Improving Stability and Accuracy of Cell Viability Evaluation by Fusion of Impedance Spectroscopy Information

Author list:

Yecheng Zhang[1], Mingji Wei[1], Rongbiao Zhang, Fei Zhang

Affiliation:

School of Electrical and Information Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China

Correspondence:

Mingji Wei, School of Electrical and Information Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China

E-mail address: 1422737125@qq.com (M. Wei)

Note:

[1]Y. Zhang and [1]M. Wei contribute to this paper equally.

Abstract

The increasing attention to precision medicine is widely paid in order to greatly improve the cure rate of cancer. Improving the stability and accuracy of cell viability evaluation is the key of precision medicine, for excess dosage of anti-cancer drugs not only kills the cancer cells, but also does harm to normal cells. Electrochemical impedance sensing (EIS) method is widely accepted as a label-free, non-invasive approach for real-time, online monitoring of cell viability. Due to the large effects of many influencing factors, the existing EIS methods that utilized single-frequency impedances show poor stability and low accuracy of cell viability evaluation. In this paper, we proposed a multi-physical information fusion method based on least squares support vector machine (LS-SVM) for improving the stability and accuracy of cell viability evaluation. The results show that the mean relative error of single-frequency method is about 0.08, while that of fusion method is about 0.04. It means that the prediction results of fusion method are more accurate than that of the single-frequency method. Moreover, the maximum relative error of single-frequency method is up to 0.5 due to the influencing of cell micromotion, while that of fusion method is below 0.07, showing that the fusion method is more stable than single-frequency method.

KEYWORDS

Cell viability, Electrochemical impedance spectroscopy (EIS), Information fusion, LS-SVM.

1 | INTRODUCTION

With the rapid development of precision medicine, the strict requirements for more stable and accurate evaluation of cell viability are of great significance to improve the cure rate of cancer (Bashraheel, Domling, & Goda, 2020; L. Chen, Manautou, Rasmussen, & Zhong, 2019; Cotchim, Thavarungkul, Kanatharana, & Limbut, 2020; Mahomed, Padayatchi, Singh, & Naidoo, 2019; Song, Kong, Huang, Luo, & Zhu, 2020; Tan, Huang, Zhang, & Li, 2019; Vetsch et al., 2019; Vo, Parsons, & Seibel, 2019). Cell viability defined as the percentage of live cells in total cells (G. Li et al., 2018), is one of the most important segments of precision medicine, for excess dosage of anti-cancer drugs not only kills cancer cells, but also does harm to normal cells (Wei, Zhang, Zhang, et al., 2019). Electrochemical impedance sensing (EIS) method using cellular impedance spectroscopy (CIS) is widely accepted as a label-free, non-invasive approach, which can realize real-time, online monitoring of cell viability (Anh-Nguyen, Tiberius, Pliquett, & Urban, 2016; Daza, Olmo, Cañete, & Yúfera, 2013; Pradhan, Mandal, Mitra, & Das, 2014; Pui et al., 2013; Qiu, Liao, & Zhang, 2008; Ren & Chui, 2018; Wang et al., 2010; Xiao, Lachance, Sunahara, & Luong, 2002; Yang, Arias, Lane, Yancey, & Mamouni, 2011). Many researchers commonly utilized impedances at a constant (Anh-Nguyen et al., 2016; Daza et al., 2013; Keese, Wegener, Walker, & Giaever, 2004; Pradhan et al., 2014; Xiao et al., 2002; Yang et al., 2011) or at the most sensitive frequency (Arndt, Seebach, Psathaki, Galla, & Wegener, 2004; Wang et al., 2010) to monitor the cell viability. However, this single-frequency approach in many situations like anti-cancer drug test, cell dynamic event monitoring, shows a poor stability and low accuracy. The investigation shows that many influencing factors, such as the changes of cell status during drug test, the resistance between cells at high density, the impedance fluctuations caused by cell micromotion (Opp et al., 2009) and the drifts of the most sensitive frequency caused by electrode properties (Daza et al., 2013), can lead to the instability and inaccuracy of cell viability evaluation. The solutions on how to decrease the effects of those factors talked above to improve the stability and accuracy of cell viability assessment still remain difficult.

Among those influencing factors, cell adhesion status during drug test greatly affects the cell viability evaluation using CIS. Because cell adhesion area and cell-electrode distance, that are changed with the time course of dose-response assay of anti-cancer drugs in cell adhesion system, alters electric current path, finally leading to the changes of cell impedance

(Giaever & Keese, 1991). The linear relationship between cell number and cell impedance (Xiao et al., 2002) is largely affected by cell density due to the influencing of resistances between crowded cells (Arndt et al., 2004; Giaever & Keese, 1991). Because the spaces of cell spreading are largely constricted by each other at high cell density, ultimately causing the changes of cell impedance (Giaever & Keese, 1991). Meanwhile, cell micromotion that alters available areas of current flow leads to the impedance fluctuations in cell-electrode system (Giaever & Keese, 1991; Opp et al., 2009). Besides, the electrode-electrolyte interface (equivalent to constant phase element, CPE) determined by electrode properties such as electrode sizes and materials also contributes to the changes of CIS (Abdur Rahman, Price, & Bhansali, 2007; X. Zhang et al., 2017). Theoretically, the changes of CPE that dominates the cell impedance at low frequency (LF) band (< 10^3 Hz) will cause the drift of the most sensitive frequency in CIS. In summary, there are too many influencing factors talked above, proving that evaluating cell viability using impedances at a constant or the most sensitive frequency is quite instable and inaccurate. Therefore, a more stable and accurate approach for cell viability evaluation is required to improve the precision medicine and the cure rate of cancer.

In this paper, the multi-physical properties of cell-electrode system were firstly extracted to fuse together for cell viability evaluation based on least squares support vector machine (LS-SVM). Compared with single-frequency method, information fusion method has the ability to decrease the effects of those factors talked above. Theoretically, the CPE of electrode-electrolyte interface, resistance and capacitance of adhered cells and solution resistance dominate the impedance spectroscopy in the whole LF band (< 10^3 Hz), the part of LF and the whole intermediate frequency (MF) band (10^2-10^5 Hz), and high frequency (HF) band (> 10^5 Hz), respectively (Ren & Chui, 2018). Multiple impedances at different frequencies extracted from CIS were assumed as the input of LS-SVM model. Meanwhile the cell number calculated by manual observations was assumed as the output of LS-SVM model (see Figure 1). The results indicate that our proposed fusion method greatly improves the stability and accuracy of cell viability evaluation, which has significant meanings for the advance of biosensors based on CIS and precision medicine.

2 | MATERIALS AND METHODS

2.1 | Cell culture and assays

Human gastric cancer cells (SGC-7901 and BGC-823) provided by Medical School, Jiangsu University (China) are used in cellular assay in this paper. The two types of cells are cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) at 37 °C and 5% CO_2 inside a Water Jacketed CO_2 Incubator (Heal Force®). Cell culture medium (DMEM with 10% FBS) is changed every 48 h. Cells are detached from cell culture dish using try-EDTA (Life Technologies GmbH, Darmstadt, Germany) for 2 min, and then cell culture medium is centrifuged at a speed of 600 rpm for 5 min. The supernatant solution is sucked away and changed for 3 mL new medium, and the new medium containing cells is mixed uniformly.

Eight groups of cell medium with SGC-7901 cells (10^4 cells/mL, 400 μL) were dropped into 8W10E+ PET ECIS culture ware, which was measured for 20 h to monitor cell dynamic events from cell seeding to cell proliferation (see Figure 2(c)).

SGC-7901 or BGC-823 cells (10^4 cells/mL, 400 μL) can be attached to the electrodes of 8W10E+ PET ECIS after cultured for 12 h at 37 °C and 5% CO_2. Then, eight groups of cell medium (SGC-7901, BGC-823) were monitored by impedance analyzer for 20 min to obtain the data of cell micromotion (see Figure 2(d)).

Eight groups of cell medium with different concentrations of SGC-7901 or BGC-823 cells (from 10^2 to 10^6 cells/mL, 400 μL) cultured for 8 h in 8W10E+ PET ECIS were measured by impedance analyzer at 10 kHz to obtain the relationship between cell number and normalized impedance (*NI*) (see Fig 4(d)).

Eight groups of culture medium with SGC-7901 cells (10^4 cells/mL, 400 μL) were dropped into our designed ECIS with different electrode sizes (width of 25, 50, 100 μm, the same spacing of 50 μm), which was cultured for 8 h and was scanned to obtain the CISs (see Fig 4(f) and (g)).

Eight groups of culture medium with SGC-7901 cells (10^4 cells/mL, 400 μL) were dropped into 8W10E+ PET ECIS cultured for 8 h, and the CISs were scanned from 1 to 10^6 Hz. Meanwhile, the corresponding cell number can be obtained by manual observations. Then, after eight groups of cell medium was cultured for 12 h, 20 μL cisplatin (50 μM) was dropped into each culture ware and eight groups of cell medium were monitored for 10 h (Liu et al., 2009). Those 150 groups of data obtained by assays above constitute the training set of LS-SVM model.

2.2 | Experimental equipment

The impedance analyzer (Chi660E electrochemical workstation) was purchased from Chenhua Instrument (Shanghai, China), and 8W10E+ PET ECIS was purchased from Applied BioPhysics (America). The ECIS with different electrode sizes (25, 50, 100 μm) were fabricated in Wenhao Chip Technology Co., Ltd. (Suzhou, China).

2.3 | Dynamic monitoring of cell events

Cell viability defined as the percentage of live cells in total cells can be evaluated by electrochemical impedance sensing (EIS) method which is widely accepted as a label-free and non-invasive approach (R. Zhang et al., 2018). However, cell events like cell adhesion status, cell proliferation (Xiao et al., 2002), cell micromotion (Giaever & Keese, 1991; Opp et al., 2009) will seriously influence the electrical signal, causing the large inaccuracy of cell viability assessment.

The process of cell adhesion in ECIS was monitored in real time by impedance analyzer and the diagram of this process is shown in Figure 2(a) and (b). P1 represents cell seeding, which causes little changes in impedance of electrodes. In fact, the electrode-electrolyte interface and adherent cell membrane dominate the overall impedance at low and intermediate frequencies (Yang et al., 2011). Cell seeding (cell-electrode distance d > 1 μm) actually increases little resistance of solution, thus hardly influencing the overall impedance. When cells attach and spread on the electrodes (from P2 to P4), the overall impedance largely increases (see Figure 2(b) and (c)) (Giaever & Keese, 1991) and the changes of impedance are determined by adhesion area of individual cells and cell number (Xiao et al., 2002). Cell adhesion theoretically is a reversible process mediated by specific interactions between receptors and ligands (Li, Tang, Wang, Lin, & Yao, 2018). The effective area available for current flow is altered by those receptor-ligand bonds, ultimately causing a large increase in overall impedance (Giaever & Keese, 1991). Besides, the cell-electrode distance d decreasing from micron level to about 60 nm with the time course of cell adhesion also affect the overall impedance a lot (Seriburi, McGuire, Shastry, Böhringer, & Meldrum, 2008). Commonly, cell viability should be evaluated after cells are cultured for about 8 h, i. e., the measured status of cell adhesion is P4 shown in Figure 2(a). Meanwhile, cells proliferating on electrodes all belong to the status of P4. Although individual cell adhesion statuses are slightly different with each other in cell population, cellular impedances are still approximately linearly related with adherent cell number (Xiao et al., 2002). Thus, cell proliferation can be monitored in real-time using EIS approach as is shown in Figure 2(c). However, in dose-response assay of anti-cancer drugs,

completely adhered cells are gradually killed and their adhesion status will change from P4 to P1, which has great effects on CIS.

Besides, cell micromotion (Opp et al., 2009) also affects the measurement of cell viability as is shown in Figure 2(d). Cell micromotion leads to the fluctuations of impedance, even cell layer becomes confluent (Giaever & Keese, 1991). Besides, cells moving around lead to the changes of relative position with electrodes, causing the fluctuation of impedance magnification and enhancing the difficulty of accurate measurement of cell viability (Seriburi et al., 2008).

2.4 | Fusion approach of cell impedance spectroscopy information

Cellular impedance spectroscopy contains some physical information of cell sensing system like CPE of electrode-electrolyte, capacitive and resistive properties of cell membrane, cytoplasm resistance and resistance of cell culture medium (Ren & Chui, 2018; Y. Xu et al., 2016). As can be seen from the equivalent circuit model (see Figure 3(a)), cell, electrode-electrolyte interface and medium impedance dominate the total impedance. However, capacitive and resistive elements contribute differently to the overall impedance at different frequency bands (Ren & Chui, 2018). In this paper, the whole frequency interval can be divided as low-frequency (LF < 10^3 Hz) band, intermediate-frequency (10^3 < MF < 10^5 Hz) band and high-frequency (10^5 < HF <10^7 Hz) band. Theoretically, capacitive elements in series like CPE dominate overall impedance in LF band, while those in parallel like capacitance of cell membrane C_c dominate overall impedance in MF band. Besides, resistive elements in series like solution resistance R_s dominate the total impedance in HF band, while those in parallel like resistance of cell membrane R_c dominate the total impedance in MF band (see Figure 3(b) and (c)). Therefore, evaluating cell viability using impedances at a single frequency is incomplete and inaccurate.

Here, a more complete and accurate approach based on information fusion for cell viability assessment is proposed in this paper. Firstly, the impedances at 10^2 (in LF band), 10^4 (in MF band), 10^6 (in HF band) Hz were extracted and defined as Z_1, Z_5, Z_9, respectively. Then, three impedances at $10^{2.5}$, 10^3, $10^{3.5}$ Hz were extracted by exponentially evenly dividing the interval of frequency from 10^2 to 10^4 Hz, and they were defined as Z_2, Z_3, Z_4. Likewise, Z_6, Z_7 and Z_8 at $10^{4.5}$, 10^5, $10^{5.5}$ Hz were acquired and all extracted impedances are shown in Figure 1(b). Therefore, define that $\mathbf{X} = [Z_1, Z_2, Z_3, Z_4, Z_5, Z_6, Z_7, Z_8, Z_9]$ and $\mathbf{Y} = [CI]$, where CI denotes cell viability index. The formula of cell viability index is given as:

$$CI = \frac{\text{Sample } NUM}{\text{Control } NUM} \times 100\% \tag{1}$$

where *NUM* represents the total number of live cells, the sample group represents the total live cell number in ECIS in cellular assays, the control group is the total live cell number when cells overgrow in ECIS (about 5000 cells) (G. Li et al., 2018).

In this paper, LS-SVM was used to fuse multiple physical properties of cell-electrode system for more accurate evaluation of cell viability. We define that $T = \{(x_k, y_k) | x_k \in \mathbf{X}, y_k \in \mathbf{Y}, k = 1, 2, \ldots, N\}$ is the training set, where x_k represents input, y_k represents the output, N denotes the sample size, $\mathbf{X}$ denotes the input space, $\mathbf{Y}$ denotes the output space (S. Zhou, Chu, Cao, Liu, & Zhou, 2020). The decision function is described as:

$$f(x) = \boldsymbol{w}^T \boldsymbol{\varphi}(x) + b \tag{2}$$

where $\boldsymbol{w}$ is a weight matrix; $\boldsymbol{\varphi}(x)$ is mapping function that maps the training data, b is the bias term. The optimization process of LS-SVM can be considered as solving the following equation with constraints.

$$\begin{aligned} &\min_{\boldsymbol{w},b,e} J(\boldsymbol{w}, e) = \frac{1}{2}\boldsymbol{w}^T\boldsymbol{w} + \frac{C}{2}\sum_{k=1}^{N} e_k^2 \\ &s.t. \quad y_k = \boldsymbol{w}^T\boldsymbol{\varphi}(x_k) + b + e_k, \quad k = 1, 2, \ldots, N \end{aligned} \tag{3}$$

where J denotes the structure risk, y_k denotes the constraints, C denotes the relaxation factor, $\boldsymbol{w}$ and b denote the decision function parameters and e_k denotes the error variable (S. Zhou et al., 2020). Lagrange function is built as:

$$L(\boldsymbol{w}, b, e, \alpha) = J(\boldsymbol{w}, e) - \sum_{k=1}^{N} \alpha_k (\boldsymbol{w}^T \boldsymbol{\varphi}(x_k) + b + e_k - y_k) \tag{4}$$

where $\alpha = [\alpha_1, \alpha_2, \ldots, \alpha_n]$ denotes the Lagrange multiplier. According to the Karush-Kuhn Tucker conditions (KKT) (Sutulo, Moreira, & Guedes Soares, 2002). Lagrange function can be derived as follows (H. Xu & Soares, 2019):

$$\begin{aligned} &\frac{\partial L}{\partial \boldsymbol{w}} = 0 \rightarrow \boldsymbol{w} = \sum_{k=1}^{N} \alpha_k \boldsymbol{\varphi}(x_k) \\ &\frac{\partial L}{\partial b} = 0 \rightarrow \sum_{k=1}^{N} \alpha_k = 0 \\ &\frac{\partial L}{\partial e_k} = 0 \rightarrow \alpha_k = C e_k, k = 1, 2, \ldots, N \\ &\frac{\partial L}{\partial \alpha_k} = 0 \rightarrow \boldsymbol{w}^T \boldsymbol{\varphi}(x_k) + b + e_k - y_k = 0, k = 1, 2, \ldots, N \end{aligned} \tag{5}$$

The result after derivation is shown as follows:

$$\begin{bmatrix} 0 & 1 & \cdots & 1 \\ 1 & K(x_1, x_1) + 1/C & \cdots & K(x_1, x_l) \\ \vdots & \vdots & \vdots & \vdots \\ 1 & K(x_l, x_1) & \cdots & K(x_l, x_l) + 1/C \end{bmatrix} \begin{bmatrix} b \\ \alpha_1 \\ \vdots \\ \alpha_l \end{bmatrix} = \begin{bmatrix} 0 \\ y_1 \\ \vdots \\ y_l \end{bmatrix} \tag{6}$$

where $K(x_k, x_l) = \varphi(x_k)^T \varphi(x_l)$, $(k, l = 1, 2, \ldots, N)$ denotes the kernel function representing an inner production. The linear kernel function is used for parameter estimation. Thus, the final LS-SVM model for the regression can be described as:

$$y(x) = \sum_{k}^{N} \alpha_k K(x, x_k) + b \tag{7}$$

Besides, the LS-SVM model is suitable for small data groups, because of its low ability to solve large training set (H. Xu & Soares, 2019).

3 | RESULTS AND DISCUSSION

3.1 | Theoretical analysis of cell viability detection model

There are many theoretical models about cell adhesion on electrodes proposed in cell viability assays (Anh-Nguyen et al., 2016; El Hasni et al., 2017; Pradhan et al., 2014; Xiao & Luong, 2010; Yang et al., 2011). Because the cell-electrode system contains many specific modules, such as electrode-electrolyte interface, cell impedance, cell adhesion area and cell-electrode distance. Meanwhile, the influencing factors like the parasitic capacitance between electrodes (Beltrán, Finger, Santiago-Aviles, & Espinoza-Vallejos, 2003), the gap resistance between cell and electrode (Y. Chen et al., 2012; Daza et al., 2013) and the charge transfer resistance (Anh-Nguyen et al., 2016) need to be carefully considered too. Actually, the research targets they focused on determine the simplification of theoretical model. If the target parameters need more accuracy, some parameters which are difficult to measure or calculate should be eliminated in circuit model of cell-electrode system. Besides, the similar and constant elements should be built as one component as possible for the convenience of variable calculations. Because the fitting results of constant elements, such as CPE, solution resistance and protein coating, are different in the dynamic monitoring process of cell events. Moreover, the series or parallel combinations should be adjusted according to the physical situation and experimental data, for the series or parallel elements contribute to total impedance in different frequency bands.

According to the discussion talked above, the circuit model of cell-electrode system is simplified in this paper as shown in Figure 3(a). The diagram of main current paths passing through the equivalent circuit system is shown in Figure 3(b). Then the formula of impedance without cells and with cells can be acquired as follows:

$$\begin{cases} Z_{cells} = R_s + \dfrac{K}{(j\omega)^{\alpha}} + \dfrac{R_c}{1 + j\omega R_c C_c} \\ Z_{no\text{-}cell} = R_s + \dfrac{K}{(j\omega)^{\alpha}} \end{cases} \tag{8}$$

where $Z_{no\text{-}cell}$ and Z_{cells} denote the total impedance without cell and with cells, respectively, R_s denotes the solution resistance, K and α are the parameters determined by the properties of electrolyte and electrode (Wang et al., 2010), R_c and C_c denote the adhered cell impedance (Mamouni & Yang, 2011; Yang et al., 2011; F. Zhang, Jin, Hu, & He, 2018). As can be obviously seen from Figure 3(c), the range of R_s is about 200-300 Ω, far less than CPE and cell impedance at LF and MF bands. Meanwhile, cell impedance starts to have resolution at above 10 Hz compared with cell-free impedance spectroscopy. Because the impedance of CPE is far larger than cell resistance R_c in parallel with cell capacitance C_c which nearly is open circuit at below 10 Hz. Moreover, R_c and C_c dominates the total impedance from 10^2 to 10^4 Hz, where are suitable for cell viability evaluation. However, the influencing frequency intervals of R_c and C_c are also different with each other. Generally, R_c has more effects on the total impedance at about 10^2-10^3 Hz, while C_c has more effects at about 10^3-10^4 Hz. Physically, it performs as the electric current flowing beneath cell layer (R_c) more at lower frequency and through cell membrane (C_c) more at higher frequency (see Figure 4(a)) (Giaever & Keese, 1991; R. Zhang et al., 2018). Thus, it is quite inaccurate to evaluate cell viability using impedances at a single frequency.

In this paper, multiple physical information of cell adhesion on electrodes were extracted based on normalized impedance spectroscopy. And the normalized impedance spectroscopy can be acquired by the formula (Wei, Zhang, Zhang, et al., 2019):

$$NI = \frac{Z_{cells} - Z_{no\text{-}cell}}{Z_{no\text{-}cell}} \tag{9}$$

where NI represents normalized impedance, Z_{cells} represents the impedance with cells, $Z_{no\text{-}cell}$ represents impedance without cells.

3.2 | Influencing factors on detection range and accuracy of cell viability

The accuracy of cell viability evaluation using EIS method is largely affected by many factors, such as the changes of cell adhesion status during drug test, resistance between cells at high cell density, impedance fluctuations caused by cell micromotion and drift of the most sensitive frequency. As was discussed above, the process of cell adhesion usually includes cell seeding (P1), initial adhesion (P2), cell adhesion (P3) and cell spreading (P4) (see Figure 2(a)) (R. Zhang et al., 2018). The distance between cell and electrode decreases with time course of cell adhesion and spreading, meanwhile the impedance largely increases as can be seen in Figure 2(b) (Qiu et al., 2008; Seriburi et al., 2008). The distance between cell and electrode also affects the total impedance, for the cell resistance is directly proportional to the distance as (Ren & Chui, 2018):

$$R_c = \frac{\rho_s d}{A_{cell}} \tag{10}$$

where R_c denotes the cell resistance, ρ_s denotes the solution resistivity, d denotes the cell-electrode distance, A_{cell} denotes the cell-covered area. Generally, cells used for impedance detection need to be cultured for about 8-12 h, thus the final status of cell adhesion is P3 or P4. Therefore, the adhesion status has little effect on CIS. However, in the drug test, the status of adhered cells that are harmed by anti-cancer drugs is from P4 to P1, greatly influencing the cell viability evaluation.

The impedance is linearly related with the cell adhesion areas rather than cell number (Seriburi et al., 2008). Although the adhesion areas of individual cells are quite different with each other, the total areas of cell population are approximately related with cell number (Xiao et al., 2002). Usually, cell adhesion areas are also closely related with cell density (Seriburi et al., 2008). The spaces of cell spreading are largely constricted by each other at high cell density, ultimately causing the increasing of mean adhesion area of cells, which damages the linear relationship between cell number and impedance. As can be seen from Figure 4(a)-(d), both the resistance between cells and the constricted area of cell adhesion have large effects on evaluation of cell viability at single frequency (10 kHz). The effect of resistance between cells was given as (Giaever & Keese, 1991):

$$\frac{1}{Z_c} = \frac{1}{Z_n}\left(\frac{Z_n}{Z_n + Z_m} + \frac{\frac{Z_m}{Z_n + Z_m}}{\frac{i\gamma r_c}{2}\frac{I_0(\gamma r_c)}{I_1(\gamma r_c)} + 2R_b(\frac{1}{Z_n} + \frac{1}{Z_m})} \right) \tag{11}$$

where Z_c denotes the impedance of cells, j is $\sqrt{-1}$, I_0 and I_1 are the modified Bessel functions of the first kind of order 0 and 1; r_c represents the radius of individual cells, Z_n denotes the specific impedance of the electrode-electrolyte interface and Z_m denotes the specific membrane impedance of cells; R_b is the resistivity between adherent cells (see Figure 4(a)). Besides, the γ is defined as:

$$\gamma = \sqrt{\frac{\rho}{d}\left(\frac{1}{Z_n} + \frac{1}{Z_m} \right)} \tag{12}$$

where ρ denotes the resistivity of solution and d denotes the distance between the ventral surface of individual cells and the electrode. Here, the theoretical model talked above is only used for qualitative analysis, not for quantitative analysis. We can know from the Equation (11) and (12), except for the specific membrane impedance and individual cell radius, the impedance of cells adhered on electrodes is closely related with many other factors, like electrode-electrolyte interface, resistivity of solution, distance between cell and electrode, and resistance between cells. Among those factors, r_c and R_b are the only adjustable parameters in Equation (11) (Giaever & Keese, 1991). In fact, r_c will decrease and R_b will increase with cell growth at high cell density, causing large effects on linear relationship at about 4000-5000 cell number (see Figure 4(d)). Commonly, cells spread freely on electrodes without huddling together if the space and nutrition are enough for cells to grow. As can be seen from Figure 4(b), R_b is assumed as 0 at low cell density (< 3000 cells in ECIS), and the impedance is assumed as linearly related with cell number. However, cell viability evaluation for dose-response assay of anti-cancer drugs is generally based on the condition that electrodes are filled with cells. Thus, it is quite inaccurate for cell viability assessment to use single-frequency method.

Cell micromotion that leads to impedance fluctuation also is one of the important influencing factors on cell viability evaluation (see Figure 2(d)) (Opp et al., 2009). Cell motions, that physically performs as the dynamic binding-unbinding

process of receptor-ligand bond (L. Li et al., 2018), alter available areas of current flow, ultimately causing cellular impedance fluctuations (Giaever & Keese, 1991).

In addition, the effects of electrode properties on cellular impedance spectroscopy provide the convincing evidence that evaluating cell viability using a single-frequency is inaccurate (Daza et al., 2013) (Abdur Rahman et al., 2007; Asphahani et al., 2008; X. Zhang et al., 2017). As can be seen from Figure 4(e)-(f), the impedance measured at 10 kHz decreases with the increasing of electrode size. However, the CPE of electrode-electrolyte interface increases with the decreasing of electrode size, ultimately causing the drifts of the most sensitive frequency (see Figure 4(g)) (Daza et al., 2013; R. Zhang et al., 2018). Theoretically, the increasing of CPE that dominates the impedance at LF band will postpone the most sensitive frequency of impedance spectroscopy. In fact, the CPE of electrode-electrolyte interface which is repeatedly used also increases, causing the changes of impedance spectroscopy. In summary, many factors, like cell adhesion status, cell density, cell micromotion and electrode properties, proved to largely influence the evaluation of cell viability using single-frequency method. Thus, a more accurate approach for cell viability evaluation should be proposed to improve the dose-response assay.

3.3 | Cell viability evaluation by fusing the impedance spectroscopy information

In order to improve the accuracy of cell viability assessment, a LS-SVM-based approach that utilizes multiple physical properties of cell adhesion on electrodes instead of single-frequency impedances was proposed in this paper. As was talked above, there are many influencing factors on the accuracy of cell viability assessment, such as cell adhesion status, cell density, cell micromotion, electrode properties, etc. Thus, the accuracy of cell viability evaluation based on single-frequency method has no longer met the strict requirements of precision medicine. Here, multiple impedances (at 10^2, $10^{2.5}$, 10^3, $10^{3.5}$, 10^4, $10^{4.5}$, 10^5, $10^{5.5}$, 10^6 Hz) with physical meanings instead of single-frequency impedances were extracted to fuse together based on LS-SVM to improve the accuracy of cell viability evaluation (see Figure 1).

The LS-SVM prediction model can solve the effects of the factors like cell adhesion status during drug test, resistance between cells at high density, impedance fluctuations caused by cell micromotion, and drift of the most sensitive frequency. Cell viability obtained by manual cell counting was assumed as the output of prediction model. By the way, not all interdigitated electrode structures (IDES) are exposed to the out space. In fact, a layer of insulated thin film is attached to

the surface of 8W10E+ PET ECIS IDES, and the insulated film has five round holes (diameter = 250 μm) above each electrode allowing the electrode to be exposed to the outside environment (Wei, Zhang, Li, et al., 2019). Thus, the number of cells overgrowing on exposed electrode are about 150, which is easily counted by manual observations.

The experiment data (training set $T = [\mathbf{X}, \mathbf{Y}]$, 180 groups in total) were divided into two groups, 150 groups are used for model training while 30 groups are used as the test groups. Besides, LS-SVM Lab toolbox in MATLAB2016a was used as the training tool, and the training results is shown in Figure 5(a). The prediction values that obtained by utilizing LS-SVM model seem consistent with the actual values that obtained by manual observations. Furthermore, the maximum of errors is about 0.07, and except for the two special groups (13th and 25th groups), the errors of other groups are all below 0.04. We can see from the Figure 2(d), the impedance of cell micromotion measured at 4 kHz has large fluctuations in 20 min, and simultaneously causes larger relative errors (SGC-7901: ~ 0.5, BGC-823: ~ 0.3) than LS-SVM approach. Thus, it seems that evaluating cell viability using LS-SVM model is more stable and more accurate than single-frequency method. Moreover, the comparisons between fusion method and single-frequency calibrated with standard method will be discussed in detail in next section.

3.4 | Comparison on accuracy between fusion method and single-frequency method

Improving accuracy of cell viability evaluation is of great importance for precision medicine (Cotchim et al., 2020; Vo et al., 2019). However, the single-frequency method is largely affected by many factors such as the changes of cell adhesion status during drug test, resistance between cells at high cell density, impedance fluctuations caused by cell micromotion and drift of the most sensitive frequency. In this paper, a fusion approach for cell viability assessment based on LS-SVM was proposed by fusing multiple impedances which represent various physical properties of cell-electrode system.

Furthermore, the correlation coefficient r_1 between the single-frequency method and standard method (cell counting method) is about 0.981, while the correlation coefficient r_2 between the fusion method and standard method is 0.996. It means that the prediction values of fusion method are closer to the standard method than the single-frequency method. Besides, as can be seen from Figure 6(a) that the error bar of single-frequency method is larger than the fusion method, which can convincingly prove a good stability of fusion method. The relative errors of single-frequency method and fusion

method are about 0.08 and 0.04, respectively, as shown in Figure 6(b). In fact, the errors of single-frequency method will be larger if cell micromotion is taken into consideration (see Figure 2(d)). The decreasing of relative errors using fusion method contributes to the improvement of cell viability evaluation. For example, the adhesion and detachment of about 5 cells can be detected using fusion method in ECIS, while that of only about 20 cells can be distinguished using single-frequency method. However, the fusion approach based on LS-SVM cannot improve the detection limitation (about 10-5000 cells) compared with general single-frequency method. In fact, the detection limitation is usually determined by the properties of device, not related with the performance of algorithm. By the way, the error bar of standard method represents the situation that part of individual cells is adhered on bared electrode and other part of cells is on electrode with insulated film.

Besides, the LS-SVM model cannot solve the calculations of large number of samples, because its computation time will also increase with the increasing of sample number (S. Zhou et al., 2020). Thus, some high-throughput detections like characterizing individual cell properties based on microfluidic chip (Zhao et al., 2013; Zhao et al., 2018; Y. Zhou et al., 2018), need some deep-learning methods based on large data, like RBF, BP neural networks.

4 | CONCLUSION

In this paper, an approach for improving the stability and accuracy of cell viability evaluation was proposed by fusing multi-physical information of cell-electrode system utilizing LS-SVM model. Firstly, the influencing factors on cellular impedance spectroscopy were analyzed showing that cell adhesion status cultured for above 8 h has little effect on CIS, however the cell adhesion status during drug test has large influences on CIS. In addition, the high cell density largely affects the linear relationship between cell number and single-frequency impedance due to the influencing of resistance between cells. Besides, cell micromotion causes about 0.5 (SGC-7901, normalized impedance at 4 kHz) and 0.3 (BGC-823, normalized impedance at 4 kHz) maximum relative errors, ultimately leading to the instability of cell viability evaluation. Moreover, electrode properties like electrode sizes and electrode materials change the CPE of electrode-electrolyte interface, which can cause the drift of the most sensitive frequency. By theoretical analysis according to the equivalent circuit model, we know that CPE of electrode-electrolyte interface, resistance and capacitance of adhered cells, and solution

resistance, dominate the impedance spectroscopy in the whole LF band (< 10^3 Hz), the part of LF and the whole MF band (10^2-10^5 Hz), and HF band (> 10^5 Hz), respectively.

The results indicate that the maximum relative error of single-frequency method is about 0.5 due to the effects of cell micromotion, while that of fusion method is below 0.07. It means that the fusion method is more stable than single-frequency method. Furthermore, the correlation coefficient of single-frequency method with standard method is 0.981, while that of fusion method is 0.996. Meanwhile, the mean relative error of single-frequency is about 0.08, while that of fusion method is about 0.04. It indicates that the prediction results are more accurate than the single-frequency method.

Although the fusion method can improve the resolution rate from 20 cells to 5 cells, it cannot improve the detection limitation (about 10-5000 cells) compared with general single-frequency method. Actually, the detection limitation is usually determined by the properties of biosensors, not related with the performance of algorithm. Thus, the biosensor properties can be studied to improve the detection limitation of cell viability assessment.

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DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interest.

REFERENCES

Abdur Rahman, A. R., Price, D. T., & Bhansali, S. (2007). Effect of electrode geometry on the impedance evaluation of tissue and cell culture. *Sensors and Actuators B: Chemical, 127*(1), 89-96. doi:https://doi.org/10.1016/j.snb.2007.07.038

Anh-Nguyen, T., Tiberius, B., Pliquett, U., & Urban, G. A. (2016). An impedance biosensor for monitoring cancer cell attachment, spreading and drug-induced apoptosis. *Sensors and Actuators A: Physical, 241*, 231-237. doi:https://doi.org/10.1016/j.sna.2016.02.035

Arndt, S., Seebach, J., Psathaki, K., Galla, H.-J., & Wegener, J. (2004). Bioelectrical impedance assay to monitor changes in cell shape during apoptosis. *Biosensors and Bioelectronics, 19*(6), 583-594. doi:https://doi.org/10.1016/S0956-5663(03)00269-0

Asphahani, F., Thein, M., Veiseh, O., Edmondson, D., Kosai, R., Veiseh, M., . . . Zhang, M. (2008). Influence of cell adhesion and spreading on impedance characteristics of cell-based sensors. *Biosensors and Bioelectronics, 23*(8), 1307-1313. doi:https://doi.org/10.1016/j.bios.2007.11.021

Bashraheel, S. S., Domling, A., & Goda, S. K. (2020). Update on targeted cancer therapies, single or in combination, and their fine tuning for precision medicine. *Biomedicine & Pharmacotherapy, 125*, 110009-110024. doi:https://doi.org/10.1016/j.biopha.2020.110009

Beltrán, N. H., Finger, R. A., Santiago-Aviles, J., & Espinoza-Vallejos, P. (2003). Effect of parasitic capacitances on impedance measurements in microsensors structures: a numerical study. *Sensors and Actuators B: Chemical, 96*(1), 139-143. doi:https://doi.org/10.1016/S0925-4005(03)00516-1

Chen, L., Manautou, J. E., Rasmussen, T. P., & Zhong, X.-b. (2019). Development of precision medicine approaches based on inter-individual variability of BCRP/ABCG2. *Acta Pharmaceutica Sinica B, 9*(4), 659-674. doi:https://doi.org/10.1016/j.apsb.2019.01.007

Chen, Y., Wong, C. C., Pui, T. S., Nadipalli, R., Weerasekera, R., Chandran, J., . . . Rahman, A. R. A. (2012). CMOS high density electrical impedance biosensor array for tumor cell detection. *Sensors and Actuators B: Chemical, 173*, 903-907. doi:https://doi.org/10.1016/j.snb.2012.07.024

Cotchim, S., Thavarungkul, P., Kanatharana, P., & Limbut, W. (2020). Multiplexed label-free electrochemical immunosensor for breast cancer precision medicine. *Analytica Chimica Acta, 1130*, 60-71. doi:https://doi.org/10.1016/j.aca.2020.07.021

Daza, P., Olmo, A., Cañete, D., & Yúfera, A. (2013). Monitoring living cell assays with bio-impedance sensors. *Sensors and Actuators B: Chemical, 176*, 605-610. doi:https://doi.org/10.1016/j.snb.2012.09.083

El Hasni, A., Schmitz, C., Bui-Göbbels, K., Bräunig, P., Jahnen-Dechent, W., & Schnakenberg, U. (2017). Electrical impedance spectroscopy of single cells in hydrodynamic traps. *Sensors and Actuators B: Chemical, 248*, 419-429. doi:https://doi.org/10.1016/j.snb.2017.04.019

Giaever, I., & Keese, C. R. (1991). Micromotion of mammalian cells measured electrically. *Proceedings of the National Academy of Sciences, 88*(17), 7896-7900. doi:https://doi.org/10.1073/pnas.88.17.7896

Keese, C. R., Wegener, J., Walker, S. R., & Giaever, I. (2004). Electrical wound-healing assay for cells <em>in vitro</em&gt. *Proceedings of the National Academy of Sciences of the United States of America, 101*(6), 1554-1559. doi:https://doi.org/10.1073/pnas.0307588100

Li, G., Zhang, R., Yang, N., Yin, C., Wei, M., Zhang, Y., & Sun, J. (2018). An approach for cell viability online detection based on the characteristics of lensfree cell diffraction fingerprint. *Biosensors and Bioelectronics, 107*, 163-169. doi:https://doi.org/10.1016/j.bios.2018.01.047

Li, L., Tang, H., Wang, J., Lin, J., & Yao, H. (2018). Rolling adhesion of cell in shear flow: A theoretical model. *Journal of the Mechanics and Physics of Solids, 119*, 369-381. doi:https://doi.org/10.1016/j.jmps.2018.07.013

Liu, Q., Yu, J., Xiao, L., Tang, J. C. O., Zhang, Y., Wang, P., & Yang, M. (2009). Impedance studies of bio-behavior and chemosensitivity of cancer cells by micro-electrode arrays. *Biosensors and Bioelectronics, 24*(5), 1305-1310. doi:https://doi.org/10.1016/j.bios.2008.07.044

Mahomed, S., Padayatchi, N., Singh, J., & Naidoo, K. (2019). Precision medicine in resistant Tuberculosis: Treat the correct patient, at the correct time, with the correct drug. *Journal of Infection, 78*(4), 261-268. doi:https://doi.org/10.1016/j.jinf.2019.03.006

Mamouni, J., & Yang, L. (2011). Interdigitated microelectrode-based microchip for electrical impedance spectroscopic study of oral cancer cells. *Biomedical Microdevices, 13*(6), 1075-1088. doi:https://doi.org/10.1007/s10544-011-9577-8

Opp, D., Wafula, B., Lim, J., Huang, E., Lo, J.-C., & Lo, C.-M. (2009). Use of electric cell–substrate impedance sensing to assess in vitro cytotoxicity. *Biosensors and Bioelectronics, 24*(8), 2625-2629. doi:https://doi.org/10.1016/j.bios.2009.01.015

Pradhan, R., Mandal, M., Mitra, A., & Das, S. (2014). Monitoring cellular activities of cancer cells using impedance sensing devices. *Sensors and Actuators B: Chemical, 193*, 478-483. doi:https://doi.org/10.1016/j.snb.2013.12.003

Pui, T. S., Chen, Y., Wong, C. C., Nadipalli, R., Weerasekera, R., Arya, S. K., . . . Rahman, A. R. A. (2013). High density CMOS electrode array for high-throughput and automated cell counting. *Sensors and Actuators B: Chemical, 181*, 842-849. doi:https://doi.org/10.1016/j.snb.2013.02.065

Qiu, Y., Liao, R., & Zhang, X. (2008). Real-Time Monitoring Primary Cardiomyocyte Adhesion Based on Electrochemical Impedance Spectroscopy and Electrical Cell−Substrate Impedance Sensing. *Analytical Chemistry, 80*(4), 990-996. doi:https://doi.org/10.1021/ac701745c

Ren, D., & Chui, C. O. (2018). Feasibility of Tracking Multiple Single-Cell Properties with Impedance Spectroscopy. *ACS Sensors, 3*(5), 1005-1015. doi:https://doi.org/10.1021/acssensors.8b00152

Seriburi, P., McGuire, S., Shastry, A., Böhringer, K. F., & Meldrum, D. R. (2008). Measurement of the Cell−Substrate Separation and the Projected Area of an Individual Adherent Cell Using Electric Cell−Substrate Impedance Sensing. *Analytical Chemistry, 80*(10), 3677-3683. doi:https://doi.org/10.1021/ac800036c

Song, C., Kong, Y., Huang, L., Luo, H., & Zhu, X. (2020). Big data-driven precision medicine: Starting the custom-made era of iatrology. *Biomedicine & Pharmacotherapy, 129*, 110445. doi:https://doi.org/10.1016/j.biopha.2020.110445

Sutulo, S., Moreira, L., & Guedes Soares, C. (2002). Mathematical models for ship path prediction in manoeuvring simulation systems. *Ocean Engineering, 29*(1), 1-19. doi:https://doi.org/10.1016/S0029-8018(01)00023-3

Tan, A., Huang, H., Zhang, P., & Li, S. (2019). Network-based cancer precision medicine: A new emerging paradigm. *Cancer Letters, 458*, 39-45. doi:https://doi.org/10.1016/j.canlet.2019.05.015

Vetsch, J., Wakefield, C. E., Techakesari, P., Warby, M., Ziegler, D. S., O'Brien, T. A., . . . Tucker, K. (2019). Healthcare professionals' attitudes toward cancer precision medicine: A systematic review. *Seminars in Oncology, 46*(3), 291-303. doi:https://doi.org/10.1053/j.seminoncol.2019.05.001

Vo, K., Parsons, D., & Seibel, N. (2019). Precision Medicine in Pediatric Oncology. *Surgical Oncology Clinics of North America, 29*, 63-72. doi:https://doi.org/10.1016/j.soc.2019.08.005

Wang, L., Wang, L., Yin, H., Xing, W., Yu, Z., Guo, M., & Cheng, J. (2010). Real-time, label-free monitoring of the cell cycle with a cellular impedance sensing chip. *Biosensors and Bioelectronics, 25*(5), 990-995. doi:https://doi.org/10.1016/j.bios.2009.09.012

Wei, M., Zhang, R., Zhang, F., Zhang, Y., Li, G., Miao, R., & Shao, S. (2019). An Evaluation Approach of Cell Viability Based on Cell Detachment Assay in a Single-Channel Integrated Microfluidic Chip. *ACS Sensors, 4*(10), 2654-2661. doi:https://doi.org/10.1021/acssensors.9b01061

Wei, M., Zhang, Y., Li, G., Ni, Y., Wang, S., Zhang, F., . . . Wang, P. (2019). A cell viability assessment approach based on electrical wound-healing impedance characteristics. *Biosensors and Bioelectronics, 124-125*, 25-32. doi:https://doi.org/10.1016/j.bios.2018.09.080

Xiao, C., Lachance, B., Sunahara, G., & Luong, J. H. T. (2002). An In-Depth Analysis of Electric Cell−Substrate Impedance Sensing To Study the Attachment and Spreading of Mammalian Cells. *Analytical Chemistry, 74*(6), 1333-1339. doi:https://doi.org/10.1021/ac011104a

Xiao, C., & Luong, J. H. T. (2010). A simple mathematical model for electric cell-substrate impedance sensing with extended applications. *Biosensors and Bioelectronics, 25*(7), 1774-1780. doi:https://doi.org/10.1016/j.bios.2009.12.025

Xu, H., & Soares, C. G. (2019). Hydrodynamic coefficient estimation for ship manoeuvring in shallow water using an optimal truncated LS-SVM. *Ocean Engineering, 191*, 106488-106500. doi:https://doi.org/10.1016/j.oceaneng.2019.106488

Xu, Y., Xie, X., Duan, Y., Wang, L., Cheng, Z., & Cheng, J. (2016). A review of impedance measurements of whole cells. *Biosensors and Bioelectronics, 77*, 824-836. doi:https://doi.org/10.1016/j.bios.2015.10.027

Yang, L., Arias, L. R., Lane, T. S., Yancey, M. D., & Mamouni, J. (2011). Real-time electrical impedance-based measurement to distinguish oral cancer cells and non-cancer oral epithelial cells. *Analytical and Bioanalytical Chemistry, 399*(5), 1823-1833. doi:https://doi.org/10.1007/s00216-010-4584-9

Zhang, F., Jin, T., Hu, Q., & He, P. (2018). Distinguishing skin cancer cells and normal cells using electrical impedance spectroscopy. *Journal of Electroanalytical Chemistry, 823*, 531-536. doi:https://doi.org/10.1016/j.jelechem.2018.06.021

Zhang, R., Wei, M., Chen, S., Li, G., Zhang, F., Yang, N., & Huang, L. (2018). A cell viability assessment method based on area-normalized impedance spectrum (ANIS). *Biosensors and Bioelectronics, 110*, 193-200. doi:https://doi.org/10.1016/j.bios.2018.03.041

Zhang, X., Wang, W., Nordin, A. N., Li, F., Jang, S., & Voiculescu, I. (2017). The influence of the electrode dimension on the detection sensitivity of electric cell–substrate impedance sensing (ECIS) and its mathematical modeling. *Sensors and Actuators B: Chemical, 247*, 780-790. doi:https://doi.org/10.1016/j.snb.2017.03.047

Zhao, Y., Chen, D., Li, H., Luo, Y., Deng, B., Huang, S.-B., . . . Chen, J. (2013). A microfluidic system enabling continuous characterization of specific membrane capacitance and cytoplasm conductivity of single cells in suspension. *Biosensors and Bioelectronics, 43*, 304-307. doi:https://doi.org/10.1016/j.bios.2012.12.035

Zhao, Y., Wang, K., Chen, D., Fan, B., Xu, Y., Ye, Y., . . . Huang, C. (2018). Development of microfluidic impedance cytometry enabling the quantification of specific membrane capacitance and cytoplasm conductivity from 100,000 single cells. *Biosensors and Bioelectronics, 111*, 138-143. doi:https://doi.org/10.1016/j.bios.2018.04.015

Zhou, S., Chu, X., Cao, S., Liu, X., & Zhou, Y. (2020). Prediction of the ground temperature with ANN, LS-SVM and fuzzy LS-SVM for GSHP application. *Geothermics, 84*, 101757-101767. doi:https://doi.org/10.1016/j.geothermics.2019.101757

Zhou, Y., Yang, D., Zhou, Y., Khoo, B. L., Han, J., & Ai, Y. (2018). Characterizing Deformability and Electrical Impedance of Cancer Cells in a Microfluidic Device. *Analytical Chemistry, 90*(1), 912-919. doi:https://doi.org/10.1021/acs.analchem.7b03859

FIGURE LEGENDS

FIGURE 1 Comparison diagram of the fusion method and single-frequency method. (a) Single-frequency method utilizes an impedance at a constant frequency or the most sensitive frequency for cell viability evaluation. The accuracy of single-frequency method is about 98.1%, the distinguishing resolution is about 20 cells and the range of relative errors is from 0.08 to 0.5. (b) Fusion method based on LS-SVM utilizes multi-frequency impedances for cell viability evaluation. The accuracy of fusion method is about 99.6%, the distinguishing resolution is about 5 cells and the range of relative errors is from 0.04 to 0.07.

FIGURE 2 Monitoring of cell dynamic events. (a) The process of cell adhesion with course of time. P1 to P4 represent cell seeding, initial adhesion, cell adhesion and cell spreading, respectively. (b) Diagram of normalized impedance spectroscopy with different cell adhesion statuses. (c) Normalized impedance of cell dynamic events from cell seeding to cell proliferation. (d) Normalized impedance of cell movement at 4 kHz.

FIGURE 3 Theoretical model of cell adhesion. (a) The equivalent circuit model of cell adhesion on electrodes. (b) The diagram of main current paths in different frequency bands. (c) The contribution area of elements to CIS. CPE dominates to the CIS in LF band, cell impedances (R_c, C_c) dominate to CIS in LF and MF bands, while R_s mainly dominates to CIS in HF band.

FIGURE 4 The influencing factors on overall impedance. (a) Diagram of the adhesion cells emphasizing the spaces between the cell and the substratum. (b) Cells adhered on interdigitated electrodes with low density ($R_b = 0$). (c) Cells adhered on interdigitated electrodes with high density ($R_b > 0$). (d) The relationship between cell number and normalized impedance (NI) at 10 kHz. (e) Diagram of interdigital electrode with different widths (w = 25, 50, 100 μm) and same spacing (s = 50 μm). (f) The impedance of interdigital electrode with different widths measured at 10 kHz. (g) The contributions of different CPEs (caused by different electrode sizes) to the most sensitive frequency.

FIGURE 5 Results of cell viability evaluation based on LS-SVM. (a) The training results compared with actual values. (b) The relative errors between prediction value and actual value.

FIGURE 6 (a) Comparison analysis among the standard method, single-frequency method and fusion method. (b) The relative errors of two methods compared with standard method.