, t_{Au} = 0.5 μm, t_{SU8} = 55 μm, and h = 700 μm)

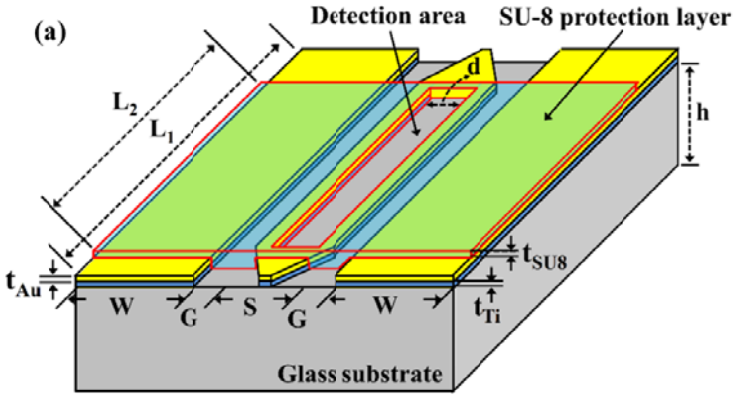
Fig. 2. Photograph of cultured HepG2 cells on the biosensor.

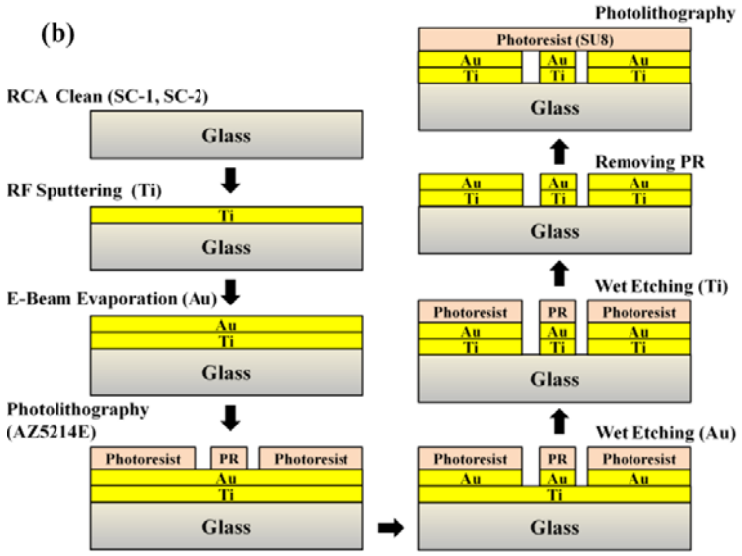
Fig. 3. Experimental set-up for the HepG2 cell dielectric characterization.

Fig. 4. S_{21}-magnitude measurements at different HepG2 cell densities.

Fig. 5. Measured and calculated frequency-dependent cell-based microwave attenuation $\alpha(f)_{cell}$ at different HepG2 cell densities.

Fig. 6. Frequency-dependent cell-based dielectric constant $\varepsilon_r(f)_{cell}$ of the HepG2 cells.





(c)

Base pressure (torr)	Substrate temperature (°C)	Voltage (KV)	Current (mA)
5×10^{-4}	28	4.7	80

(d)

Base pressure (torr)	Working pressure (mtorr)	Substrate temperature (°C)	Ar flow (sccm)	RF power (W)
5×10^{-6}	10	30	60	200

Fig. 1

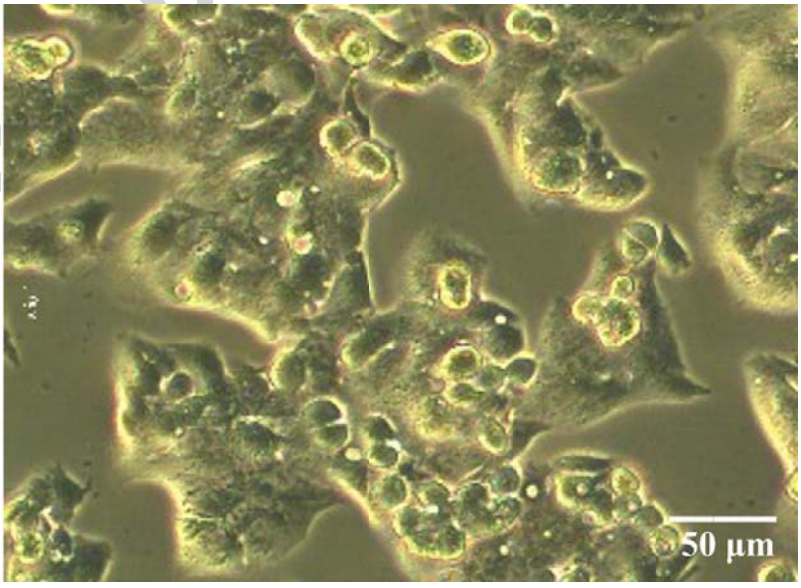


Fig. 2

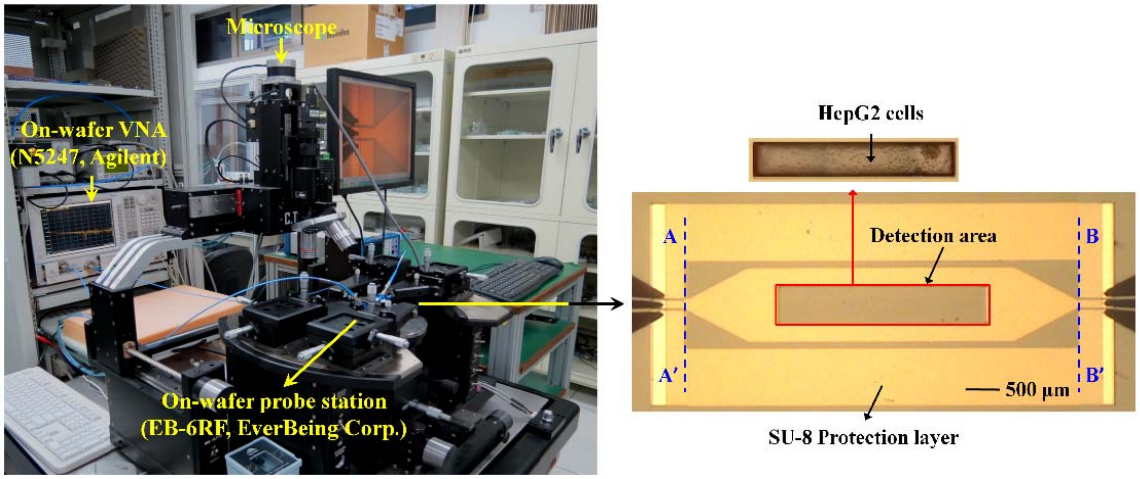

Microscope
On-wafer VNA
(N5247, Agilent)
On-wafer probe station
(EB-6RF, EverBeing Corp.)
HepG2 cells
A
Detection area
B
A′
B′
500 μm
SU-8 Protection layer

Fig. 3

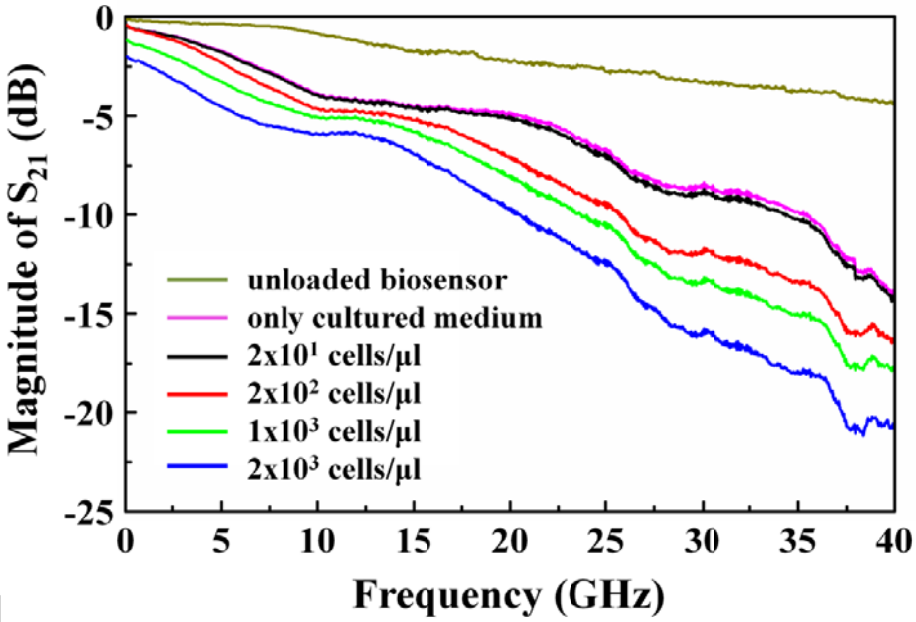

Magnitude of S21 (dB)
Frequency (GHz)
unloaded biosensor
only cultured medium
2x10¹ cells/μl
2x10² cells/μl
1x10³ cells/μl
2x10³ cells/μl

Fig. 4

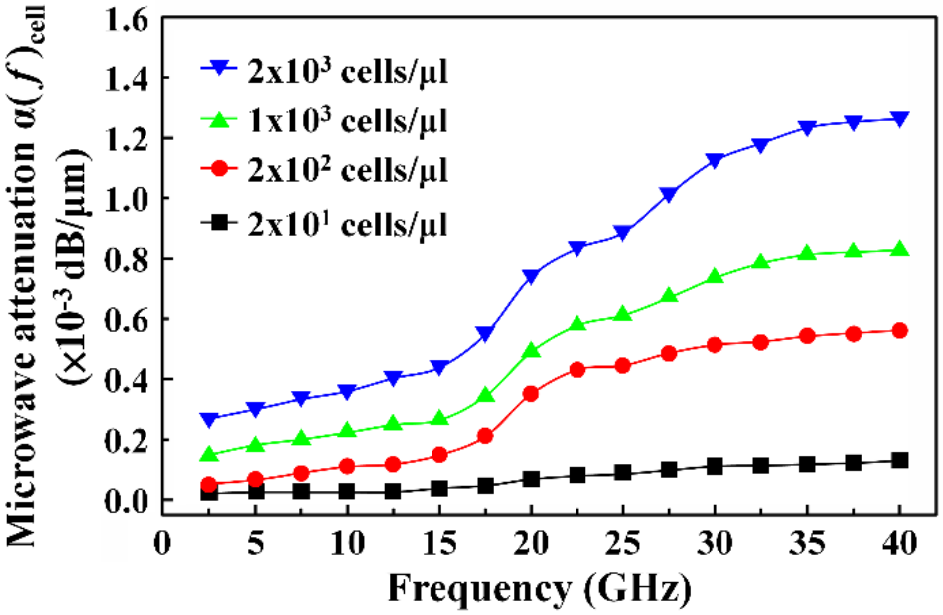


Fig. 5

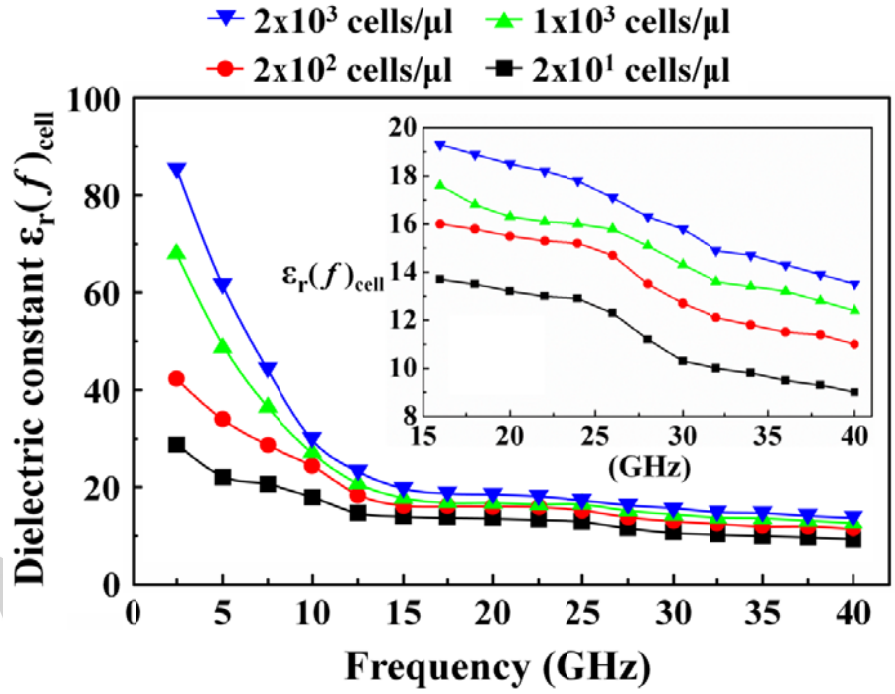


Fig. 6

> It is the first attempt to study the effect of combining TRAIL with dasatinib for the therapy of CML.

> The TRAIL combined with dasatinib was proven to be a more effective potential antileukemic agent than either of them alone.

> It is the first work to use enzymatic reaction for detection of caspase-3 activity, enhancing the specifity of the sensor.

> The environmentally friendly electrochemical platform could be used for retest without strong acid or heavy metal ions.