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# 3D cell-based biosensor for cell viability and drug assessment by 3D electric cell/matrigel-substrate impedance sensing

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ABSTRACT

Preclinical efficacy and toxicity assessment of drug candidates plays a significant role in drug discovery and development. Traditional planar cell culture is a common way to perform the preclinical drug test, but it is difficult to correctly predict the drug efficacy and toxicity due to the simple two-dimensional (2D) extracellular microenvironment. Compared to the planar cell culture, three-dimensional (3D) cell culture system can better mimic the complex extracellular microenvironment where cells reside in the 3D tissues/organs *in vivo*. However, the conventional imaging techniques are difficult to achieve the dynamic and label-free monitoring of cellular behavior in thick sample by 3D cell culture. Here, 3D electric cell/matrigel-substrate impedance sensing (3D-ECMIS) is developed for real-time and non-invasive monitoring of 3D cell viability and drug susceptibility. In this study, human hepatoma cells (HepG2) are encapsulated in the matrigel scaffold and cultured in a 3D ECMIS chip which involves a pair of vertical golden electrodes on the opposite sidewalls of the culture chamber for the *in-situ* impedance measurement. Moreover, a portable multichannel system is developed to monitor the 3D cell/matrigel construct. The number of 3D-cultured cells was inversely proportional to the impedance magnitude of the entire cell/matrigel construct. Furthermore, anti-cancer drug screening will be conducted on the 3D-cultured cells when the cell proliferation reaches to a plateau phase. To validate the performance of 3D-ECMIS for the cell viability and drug susceptibility, the cell live/dead staining are utilized to confirm the results of drug susceptibility by this 3D-cell-based biosensor system. It is demonstrated that the 3D cell-based biosensor and 3D-ECMIS system will be a promising platform to improve the accuracy of cell-based anti-cancer drug screening *in vitro*.

## 1. Introduction

Traditional drug screening usually adopts animal experiments to analyze the efficacy and toxicity of novel drug candidates (Alley et al., 1988). However, these animal bioassay suffers from massive and cost-intensive animal experiments, which hampers the high-throughput and high-efficiency. Consequently, cell-based assays are widely used in drug screening processes. Up to now, the majority of cell-based assays use traditional two-dimensional (2D) monolayer cells cultured on the flat substrates. However, 2D cell culture has contact inhibition among cells and mismatches the *in vivo* three-dimensional (3D) environment. The original properties of cell morphology and heterogeneity might change due to the 2D cell culture (Mulhall et al., 2013). Therefore, 2D cell-based assays provide misleading and false positive results for *in vivo* assays (Bhadriraju and Chen, 2002; Weaver et al., 1997). Although some novel drug candidates show good efficacy *in vitro*, they might not be effective *in vivo* due to the differences between *in vitro* and *in vivo* microenvironments (Edmondson et al., 2014). Currently, 3D cell culture have drawn great public attention for its advantages in simulating the complex *in vivo* microenvironments (Abbott, 2003; Mueller-Klieser, 1997). Cell–cell and cell–extracellular matrix (ECM) interactions of cells are reconstructed by encapsulating cells in the 3D scaffolds (Tibbitt and Anseth, 2010). Therefore, 3D-cultured cells can better reflect *in vivo* cellular behavior or drug responses such as morphogenesis and cell metabolism (Thoma et al., 2014).

Conventionally, cells are usually cultured on transparent glass or

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polystyrene substrate, and the morphology and number of 2D-cultured cells can be observed by an optical microscope easily. For cell proliferation analysis, it is performed by cell counting using the microscope. For further cell viability evaluation, the live/dead fluorescent staining and microscopic observation were applied. However, fluorescent staining will cause irreversible damage to cells. As an endpoint detection, fluorescent staining can only provide the static and limited data for kinetics of cellular responses. On the other hand, 3D-cultured cells are typically encapsulated in the several hundred micrometers thick matrigel, which is difficult for conventional optical microscope to observe. In fact, a quantitative analysis of 3D-cultured cells require a confocal fluorescence microscope, which have an excellent spatial resolution and great optical sectioning capability (Pampaloni et al., 2007). However, it is labor-intensive and time-consuming to operate confocal fluorescence microscope for 3D-cultured cells observation. Furthermore, the complex operations will restrict the efficiency and throughput of the 3D cell-based assay for drug screening.

In these decades, electric cell-substrate impedance sensing (ECIS) have been well developed for dynamic, real-time, non-invasive, and label-free monitoring of cell growth and viability (Hu et al., 2015; Liu et al., 2014; Nguyen et al., 2013; Zhou et al., 2013; Zou et al., 2015). ECIS applies a sinusoidal voltage (typical 10 kHz) on the interdigitated electrodes (IDEs), and then ion current is formed between the IDEs. Basically, cells are considered as non-conductors at the low frequency. When cells attach and grow on the IDEs, the ion current is impeded and the impedance of IDEs will increase. When cells died, they are detached from IDEs and the impedance will decrease. However, conventional IDEs is difficult to monitor the 3D cultured-cell encapsulated in the matrigel due to the cells cannot attach on or detach from the IDEs directly. Therefore, the traditional methods for monitoring 2D-cultured cells are not applicable in the 3D cell culture. It is demanded to develop a high throughput, real-time, non-invasive and label-free approach of 3D cultured cell.

In this study, we report a 3D electric cell/matrigel-substrate impedance sensing (3D ECMIS) in the dynamic, real-time, high-throughput, non-invasive, and label-free way. The 3D ECMIS consists of a pair of vertical golden electrodes and applies alternating current (AC) mode to measure the impedance of 3D cell/matrigel construct. Meanwhile, an 8-channel 3D ECMIS detection system is developed to record the impedance changes of 3D cell/matrigel construct. As expected, cellular activities were monitored by measuring the impedance changes between vertical electrodes. To improve the sensitivity of 3D ECMIS, we optimized the vertical electrode size and working frequency. In addition, a comparison between 3D ECMIS and traditional optical detectors were performed. A good correlation was found between results of 3D ECMIS measurements and endpoint assays. The results show the 3D ECMIS will be a promising measurement method for 3D-cultured cell-based drug screening.

## 2. Materials and methods

### 2.1. Detection principle of 3D electric cell/matrigel-substrate impedance sensing

3D ECMIS can monitor 3D cell proliferation noninvasively and dynamically. Firstly, cells mixed with matrigel are gelated in the culture chamber. After cultured for a few days, the original scattered globular cells will form 3D structures with the cell-cell interaction to stimulate the *in vivo* microenvironment (Fig. 1a). The initial impedance is high because the gel acts as a type of non-conductor. When live cells are mixed into the gel, the impedance drops dramatically (Fig. 1b). This phenomenon suggests that the electrical conductivity of gel is improved after mixed with living cells. Because of the existence of cell membranes, cells are basically considered non-conductors at low frequency (Ehret et al., 1997). However, the gap junctions of cells act as channels between cell membranes that allow electrical connections among neighboring cells. When external electrical field is applied to the cell/matrigel construct, the cells act as conductor, causing the impedance decrease of the entire construct (Fear and Stuchly, 1998; Knedlitschek et al., 1994). Based on these, the more cells exist, and the conductivity is higher in the 3D cells/matrigel construct (Jeong et al., 2012). When drugs/compounds are administrated on 3D cells/matrigel construct, the impedance of entire construct would change due to the different drug efficacy on 3D-cultured cells (Fig. 1c). Here, cell index (CI) is introduced to normalize the impedance values, which is defined as the ratio of cell growth induced impedance changes $\triangle Z$ to background impedance ($Z_0$). The equation is $CI = \triangle Z/Z_0$. Based on the 3D ECMIS, we can evaluate the efficacy of anti-cancer drugs on the 3D-cultured tumor cells.

### 2.2. Fabrication of 3D impedance biosensor and detection system

An 8-channel 3D impedance biosensor was developed to monitor the activities of 3D-cultured cell, which is consisted of eight individual sensor units. Each unit contains a pair of vertical electrodes, culture chamber and glass substrate. To fabricate the vertical electrodes, the gold electrodes were laser-cut. Subsequently, they were bent to the right angle and attached to the inner surface of the polyethylene terephthalate (PET) culture chamber, which was fabricated by the numerical control machine. To ensure the biocompatibility, 4-in. Pyrex Corning 7740 glass (500 μm) was chosen as the substrate material, and Henkel Loctite HY4090 structural hybrid adhesive was used to fix and seal the PET chamber. To optimize the parameters of 3D impedance biosensor, we assembled several 3D impedance biosensors with electrodes of different sizes and distances (Fig. 2a). According to the experimental results, we chose the optimal one to assemble an 8-channel sensing chip (Fig. 2b). The 3D ECMIS detection system contains 3D vertical impedance biosensor, signal-conditioning module and DAQ card USB-6255 (National Instruments Inc., USA) (Fig. 2c). When 3D-cultured cells were seeded in the chip, a sinusoidal voltage with amplitude of 30 mV and frequency of 10 kHz was generated by signal generator module and was applied on the vertical gold electrodes (Fig. 2d). The DAQ card was controlled by the computer terminal and measure the impedance signals. Finally, the digital signals were transferred to computer to calculate the impedance values by the signal processing. All the data were analyzed by LabVIEW software (National Instruments, USA) or Graph Pad Prism 6 (GraphPad software, USA).

### 2.3. 3D Cell culture

Human hepatoma cells (HpeG2) and human epithelial carcinoma cells (HeLa) (American Type Culture Collection), were maintained in Eagle's Minimum Essential Medium (EMEM, ATCC, USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA) in the 37 °C and 5.0% $CO_2$ humidified incubator (Thermo Fisher Scientific, USA). The medium was renewed every 2 days, and cells were passaged using 0.25% trypsin/EDTA solution (Gibco, USA) after reached the confluence of 90%. 3D cell culture processes was carried out according to Lee's protocol (Lee et al., 2007). Detached HepG2 cells and HeLa cells were transferred to a 1.5 mL microcentrifuge tube with prechilled matrigel solution (BD Biosciences, USA) to a concentration of $5 \times 10^6$ cells/mL. 100 μl of the cold matrigel-cells mixture was gelated in the culture chamber in the 37 °C and 5.0% $CO_2$ humidified incubator. After 30 min incubation, 50 μl medium was added into culture chamber. The medium was changed every 2 days.

### 2.4. Pharmacological treatments

To evaluate the anti-cancer drug screening ability of 3D ECMIS, cytotoxicity studies were performed by treating cisplatin (Solarbio, China), Taxol (Solarbio, China), sorafenib (Solarbio, China) to 3D cultured HepG2 cells for 48 h after the 3D cells cultured in the culture

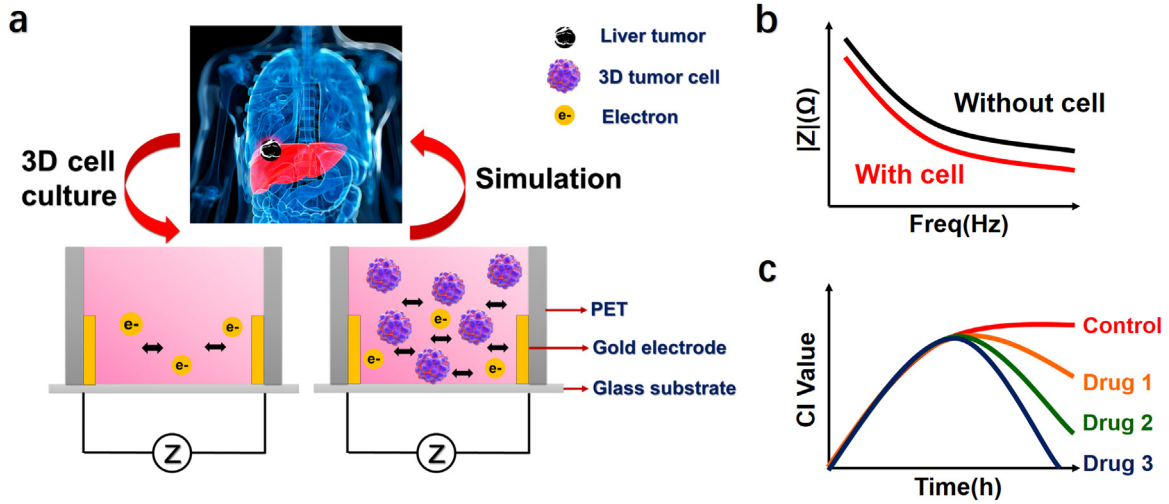


**Fig. 1.** (a) The working principle of 3D ECMIS. Cells mixed with matrigel were gelated between the vertical electrodes. (b) Different aspects of electrical signal between groups with and without cell. (c) The response characteristic of 3D-cultured cells to different drugs.

chambers for 2 days. For further comparison of drug susceptibility between 2D ECIS and 3D ECMIS, the same treatment was performed to 2D cultured HepG2 cells on the 2D ECIS chips for 24 h.

### 2.5. Impedance measurements and data analysis

A.C. impedance measurements were implemented by the electrochemical analyzer CHI 660e (CH Instruments, China) to determine an optimal frequency for the following impedance measurement. The initial potential of 30 mV was applied across the vertical electrodes and the impedance was measured from 100 Hz to 50 kHz. The high-throughput impedance measurements of the 3D cultured cell were performed by the homemade 3D ECMIS device every 10 min. When the parallel electric field passes through the 3D cell/matrigel construct and the cellular growth will be reflected by the impedance values.

### 2.6. Live/dead Staining

To directly observe cellular viability after drug treatment, live/dead staining was performed using a Calcein-AM/PI Double Stain Kit (DOJINDO, China). 2 μl of 1 mol/L Calcein-AM ( live cell staining ) and 2 μl of 1.5 mol/L PI (dead cell staining) were mixed into cell culture dish which contains 1 mL medium. Cells were incubated at 37 °C for 30 min in a dark incubator. Decant the medium and wash the samples 3 times with PBS. The images were taken by using a confocal microscope (Olympus FV1000, Japan) at 100 × magnification. The live cells showed the green fluorescence color, and the dead cells showed red fluorescence color.

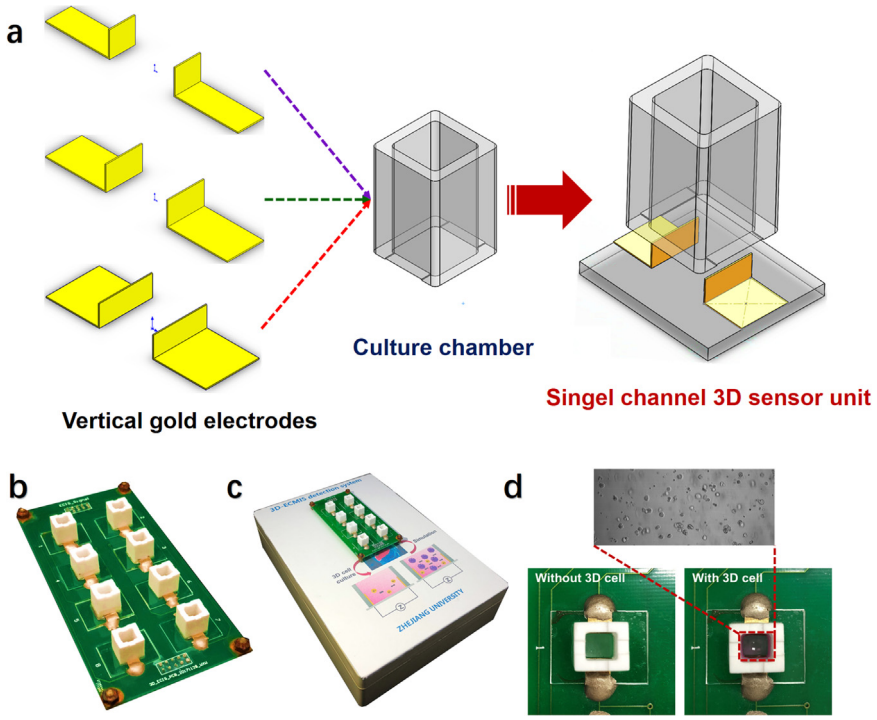


**Fig. 2.** (a) Fabrication schematic of 3D impedance biosensor. (b) Images of 8-channel 3D impedance biosensor. (c) Images of 3D ECMIS detection system. (d) The images of the 3D cell-based biosensor before and after seeding the living cells.

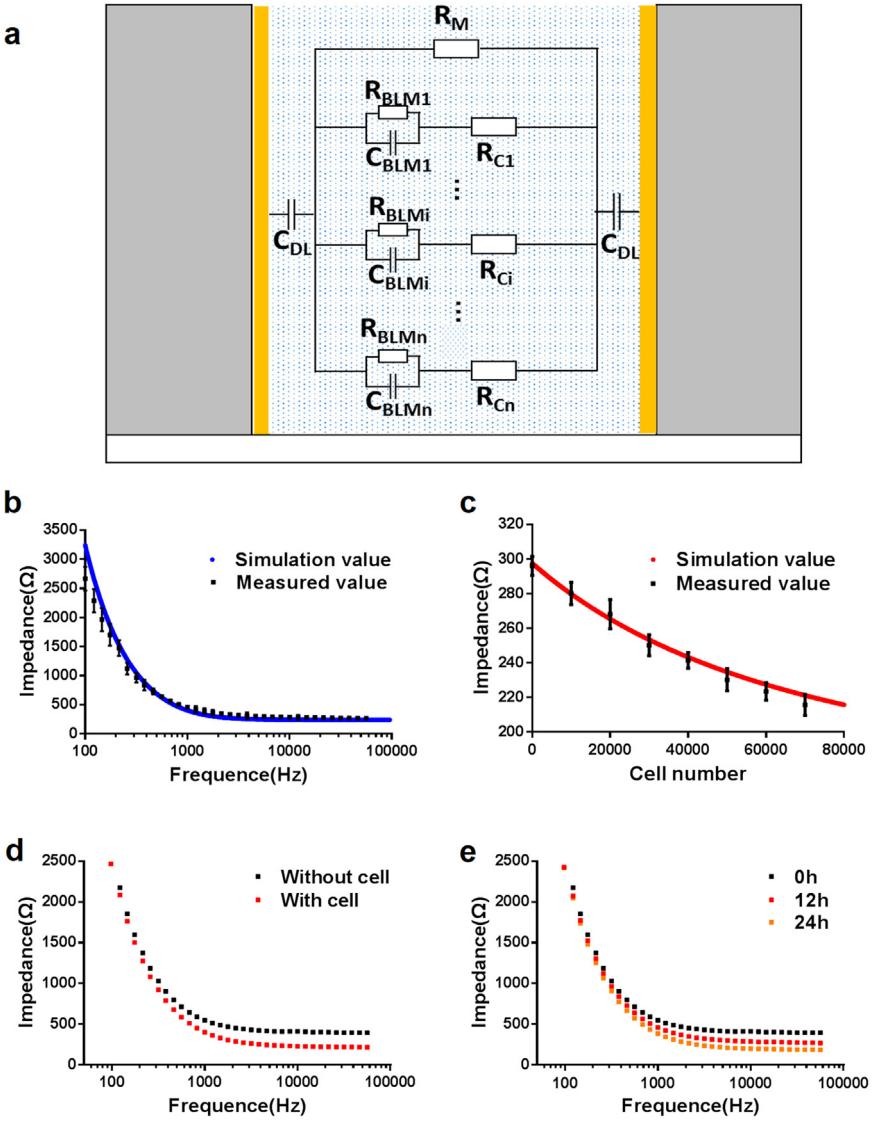


**Fig. 3.** (a) Equivalent circuit of 3D cells/matrigel construct in the culture chamber. (b) Comparison between practical measured value and simulation value of impedance-frequency response. (c) Comparison between practical measured value and simulation value of impedance with different cell numbers. (d) Bode plot of the 3D cells/matrigel construct and the construct without cells. (e) Bode plot of 3D ECMIS with cells at different time.

## 3. Results and discussion

### 3.1. Electrical simulation of 3D ECMIS

2D cultured cells directly attach to the working electrode to influence the electron transfer efficiency. The viability of cells can be estimated by analyzing the impedance between the planar working electrodes. In contrast, 3D cultured cells are encapsulated in matrigel and seldom contact with working electrode. The viability of 3D cultured cells is estimated by measuring the total impedance of cell/matrigel construct between vertical working electrodes. In this study, the equivalent circuit diagram of 3D cells/matrigel construct between two vertical electrodes is illustrated in Fig. 3a. The electrical model of 3D cell is based on Morgan's theory (Morgan et al., 2007). In the equivalent circuit, $C_{DL}$ represents the double layer capacitance between electrolyte and electrode, $R_M$ represents the resistance of the matrigel, $R_{BLM}$ represents the resistance of the cell membrane, $C_{BLM}$ represents the capacitance of the cell membrane, and $R_C$ represents the resistance of the cell. Based on the equivalent circuit diagram, the impedance of 3D cells/matrigel construct can be divided into two parts. The first part represents the impedance of the matrigel; another part represents the impedance of the 3D cultured cells, including live and dead cells. The impedance of the matrigel remains stable during the measurement. However, the electric property of live and dead cells are different, because the dead cell might lose its cell membrane (Lei et al., 2014). Therefore, the cell number and viability mainly dominate the total impedance of the 3D cells/matrigel construct.

In order to predict the total impedance of cells/matrigel construct with different numbers of cells at different frequencies, MATLAB (R2012a, Math Works, USA) is used for simulation based on the 3D cell circuit model. We measured the total impedance of the groups with and without cells at low (500 Hz) and high (50 kHz) frequency to get the values of $R_m$, $C_{DL}$, $R_{ce}$ and $C_{ce}$ ($R_{ce}$ and $C_{ce}$ are the equivalent resistance and capacity of $R_{BLM}$, $C_{BLM}$ and $R_c$) by putting these impedance values in the following equations:

$$Z_{wo} = \frac{1}{j2\pi f C_{DL}} + R_m$$

$$Z_w = \frac{1}{j2\pi f C_{DL}} + \frac{1}{\frac{1}{R_m} + \frac{1}{R_{ce} + \frac{1}{j2\pi f C_{ce}}}}$$

Where $Z_w$ and $Z_{wo}$ are the measured impedances with and without cells,

$f$ is the detecting frequency and $j$ is the imaginary unit. We simulated the trend of impedance changes at different frequencies. It was found that the total impedance decreased with the increase of the detecting frequency (Fig. 3b). Also, we simulated the trend of impedance in different cell numbers and found that the total impedance decreased as the number of cells increased (Fig. 3c). It is demonstrated that the cell number and viability mainly dominate the total impedance of the 3D cells/matrigel construct. In addition, in order to verify the accuracy of the simulation results, we compared the measured results with the simulation results. As shown in Fig. 3b and c, the measured results and simulation results present good consistency with different frequency and cell number.

Subsequently, cell experiments were also performed to verify the simulation results. At different frequencies, the impedance of the group with cell was always lower than the impedance of the group without cell (Fig. 3d). Moreover, the number of cells would increase due to the proliferation, leading to the decrease of total impedance value (Fig. 3e). These results were consistent with previous simulation results. Therefore, according to the simulation and experimental results, we confirm that the total impedance would decrease with the number of cells increase. Consequently, 3D ECMIS is a utility technique to monitor the 3D cultured cells growth.

### 3.2. Optimization for 3D ECMIS

In order to improve the performance of 3D ECMIS, the working frequency and the size of vertical electrodes were optimized. The electrochemical spectroscopy was conducted on groups with and without cells after cultured for 24 h, with the frequency ranging from 100 Hz to 50 kHz (Fig. 4a). Impedance values are usually normalized by cell index (CI) values for comparison. CI is the ratio of the cell impedance change $|\Delta Z|$ to background impedance $Z_0$, which excludes the impacts of electrode–electrolyte combination and parasitic elements. The results showed that when the working frequency was 10 kHz, the difference in CI value between groups with and without cells was the most obvious (Fig. 4b). Consequently, 10 kHz was chosen for the working frequency of 3D ECMIS detection system.

On the other hand, the sizes of working electrode were optimized for 3D ECMIS. Cell experiments were performed in the 3D ECMIS chips with different size vertical working electrodes, and the number and condition of the 3D-cultured cells between groups were consistent. As showed in Fig. 4c, the detected impedance would increase when the size of electrodes decreased. However, after calculating the CI values, no significant difference of sensitivity were presented among the three different working electrodes to monitor the 3D cultured cells proliferation (Fig. 4d). The results indicated that the size of working electrode could influence the impedance of cells/matrigel construct, but the CI value of impedance remained same. In order to analyze the stability of this sensor, we adopted conventional 2D medium and 3D matrigel on the sensor for a long time test. During the first few hours, the impedance of 3D matrigel would slightly change due to the gel solidification process. In the long time stability test, the CI value of the sensor with 2D medium and 3D matrigel was very stable (Fig. 4e).

### 3.3. Real-time monitoring of 3D cell proliferation

To verify the ability of 3D ECMIS for monitoring 3D cell proliferation in real-time, HepG2 cells (50000 cells/well) mixed with matrigel (1:2; matrigel: medium) were added into both 2D ECIS and 3D ECMIS chips. The 3D cells/matrigel construct would gelate quickly when the chips placed inside a humidified incubator. As shown in Fig. 5a 2D ECIS can only detect the proliferation of 2D cultured cells, which impeded the electron transfer rate of 2D working electrodes, while 2D ECIS is difficult to reflect the changes of 3D cultured cells (Fig. 5b). It shows no significant differences between the signal of medium, matrigel and cells/matrigel construct. Fig. 5c showed the time dependence of CI value measured at 10 kHz for HepG2 cell-encapsulated and cell-free matrigel during 96 h. The CI value of cells/matrigel construct increased with time, whereas CI value of cell-free matrigel remains low, implying that the increase of CI value was due to proliferation of HepG-2 cells. Fig. 5d showed the seeding cell density-dependence of CI value measured for HepG2 cells ranging from 10,000 cells/well to 50,000 cells/well. With the increase of the initial cell densities, the growth rates of CI value were also different, and group of 50,000 cell/well would reach a high and stable period more efficiently. Therefore, we selected 50,000 cells/well as the cell density for subsequent experiments. Furthermore, in order to explore the applicability of the 3D ECMIS, we also analyzed other tumor cells, such as human epithelial carcinoma cells (HeLa cells). As shown in Fig. 5e, both HepG2 cells and HeLa cells caused the increase of CI value with the increasing of time. Due to the different cell

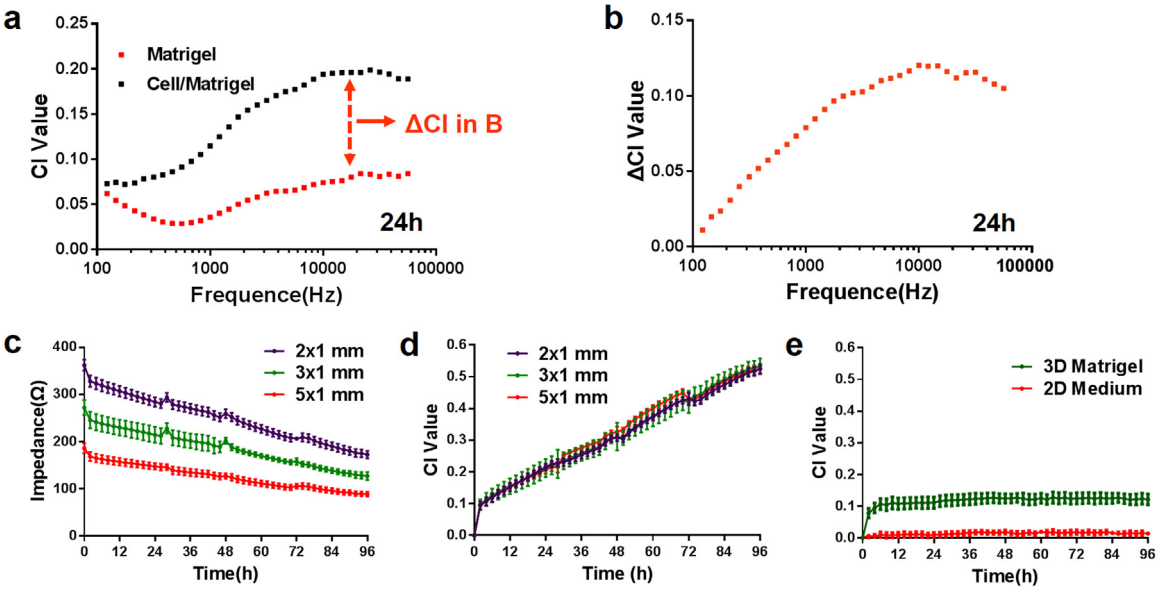


**Fig. 4.** (a) Bode plot of the 3D cells/matrigel construct and the construct without cells after 24 h culture (b) Bode plot of ΔCI value at 24 h. (c) The relation between working electrode size and impedance. (d) The relation between working electrode size and CI values. (e) The stability analysis of 3D ECMIS, which was conducted by long time test of 3D matrigel and 2D medium. All the data is represented by means ± SD (standard deviation), n = 3.

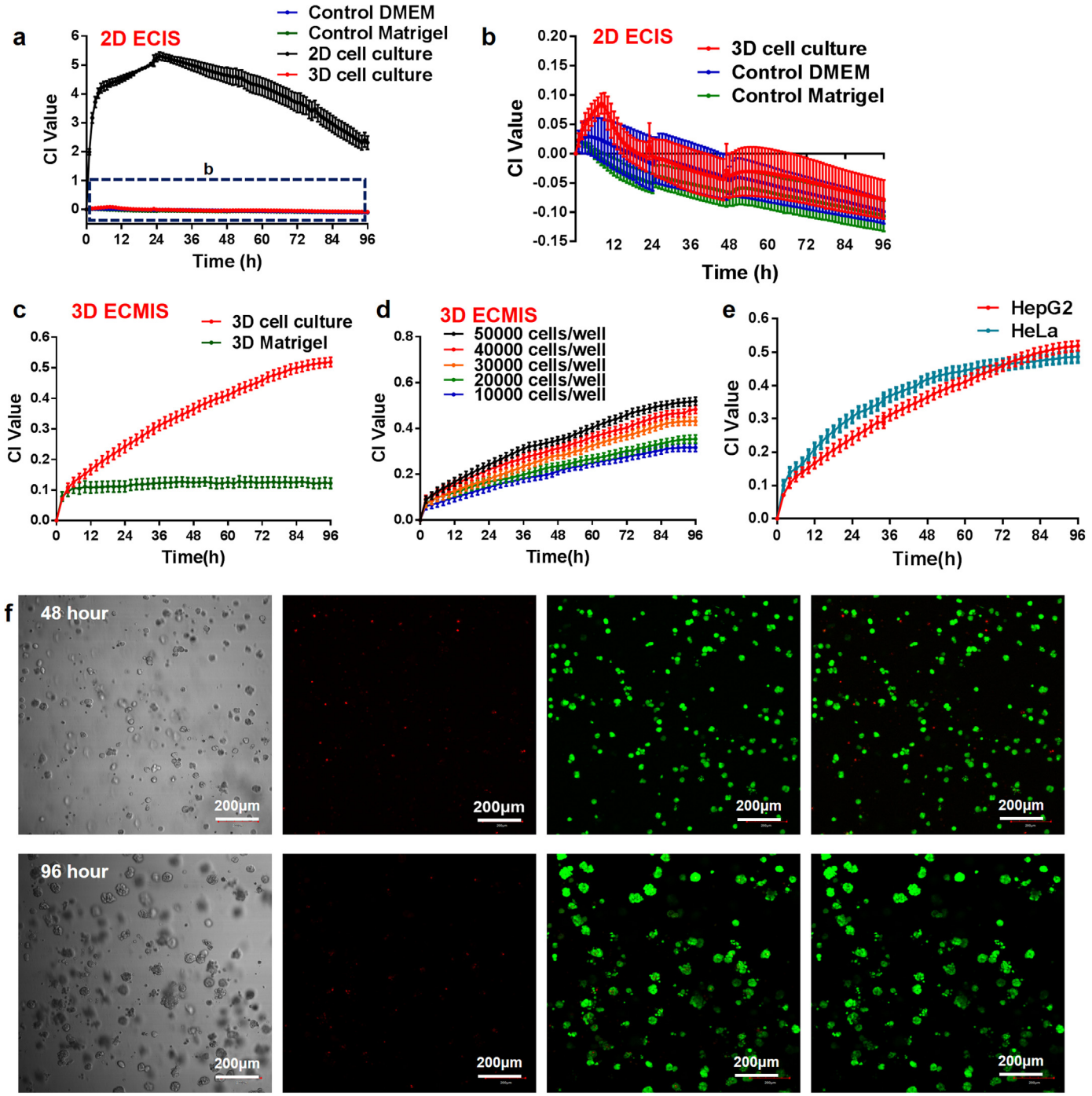


**Fig. 5.** (a) Cell growth curves of 2D-cultured HepG2 cells and 3D-cultured HepG2 cells on 2D ECIS. (b) Cell growth curves of medium, matrigel and 3D cells on 2D ECIS. (c) A group of representative cell growth curves of 3D-cultured HepG2 cells and matrigel. (d) A group of representative cell growth curves of 3D-cultured HepG2 cells at different cell numbers ranging from 10,000 to 50,000 cells/well. (e) Representative cell growth curves of 3D-cultured HepG2 cells and HeLa cells. (f) Changes in numbers and morphology of 3D-cultured HepG2 cells with time observed by Confocal fluorescence microscope. All the data is represented by means ± SD (standard deviation), n = 3.

proliferation rates, we also observed slightly different growth curves of them. In parallel, live/dead fluorescent staining was performed to validate the viability of HepG2 cell-encapsulated matrigel after 48 h and 96 h (Fig. 5f). The 3D-cultured cells had a clustered morphology, and the size and number of cell clusters both increased, which imitates the tumor growth *in vivo*. These results indicated that the 3D-cultured cells proliferation can be detected by 3D ECMIS.

### 3.4. 3D cell viability for anti-cancer drug response

Since conventional 2D cell culture does not adequately consider the natural 3D microenvironment of cells *in vivo*, the 2D cell culture based assay sometimes provides the misleading data for the drug screening. To verify whether 3D ECMIS can provide more reliable and accurate data for the drug screening, we selected three anti-cancer drugs (ovarian/breast anti-cancer drug, taxol, broad-spectrum anti-cancer drug, cisplatin, and liver anti-cancer drug, sorafenib) with different

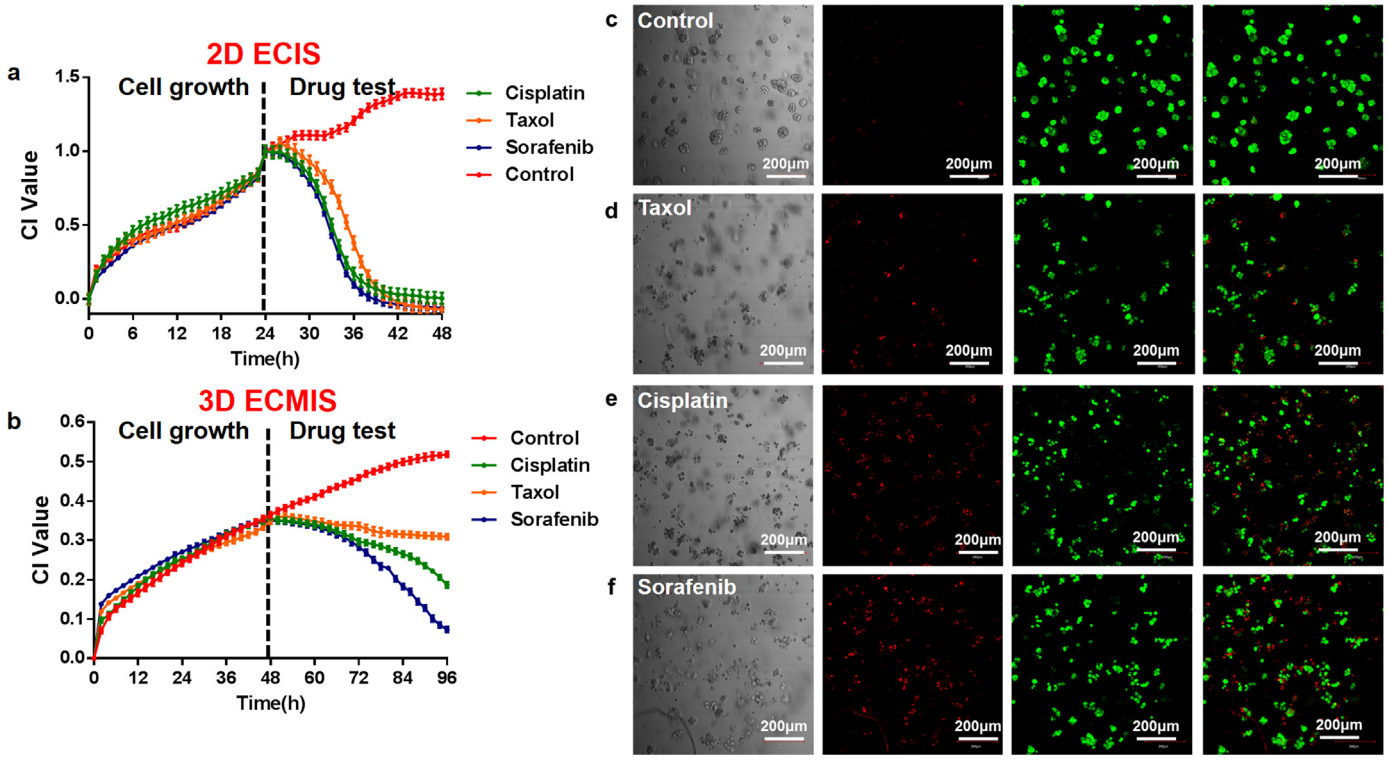


**Fig. 6.** (a) The normalized cell growth curves of 2D-cultured HpeG2 cells responding to different anti-cancer drugs (Cisplatin 10 μg/mL, Taxol 10 μg/mL, Sorafenib 10 μg/mL) on the 2D ECIS. (b) The normalized cell growth curves of 3D-cultured HpeG2 cells responding to different anti-cancer drugs (Cisplatin 10 μg/mL, Taxol 10 μg/mL, Sorafenib 10 μg/mL) on the 3D ECMIS. (c) Changes in numbers and morphology of non-treated 3D-cultured HepG2 cells observed by Confocal fluorescence microscope at 96 h. (d) Changes in numbers and morphology of Taxol-treated 3D-cultured HepG2 cells observed by Confocal fluorescence microscope at 96 h. (e) Changes in numbers and morphology of cisplatin-treated 3D-cultured HepG2 cells observed by Confocal fluorescence microscope at 96 h. (f) Changes in numbers and morphology of sorafenib-treated 3D-cultured HepG2 cells observed by Confocal fluorescence microscope at 96 h. All the data is represented by means ± SD (standard deviation), n = 3.

efficacy to administrate on 2D cultured and 3D cultured HepG2 cell model. Fig. 6a showed that both three drugs have perfect efficacy on 2D-cultured HepG2 cells, while the efficacies of the three drugs on liver cancer in patients are different. According to the pharmacology, only sorafenib is a kinase inhibitor drug approved for the targeted treatment of advanced primary liver cancer, which should have the best effect among three drugs (Keating and Santoro, 2009). Cisplatin is a chemotherapy medication used to treat a number of cancers, which might have the medium effect (Wang and Lippard, 2005). Taxol is a chemotherapy medication mainly used to treat ovarian cancer and breast cancer, and it is rarely used to treat liver cancer (Rowinsky et al., 1993). However, it was difficult to distinguish the efficacies of these three drugs by 2D ECIS. Meanwhile, 3D ECMIS could reflect different responses of the 3D cultured HepG2 cells to these drugs (Fig. 6b). As expected, sorafenib had the best effect on the 3D HepG2 cells among these drug. The results of fluorescent images in Fig. 6c–f also confirmed this conclusion. The intensity of red fluorescence is higher than other two drugs (Fig. 6f). The above experimental results showed that 3D ECMIS have better performance in anti-cancer drug screening *in vitro*.

## 4. Conclusion

In this study, we developed a dynamic, real-time, and non-invasive 3D ECMIS for cell growth/viability and drug screening. Based on this 3D cell-based biosensor, it is convenient to monitor the cell growth/viability of 3D cells and provide more dynamic data for drug screening. Moreover, we focused on improving the accuracy of cell-based anti-cancer drug screening by using this 3D cell-based biosensor. Furthermore, 3D ECMIS showed a high consistency with the conventional imaging method for monitoring 3D cell viability. Further work needs to be engaged in verifying the applicability and accuracy of the 3D electric cell/matrigel-substrate impedance sensing system: (i) A variety of 3D cell models and anti-cancer drug testing would to be carried out. (ii). Comparison of 3D ECMIS and Xenograft model in anti-cancer drug screening would be studied. It is envisioned that 3D ECMIS will be a promising high-throughput, non-invasive and real-time platform for 3D cell monitoring and anti-cancer drug screening.

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## References

Abbott, A., 2003. Cell culture: biology's new dimension. Nature 424 (6951), 870–872.
Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., Boyd, M.R., 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 48 (3), 589–601.
Bhadriraju, K., Chen, C.S., 2002. Engineering cellular microenvironments to improve cell-based drug testing. Drug Discov. Today 7 (11), 612–620.
Edmondson, R., Broglie, J.J., Adcock, A.F., Yang, L., 2014. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay. Drug Dev. Technol. 12 (4), 207–218.
Ehret, R., Baumann, W., Brischwein, M., Schwinde, A., Stegbauer, K., Wolf, B., 1997. Monitoring of cellular behaviour by impedance measurements on interdigitated electrode structures. Biosens. Bioelectron. 12 (1), 29.
Fear, E.C., Stuchly, M.A., 1998. A novel equivalent circuit model for gap-connected cells. Phys. Med. Biol. 43 (6), 1439–1448.

Hu, N., Wang, T., Wang, Q., Zhou, J., Zou, L., Su, K., Wu, J., Wang, P., 2015. High-performance beating pattern function of human induced pluripotent stem cell-derived cardiomyocyte-based biosensors for hERG inhibition recognition. Biosens. Bioelectron. 67, 146–153.
Jeong, S.H., Lee, D.W., Kim, S., Kim, J., Ku, B., 2012. A study of electrochemical biosensor for analysis of three-dimensional (3D) cell culture. Biosens. Bioelectron. 35 (1), 128–133.
Keating, G., Santoro, A., 2009. Sorafenib: a review of its use in advanced hepatocellular carcinoma. Drugs 69 (2), 223–240.
Knedlitschek, G., Noszvai-Nagy, M., Meyer-Waarden, H., Schimmelpfeng, J., Weibezahn, K.F., Dertinger, H., 1994. Cyclic amp response in cells exposed to electric fields of different frequencies and intensities. Radiat. Environ. Biophys. 33 (2), 141–147.
Lee, G.Y., Kenny, P.A., Lee, E.H., Bissell, M.J., 2007. Three-dimensional culture models of normal and malignant breast epithelial cells. Nat. Methods 4 (4), 359.
Lei, K.F., Wu, M.H., Hsu, C.W., Chen, Y.D., 2014. Real-time and non-invasive impedimetric monitoring of cell proliferation and chemosensitivity in a perfusion 3D cell culture microfluidic chip. Biosens. Bioelectron. 51 (2), 16–21.
Liu, Q., Wu, C., Cai, H., Hu, N., Zhou, J., Wang, P., 2014. Cell-based biosensors and their application in biomedicine. Chem. Rev. 114 (12), 6423–6461.
Morgan, H., Sun, T., Holmes, D., Gawad, S., Green, N.G., 2007. Single cell dielectric spectroscopy. J. Phys. D. Appl. Phys. 40 (2), 61–70.
Mueller-Klieser, W., 1997. Three-dimensional cell cultures: from molecular mechanisms to clinical applications. Am. J. Physiol. 273, C1109–C1123 (4 Pt 1).
Mulhall, H.J., Hughes, M.P., Kazmi, B., Lewis, M.P., Labeed, F.H., 2013. Epithelial cancer cells exhibit different electrical properties when cultured in 2D and 3D environments. Biochim. Et. Biophys. Acta 1830 (11), 5136–5141.
Nguyen, T.A., Yin, T.I., Reyes, D., Urban, G.A., 2013. Microfluidic chip with integrated electrical cell-impedance sensing for monitoring single cancer cell migration in three-dimensional matrixes. Anal. Chem. 85 (22), 11068–11076.
Pampaloni, F., Reynaud, E.G., Stelzer, E.H., 2007. The third dimension bridges the gap between cell culture and live tissue. Nat. Rev. Mol. Cell Biol. 8 (10), 839–845.
Rowinsky, E.K., Eisenhauer, E.A., Chaudhry, V., Arbuck, S.G., Donehower, R.C., 1993. Clinical toxicities encountered with paclitaxel (Taxol). Semin. Oncol.
Thoma, C.R., Zimmermann, M., Agarkova, I., Kelm, J.M., Krek, W., 2014. 3D cell culture systems modeling tumor growth determinants in cancer target discovery. Adv. Drug Deliv. Rev. 69–70, 29–41.
Tibbitt, M.W., Anseth, K.S., 2010. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnolo. Bioeng. 103 (4), 655–663.
Wang, D., Lippard, S.J., 2005. Cellular processing of platinum anticancer drugs. Nat. Rev. Drug Discov. 4 (4), 307–320.
Weaver, V.M., Petersen, O.W., Wang, F., Larabell, C.A., Briand, P., Damsky, C., Bissell, M.J., 1997. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J. Cell Biol. 137 (1), 231–245.
Zhou, J., Wu, C., Tu, J., Ling, Y., Hu, N., Zhang, Y., Su, K., Wang, P., 2013. Assessment of cadmium-induced hepatotoxicity and protective effects of zinc against it using an improved cell-based biosensor. Sens. Actuators A Phys. 199 (9), 156–164.
Zou, L., Wu, C., Wang, Q., Zhou, J., Su, K., Li, H., Hu, N., Wang, P., 2015. An improved sensitive assay for the detection of PSP toxins with neuroblastoma cell-based impedance biosensor. Biosens. Bioelectron. 67, 458–464.