

Electric cell-substrate impedance sensing in cancer research: An in-depth exploration of impedance sensing for profiling cancer cell behavior

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ABSTRACT

Impedance assessment in living biological cells has gained popularity as a label-free, real-time, and quantitative analytical approach for determining cellular states. Electric cell substrate impedance sensing (ECIS) provides valuable insights into cell adhesion, the intricate interactions between cells and their underlying substrate, and cellular communication. Further, the ECIS method's high sensitivity enables the observation of biological events at the single-cell level and the precise determination of cell-substrate distances at the nanoscale. Importantly, using cellular electrical properties as a valuable marker, ECIS can shed light on how cancer cells proliferate, migrate, and invade. In this article, we discuss electric cell-substrate impedance sensing as it relates to electrode design, manufacturing, and application in impedance measurement. The present review also outlines our current understanding of the advantages of ECIS in studying cancer cell behavior and drug screening and their prospective future modifications. Impedance-sensing approaches in biology have many potential applications, including point-of-need diagnostics, highly specialized devices, and seamless integration. In summary, this impedance-based technology might one day be an attractive diagnostic tool in cancer research.

1. Introduction

Living cells have dielectric properties. The cytoskeletal elements control the distinctive dielectric characteristics of living biological cell membranes [1]. The plasma membrane of a cell has two distinct roles. Its functional role is to maintain the ionic balance between its two sides while controlling the material exchange between internal and external media. Its physical function is to confine the cell and isolate it from its external microenvironment [2]. The dielectric research of cells addresses a number of important morphological and functional characteristics of cells [3]. Studies showed that the plasma membrane's

dielectric response dominates total system capacitance and cell impedance. Modifications in the morphology or functionality of the cellular membrane can be related to changes in electrical characteristics of the cellular structure. As a result, changes in membrane dielectric characteristics may signal the presence of disease or stress in aging [4].

Several assays for assessing cell behavior have been designed, including western blotting, RT-PCR, flow cytometry, and immunofluorescence assays. In the past few years, efforts have been made in developing novel methodologies for conducting label-free and straightforward analyses [5].

Electric cell-substrate impedance sensing (ECIS) is a technique that

Abbreviations: ECIS, electric cell-substrate impedance sensing; AC, alternating current; DC, direct electric current; Cm, membrane capacitance; R, resistance; ITO, Indium Tin Oxide; PVD, Physical vapor deposition; RIE, reactive ion etching; PECVD, plasma-enhanced chemical vapor deposition; MEMS, micro-electro-mechanical systems; OSCC, oral squamous cell carcinoma; CBD, cannabidiol; ECM, extracellular matrix; TME, tumor microenvironment; HFF, Human foreskin fibroblast; FAK, focal adhesion kinase; PMC, pleural mesothelial cell; HUVEC, human umbilical vein endothelial cell; EMT, epithelial-mesenchymal transition; TGF- β 1, transforming growth factor- β 1; FACS, fluorescence-activated cell sorting; ABC, ATP-binding cassette; Pgp, P-glycoprotein; MRP1, MDR-related protein 1; BCRP, breast cancer resistance protein; MDR, multidrug resistance; MBZ, Mebendazole; PTX, paclitaxel; CytD, Cytochalasin D; CNT, carbon nanotube; CTT, cancerous tongue tissue; NTT, normal tongue tissue; LIA, lock-in amplifier; DUT, device under test; SUT, signal under test.

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utilizes electrical impedance to investigate cellular behaviors within a cell culture system without the need for labeling or external markers. This method enables real-time monitoring and analysis of various cellular activities. Apoptosis, viral infections, wound healing, cytotoxicity, and cytoprotection have all been studied using ECIS in cellular biology and drug screening [6].

The objective of this review is to present a comprehensive analysis of the latest advancements in impedance measurements pertaining to cancer cells. We discussed the fundamentals of the electrical properties of cells, as well as a brief introduction of the ECIS system, along with our current understanding of how this is used to monitor cancer cell behavior and drug screening. Furthermore, we discussed briefly how this technology will evolve in the future and the challenges it will face.

2. Cells and cell membranes

The plasma membrane envelops each individual cell and functions as a semi-permeable barrier, defining the interior (or cytoplasm) of an individual cell. The composition of plasma membranes typically consists of approximately equal proportions of lipids and proteins, accounting for roughly 50 % each in terms of weight [7]. Within this composition, glycolipids and glycoproteins contribute to 5 to 10 % of the overall mass of the membrane. The lipids comprising the membrane bilayer exhibit amphipathic characteristics, denoting the presence of both a polar head and a nonpolar tail within their molecular structure [8]. Polar regions tend to be hydrophilic, whereas nonpolar regions are hydrophobic. The lipids within the cell membrane have a unique configuration due to the presence of the cell membrane, which acts as a barrier between two aqueous compartments, namely the intracellular fluid or cytoplasm and the extracellular fluid. These lipids are organized into two parallel rows, with their polar ends close to the aqueous environment and their nonpolar ends facing the nonpolar regions of molecules in the opposite row. Although lipids are the main structural components of membranes, proteins are responsible for carrying out specific functions within the membrane [9].

In 1972, Jonathan Singer and Garth Nicolson proposed the fluid mosaic model of membrane structure, a concept that has gained widespread acceptance as the fundamental principle governing the arrangement of biological membranes [10]. This hypothesis suggests that biological membranes primarily consist of lipid bilayers through which proteins pass partially or completely. The fluid mosaic model was the first to incorporate fluidity, membrane channels, and multiple protein/bilayers coupling into a single hypothesis. Studies have shown that cell membranes can store electrical charge [11]. As a result, it is plausible to expect ECIS to be able to monitor this cell membrane characteristic during cell growth and proliferation.

3. Cell membrane electrical properties

Cell membrane electrical properties determine how electrical signals travel across the plasma membrane [12]. The morphological characteristics of the membrane material and the structural and functional aspects of the ion channels embedded within the membrane contribute to the manifestation of these electrical properties. The cellular membrane operates like a fundamental electrical circuit, exhibiting properties of resistance and capacitance.

Current: An electrical current is a flow of charges calculated in coulombs per second or amperes. (Abbreviated A).

Resistance: A resistance (R) measures how difficult it is for current to flow through an electrical circuit; the greater the difficulty, the higher the resistance. The ohm (Ω) is a unit of resistance.

Conductance: The conductance, abbreviated G, is the inverse of resistance and represents how easily electrical current flows in a circuit. The Siemens (S), formerly the mho, is the accepted unit of conductance.

Voltage: Voltage, also known as potential difference, is the separation of opposing charges in space; the greater the separation, the higher

the voltage, and the greater the likelihood that the charges will flow toward one another. A voltage measurement requires the comparison of two points. Further, a voltage cannot exist at a single location in space. A volt is the unit of voltage (V). Ohm's law relates voltage, current, and resistance to one another: $V = IR$. Ohm's law can be rewritten as $I = GV$ [13].

4. Membrane impedance

Impedance, represented by the symbol Z, is a crucial parameter used to assess the resistance of electronic circuits, components, or systems to both alternating current (AC) and direct electric current (DC) [14]. The impedance and potential of various synthetic membranes have been measured. Changes in impedance are calculated as functions of current. Resistance and impedance are two distinct concepts; resistance is a DC phenomenon, while impedance describes its AC counterpart. The resistance of a conductor converts electrical energy into heat when electrons collide with its ionic lattice. The resistivities and impedances of various membranes differ from one another [11].

5. Electric cell-substrate impedance sensing (ECIS) definition

Giaever and Keese were the first to work on ECIS in 1984, when they developed their first biosensor to track cell shape [15]. This method offers a non-invasive way to measure the frequency-dependent electrical impedance of gold-film electrodes covered by cells. The measured impedance can be used for data modeling to obtain morphological information about the cells present on the surface of the electrode [16]. Mammalian cells act substantially like insulating particles when they adhere to and spread across the surface of a flat gold-film electrode [17]. This phenomenon increases the overall impedance of the electrode, as the presence of cells hinders the unrestricted flow of current from the electrode into the surrounding electrolyte. When the AC signal with a high frequency is applied, a certain amount of current can capacitive couple across plasma membranes and cross transcellular pathways [18]. However, for the majority of frequencies, the current must skip over the cellular structures. Before the current may escape through the intercellular shunt and into the bulk electrolyte along these paracellular pathways, it must first travel through the small channels between the cell and electrode surface [19] (Fig. 1A).

As a result, the intercellular cleft, cell adhesion zone, and plasma membrane all contribute varying amounts of individual resistance to trans-and paracellular currents. Since the frequency of the applied AC signal affects how much trans-and paracellular impedance is present, impedance measurements over a wide frequency range can be used to separate and quantify each component separately [20]. Giaever and Keese (1991) developed an analytical model that can be fit to experimental data to deconvolute the entire ECIS signal [32]. Three separate factors in the ECIS-model describe the impedance contributions of the adhering cell layers. The impedance contribution of the intercellular shunt is accounted for by a parameter R_b (resistance between cells) (Fig. 1B) [21]. The α parameter is employed to account for cell-substrate adhesion zone impedance contributions. α is calculated by the following formula

$$\alpha = r_c \sqrt{\frac{\rho}{d}}$$

the radius of the cell is r_c , the specific resistivity of the electrolyte underneath the cell is ρ , and the distance between membrane and electrode surface is d . The membrane capacitance C_m describes the plasma membrane's dielectric characteristics. This equation describes the overall impedance of the electrode Z that is covered by cells [19].

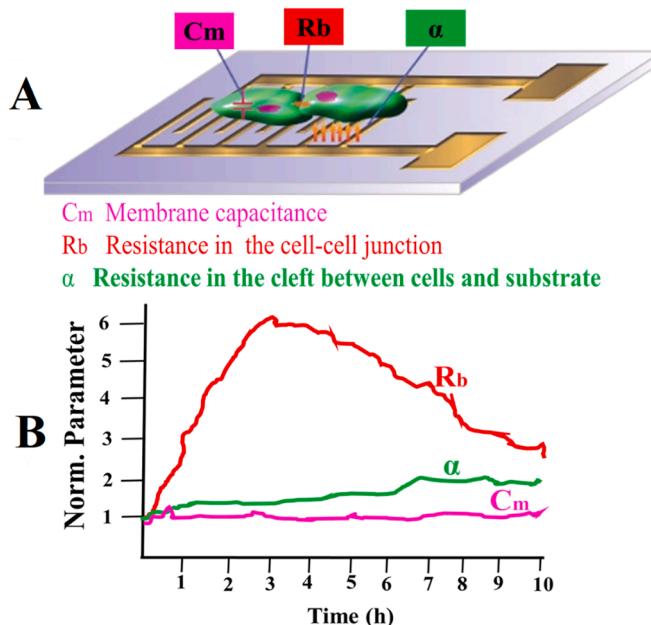


Fig. 1. Principle of ECIS. (A) Schematic of adherent cells on gold-film electrodes. (B) The parameters that can be extracted from electrochemical impedance recordings and subsequent data modeling R_b (Resistance in the cell-cell junction), α (resistance in the cleft between cells and substrate), and C_m (Membrane capacitance) are assigned to their respective cellular origin [19].

$$\frac{1}{Z_{total}} = \frac{1}{Z_n} \left\{ \frac{Z_n}{Z_n + Z_m} + \frac{Z_m / (Z_n + Z_m)}{\left(\frac{1}{2} \right) \gamma \cdot r_c \cdot \left(\frac{I_0(\gamma r_c)}{I_1(\gamma r_c)} \right) + R_b \left(\left(\frac{1}{Z_n} \right) + \left(\frac{1}{Z_m} \right) \right)} \right\}$$

with

$$\gamma = \frac{\alpha}{r_c} \left(\frac{1}{Z_n} + \frac{1}{Z_m} \right)$$

Z_m is the total impedance of the apical and basolateral plasma membranes, with Z_m = 2/iωC_m, and Z_n is the impedance of the cell-free gold-film electrode. I₀ and I₁ are modified Bessel functions of order 0 and 1, respectively, of the first kind. Using the empirical constant phase element (CPE) for modeling, the impedance of the cell-free electrode was calculated from the impedance spectra of the cell-covered electrode at the low-frequency end, resulting in Z_n = Z_{CPE}. The resistivity of the bulk electrolyte was determined independently using the high-frequency end of the spectrum. This value was then added to the impedance measurements [19].

A sinusoidal AC voltage with peak amplitudes of 10 mV (rms) was used to obtain impedance values between 1 and 106 Hz. The DC bias potential of the two identical gold electrodes was set to 0 V. All impedances were measured using a Solatron SI-1260 continuous wave impedance analyzer [19].

5.1. Electrode/cell membrane circuit model of ECIS

When exposed to an electric field, a cell exhibits the characteristics of passive electronic elements. When examining a complete layer of cells that have been cultivated on electrodes and supplemented with a cell culture medium, the basic model of a resistor and capacitor must be expanded to encompass an entire electrical network [22]. The so-called equivalent circuit requires consideration of the resistance of the culture medium (R_{Med}), as well as the capacitance (C_{Electr}) and resistance (R_{Electr}) that characterize the interaction between the electrode and electrolyte. Fig. 2 shows a simple, general equivalent circuit for an adhering growing cell layer. An advantage of applying mathematical methods to

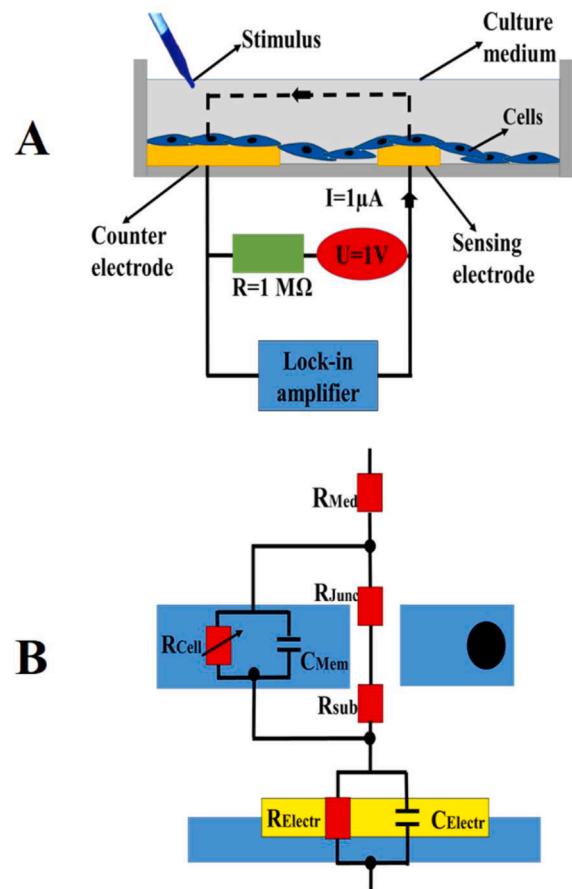


Fig. 2. Schematic of the ECIS system and representative equivalent circuit for an adherent growing cells layer. (A) Cross section of an ECIS culture well. The culture media enclose the proliferating cells on the surface of the counter electrode and sensing electrode. The electrodes are linked to a lock-in amplifier, and an AC signal is administered through a 1 MΩ resistor to establish a consistent current source. Stimuli can be introduced into the culture media at any given moment. (B) ECIS quantifies the total of all individual contributions to the impedance. The analysis must take into account the resistance of the culture medium (R_{Med}), as well as the impedance caused by the electrode/electrolyte interface. This interface can be simplified as a parallel combination of a resistor (R_{Electr}) and a capacitor (C_{Electr}). Additionally, the electrical properties of the cell membrane, which can be described as a parallel connection of resistance (R_{Cell}) and capacitance (C_{Mem}), must also be considered. The value of R_{Cell} is changeable, as it relies on the cell's permeability to the current. The analogous circuit can be expanded and improved indefinitely. Junctional resistance (R_{Junc}) and subendothelial resistance (R_{Sub}) were included in the circuit as examples [23].

describing biological systems is that the circuits can be continually improved and tailored to address specific experimental questions. For instance, one can consider the impedance caused by intra-cellular organelles or differentiate the effects of cell-cell (R_{Junc}) and cell-substrate adhesions (R_{Sub}) on the overall impedance. However, the objective of the modeling should always be to utilize the fewest elements necessary to represent all aspects of the measured impedance spectrum accurately [23].

5.2. ECIS detection equipment and signal processing

The impedance of cells is often measured using an impedance converter and a lock-in amplifier (LIA) [24]. The impedance converter is an alternating current (AC) self-balancing bridge comprising a basic operational amplifier and a feedback resistor. In this method, an alternating current (AC) excitation signal V_{in} is applied to one port of the device

under test (DUT), while the feedback resistor R_f carries the same current that passes through the DUT. In an ideal scenario where there is no phase shift in the op-amp, the current passing through the DUT is directly proportional to the voltage across [25]. Analyzing the output voltage, V_{out} , allows you to determine the DUT's complex impedance. The phase-sensitive detector, or LIA, has the ability to retrieve faint signals from a noisy background. The output voltage, V_{out} , also known as signal under test (SUT), is amplified by the in-phase and quadrature carrier signal, V_c , which has the same frequency as V_{in} . Next, the magnitude and phase of the SUT are obtained using correlative demodulation with a carrier signal at the identical reference frequency [26]. Benchtop instruments, such as LIAs created by Stanford Research Systems Inc., Zurich Instruments AG, NF Corp., SBT instruments, Sine Scientific Instruments, and Liquid Instruments, are typically connected directly or through custom printed circuit boards (PCBs) to microfluidic devices. In this context, PCBs are commonly modified with control modules, such as multiplexers, in order to activate sensing electrodes on the devices. Integrating devices reduces costs, whereas portable systems are appropriate for a broader range of circumstances. In response, researchers began incorporating impedance measurement circuitry onto PCBs [27]. AD5933 integrated circuit chip, a cost-effective impedance analyzer system, is now available in embedded portable systems. Significantly, the frequency range of this system is limited to a maximum of 100 kHz, and its precision is inferior to that of tabletop instruments [28]. Huang et al. created a digital lock-in amplifier (DLIA) with a large frequency range of up to 65 MHz [29]. This DLIA has a low input noise of 4.4 nV/ $\sqrt{\text{Hz}}$ and a dynamic reserve of 120 dB. Additionally, it maintains a phase deviation of less than 0.02° across the whole frequency range. The portability of this impedance system has been showcased by impedance measurements of three distinct sets of microbeads, each with varying sizes [29].

The signal processing unit receives a digital value representing the frequency to be analyzed and outputs the complex impedance result. The procedure can be divided into two stages: stimulation and reading. The former consists of frequency generation, which includes regulated oscillation, digital-to-analogue conversion, and amplitude adjustment. The latter pertains to impedance readout circuits, encompassing response amplification, analog-to-digital conversion, and impedance computation [30].

6. Basics of electrochemical impedance spectroscopy and electrical elements

Electrochemical impedance spectroscopy (EIS) provides valuable information about the kinetics and mechanisms of different electrochemical systems [31]. The technology finds extensive use in corrosion research, semiconductor science, energy conversion and storage technologies, as well as chemical and biosensing and noninvasive diagnostics [32]. EIS involves disturbing an electrochemical system that is in a balanced or stable state by applying a sinusoidal signal (alternating current or voltage) across a wide range of frequencies. The system's response to this disturbance, in terms of current or voltage, is then observed [33]. Given that the electrochemical system being examined is a linear time-invariant system, meaning that the output signal is directly proportional to the input signal and the system's behavior remains constant over time, EIS is a technique that models the relationship between the output signal (ac current or ac voltage) and the input signal (ac voltage or ac current) across a wide range of frequencies.

The significance of EIS, in comparison to other electrochemical techniques, stems from its capacity to differentiate and thus offer a plethora of data regarding the diverse electrical, electrochemical, and physical processes occurring within an existing electrochemical system [34]. This work is quite challenging because of the varying time behaviors exhibited by these diverse processes, ranging from rapid to sluggish [35]. The resistance of a liquid electrolyte, the conductivities in bulk and at the grain boundaries when using a solid polycrystalline

electrolyte, the charging and discharging of the electric double layer at the interface between electrolytes, the influence of the electrode surface morphology and electrolyte composition on the capacitive behavior of the electric double layer, the kinetics of charge transfer reactions at the electrode, reactions occurring uniformly and adsorption/desorption phenomena associated with charge transfer reactions, and mass transfer phenomena such as species diffusion to the electrode surface, all exhibit distinct time constants (τ), which measure the time behavior of these processes. The time constant of a process is defined as

$$\tau = RC$$

The variable R represents the resistance of a resistor, measured in ohms, whereas the variable C represents the capacitance of a capacitor, measured in farads (F) [36].

EIS can be effectively modeled using an equivalent electrical circuit. This circuit comprises standard passive components like resistors, capacitors, and inductors, as well as more intricate distributed elements that are interconnected in various configurations [37].

Each of these operations can be considered analogous to an electrical circuit with a distinct time constant [38]. Most electrochemical analyzers come with software that permits modeling impedance data to a model circuit in order to meet this objective. There are also specialized software programs available for modeling comparable circuits, such as Zview and Zplot by Scribner Associates, Inc. Before replicating the EIS data with an equivalent electric circuit, it is vital to ensure that the data is correct. The Kramers-Kronig test can be applied to accomplish this task. It is a feature commonly available in software accompanying electrochemical analyzers. The accuracy of a model circuit increases with the inclusion of more components, making it crucial to establish a scientific correlation between each component and an individual process [39]. When doing experiments in the time domain, such as with widely employed voltammetric techniques like cyclic voltammetry or chronoamperometry approaches, certain processes might be extremely challenging, if not impossible, to analyze. Conversely, in the frequency domain, EIS streamlines a complicated electrochemical system by separating it into distinct processes with varying time constants, allowing for straightforward analysis. It is possible to examine slow processes at low frequencies while quick processes at high frequencies. In practice, the frequency range is determined by the constraints imposed by the available equipment, the wiring connecting the electrochemical system to the instrument (limiting the high frequencies), and the long-term stability of the electrochemical system (limiting the low frequencies). Most commercially available electrochemical analyzers maintain a frequency range from 10 μHz to 1 MHz. Some manufacturers provide instruments that allow excitation frequencies in the MHz range for specialized purposes, such as studying ionic motion in polycrystalline solid ionic conductors [40]. These applications involve extremely rapid operations with microsecond time constants. A real-world scenario, however, restricts the minimum frequency limit to 1 MHz (sometimes even 10–100 MHz) [36]. This limitation arises due to the extensive time necessary to conduct measurements at such low frequencies. The behavior of this system in response to a specific disturbance (such as the resulting alternating current produced by a small amplitude alternating voltage disturbance, usually added to a direct current voltage associated with the formal potential E_0 of the redox couple) is determined by three mechanisms: the process of charging and discharging the electric double layer at the interface between the electrode and electrolyte, the rate of the faradaic reaction, and the movement of the redox species from the bulk solution to the electrode surface. The accuracy of the impedimetric data can be further assessed by employing the Kramers–Kronig relations. The Kramers-Kronig transformations were derived based on the assumptions of system stability, linearity of reaction to perturbations, and causality, where the response to a perturbation cannot occur before the perturbation itself [40].

These relationships are commonly employed in a validation process to detect the frequency ranges that may have been affected by instru-

ment artifacts or non-stationary behavior. The imaginary component of the impedance, \dot{Z} , can be determined by means of the real component using the following equation [41]:

$$Z(\omega) = \frac{2\omega}{\pi} \int_0^\infty \frac{Z(x) - Z(\omega)}{x^2 - \omega^2} dx$$

Here, ω represents the angular frequency, \dot{Z} represents the imaginary component of the impedance, and Z'' represents the real component of the impedance. If the impedance's high-frequency limit ($Z''(\infty)$) is known, the real part can be derived from the imaginary part using the following equation [41]:

$$Z(\omega) = Z''(\infty) + \frac{2}{\pi} \int_0^\infty \frac{xZ''(x) - \omega Z''(\omega)}{x^2 - \omega^2} dx$$

7. Comparison of electrical methods for measuring cell impedance

Impedance measurements are of three types: ECIS [42], Impedance Flow Cytometry (IFC) [43], and EIS [33]. ECIS is utilized to identify cells that are attached to a surface [42], whereas IFC is suitable for quantifying cells that are suspended and used for analyzing blood cells [42]. EIS is a technique that involves sweeping many frequencies to measure impedance. EIS provides greater insight about the cell membrane and cytoplasm than single frequency impedance testing. However, it does require a longer detection time. Hence, EIS can be employed to filter out the suitable frequencies for detection, and subsequently, specific, or particular frequencies are selected for subsequent high-speed analysis. The frequencies used for impedance sensing of single cells (2 kHz) and multiple cells (60 kHz) varied due to variations in their most responsive frequencies observed during EIS sweeps [32]. ECIS exhibits exceptional sensitivity in accurately detecting and interpreting cellular responses to various stimuli. Thus, it is appropriate for the real-time monitoring of cellular activities to evaluate several situations simultaneously. The reading system of ECIS is significantly more cost-effective compared to IFC. This procedure is easily usable by the user and does not necessitate the presence of a technician. Typically, these three impedance measurement techniques are not employed individually but rather selected based on the specific requirements of the applications [44]. Table 1 compares the three electrical approaches used to determine cell impedance.

8. Advantages of ECIS

ECIS is a powerful tool for assessing cell properties and behavior, including analyzing the influence drugs. This makes it possible to measure impedance continuously with excellent temporal precision under standard growth conditions [45]. Assessing the cells' morphological and functional state can help determine the most favorable time period for cell manipulation. Applying a low-intensity electrical current to the cells allows for noninvasive, nondestructive, and label-free assessment. However, this method only enables the evaluation of

passive bioelectrical characteristics and does not capture action potentials. An important characteristic is that several parameters can be obtained from a single test. This approach is notable because it allows investigators to attribute alterations in resistance and capacitance to specific organelles, such as cell contacts and membranes [46]. ECIS allows for the measurement of cell-cell interactions in real-time, eliminating the need for mechanical manipulations. This feature can be used to determine the ideal time to begin an intervention, such as administering a detrimental stimulus. After that, the cellular response, such as changes in impedance, can be observed, and the exposure time can be adjusted accordingly. The extent of injury can be succinctly quantified. ECIS provides continuous impedance monitoring, and this feature allows for the reproducibility of results in subsequent studies. A damaged monolayer may heal as an effect of nearby cells migrating and replicating, as determined by impedance. For instance, the alteration in impedance in primary tubular epithelial cells damaged by histones was used to determine the optimal time for adding various drugs [47]. As a result, ECIS offered precise titration and appropriate timing of injury and regeneration, thus providing a better approach. The extent of real-time monitoring and recording of the cell population might vary, spanning multiple days, contingent upon factors such as cell type, culture conditions, and damage parameters [47]. The ECIS assay is highly advantageous for early use in drug discovery due to its ability to offer further information on ligand activity and bias. ECIS measurements offer superior temporal resolution compared to most label-based assays, enabling real-time kinetic measurements of receptor-mediated signaling. This is in contrast to the restricted dynamic resolution typically observed in label-based assays. Unlike other label-based assays, this approach is non-hazardous, allowing to evaluate receptor activity and toxicity in a single experiment without incurring extra expenses. Therefore, it is a highly effective approach to assess the caliber and selectivity of compounds at an early stage of drug discovery. A screening conducted with ECIS will exhibit less "bias" compared to conventional screening methods, as the readout does not solely concentrate on one specific pathway [48]. With limited knowledge of downstream signaling pathways, the ECIS assay is suitable for screening smaller chemical libraries with established and less-studied targets. Screening with ECIS can be conducted on cell types that are more physiologically relevant and in settings that mimic physiological conditions [49]. As a result, the findings may have greater applicability, such as improved potency prediction, compared to conventional signaling assays. Using ECIS technology early in the drug development process may reduce the number of compounds that are removed later in the process since it allows for more complete testing during the *in vitro* phase. Cell monolayer barrier function is commonly studied using radioactive or fluorescence-labeled markers. Despite the extreme sensitivity of radioisotopes, various safety precautions must be taken when handling and storing them. Further, certain isotopes' short half-lives make long-term storage impossible [50]. On the other hand, fluorophore-based probes are usually less sensitive to minute variations in monolayer permeability and possibly unstable and poorly specific. Moreover, the inherent nature of tracer compounds can influence the integrity of biological barriers. Contrarily, ECIS is a label-free and non-invasive methodology that exhibits remarkable sensitivity, requires minimal exertion, and boasting a

Table 1

Three typical techniques are used for cell impedance measurement.

Technique	Frequency range	Parameters used	Cost of Read-out system	Long-time monitoring	Type of cell	Shape of sensors
ECIS	~100 Hz–~100 KHz, single frequency or sweeping	Z , resistance, capacitance, α	Low	Available	Adherent cells	electrodes of interdigital arrays
EIS	~1 Hz–~1 MHz, single frequency or sweeping	Z , Z_{RE} , Z_{IM} , Nyquist diagram	High	Available	Adherent cells	electrodes of interdigital arrays
IFC	~100 Hz–~10 MHz, single or several frequency points	Z , phase, opacity	High	Not available	suspended cells	square electrodes arranged with a parallel overlap and coplanar arrangement

|Z|: Total impedance, Z_{RE} : Real impedance, Z_{IM} : Imaginary impedance.

low degree of setup and handling intricacy. ECIS arrays have a thin structure that allows for using bright field and phase contrast imaging techniques. Consequently, this allows for the close examination of experimental cells during an ECIS procedure. By combining ECIS with fluorescence microscopy, Rother et al. obtained data continuity and efficacy [49].

9. Electrode design and fabrication

Electrode design and fabrication are crucial in electrochemistry, electronics, sensors, and energy storage devices. They have a considerable impact on efficiency, improving reaction kinetics and decreasing side reactions. Modifying the materials and shapes of electrodes enhances selectivity while improving specificity.

A lock-in amplifier measures impedance at a specific frequency, and a computer is used to gather and analyze the data. For an accurate measurement of the impedance of biological cells, it is essential for the measurement system to have a reliable sensor that can collect the relevant data. Because cells dwell within the cell culture medium, the sensor's configuration depends on two critical elements: the electrodes that administer the electric field and the device that connects the electrodes to the liquid supply. Both of these factors are necessary for the sensor to function effectively.

The electrode plays a crucial role as a fundamental component in a sensing chip. For biological cells less than 10 micrometers in diameter, the electrode size should generally be proportional to the cell size. For this reason, microelectronics-based technology is necessary to construct the sensing electrodes. The following section will address topics related to electrodes, including substrate and electrode materials and electrode fabrication [51].

9.1. Substrate and electrode materials

The two basic base materials utilized for the fabrication of impedance sensing chips are glass [52] and silicon [53]. As the sheets are transparent, they're compatible with an array of microscopes. Only the obverse direction may be seen, considering the transparent silicon chips. Nevertheless, they are more compatible with electronic circuits, particularly amplification circuits and many various structures can be constructed using only one electrode chip [54]. Electrode materials must have excellent electrochemical and biocompatible efficiency. Gold is commonly used to produce electrodes due to its superior electrochemical and biocompatible properties. In addition to this, the modification of gold is simple, and it is compatible with the procedures used to fabricate microelectronics. Other materials used, such as Ag/AgCl [55], Pt [56], indium tin oxide (ITO) [57], Ni [58], ultra-nanocrystalline

diamond [59] and electrolyte solutions such as KCl [60] or NaCl [55].

9.2. The fabrication processes

Since 1972, a wide variety of microelectronic fabrication techniques, such as Physical vapor deposition (PVD), photolithography, and reactive ion etching (RIE), have been put into use [61]. Fig. 5 depicts a flow chart of the general fabrication technique for glass-based electrodes. In this approach, a chip (at 30 nm thickness) and the Au (at 300 nm thickness) are successively placed onto a clean glass substrate. Under certain circumstances, the Chromium (Cr) is substituted by Titanium (Ti) [52]. The purpose of the Cr layer (or Ti layer) is to improve the adhesion of the gold layer to the glass. Afterwards, lithography and etching are used to pattern the electrodes, bonding pads, and connecting circuits. After that, a plasma-enhanced chemical vapor deposition (PECVD) process is used to build an insulation layer of SiO₂ (which, in certain instances, consists of SiO₂/Si₃N₄/SiO₂) onto the Au layer that is already present on the surface of the chip. In the last process, the insulation layer that covered the electrodes and bonding pads was removed using reactive ion etching. The exposed electrodes and bonding pads are illustrated in Fig. 3.

A silicon-based chip usually comes along with the fabrication of a circuit (i.e., FET) [24] or three-dimensional structures produced using micro-electro-mechanical systems (MEMS). Therefore, utilizing traditional methods for silicon microfabrication or MEMS techniques requires two additional processes that are more sophisticated than the one used for glass chips. A metal layer is often placed on top of the silicon wafer to form impedance-sensing electrodes in the traditional manner [62]. The SiO₂ layer, which is thermally produced on the silicon wafer, is coated with a metal layer (Cr/Au or Cr/Pt). An electrode is created in a similar manner to how glass chips are insulated [63]. Overall, the design and manufacture of electrodes in ECIS is fundamental to obtaining high sensitivity, reliability, and versatility in cellular monitoring. Researchers and engineers continuously refine electrode designs to address specific biological concerns and improve the general capabilities of ECIS as a powerful tool in biomedical and cell biology research.

10. ECIS and cancer study

Cell-based impedance sensing is a promising tool for analyzing cells from both a biophysical and biological standpoint. This technology can help to explore cellular toxicity, angiogenesis, cellular permeability, signal transduction, and cellular responses in the context of fluidic dynamics. Moreover, investigations have been undertaken on cellular adhesion, migration, invasion, and intercellular interactions. It is a flexible, sensitive, and probe-free technological platform. Recent

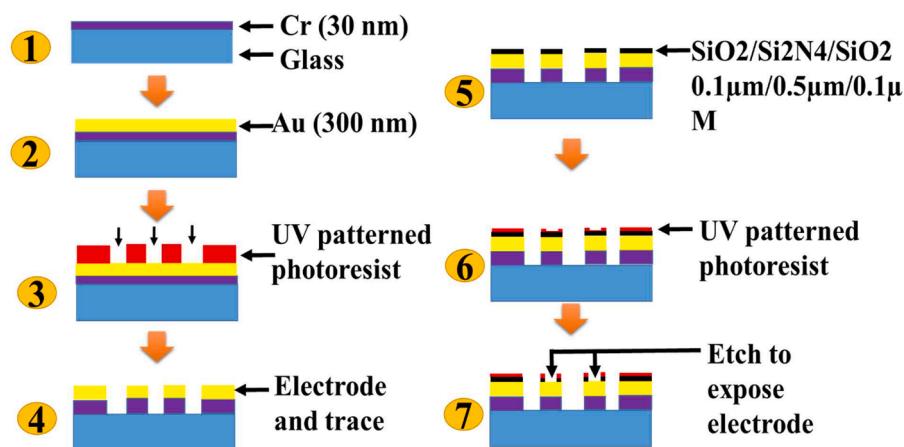


Fig. 3. Fabrication of glass-based electrodes chip [52]. 1. CR coating 2. Au coating 3. UV patterned photoresist, 4. Electrode and trace 5. Deposition of SiO₂/Si₃N₄/SiO₂ 6. UV Patterned photoresist 7. Etch to expose electrode.

technical improvements have also enabled high throughput and automated analysis. ECIS is a flexible and powerful approach for studying cancer cells. It offers the advantages of real-time monitoring, label-free detection, and the ability to investigate different aspects of cancer cell behaviors [46]. The following section describes ECIS's accomplishments in cancer research and is summarized in Table 2.

10.1. Cancer cell apoptosis detection

Apoptosis, also referred to as programmed cell death, is a natural biological process in which cells self-destruct. It is a highly intricate and multifaceted phenomenon, encompassing various pathways [64]. Deficiencies may manifest at various stages within these biological pathways, ultimately resulting in the malignant conversion of the impacted cells, the dissemination of tumors to distant sites, and the development of resistance toward therapeutic agents designed to combat cancer. Apoptosis, a fundamental process in cancer therapy, represents a frequently targeted mechanism across diverse treatment modalities. Accumulating evidence indicates that targeting apoptosis is becoming increasingly popular in cancer research [65]. However, novel drugs or treatment techniques targeted at increasing apoptosis raise a number of issues. Apoptotic cells undergo a reduction in size while maintaining the structural integrity of their plasma membrane. Further, apoptotic cells exhibit various morphological features, including detachment from adjacent cells, the formation of cytoplasmic vesicle, nuclear and plasma membrane shirking, chromatin condensation, and DNA degradation [66]. While the Annexin/PI assay, RT-PCR assay, fluorescence microscopy, and western blotting are helpful for studying cell apoptosis, they are typically complicated, indirect, and time consuming [67]. Impedance sensing provides a promising approach to enhance our comprehension of the cellular apoptosis phenomenon [68].

Arendt and colleagues employed ECIS to examine the impact of cycloheximide on the capillary endothelial cells [19]. The researchers utilized a non-invasive electrochemical method known as ECIS to observe and measure the alterations in cell shape caused by apoptosis. This methodology provided a comprehensive and precise analysis with a time resolution of minutes. The cells in ECIS are cultivated directly on the surface of small gold-film electrodes with a diameter of 2 mm. By analyzing the electrical impedance of the electrode covered with cells using non-invasive, low amplitude sensing voltages, it is feasible to infer changes in the connections between cells and between cells and the substrate. In order to enhance the sensitivity of this impedance experiment, they employed endothelial cells obtained from cerebral micro-vessels as cellular model systems. This choice was made since these cells are widely recognized for their ability to exhibit electrically tight intercellular connections. Apoptosis was triggered by the administration of cycloheximide (CHX) and confirmed using biochemical and cytological tests. The temporal progression of cellular morphological alterations was monitored with unparalleled temporal precision using impedance measurements at a frequency of 1 kHz, and subsequently compared with biochemical markers. By analyzing impedance values throughout a wide frequency range of 1–106 Hz, they were able to attribute the observed changes in impedance to modifications at the subcellular level. Their observation indicates that the breakdown of tight junctions, which operate as barriers, occurs before any alterations in cell-substrate interactions and is highly associated with the timing of protease activation [19].

Chiu et al. studied the effects of SAHA and andrographolide on Uppsala 87 Malignant Glioma (U-87 MG) cell migration and viability compared to temozolomide [69]. They used the electric cell-substrate impedance sensing (ECIS) system to monitor the migration of U-87 MG cells and observed concentration-dependent changes in viability and apoptosis. Results showed that both compounds significantly inhibited GBM cell migration, induced higher expression levels of apoptosis-related proteins such as caspase-3, BAX, and PARP, and downregulated the expression of the antiapoptotic protein Bcl-2. The

Table 2
Application of ECIS in cancer study.

Analysis	Cell type	Results/Observation	Reference
Apoptosis Monitoring	U-87 MG	Andrographolide reduced resistance at the frequency of 4 kHz	[69]
	HT-29	Aspirin decreased impedance at 40 kHz	[70]
	MCF-7	Cisplatin reduced impedance	[71]
	T47D	ZD6474 reduced the magnitude of resistance across the frequency spectrum from 10 Hz to 100 kHz	[73]
	OECM1	Cannabidiol decreased the impedance in a concentration-dependent manner	[74]
	BS-C-1	ECIS was used to conduct a wound-healing assay to examine cell migration, and the results were comparable to standard assessments	[82]
	NRK		
	MDCK		
	MDA-MB231 ^{WT}	EPLIN-overexpression resulted in a considerable decrease in resistance and capacitance	[85]
	MDA-MB231 ^{PEF/His}		
Cell migration	HFF	Real-time and label free monitoring of cell migration	[86]
	HaCaT		
	U-87 MG	Decreased glioblastoma cell migration after paxilline treatment	[87]
	hCMVEC	Quantified the dynamics of human melanoma cell infiltration through human brain endothelial cells	[91]
	NZM		
	PBMC		
	SKOV-3	HGF increased SKOV-3 cell invasion with reduced junctional resistance (R _b), SU11274 decreased the effects of HGF on R _b .	[92]
	HUVEC		
	K12	Significant variations in impedance are caused by the breakdown of endothelial connections, contraction of the monolayer, and replacement of tumor cells. These alterations were directly related to tumor cell invasiveness	[93]
	HUVEC		
Cell invasion	HCT116	ECIS provided real-time data on fibroblast-mediated migration and invasion kinetics of colon cancer cells	[94]
	HDF		
	HUVEC	Detected metastatic cells in patient samples	[95]
	MDA-MB-231		
	MDA-MB-468		
	16HBE14o	Characterization of cell adhesion in airway epithelial	[21]
	BEAS-2B		
	NCI-H292		
	A549		
	V79	Optimized the attachment and spreading of fibroblast cells on a gold surface coated with fibronectin or ovalbumin	[97]
Cell adhesion	PC3	The RT-CES system offered a convenient and quantitative means of assessing the kinetics of	[98]

(continued on next page)

Table 2 (continued)

Analysis	Cell type	Results/Observation	Reference
EMT monitoring	OVCA429	cell adhesion in a high-throughput manner Characterized cell adhesion in ovarian cancer	[99]
	NMuMG	Distinguished three-time domains during EMT.	[104]
	A549 MDA-MB-231	Initially, all cell lines displayed an increase in micromotion lasting 4 to 9 h termed transitional state I	
Tumor microenvironment monitoring	MRC-5	Impedance signal differences were examined to determine the distance effect between MRC-5 and A549 cells in a co-culture setting. Significant suppression in direct fibroblast-cancer interaction, which was greater than that imparted by indirect interaction, particularly under drug treatment circumstances	[107]
	A549		
Drug Resistance	Doxorubicin-resistant HT-29 wild type HT-29	Characterized chemo-resistant colon cancer cells. Impedance-based curve analysis revealed distinct tendencies from chemo-sensitive and chemo-resistant cells	[112]
	MCF-10A MCF-7 MDA-MB-231 MDA-MB-468	Compared to anti-actin medicines, anti-tubulin medicines produced distinct bioelectrical responses in breast cancer cells that contained semiconductive microtubules and conductive actins	[113]
	A431 HaCaT	distinguished skin cancer cells (A431) and normal cells (HaCaT) using electrical impedance spectroscopy	[115]
	SW48	CNT-ECIS detected the cancer cells with the concentration as low as 4000 cells cm ⁻² on its surface and a sensitivity of $1.7 \cdot 10^3 \text{ U cm}^2$	[116]
Cancer Detection	SW48 HT-29	Detected human colon invasive cancer cells (SW48) in a mixed cell culture of primary cancerous colon cells (HT29) without any biochemical labels	[117]
	CAL 27 Het-1A	Distinguished oral cancer cells and non-cancer oral epithelial cells based on their cellular activities on the microelectrodes in a real-time and label-free manner	[118]
	HeLa	Real-time impedance sensing clearly mirrored the progression of the cell cycle	[122]
Cell cycle monitoring	MRC-5 QUDB	The device monitored the electrical changes caused by spreading between malignant and normal lung cells	[123]

study suggests that SAHA and andrographolide are potential therapeutic agents for inhibiting cancer cell migration [69].

Finally, SAHA and andrographolide inhibited cell migration and motility exceptionally well. The study suggests that ECIS wound healing test is an effective method for identifying and screening possible therapeutic compounds that can prevent cancer cell migration.

Yin and colleagues used the ECIS technique to monitor alterations in the morphology of HT-29 human colon cancer cells following treatment with aspirin [70]. The ECIS provided a detailed and quantitative analysis of the alterations, with a time resolution of minutes. Whole-cell biosensors involve the cultivation of cells directly on the surface of tiny gold-film electrodes. By analyzing the electrical impedance of the electrode covered with cells, they inferred changes caused by aspirin in the interactions between HT-29 cells, both in cell-to-cell and cell-to-substrate connections. Also, the apoptosis was confirmed with the use of a transmission electron microscope. The data provided in their study extensively confirmed the correlation between apoptosis and the dielectric properties change of cells [70].

Anh-Nguyen et al. employed ECIS to monitor apoptosis in the MCF-7 breast cancer cell line following treatment with cisplatin [71] (Fig. 4A). An impedance biosensor was developed for label-free monitoring of MCF-7 breast cancer cells. They also assessed how these cells responded to the anticancer drug cisplatin. It was found that during the 23 h of incubation, cell spreading significantly increased the impedance magnitude in the frequency range between 10 kHz and 100 kHz. This increase was reversed after the 24 h of Cisplatin therapy. Cell apoptosis is the cause of this reversal, which was confirmed by microscopic observation of the cells (Fig. 4B). The equivalent circuit model and the impedance spectra suggested that the electrical properties in the circuit might distinguish between cellular processes such as cell death, adhesion, attachment, and spreading. Importantly, the responses of MCF-7 cells to various Cisplatin concentrations were successfully tracked. [71].

Yang et al. used electrical impedance-based measurements to distinguish oral cancer cells and non-cancer oral epithelial cells on microelectrodes in real-time and label-free manner [72]. CAL 27 and Het-1A cell lines were used as models. Cell adhesion, spreading, and proliferation were monitored. The study found that both kinetics of cell spreading, and static impedance-based cell index could distinguish the two cell types. CAL 27 cells showed a smaller cell index change rate than Het-1A cells, and when fully spread, they generated a cell index more than four times greater than Het-1A cells.

Further, the results showed that cisplatin significantly decreased impedance in OSCC in a concentration and time-dependent manner. The widely used anticancer drug cisplatin caused oral squamous cell carcinoma (OSCC) cells to undergo apoptosis, which was successfully monitored by the impedance-based technique [72]. The results indicate that the impedance-based method, which detects cell spreading and attachment in the first few hours of microelectrode culture, could be a rapid, non-invasive method for distinguishing oral cancer cells from non-cancer oral epithelial cells.

Pradhan et al. used impedance sensing devices to evaluate the cellular activity of T47D cells treated with ZD6474, an anticancer medication [73]. They created four different device types using micro-machining technology, which were spread in five hours using real-time impedance monitoring data. The cytotoxic effect of ZD6474 was also evaluated by studying the frequency response characteristics of drug-treated cells. The drug-treated samples showed a significant drop in impedance data compared to control data above 10 M dosage, indicating an inverse relationship between drug dosage and cell impedance [73].

Using ECIS, Hung et al. monitored the apoptosis of oral cancer cells following cannabidiol (CBD) treatment [74]. They analyzed the effects of cannabidiol (CBD) on the viability, cellular apoptosis, cell morphology, and migration of OEC-M1 cells. ECIS was used to measure the change in cell impedance over 24 h for cells exposed to a series of CBD concentrations. Alamar Blue and Annexin V/7AAD tests revealed

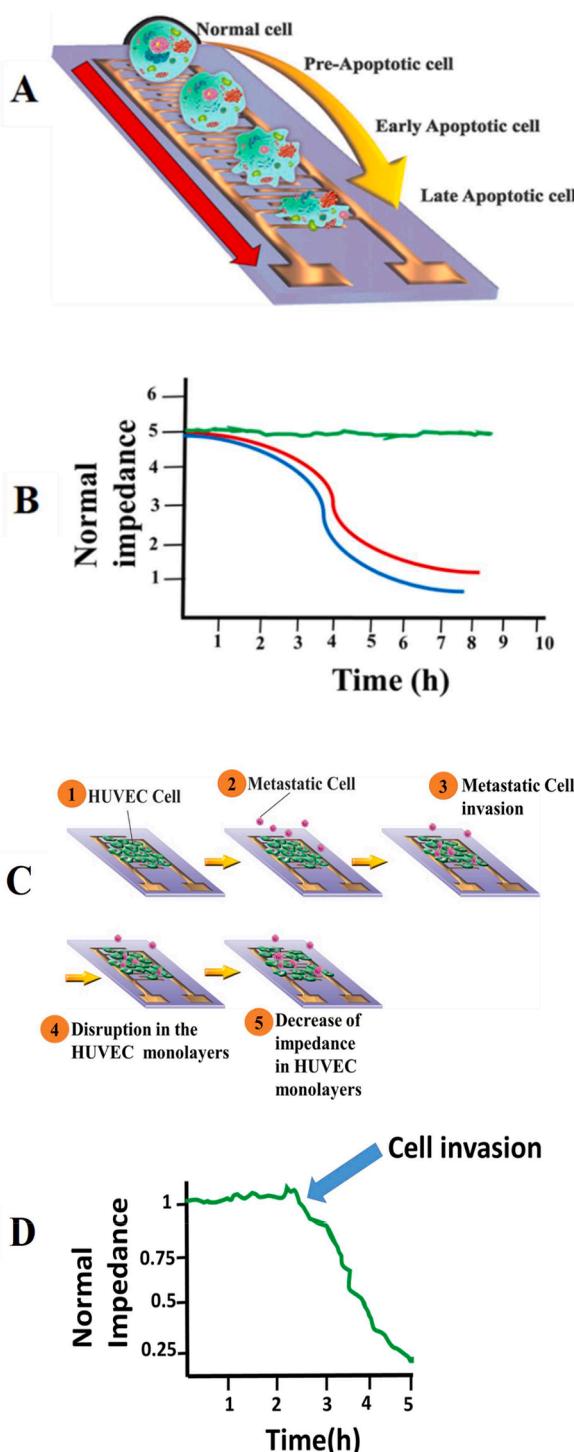


Fig. 4. ECIS system usage in metastasis and apoptosis detection. (A) Apoptosis detection after drug treatment by an impedance sensor, the red arrow shows the decrease in impedance after apoptosis induction. (B) Diagram of impedance after drug treatment. Cell impedance decreases after drug treatment in a time-dependent manner [71]. (C) The invasion experiment measures changes in impedance at the electrode/cell interface after metastatic cells invade a HUVEC monolayer (1. HUVEC monolayer formation, 2. Adding metastatic cells, 3. The beginning of attachment and invasion of metastatic cells to the HUVEC cell, 4. Invasion of metastatic cells and disruption of the HUVEC monolayer, 5. HUVEC monolayer destruction and impedance reduction) (D) Diagram of the impedance of HUVEC monolayer after the invasion [93].

that CBD decreased cell viability and induced apoptosis. According to the ECIS analysis, CBD reduced the overall resistance and the morphological characteristics at 4 kHz in a concentration-dependent way. The study demonstrated that ECIS is superior to traditional biochemical assays in terms of sensitivity, function, and broadness and can be used to validate novel treatments *in vitro* [74].

In summary, ECIS is a valuable tool for studying apoptosis in cancer cells due to its ability to provide real-time, dynamic information on cellular behavior. Researchers can use ECIS to assess the effects of various treatments on cancer cells, monitor changes in impedance associated with apoptosis, and gain insights into the mechanisms of cell death in a time-dependent manner.

10.2. Cancer cell metastasis detection

The primary cause of cancer death and morbidity is metastasis. The metastatic cascade involves detaching cancer cells and allowing them to enter the circulating and lymphatic systems, evading immune attack, extravasating into distant capillary beds, and proliferation in distant organs [75]. Both intravasation and extravasation, which are invasion mechanisms, require the extracellular matrix (ECM) [76]. The extracellular matrix (ECM) is a critical component of the tumor microenvironment (TME), serving as a reservoir and a source of molecular information cue. It anchors soluble proteins and provides a strong structural framework for resident and visiting cells. The mechanical and molecular properties of the ECM influence the behavior of a cell exhibiting specific trends under a variety of conditions, such as durotaxis, haptotaxis, or chemotaxis. The tools available for researchers to employ in the laboratory to quantify the ability of tumor cells to migrate and invade are quite limited [77]. Typically, optical-based approaches are used in most research to measure tumor cell invasion capacity. Traditional methods for studying cell migration involve the use of scratch or wound healing assays, which necessitate time-lapse microscopy equipment. The Boyden chamber/transwell assay detects cell invasion using one opaque, permeable membrane [78]. Metastasis research requires a fluorescent tag, but these fluorescent tags are cost-effective [79]. Presently, research and development efforts focus on developing an ECIS system that is non-invasive, label-free, capable of real-time monitoring, and has high throughput for parallel testing and screening. The ECIS system has conducted considerable studies on cell migration, invasion, and adhesion; these cell-biological processes play a fundamental role in cancer metastasis [80].

10.2.1. Cell migration

Cell migration is crucial for cancer metastasis and is a key target for evaluating anticancer medication and cancer treatment [81]. ECIS is useful for analyzing cancer cell movement. ECIS monitors the impedance across a cell-covered electrode surface, delivering real-time data on cell behavior changes.

Keese and colleagues used an ECIS-based wound healing assay to perform automated cell migration assessment. The ECIS signal was applied to monitor the migration of live cells [82]. The highly regulated measurement makes this ECIS-based method better than the conventional "Scrape" method. As a result, this approach has often been applied in more recent studies, particularly in pathophysiology [83]. Earley and colleagues found that focal adhesion kinase (FAK) upregulation increases the transendothelial migration of AU-565 breast cancer cells due to a considerable decrease in monolayer resistance [84].

Jiang et al. conducted an investigation pertaining to the expression and activity of Eplin- α within breast cancer cells [85]. Cell migration was evaluated utilizing the ECIS technique. Eplin- α exhibited potential tumor suppressor properties by effectively impeding the proliferation and migration of cancer cells. The findings presented in this study underscore the significance of employing ECIS-based migration assays as a valuable tool in cancer research [85].

Cui and colleagues created an ECIS device with nanostructures in the

shape of grooves and ridges with a depth of 75 nm and a breadth of 200 nm [86]. Human foreskin fibroblast (HFF) and human keratinocyte (HaCaT) cells were grown, but only HFF cells could align along the nano-grooves. Both HFF and HaCaT cells had higher normalized impedance (NI) values at their respective frequencies of 977 Hz and 1465 Hz. In contrast to flat electrodes, nano-groove electrodes resulted in lower impedance signals during HFF and HaCaT cell migration and proliferation. However, the nano-groove electrodes showed more pronounced impedance signals during HaCaT cell proliferation [86].

In a different experiment, ECIS was utilized to show how paxilline therapy reduced the migration of glioblastomas [87]. Data showed that ECIS sensors could detect migration decreasing after treatment with paxilline. These investigations demonstrated that ECIS could differentiate between cell types' attachment and migration [87].

10.2.2. Cell invasion

One of the most important stages in the metastasis of cancer is invasion, a complicated process characterized by the intrusion of malignant cells into neighboring tissues. ECIS is a powerful approach for studying cancer cell invasion.

The process of cancer cell invasion is more intricate than two-dimensional cell migration, and there is a great deal of study being done on the interactions that cancer cells have with the extracellular matrix (ECM) or with epithelial/endothelial cells [88]. Therefore, a confluent monolayer of cells must be pre-planted on the electrode surface in order to perform invasion tests with an ECIS system. The pre-planted cell monolayer is then invaded by post-seeded cancer cells, destroying its strong bond to the substrate and leading to a drop in cellular impedance [80].

Following this principle, pleural mesothelial cell (PMC) monolayers were penetrated by and attached to ovarian epithelial cancer cells, which resulted in a breakdown of the PMC barrier and a reduction in cellular impedance [89].

Human umbilical vein endothelial cells were seeded with hepatocellular carcinoma cell lines to examine leptin's effect on invasion, and a mechanism that mediates leptin's effect was identified [90]. The results showed that leptin stimulates hepatocellular carcinoma cell invasion in matrigel invasion and electric cell-substrate impedance-sensing tests. The study further revealed the JAK/STAT pathway as a critical mediator of leptin action [90].

Anchan et al. employed ECIS to analyze the kinetics of human melanoma cells invading the endothelium of the human brain [91]. Moreover, the study determines if the paracellular and basolateral endothelium barriers are affected and when to analyze ECIS data statistically. The findings demonstrated that a range of human melanoma cells can induce disruption of the human brain endothelium, primarily via the paracellular pathway. The sensitivity of ECIS also shows that the paracellular barrier weakens within 30 to 60 min of adding melanoma cells to the endothelial cells' apical face. Time-lapse imaging also showed that the invasive melanoma cells open junctions and damage the endothelium monolayer over a long period. They asserted that ECIS is a potent tool for future research into invasive processes of cancer cells [91].

Lo et al. utilized ECIS technique to observe the infiltration process of SKOV-3 ovarian cancer cells across a cohesive layer of human umbilical vein endothelial cells (HUVECs) [92]. The study observed that the penetration of SKOV-3 cells across a HUVEC monolayer was characterized by a rapid decline in transendothelial electrical resistance, as observed in a dynamic and real-time manner by impedance measurements at a frequency of 4 kHz alternating current. The presence of hepatocyte growth factor (HGF) promoted invasion. In contrast, c-Met inhibitor (SU11274) blocked HGF's stimulating action. By analyzing the frequency-dependent impedance of SKOV-3 cells over time, the study discovered that HGF-enhanced SKOV-3 cell invasion was associated with reduced junctional resistance (R_b), larger average cell-substrate separation (h), and increased micromotion. In the SKOV-3 monolayer,

SU11274 reduced the effects of HGF on R_b, h, and micromotion. By increasing R_b and reducing h, SU11274 improved the barrier function of the HUVEC monolayer. Changes in cellular biophysical parameters (R_b, h) were linked with cancer transendothelial invasion [92]. In conclusion, the study demonstrated a better approach for real-time monitoring and investigating the interactions between cancer cells and the underlying adhesive cells during invasion.

Rahim and Üren employed ECIS to assess cancer cell invasion of an endothelial cell monolayer [93]. Their invasion experiment is based on changes in impedance at the electrode/cell interphase as a population of metastatic cells invades a HUVEC monolayer (Fig. 4C). Endothelial connection breakdown, endothelial monolayer contraction, and tumor cell replacement all resulted in substantial variations in impedance. These alterations are strongly correlated with the ability of tumor cells to invade, which means that invasion by cells with high levels of aggression results in significant alterations in cell impedance and vice versa (Fig. 4D). This method offers two advantages over other invasion measurement techniques like the Boyden chamber and matrigel test: The interaction between endothelial cells and tumor cells is more similar to the in vivo process, and the data are obtained in real-time and easier to quantify than other types of studies [93].

Dowling et al. utilized ECIS to track how fibroblast-derived conditioned medium affected colon cancer cells' adhesion, proliferation, migration, and invasion [94]. They demonstrated that HCT116 cells adhere, multiply, and migrate noticeably more quickly in the presence of medium from human fibroblasts using various complementary and unique experimental techniques. Furthermore, they revealed that when stimulated with medium derived from human fibroblasts, HCT116 cells migrated aggressively and infiltrated matrigel layers using the xCELLigence CIM plates system (also known as the ECIS system). These findings overwhelmingly pointed to fibroblasts' capacity to enhance the migratory and invasive abilities of HCT116 cells. The kinetics of colon cancer cell migration and invasion mediated by fibroblasts were studied for the first time in this work [94]. Identifying micrometastasis is a significant challenge in patient survival. Because of the tiny volume of biopsied tissue, there are fewer histopathological samples, which may affect the likelihood of precise diagnosis even using molecular approaches. Nikshoar et al. introduced a microelectronic biochip (named Metas-Chip) to detect the micrometastasis in unprocessed liquid or solid samples [95]. It operates on the premise that cancerous cells tend to track individual human umbilical vein endothelial cell (HUVEC)-sensing traps. By triggering membrane retraction and blebbing, these cells separate from the biopsied material and infiltrate the sensing traps, which causes abrupt changes in the electrical response of the sensing elements. Metas-Chip diagnosed more than 70 breast cancer patients in less than five hours [95]. With a quick, easy, and chemical-free process, this methodology makes it possible to collect metastatic cells efficiently and specifically in small biopsy samples. This improves the diagnostic potential of CNB and FNA prior to surgery or medical therapies.

10.2.3. Cell adhesion

Cell adhesion is a fundamental cellular process that is pivotal in cancer dissemination. In 1986, Giaever and Keese conducted a study wherein they employed an ECIS system to assess and compare the properties of four distinct protein layers applied to electrodes [95]. These protein layers included bovine serum albumin, gelatin, bovine fetuin, and human plasma fibronectin [96]. They discovered that fibronectin and gelatin had superior biological compatibility for cell adhesion. One study used the ECIS method to analyze adhesion/spreading, barrier function, and wound healing in four epithelial cell lines. The study discovered that epithelial cell types exhibit significant phenotypic variations [21].

Using ECIS, Xiao and coworkers investigated how mammalian cells connect and spread [97]. Their device included a detecting gold electrode (surface area 0.057 mm²) and a gold counter electrode (18 mm²) for the cultivation of cells. In an extensive theoretical framework, a

precise determination of the electrode-electrolyte interface impedance and a cell layer impedance was made for frequencies ranging from 1 to 10 kHz. A surface plasmon resonance biosensor was used to measure the protein surface concentrations adsorbed on the gold surface. The number of fibroblast cells adhering to the detecting electrode increased linearly with the resistance variation of the electrode-electrolyte interface at 4 kHz. The type of coating protein appeared to affect the slope of the linear connection. The alteration in resistance exhibited a direct correlation with the extent of cellular coverage, as the surface area encompassed by the cells was directly proportional to the number of cells present [97].

Atienza and coworkers utilized ECIS to monitor cell adherence and spreading on impedance sensor arrays. Their study presented a label-free, real-time method for measuring and monitoring cell adhesion on specialized microplates equipped with electronic cell sensor arrays. The real-time cell electronic sensing (RT-CESTM) system was used to dynamically and quantitatively measure the specific interaction of fibroblasts with extracellular matrix (ECM) proteins and compare it to standard adhesion techniques. Chemicals that prevent ECM from engaging with cell surface receptors can prevent cell binding to ECM-coated cell sensor arrays, which depends on the amount of ECM protein on the surface. The study further revealed that the stability of the actin cytoskeleton is needed for cells to stick to and spread on ECM-coated microelectronic sensors. The results showed that inhibiting Src expression or activity with siRNA or a small chemical prevents BxPC3 cells from sticking together and spreading. The study further revealed that the RT-CES method is a quick and easy way to measure the rate of cell adhesion in a high-throughput manner [98]. Rahman et al. reported a detailed model for impedance characterization of ovarian cancer epithelial cell layer using ECIS electrodes. The OVCA429 ovarian cancer epithelial cell layer was described in electrical equivalent circuit elements. Impedances of ECIS-8W1E array were recorded with cell culture medium (without cells) and with OVCA429 cell layer in the culture medium between 100 Hz and 10 MHz frequency. The trans-layer resistance (R_b) and capacitance (C_m) of ovarian cancer cells were determined to be $R_b=152 \pm 59 \Omega \cdot \text{cm}^2$ and $C_m=8.5 \pm 2.4 \mu\text{F}/\text{cm}^2$, respectively [99].

To summarize, ECIS provides a robust and diverse platform for investigating the complexities of cancer spread. Because of its potential to provide real-time, label-free, quantitative data, it is a powerful tool for gaining insight into the mechanisms behind metastatic processes and developing targeted treatment therapies.

10.3. ECIS and epithelial-to-mesenchyme transition (EMT)

The epithelial-mesenchymal transition (EMT) is a biological phenomenon in which epithelial cells change, losing their distinguishing characteristics and acquiring those of mesenchymal cells. During EMT, epithelial cells lose cell-cell connections, apical-basal polarity, and epithelial markers while gaining cell motility, a spindle-cell shape, and mesenchymal markers [100]. This phenotype has an increased tendency for migration, metastatic potential, resistance to apoptosis, and extracellular matrix protein synthesis.

EMT is crucial for embryogenesis and wound healing, but it has also been connected to the growth and spread of cancers and triggering the development of metastases [101]. The transition process induces significant and malignant consequences such as increased metastatic potential, invasiveness, and motility of cancer cells. The transforming growth factor- β 1 (TGF- β 1), a multifunctional cytokine, is a widely recognized stimulant of EMT [102]. It binds to specific transmembrane serine/threonine kinase receptors with a heteromeric structure. The activation of T β R-I through phosphorylation via T β R-II occurs as a result of the receptor formation between two TGF- β type I (T β R-I) and two TGF- β type II (T β R-II) receptors. Despite the growing body of molecular evidence indicating that TGF- β exposure elicits a complex and dynamic response in epithelial cells, characterized by an initial active state

followed by a later mesenchymal-like state marked by reduced motility and the formation of stress fibers, there is currently no functional study that offers quantitative data on the dynamics of epithelial-mesenchymal transition [103].

ECIS can offer real-time information about cell morphological changes during EMT. An impedance measurement may reflect changes in cell shape, size, and adhesion properties during EMT. Schneider and colleagues employed ECIS to track how the cytokine TGF- β 1 affected the NMuMG, A549, and MDA-MB-231 mammalian epithelial cell lines' shape, motility, cytoskeletal dynamics, and long-term correlations (Fig. 5A). The three cell lines exhibited significant variations in concentration dependence, cellular morphology, and dynamics in response to TGF- β 1. Following cytokine treatment, A549 cells and the non-tumor mouse epithelial cell line NMuMG displayed a significant change in morphology reflected in stepwise alterations in their phenotypic. Data showed that ECIS could detect EMT in cancer cells (Fig. 5B) [104].

Overall, ECIS offers real-time, quantitative data on EMT alterations, providing a versatile platform for studying complex cellular changes in various biological situations, including cancer progression.

10.4. ECIS and tumor microenvironment monitoring

The role of the tumor microenvironment, which comprises stromal cells, adjacent blood vessels, and extracellular matrix components, is crucial in regulating malignant epithelial cell proliferation, drug resistance, invasion, and metastasis [105]. The promotion and progression of cancer have been reported to be influenced by various factors, including the communication and interaction between stromal cells and cancer cells. The conventional techniques for detection, such as the RT-PCR assay and fluorescence-activated cell sorting (FACS), Fluorescence microscopy, and Western blotting, can investigate cellular interactions within the tumor microenvironment. Typically, these processes are characterized by being arduous and lacking temporal resolution. Impedance sensing offers a viable approach to elucidate the kinetics of cellular interactions [106]. Tran and colleagues utilized ECIS to examine the cellular interactions between MRC-5 human lung epithelial cells and A549 human lung carcinoma cells under both untreated and treated circumstances [107] (Fig. 5C). The cell patterning process was conducted in a co-culture system composed of two independent fluidic chambers divided by a fence of 100 m. Microelectrode arrays with electrodes at varied distances from the confrontation line were put within each chamber for electrochemical impedimetric sensing evaluation of cell-to-cell influence. When the barrier was removed and cell-to-cell contact occurred, direct and indirect cell-to-cell interactions through conditioned media were investigated. The study analyzed the impedance signal responses, which depicted the state and behavior of the cell. The question addressed in this study was the role of distance in the differential suppression of tumor cell proliferation by human fibroblasts (Fig. 5D). [107].

In summary, ECIS is a valuable tool for studying the tumor microenvironment, a complex network of cells, blood vessels, and extracellular matrix. ECIS's real-time monitoring enables the observation of dynamic changes in the tumor microenvironment over time, advancing our understanding of cancer biology and developing targeted therapeutic strategies.

10.5. ECIS and drug resistance

Drug resistance is a significant problem in the treatment of cancer because cells might develop mechanisms to circumvent the effects of drug therapy. While chemotherapy is a prevalent approach in cancer treatment, the persistence of drug resistance poses a formidable obstacle, stemming from both inherent and acquired resistance mechanisms [108]. Genetic and epigenetic factors are involved in chemoresistance. Drug-resistant cancer cells harbor a mutation in the p53 protein and aberrant expression of ATP-binding cassette (ABC)

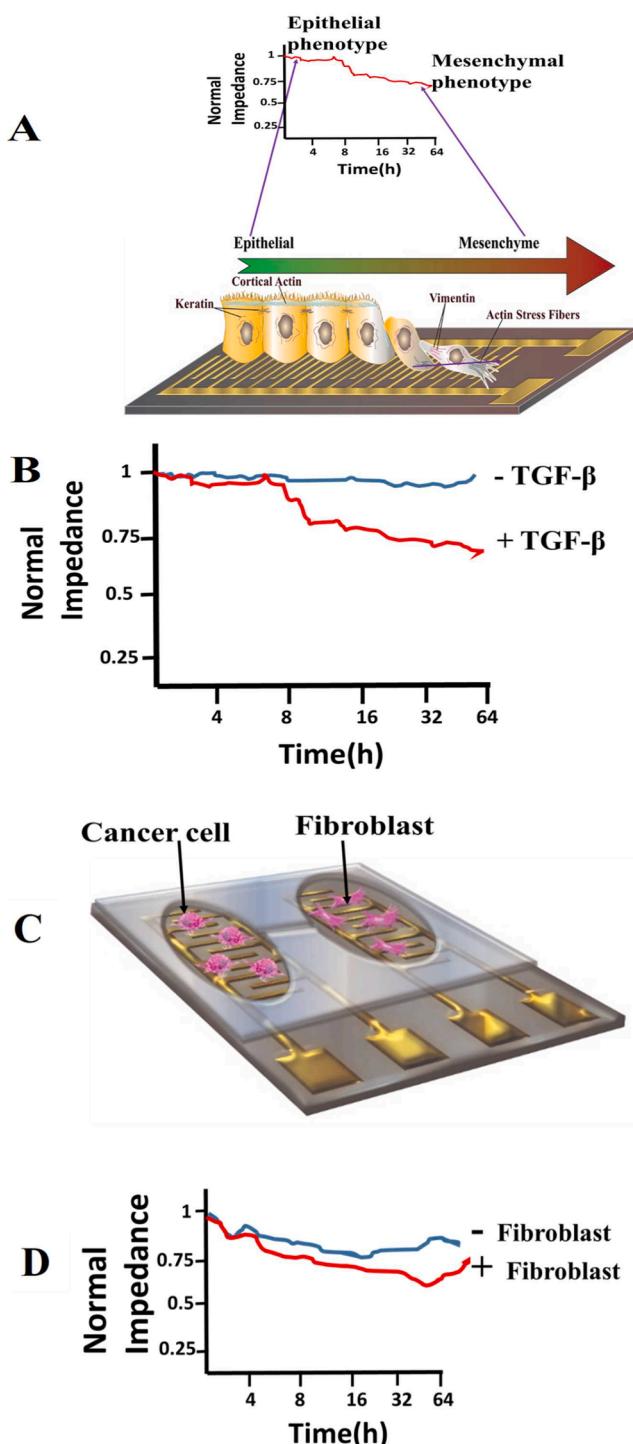


Fig. 5. ECIS system applications for tumor microenvironment monitoring and EMT identification. (A) EMT monitoring after the addition of TGF- β on cancer cells. The impedance decreases after adding TGF- β . (B) Cell impedance in the control (-TGF- β) and treatment (+TGF- β) groups was compared [104]. (C) Co-culture of cancer cells and fibroblast on ECIS sensor for tumor microenvironment monitoring. (D) The effect of fibroblast on impedance of cancer cells in tumor microenvironment [107].

transporter and anti-apoptotic protein. P-glycoprotein (Pgp/ABCB1), MDR-related protein 1 (MRP1/ABCC1), and breast cancer resistance protein (BCRP/ABCG2) are the ABC transporters clinically associated with multidrug resistance (MDR) [109]. Chemotherapy failure arises due to the upregulation of these transporters in cancer cells, thereby

augmenting the efflux of drugs from the cellular milieu while concurrently reducing intracellular accumulation. Identifying drug resistance before commencing treatment will result in time savings and the prevention of adverse effects stemming from the administration of drugs. The timely identification and comprehension of the mechanisms underlying drug resistance hold the potential to facilitate the advancement of novel therapeutic approaches for cancer. Cancer biomarker investigations, employment of positron emission tomography, and utilization of fresh tumor cell culture assays represent conventional methodologies employed in assessing drug resistance [110].

Next-generation sequencing, atomic force microscopy, and live-cell imaging are cutting-edge technologies for characterizing multidrug resistance [111]. As ECIS offers impressive benefits, such as label-free, low-cost, non-invasive, non-destructive, quantitative, and real-time monitoring, impedance-based cellular assays are gaining more and more attention to evaluate their potential for chemoresistance in early detection [111]. Through ECIS, real-time monitoring of a cell's response to drugs is possible. Using therapeutic compounds and monitoring impedance changes over time, researchers can analyze how drugs affect cell activity, including drug resistance.

Fuentes-Vélezand et al. used ECIS to monitor colon cancer cell drug resistance [112]. They employed both wild-type HT29 cells (without doxorubicin resistance) and doxorubicin-resistant HT-29 cells. They developed a brand-new, specially designed impedance measuring device ideal for long-term impedance measurements (Fig. 6A). They used cell impedance analysis to identify colon cancer cells that resisted chemotherapy. For this, 140 h of observation were carried out on doxorubicin-resistant HT-29 cells (Fig. 6B). Impedance-based curve analysis showed that chemo-sensitive and chemo-resistant cells exhibit distinct patterns [112].

Gharooni et al. employed ECIS to track the cytoskeleton's conducting changes and detect drug resistance [113]. Due to its higher interaction surface and compliance with the electronic fabrication process, they used doped silicon nanowires as electrodes. They discovered that the microtubule-destabilizing drug Mebendazole (MBZ) causes a reduction in electrical resistance, whereas the microtubule-stabilizing drug paclitaxel (PTX) leads to an increase in electrical resistance. Phalloidin, an actin-stabilizing agent, increases electrical resistance, while cytochalasin D (CytD), a destabilizing agent, decreases it. Thus, their study offers proof-of-concept for cytoskeletal compartments' electrical functions for cancer grading and drug resistance assessment [113].

On the whole, ECIS allows researchers to track real-time changes in cell behavior. Drug treatment may cause changes in cell adhesion, proliferation, and migration. Impedance profile changes can provide information about drug efficacy and resistance.

10.6. ECIS and cancer detection

Most cancer detection relies on pathological diagnosis, necessitating complex procedures and meticulous examination by highly qualified pathologists under a microscope. Moreover, when the cancer is detected, it may already be in a terminal phase. Recent years have seen several initiatives aimed at developing optical and electrochemical strategies to detect cancer cells. However, the majority of them were unable to monitor cells continuously. Therefore, developing continuous, real-time, and non-invasive detection methods for cancer cell differentiation is essential [114].

Zhang et al. utilized electrical impedance spectroscopy to differentiate between skin cancer cells and healthy cells [115] (Fig. 6C). The normalized impedance was measured at 1465 Hz with simultaneous microscopic imaging to help describe the two cell types' proliferative activities throughout a 5-day culture period. The findings showed that these two cell types could be discriminated against using ECIS based on differences in the levels and variation patterns of Rb and Cm during the proliferation process in real-time and without a label. The results of their study offered a valuable analytical framework for investigating skin

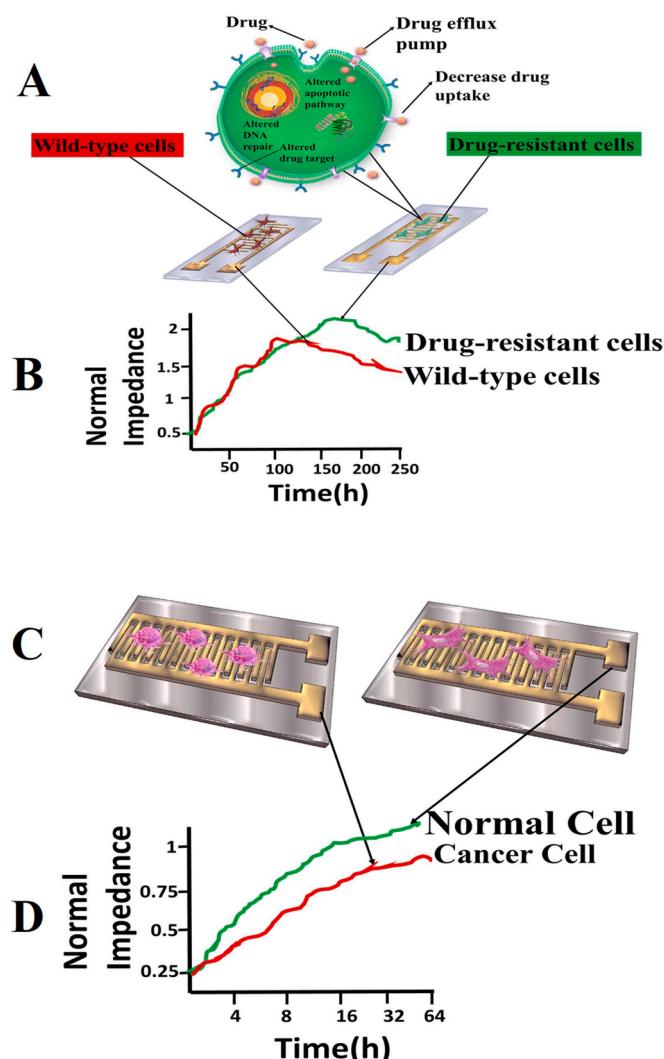


Fig. 6. Applications of the ECIS system in drug resistance and cancer detection. (A) Monitoring of drug resistance in colon cancer cells cultured on ECIS sensors. (B) The impedance diagram of drug -resistance cells and wild-type cells [112]. (C) Cancer cell detection with impedance sensors. (D) The impedance diagram of normal cells and cancer cells [115].

cancer cells and may aid in the early detection of skin cancer (Fig. 6D) [115].

Abdolahad and coworkers developed a fast and highly sensitive impedance-sensing biosensor based on carbon nanotubes that is vertically oriented [116]. Their biosensor relies on the rapid entrapment of cancer cells on arrays of vertically aligned carbon nanotubes, which causes mechanical and electrical interactions between carbon nanotube (CNT) tips and entrapped cell membranes and alters the biosensor's impedance. SW48 colon cancer cells were transferred across the surface of CNT-coated electrodes to be precisely entrapped on elastic nanotube beams. CNT arrays function as sticky and conductive agents, with impedance changes occurring in as little as 30 s (for the entire trapping and signaling operations). CNT-ECIS identified cancer cells at a surface concentration of as few as $4000 \text{ cells cm}^{-2}$ [116].

In a different study, Abdolahad and colleagues developed an impedance biosensor based on silicon nanograss for the label-free identification of metastatic cells among non-metastatic colon cells. The detection method relies on the difference in current flow blockage between benign and metastatic cell membranes caused by the beta dispersion phenomenon, which changes the sensor's impedance. The sensor exhibited the capability to detect low concentrations of

metastatic colon cells within the entirety of the sample instead of utilizing costly marker-based pathological approaches. The results indicate that bioimpedance sensing exhibits the potential cancer cell detection [117].

Yang and colleagues employed ECIS to separate oral cancer cells from non-cancer oral epithelial cells based on their biological activity on the microelectrodes in a real-time, label-free approach [71]. The models for oral cancer and non-cancer oral epithelial cells used were CAL 27 and Het-1A cell lines. They studied cellular activities such as cell adhesion, spreading, and proliferation. Further, the study determined that both the kinetics of cell spreading and the static impedance-based cell index were viable methods for discriminating between the two types of cells [72].

Ching et al. conducted preliminary research using bioimpedance to identify squamous tongue cancer. The study employed a disposable probe equipped with four silver electrodes to measure the electrical characteristics of cancerous tongue tissue (CTT) and adjacent normal tongue tissue (NTT) in five patients with squamous cell carcinoma. The measurements were taken at six distinct frequencies, namely 20 Hz, 50 kHz, 1.3 MHz, 2.5 MHz, 3.7 MHz, and 5 MHz. The study evaluated four measurement parameters, namely impedance, phase angle, essential part of impedance, and imaginary part of impedance, to determine whether there were any significant differences in the values obtained from the tongue using CTT and NTT. CTT and NTT exhibit notable differences at 50 kHz [118].

Prakash et al. employed a linear four-electrode impedance probe to assess electrical impedance spectroscopy on excised hepatic tissue obtained from human patients diagnosed with metastatic colorectal cancer. According to the study, tumor tissue has an electrical conductivity that is 2–5 times greater than normal tissue, within a frequency range of 100 Hz–1 MHz. Most frequencies showed statistically significant changes in tissue electrical permittivity. Furthermore, both healthy and cancerous tissue displayed intricate impedance patterns [119].

While ECIS is not an independently operated cancer detection technique, the insights gained by analyzing cellular behavior with ECIS can lead to a better knowledge of cancer biology, which might assist in the development of new diagnostic and treatment approaches.

10.7. ECIS and cell cycle monitoring

Researchers have paid close attention to cell cycle in recent years since it is involved in many pathophysiological or pharmacological processes. Studies involving the cell cycle tend to utilize specific procedures to assess the status of cells during different stages of cell cycle [120]. The methods used to research the cell cycle and related events have largely stayed unaltered for a long time despite significant advancements in our understanding of the process. Early strategies that relied on autoradiography proved to be a viable method of estimating the duration of the cell cycle phase. Methods, including flow cytometry, fluorescence microscopy, and western blotting, are frequently used to examine the cell cycle. These procedures cost a significant amount of time and money. Therefore, a more practical real-time cell cycle tracking technology is essential [121]. By detecting alterations in cellular activity and impedance connected to distinct stages of the cell cycle, Electric Cell-Substrate Impedance Sensing (ECIS) can be used to monitor the cell cycle.

Wang and colleagues utilized a cellular impedance sensing device to monitor the cell cycle in real-time without using labels [122]. They have created a bioelectronic chip-based system that uses non-invasive cellular impedance sensing to do a real-time dynamic analysis of the cell cycle in living cells (Fig. 7). The cellular impedance sensing chip, which consisted of microfabricated interdigitated electrode structures, was utilized to culture the cells. Throughout the five-day experiment, HeLa cells were synchronized with a double thymidine block, and the cellular impedances were recorded with minute-level precision. Their findings indicate that real-time impedance sensing could mirror the cell cycle's progression; cellular impedance increased over time in the G1 and S

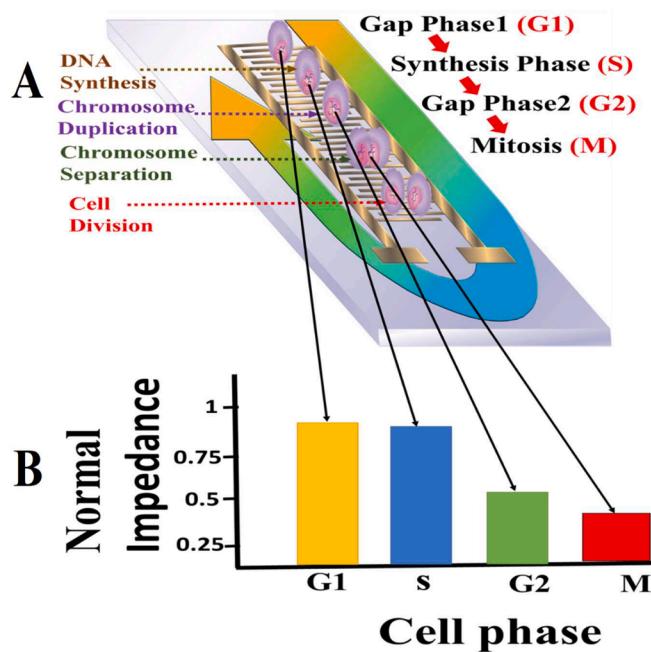


Fig. 7. Applications of the ECIS system in cell cycle monitoring. (A) Schematic of cell cycle monitoring using ECIS. (B) Diagram of the impedance of cell cycle phases using ECIS [122].

phases while decreasing in the G2 and M phases. Flow cytometry and microscopy were used to confirm the cell cycle data provided by the cellular impedance [122].

To track the cell cycle stage of lung cancer cells, Abiri and colleagues used silicon nanowire impedance sensors [123]. They developed a silicon nanowire sensor to detect malignant cultured live lung cells by monitoring the point at which the cells stretch and extend on nanowires during their spreading phase. The cell cycle details provided by the cellular impedance data was confirmed using flow cytometry and microscopy [123].

In conclusion, ECIS is a useful technique for investigating cell cycle dynamics because it provides real-time, non-invasive monitoring of impedance changes associated with distinct stages of the cell cycle. Its adaptability enables researchers to explore many elements of cell cycle progression and proliferation, as well as the impact of external variables on these processes.

11. New types of impedance devices

Several intriguing emerging advancements in cellular impedance biosensors are broadening the potential of this robust, label-free approach [124,125]. One exciting development is the expansion of the ECIS method to 3D cell cultures, and the combination of with microfluidic methods [126].

11.1. Three-dimensional (3D) cellular impedance biosensor

Cells are commonly cultured on flat, two-dimensional surfaces [127]. The convenience and efficiency of preparing large quantities of cell cultures on flat surfaces has enhanced our understanding of cell physiology [127]. However, these models have certain drawbacks, such as their inability to accurately mimic drug penetration into tissues and the intricate structure of actual tissue [124]. A 3D cell culture approach can more effectively model the complex extracellular milieu in which cells reside. Tracking 3D cell culture cells as time progresses has been made possible by integrating electrodes into the system [128]. Researchers are making great strides in developing *in situ* sensors that use EIS to monitor cell proliferation and viability. They have additionally

developed devices with customized electrode surfaces for measuring the metabolic activity of 3D cell cultures [129]. In addition, the 3D impedance cell sensors offer valuable insights into cellular activities in the vertical direction [126]. 3D impedance biosensor fabrication, optimization of culture conditions, and interpretation of impedance data are challenging tasks. Current improvements in microfabrication processes, biomaterials, and sensor technologies attempt to overcome these problems while also improving the reliability and adaptability of 3D cellular impedance biosensors.

To summarize, 3D cellular impedance biosensors provide a more realistic and informative platform for cancer research, allowing researchers to obtain more thorough understanding into cancer cell activity and contributing to the formulation of more effective therapies.

11.2. Impedance biosensors integrated with microfluidic technology

The combination of impedance sensing and microfluidics enables real-time monitoring of cellular responses and molecular interactions. The combined sensors possess notable attributes such as enhanced sensitivity, decreased reagent usage, rapid analysis duration, compact instrument dimensions, and straightforward operation [125]. Furthermore, the utilization of microfluidics enables automation, resulting in improved reliability and efficiency of analyses. Impedance biosensors combined with microfluidic technology are highly effective instruments for comprehending electrical data in cancer cells [130]. The electrical properties of single cells were measured using a microfluidic device combined with electric impedance flow cytometry (IFC) and electric impedance spectroscopy (EIS) [130]. Passively trapping single cells allowed the device to obtain data on the impedance spectrum and discrete impedance points of the cells. Additionally, the device demonstrated the individual functionality of IFC and EIS, revealing impedance magnitude differences and quantifying area-specific membrane capacitance and cytoplasm conductivity for cancer cells [130]. Microfluidic technology has also enabled complex biological processes to be consolidated and incorporated into one system [131]. Breast cancer stem cells cultured in 3D matrix-based organ-on-chip platforms allowed precise and reproducible multi-analyte metabolite monitoring. This microsensor-based method may enable efficient and personalized cancer therapy, drug standardization, and screening [131]. The integration of impedance biosensors with microfluidic technology is still an active research area, and future improvements are likely to increase these platforms' capabilities in a variety of fields including cancer.

12. Conclusions, limitations, and future perspectives

An ECIS system is capable of fully recognizing cellular activity in response to a variety of stimuli. As a result, it is appropriate for screening various diseases, including cancer, by monitoring cellular activities in real-time. In this review, we provided an overview of the impedance measurement theory, the impedance-sensing electrode design, and the applications of impedance-sensing electrode in cancer research.

ECIS offers real-time, label-free, noninvasive, and continuous measuring features. Apart from the convenient homogeneous assay format, noninvasive and kinetic measurement modes, minimal interference with normal cellular activity, and short assay times, all these factors are also advantageous. The ECIS system also enables on-chip drug screening of molecular processes, such as cellular adhesion, spreading, proliferation, drug-induced apoptosis, and apoptosis inhibition of cancer cells (Table 1). Based on the findings highlighted here, it is reasonable to assume that ECIS is a traditional systematic method with great sensitivity that allows for quick approaches to disease prediction, diagnosis, and therapy. ECIS can detect cellular activity in response to a variety of new bioactive substances or medications [132].

Limitation: When used in cancer research, ECIS has drawbacks and challenges much like any other scientific approach. ECIS is built primarily for usage in 2D cell culture setups. Cancer cells frequently exhibit

different traits in 3D environments that more accurately replicate *in vivo* conditions. In a three-dimensional context, ECIS may not adequately represent the intricacy of cell behavior. ECIS electrodes can interact with cells and influence their behavior. It is crucial to carefully evaluate the results and consider potential distortions because this interaction may change the biological response.

Although ECIS is capable of identifying behavioral alterations in cells, it may not offer comprehensive insights into the underlying cellular processes. A more comprehensive understanding of observed effects may require additional molecular and imaging studies to complement ECIS data. The observed effects may require more detailed molecular and imaging studies to complement ECIS data.

An ECIS measures the impedance of a cell layer to calculate an average response. Because it lacks spatial resolution, it cannot distinguish between individual cells or subpopulations within a culture, making it challenging to study cellular heterogeneity. ECIS is largely an *in vitro* approach, which implies that it analyzes cells in a laboratory setting not within the body. Although this is helpful in grasping the fundamental behaviors of cells, it might not adequately represent the intricacy of interactions that occur within a living thing, such as the impact of the cancer microenvironment. Analyzing ECIS data can be difficult, especially for investigators who are unfamiliar with electrical impedance fundamentals. Lastly, the analysis involves relating experimental data to mathematical models, and interpretation errors or faulty analysis can lead to untrue findings.

Future Perspectives: Integrating biosensors with microfluidic technology presents a highly efficient approach for elucidating electrical information within cellular systems. The primary benefits of integrated sensors include enhanced sensitivity, decreased reagent usage, quick analysis times, smaller instruments, and straightforward operation. Two promising development avenues for microfluidic impedance sensor glass chips are electrochemical impedance spectroscopy and electrical impedance flow cytometry. The cells are stationary in an electrochemical impedance spectroscopy. The status of the cells in an electrical impedance flow cytometer is dynamic. The primary method for measuring the impedance of other organisms depends on the modification of electrodes and the fusion of antigens and antibodies. The immobilization of aptamer or antibody on the ECIS sensor will be used for the detection of the cancer-associated metabolite in the clinical sample [133].

The integration of ECIS with other liquid handling models and microfluidics will enhance high-throughput screening capabilities in the future. It may lead to the evolution of screening techniques like drug candidate and gene function screening by improving the quality and reproducibility of measured data. Developing a variety of ECIS units with specific functionalities could also be a future option. An ECIS device, for instance, has been made available for transwell assays. This could replace conventional transwell assays as a primary tool in research. The combination of ECIS and microfluidic can separate cells from each other [134]. Nanoelectromechanical Chip (NELMEC) is a hybrid device combining nanoelectronics and microfluidics to distinguish mesenchymal circulating tumor cells from leukocytes [135].

The Digital Microfluidic Platform (DMP) is a system that uses electrowetting to handle liquid by manipulating individual microdroplets on an array of electrodes [136]. This includes tasks such as dispensing droplets from a reservoir, moving them, separating them, and combining them. The integration of biological detection systems into DMP devices can act as a roadmap for future progress towards point-of-care (POC) applications [137].

The pursuit of creating portable diagnosis devices is a crucial objective in this field, as it will aid in identifying health- or disease-associated biomarkers. This advancement offers an automated, highly sensitive, and economically viable tool for use in clinical study [138].

Even though ECIS was designed for *in vitro* research, technological advances may make it applicable to *in vivo* applications in the future. This might entail creating non-invasive methods to track changes in

impedance in living things, giving researchers a more dynamic perspective on how cancer spreads.

Combining ECIS data with AI and machine learning techniques could improve complex dataset analysis. Within large-scale ECIS datasets, AI applications may aid in tracking subtle trends, forecasting treatment responses, and uncovering novel relationships. Further, incorporating ECIS data with other "omics" databases, such as genomics, transcriptomics, and proteomics, can provide a broader view of cancer biology. This multi-omics approach has the potential to provide a more complete knowledge of the molecular pathways underlying cancer growth.

To further advance the field of cancer research and patient care, collaboration amongst researchers, physicians, and technology developers is crucial in bridging the gap between laboratory findings and clinical applications.

In conclusion, ECIS investigations have provided useful insights into cancer cell activity. These results add to our understanding of cancer and could open the way for new ways to diagnosis, therapy, and monitoring. The dynamic nature of ECIS technology makes it a strong tool for future research, with the potential to uncover even more complications within the convoluted landscape of cancer biology.

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Hassan Moghtaderi: Writing – review & editing, Writing – original draft, Software, Resources, Conceptualization. **Golfam Sadeghian:** Writing – review & editing, Writing – original draft, Software. **Hamed Abiri:** Writing – review & editing, Writing – original draft, Software. **Faizullah Khan:** Writing – review & editing, Writing – original draft. **Md Mizanur Rahman:** Writing – review & editing, Writing – original draft, Software. **Ahmed Al-Harrasi:** Writing – review & editing, Resources. **Shaikh Mizanoor Rahman:** Writing – review & editing, Writing – original draft, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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