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Assessing Microfluidic Hydrogel Multiplexed Devices in Comparison with Conventional 2D Cell Cultures for Enhanced Performance and Cell Behavior

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Abstract:- Scientists have been operating biomedical research by relying on flat monolayer 2D cell culture for a very long time. The more innovative the researches have become, the more efficiently the diseases can be cured, and inevitably higher the demand in research methods and tools. In our modern era, cancer is undeniably a threat in society and understanding the intricate interplay of tumor cell heterogeneity and plasticity holds the key to cure cancer. Since 2D models are too plain, especially in complicated studies like cancer, applying 2D cell-culture to study cancer research can be a huge obstacle. In order to decode the dynamic response of tumor cells to their microenvironment, a more physiologically relevant environment for studying cell-cell interaction and drug responses is required. Thus, 3D cell culture method is introduced to optimize the research requirement. In this paper we present a 3D microfluidic device integrated with impedance micro-sensing which is an advanced fusion of microfluidic technology with a 3D cell culture method and micro-sensing. Additionally, 3D cell culture methods as presented in this paper prove to be a better technique to obtain live cells in viability assays than 2D cell cultures. Through this paper, we hope to present an enhancing technology and showcase how 3D cell cultures are the next big thing in tumor cell research and the treatment of cancer.

Keywords:- *2D Cell Cultures; 3D Cell Cultures; Microfluidic Devices; Impedance Micro-Sensing; MCF-7(Breast Cancer Cell).*

I. INTRODUCTION

Over the past decade, there has been a rapid increase in cancer cases among every age group, from children to adults. According to the International Agency for Research on Cancer (IARC), there were approximately 19.3 million active cases of cancer in the latest report from 2020. Among them, there is an estimation of an average of 144.1 deaths per 100,000 people. Approximately 25% to 50% of cancer treatments that reach the stage of assessment in randomized clinical trials are successful[1]. These days, a vast number of populations are diagnosed with recurrent cancer cases, even though there are quality treatments available, such as chemotherapy. For long, advancements in cancer research have been due to cell cultures and the study of micro-environments. Cell culture is the process by which cells are grown under controlled conditions, generally outside of their natural environment. Cell culture models are widely used in vitro tools for improving our understanding of cell biology and cell behavior.

The first cell culture was carried out by Ross Harrison in 1907 A.D. Since then, the method has improved and is now used in various advanced biological studies, including cancer research. These in vitro models closely mimic the in vivo features of cells, making it easier to perform experiments such as tumor cell growth, behavior, interaction, and adverse responses to changes in their microenvironment. This technology is considered revolutionary and potentially ground-breaking for cancer cure and personalized treatment. Currently, there are two methods of cell culture: 2D culture and 3D culture.

The most commonly used type of cell culture is the 2D model. Most research regarding cancer biology is based on experiments using two-dimensional cell culture due to its simplicity and high throughput. However, researchers have been aware of the limitations of 2D compared to 3D culture. 2D cell culture assays may provide misleading and non-predictive data due to the lack of physiological relevance to represent the anatomical and biochemical complexities of cell-cell and cell-environment interactions since it depicts an unrealistic flat surface. As a result, applying 2D cell culture in studies regarding the cell interactions, such as tumor microenvironment (TME), may result in inaccurate data. Additionally, 2D cell culture relies on animal testing for drug experiments, which is quite expensive and controversial. These outcomes clearly indicate that 2D cell culture is an unreliable method for cancer therapies like ICI (immune checkpoint inhibitor therapy) or ACT (adoptive cell therapy).

On the other hand, 3D cultures seem to be rising in popularity. 3D cell culture models closely mimic tissues and organ-specific microarchitecture. Cells naturally grow and mature in a three-dimensional environment just as they would in vivo. Unlike 2D cultures, 3D cell cultures allow the cells to interact with each other in a more physiologically relevant manner. They also provide more accurate data and even eliminate the reliance on animal

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testing. Furthermore, with the use of easy-to-use matrices like composite hydrogels and 3D bioprinting, it provides more similarity towards the natural architecture and environment of the tissue. Such microfluidic devices are a valuable tool for studying cells under dynamic conditions. Therefore, using a 3D model is a viable option for research towards personalized treatment and drug developments for cancer cure.

To introduce intricacy and improve physiological fidelity, the integration of 3D tumor models with microfluidic devices can be employed to facilitate perfusion or replicate vascularization[2]. Microfluidic systems, which integrate cells, an extracellular matrix, and perfusion, serve as effective replicas of the in vivo tumor microenvironment. They offer advantages such as affordability, reproducibility, the ability to utilize small culture volumes, and a customizable design that can be optimized for specific applications[3].

In addition, incorporating micro-sensing devices with the 3D microfluidic devices makes it much more effective to study cell migration. Hydrogels are used to form a 3D structure that closely imitates the actual cellular environment. They are highly useful and efficient during experiments for analysing various cellular events such as attachment, adhesion, growth, and motility through the monitoring of electrical alterations between the cell and electrode. Integration of 3D micro-fluidic and micro-sensing devices certainly improves monitoring of cell-cell and cell-environment interaction within the ECM, allowing more accurate results and conclusions for the researches. It further fulfils the gap of traditional 2D models, making the future of cancer research much more advanced.

In this report, we therefore aimed to present the shortcomings of the 2D cell culture models and showcase the dynamic responses of tumor cells within the TME with hydrogel-based 3D micro-fluidic device under the monitoring of impedance micro-sensing device. We focused on conducting a few hypothetical experiments related to characterization of cell cultures, pointing out their differences, design and fabrication of micro-fluidic device based on hydrogel tissue culture and integrated with ECIS, proving the growth of cells within the matrix. We try to design an innovative assay and a strategic framework to unravel tumor cell responses to their microenvironment.

II. MATERIALS AND METHODS

➢ *Characterization between 2D Cell Cultures & 3D Cell Cultures*

To get a better understanding of our experiment, it is better to understand how 2D and 3D cell cultures are implemented and what results they showcase. Therefore, we perform a characterization experiment first.

- *Principles of 2D & 3D Cell Cultures*

A 2D cell culture involves transferring cells onto a plate, where they spread, and develop in a single layer. The culture medium provides nutrients for cell survival and growth, making it a Simplified model for studying cell behaviour and also for laboratory experiments.

Meanwhile, 3D cell cultures mimic tissues and organ specific microarchitecture. Cells naturally grow and mature in a three-dimensional environment just as they would in vivo. 3D cell cultures are generally classified into two categories: d-based methods using hydrogels or structural scaffolds and scaffold free approaches using freely Floating cell aggregates (spheroids).

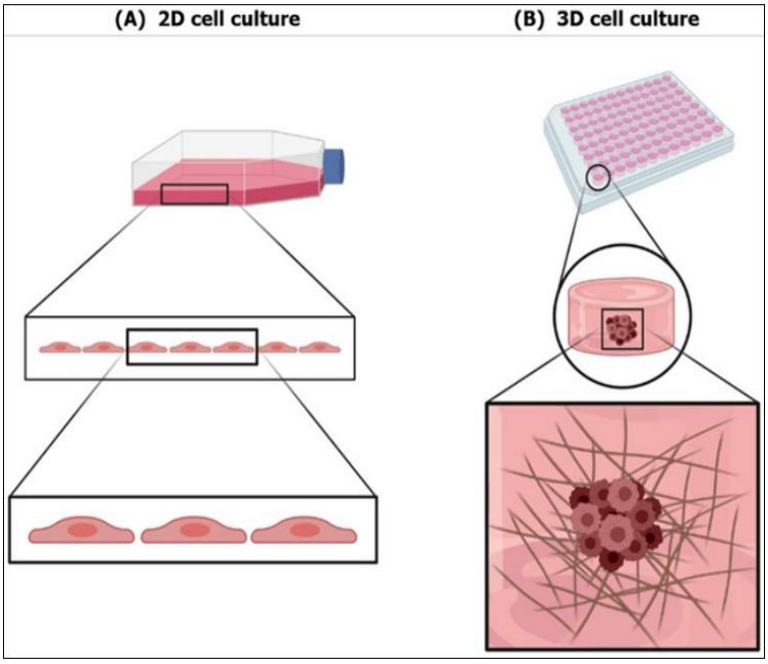

Fig 1 (A) Cell behavior in 2D conventional cell culture. Cells cultured in a 2D manner tend to have a flat shape that does not represent the real physiological cell morphology. (B) Cell behavior in 3D cell culture. Cells cultured in a 3D system are present in a microenvironment similar to that in vivo; therefore, they have a more representative morphology and behavior.

- *Materials*

Cells (MCF-7 cells in our case), Cell culture medium (RPMI-1640 supplemented with PBS and antibiotics), Plate of Choice, Conical Tubes, Trypsin-EDTA solution, Micropipettes[4]

MCF-7 cells (previously cultured and harvested), Cell culture medium (RPMI-1640 supplemented with FBS and antibiotics), Well Plate, Hydrogel solution, Conical tube (15ml), Micropipettes[5]

- *Methodology*

We will be exploiting preparation techniques from [6] and [7] for the preparation of the cell cultures to proceed further with our characterization.

Table 1 Preparation: 2D Cell Cultures 3D Cell Cultures

2D Cell Culture	3D Cell Culture
Prepared fresh cell culture medium (RPMI-1640 supplemented with PBS and antibiotics) Collected MCF-7 cells and centrifuged them for the required amount of time. Re-suspended cell in 5 ml of medium. Then, Placed the required number of cells per well in a plate, also added 200µl of medium which contains the necessary supplements. Filled the outer and additional empty wells with 200µl of PBS (PBS is a balanced salt solution) before placing the plates in a humidified incubator at 37 °C and 5% CO2.	Added 500µl cell suspension to an empty conical tube. Then added 1ml of Matrigel to the tube and mix it properly. Transferred 50µl Matrigel mixture to a well plate and then later Incubated it at room temperature for 15 minutes. Added 50µl cover medium to each well. Placed the well plate in an incubator and changed the cover medium every 48 hours.

➢ *Fabrication of Micro-Sensing 3D Microfluidic Device*

- *Materials*

Silicone elastomer kit (referred to as polydimethyl siloxane or PDMS) with Y-shaped microchannels. Polyester (PETE) membrane filters (transparent, 0.2 µm pore size, 12 µm thickness, 2e6 pores/cm2); pre-cleaned 75 mm×50 mm×1 mm plain glass slides; INTRAMEDIC polyethylene (PE) tubing (I.D. 1.40 mm, O.D. 1.90 mm; and I.D. 1.14 mm, O.D. 1.57 mm); Silicon (SI) wafers; SU-8 2025 photoresist and SU-8 developer (98–100% 1-Methoxy-2-propyl acetate); WK6500B impedance Analyzer; Matrigel solution; Syringe pumps.

- *Fabrication of Microfluidic Device*

A PDMS layer with a Y-shaped microchannel and a glass substrate with electrodes were used to create a microfluidic chip. After fabricating the PDMS substrate with monomer and curing agents, the PDMS layer was created using soft lithography, and a PMMA mold with a negative pattern of microchannels was machined. Following that, the PDMS prepolymer and curing agents were combined, and the mixture was cured for 1 hour at 70°C. The cured PDMS layer had been peeled off the mold and was ready for use. The Au electrodes were created using a standard micro-fabrication technique that included thermal evaporation and lift-off. The chip was put together by bonding two layers together with oxygen plasma. The PDMS layer and glass substrate were manually bonded, and electrodes were used to align the microchannel sidewalls. The chip was sterilized in 70% ethanol for 6 hours before being exposed to ultraviolet light overnight.

- *Cell Seeding & Cell Culture*

Metastatic MDA-MB-231 and less-metastatic MCF-7 cell lines were acquired from ATCC. These cells were cultured in Gibco Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) under standard conditions (37 °C, 5% CO2). Sub-cultivation was carried out at a 1:10 ratio. To detach the cells from culture flasks, trypsin−EDTA treatment for 2 minutes was employed. Following detachment, cells were resuspended in DMEM to halt any remaining trypsin activity. After centrifugation for 10 minutes, they were re-suspended in Gibco CO2 independent medium supplemented with 4 mM L-glutamine to achieve a concentration of 1 million cells per milliliter. We seeded cells in Matrigel by mixing the cells with the diluted Matrigel solution to create a 3D matrix that mimics the extracellular environment. Once the mixture solidified, we added DMEM to provide a supportive environment for cell growth.

- *Integration with Micro-Sensing Technology*

For this experiment, we will be using the ECIS (Electric Cell-Substrate Impedance Sensing) method to measure the impedance. The method includes the dispersion of cells on the sensing electrodes' surface, leading to disruption in electrical current flow and a significant alteration in the measured impedance[9] . ECIS utilizes 2 gold electrodes, namely, working electrode and counter electrode placed at the bottom of cell culture well and immersed in culture medium which is electrolyte[10] We can also use Fluorescence Microscopy or Time-Lapse Imaging, but to prove our hypothesis we will focus on ECIS method because it provides Real-time monitoring of cellular activity within the matrix, Label-free or non-invasive monitoring of cells which allow accurate measurements and natural observation, and it is highly sensitive detecting subtle changes in the cell behavior. Conventional methodologies for evaluating cell migration in 3D ECM, such as the Boyden chamber are label-based assays[11]. The data collection process, being only partially computable, and the challenge of determining endpoints make it difficult to visually understand the migration process. By incorporating the impedance sensing method into the design of a microfluidic device, impedance measurement devices like WK6500B could provide kinetic information about the cell migration and invasion process in a 3D microenvironment[12].

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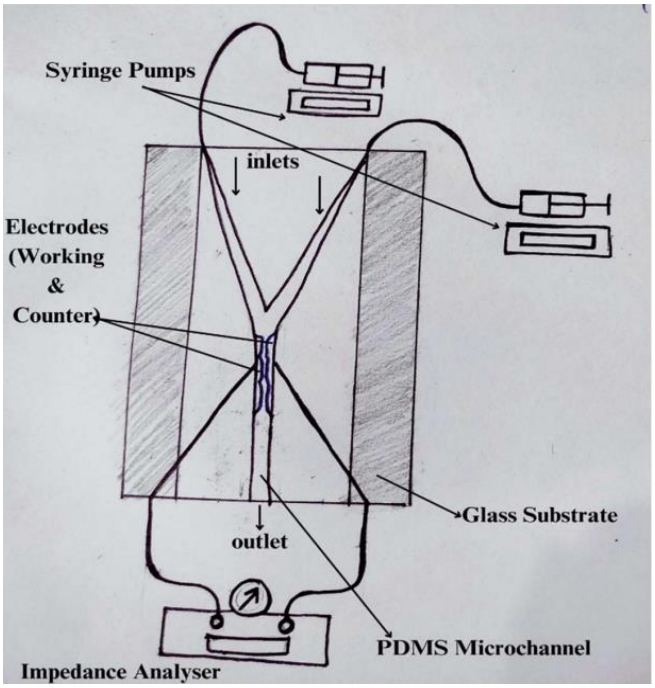

Fig 2 Shows the Hand-Drawn Design for Microfluidic Design Integrated with ECIS Micro-Sensing Methods; Involves Two Gold Electrodes in Microfluidic Channels to Catch Impedance Signals.

We will be exploiting fabrication protocols from [8] and [13] for integration of micro-sensing device. In our design of the Y- shaped microfluidic device, 2 gold electrodes were inserted along the microchannel to facilitate the measurement of impedance.

Firstly, our main goal was to keep the micro-sensor clean, for that the chip underwent a 5-minute ultrasound cleaning in acetone, followed by isopropyl alcohol and a rinse in DI water, before being affixed to a PC board. Au electrode surface was further cleaned using an oxygen plasma for 10 mins. A self-assembled monolayer was created on the Au electrodes by immersing them in an ethanol solution containing 95% 11-MUA (11-mercaptoundecanoic acid) at room temperature for 15 hrs. Later, the chips were removed from the ethanol solution, and each chip was cleaned with 70% ethanol for 30 seconds and then dried with nitrogen. The PDMS layers of the microfluidic device were cleaned with isopropyl alcohol. For better surface properties for cell adhesion, fibronectin (FN) dissolved in phosphate-buffered saline (PBS) at a pH of 7.4 (1 mg/mL) was injected into the microchannels using a syringe pump. This facilitated FN adsorption onto the SAM-modified Au substrate, followed by an hour-long incubation at room temperature. Before proceeding with cell trapping, the microchannel was cleaned with PBS through a passive pumping method. The droplets containing PBS with FN at the inlet and outlet of the microchannel were completely aspirated, and a fresh PBS droplet was introduced at the inlet to induce dynamic flow inside the channel. Cells, suspended in a solution with a concentration of 5×107 cells/mL, were then introduced into the microchannel. After a day of culture in a 37 °C, humidified atmosphere, and 5% CO2 incubator, the cells spread and formed a monolayer on the microchannel's bottom surface. Employing the syringe pumping method, a cold Matrigel solution (2.6 mg/mL at 4 °C) was pumped into the microchannel to replace the cell medium while simultaneously covering the attached cells on top of the microelectrodes. The chip was subsequently incubated inside an incubator at 37 °C for an additional 15 minutes. Following Matrigel polymerization, a porous membrane with a 0.2µm pore size was meticulously attached to a tape with a thickness of 100µm beside the SU-8 structure. This configuration resulted in the formation of a thin layer of Matrigel between the membrane and the substrate. Finally, a substantial drop of CO2-independent medium with 10% FBS was placed on top of the membrane to establish a chemoattractant gradient along the thin Matrigel layer.

- *Impedance Measurement Experimental Protocols*

To explore cell migration and its impact on electrical impedance, impedance measurements were conducted every 12 hours throughout the cell migration process. To ensure consistent temperature and pH conditions, fresh medium at 37 °C was introduced into the microchannel before each measurement. The WK6500B impedance analyzer facilitated electrical impedance spectroscopy measurements, connected to a computer via a GPIB card, and controlled using Zplot software. The acquired data were analyzed using Zview software. For impedance spectrum measurements, the WK6500B applied an alternating voltage with a 10-mV amplitude across a frequency range of 100 to 106 Hz. In real-time measurements, the WK6500B delivered an alternating voltage of 1V or 20mA at 4 kHz. A potential of 0.1Vrms was applied across a pair of electrodes, and impedance was measured from 50 Hz to 1MHz.

➢ *Cell Viability Assay Comparison between 2D and 3D Cultured Cells*

As we move forward with the design of our device we need to observe if the device is able to sustain living cells better than 2D cell culture. Hence, to assess the number of viable and non-viable cells in our microfluidic device, we will be using the MTT technique. MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], a cheap soluble powder, works by measuring metabolic activities of cells. If cells were living and growing inside a microfluidic chip, it must be metabolizing in there as well[14]. Any metabolizing cell contains NADPH related enzymes that reduce MTT which results in insoluble purple crystals[15]. Hence, the higher the percentage of living cell, the stronger the purple color of the tested subject.

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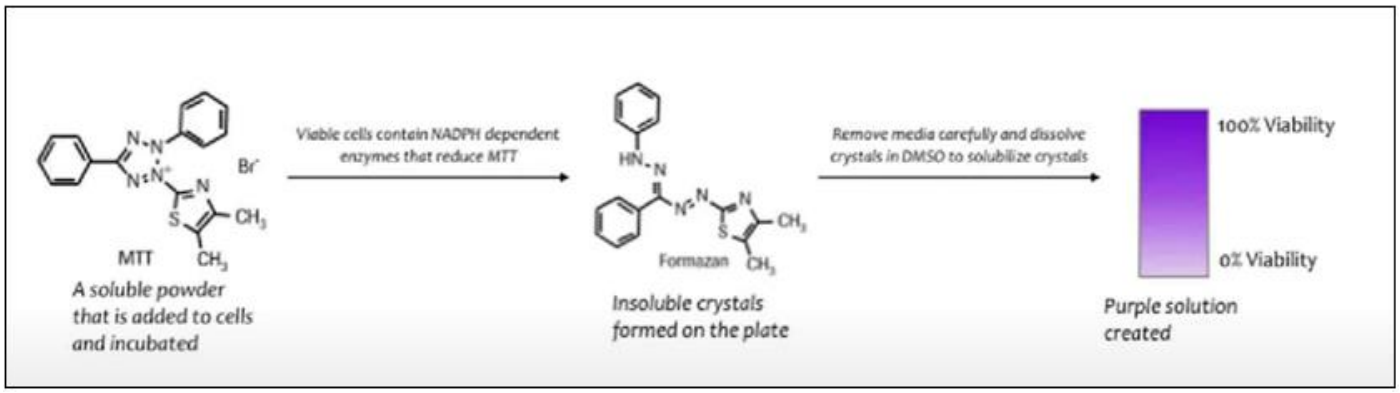

Fig 3 Showcases the Linear Progression in the Working of MTT and Methods Employed to Understand MTT Assay Results

- *Materials*

1.6-well-plate, Cells from the microfluidic device, Cells from 2D culture, Cell culture medium, Trypsin powder solution, Hemocytometer, Sterile water, Pipettes, MTT reagent, Cell culture plates, PBS (Phosphate-buffered saline), DMSO (Dimethyl sulfoxide), Incubator, and Plate reader were used.

- *Experimental Protocols*

In this experiment, all materials used in the assay process were sterilized since cell cultures are susceptible to contamination by bacteria, fungi, viruses, and other microorganisms present in the environment. Contamination could have affected cell growth, viability, and introduced confounding variables in the assay results. Therefore, sterilization procedures were employed to eliminate or reduce the risk of contamination, ensuring accurate results.

To transfer cells from the 3D microfluidic device, MCF-7 breast cancer cells were first transferred to a standard culture plate for the cell viability assay. To maintain an inhabitable environment for the cells, the proteolytic enzyme was dissolved in PBS. Subsequently, the solution was sterile-filtered, and the enzyme solution was carefully pipetted into the designated areas, channels, or compartments as required. Gentle pipetting helped break up clumps and achieved a more uniform cell suspension, avoiding the introduction of bubbles or uneven distribution. The enzyme was incubated at the required temperature for the necessary duration, depending on the solution type. Afterward, the cell suspension was transferred to a fresh tube and washed with PBS before proceeding with the subsequent steps. The recovered cell suspension was systematically distributed evenly into three replicates within a 6-well plate using a clean syringe with extreme care[16] . The goal was to optimize margin errors that could have emerged due to fluctuations between cells in each replicate. To maintain balance, the same number of cells (approximately 3000 cells per well) were seeded into the three wells[17] .

Similarly, for transferring cells from our previously characterized 2D cell culture, MCF-7 cancer cells were used. The process involved carefully removing the culture medium from the 2D cell culture dish, which was cultured with the same MCF-7 cancer cells, without disturbing the adherent cell layer. Any residual medium that could have interrupted the final results was removed by rinsing the cells once with sterile PBS. The proteolytic enzyme trypsin (EDTA) was then added to dissociate the cells together inside a cell plate, and the cells were incubated at 37°C for 10 minutes. Careful and systematic transfer processes were done to avoid disrupting the cells' uniform condition. Afterward, a pipette was used to load a small volume of the diluted cell suspension into the hemocytometer's counting chamber for cell counting. The same well-plate transfer procedure performed for 3D cells was also applied to 2D cells, and the same number of cells (3000 per well) were seeded into three plates evenly in a column within a 6-well plate to produce three replicates.

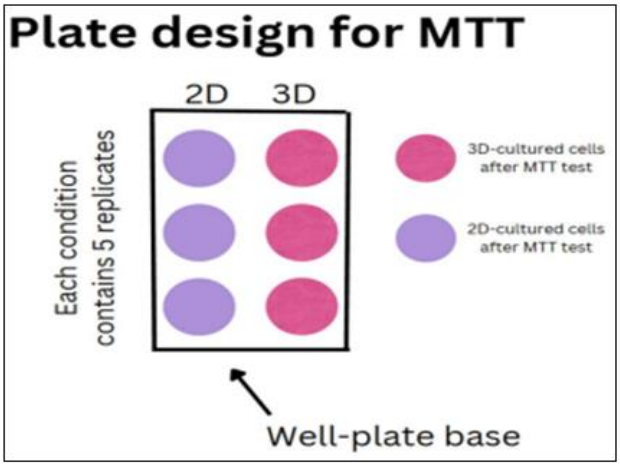

Fig 4 A 2D Representation of the Plate Design for MTT for Both 2D and 3D Cell Cultures

For MTT assay preparation, the MTT stock solution was prepared by dissolving MTT powder in PBS to make a 5 mg/mL solution[18]. The MTT solution was protected from light as it was light-sensitive. Before adding the MTT medium to the respective cells, the culture medium was carefully removed. The cells were then incubated with the MTT solution at 37°C for 4 hours. Viable cells reduced the MTT to form a crystal-like purple formazan product.

During the solubilization step, the MTT solution was carefully removed with a pipette to avoid disturbing the crystals. Then, 100µL per well of DMSO was added, and the plate was gently shaken to ensure complete solubilization of the formazan crystals. The final plate was incubated for 10 minutes at 37°C temperature in the incubator.

III. HYPOTHESIZED RESULTS

➢ *Comparative Analysis: 2D and 3D Cell Culture Characteristics*

We obtained and complied characteristics that we observed in our characterization of 2D and 3D cell cultures with reference to an already compiled work by [19].

Table 2 Comparative Analysis: 2D and 3D Cell Culture Characteristics

Type	2D Cell Culture	3D Cell Culture
Time of culture Formation	Within minutes to a few hours	From a few hours to a few days
Culture quality	High performance, reproducibility, long-term culture, easy to interpret, simplicity of culture	Worse performance and reproducibility, difficult to interpret, cultures more difficult to carry out
In vivo imitation	Do not mimic the natural structure of the tissue or tumour mass	In vivo tissues and organs are in 3D form
Cells Interactions	Deprived of cell-cell and cell extracellular environment interactions, no in vivo-like microenvironment and no "niches"	Proper interactions of cell-cell and cell-extracellular environment, environmental "niches" are created
Characteristics of cells	Changed morphology and way of divisions; loss of diverse phenotype and polarity	Preserved morphology and way of divisions, diverse phenotype and polarity
Access to essential compounds	Unlimited access to oxygen, nutrients, metabolites and signalling molecules	Variable access to oxygen, nutrients, metabolites and signalling molecules
Molecular mechanisms	Changes in gene expression, mRNA splicing, topology and biochemistry of cells	Expression of genes, splicing, topology and biochemistry of cells
Cost of Maintaining a culture	Cheap, commercially available tests and the media	More expensive, more time-consuming, fewer commercially available tests

➢ *Impedance Spectroscopy and Real-Time Cell Migration Analysis in 3D Microenvironment*

To understand the interaction between cells and electrodes in a 3D microenvironment, impedance spectra are initially measured and then matched to an equivalent circuit model. Following previous protocols, a chemoattractant gradient (10% FBS) is created across the thin Matrigel layer to induce cell migration within the 3D matrix. Figure 1 illustrates the measured impedance spectra along with their corresponding fitting curves. The baseline spectrum (red circles) is obtained using the reference electrode without cells. The impedance spectrum (blue squares) is recorded by the working electrode immediately after establishing a chemoattractant gradient. The impedance spectrum (pink stars) is recorded by the working electrode after a 2-hour incubation with cells.

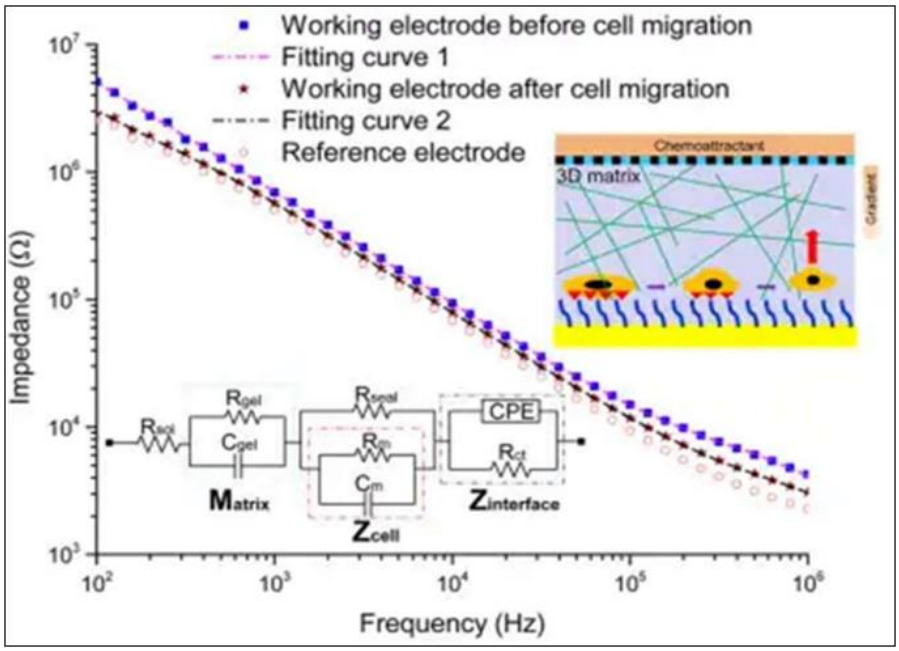

Fig 5 Bode impedance spectra acquired from a working electrode containing a single cell, both before and after cell migration. The inset diagram illustrates the process of conducting an upward cell migration assay in 3D matrices utilizing the newly introduced sensor chip.

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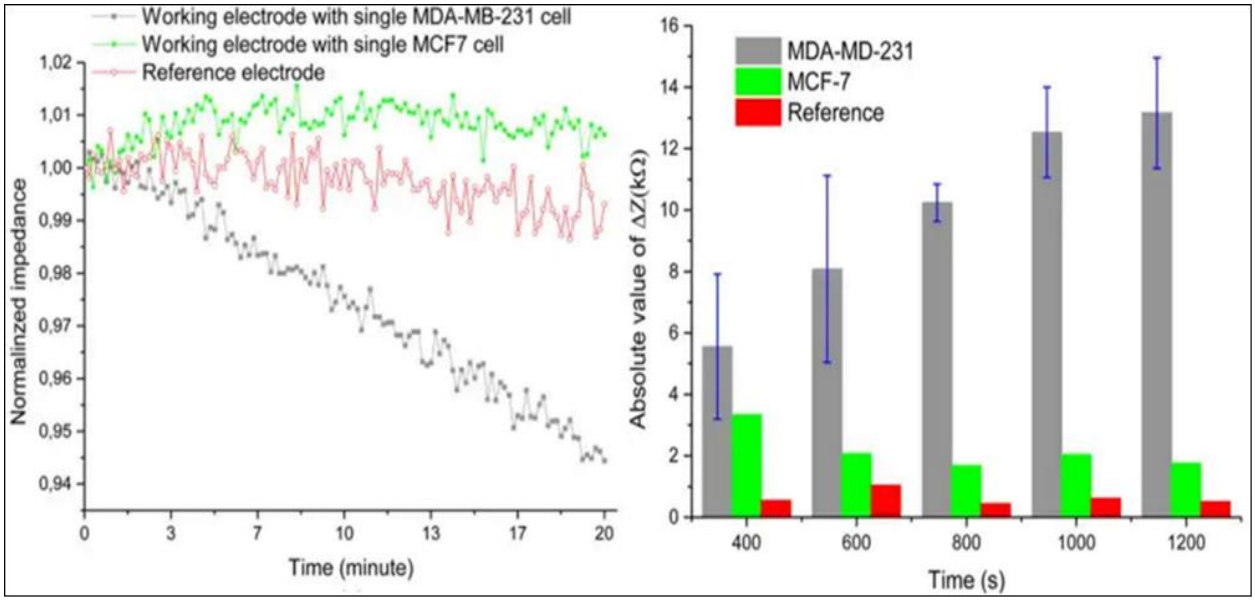
- *Real-Time Monitoring of Single-Cell Migration*

Using the chips, A real-time measurement of cell migration in Matrigel is further performed. Following the experimental protocol described earlier, , the chips were placed inside a temperature control box at 37 °C for 5 minutes to reach equilibrium before initiating real-time monitoring of impedance changes . Figure 2a depicts three representative normalized signals obtained from two working electrodes—one hosting a single MDA-MB-231 cell, the other a single MCF-7 cell—and a reference electrode devoid of cells. Notably, the impedance values of the reference electrode and the working electrode with MCF-7 exhibit similarity, whereas the impedance value of the working electrode with MDA-MB-231 gradually decreases over time. The curves illustrate that the MDA-MB-231 cell promptly responds to the chemoattractant following the diffusion of FBS.



(a) (b)

Fig 6 (a) Two working electrodes showcasing normalized signals with a single MDA-MB-231 cell and single MCF-7 cell, respectively, and a reference electrode in the upward cell migration assay. (b) The change in impedance value every 200s caused by the cell migration. The data represents either the mean of three repeated results or mean ± standard deviation (SD), n = 3. Migration of single MDA-MB231 cells induced a rapid variation of impedance magnitude with a rate of approximately 10 Ω/s, whereas no prominent impedance change was observed for less-metastatic MCF-7 cells.

Figure 2b shows the alteration in impedance values occurring every 200 secs due to the migration of individual cells. It is evident that the movement of MDA-MB-231 cells induces swift fluctuations in impedance magnitude at a rate of around 10 Ω/s, while no discernible impedance change is observed for MCF-7 cells. The unimpeded diffusion of FBS into the thin Matrigel layer can be straightforwardly approximated using a one-dimensional diffusion model grounded on Fick's law.

$$p(x,\,t) = \frac{1}{\sqrt{4\pi Dt}}\exp\left[\frac{-x^2}{4Dt}\right] \qquad t > 0$$

Where p (x, t) is the probability of finding a molecule at position x at time t and D is the diffusion coefficient.

The probability distribution follows a Gaussian distribution with a variance of 2Dt, where "D" is the diffusion coefficient of FBS in Matrigel (6.41 × 10−11 m2/s) and "t" is time. Consequently, the root-mean-square displacement(xrms) can be calculated as (2Dt)1/2. Considering the thickness of Matrigel in the proposed chip (approximately 100μm) and the diffusion coefficient, a chemoattractant gradient can swiftly develop within the Matrigel thickness in just one minute. This rapid formation of the gradient elucidates the prompt response of MDA-MB-231 cells to the chemoattractant. While there have been multiple suggestions for microfluidic devices aimed at real-time monitoring of cell migration in 3D matrices, the optical microscopy-based detection method has notably constrained the resolution and data acquisition capabilities of these devices[20-22]. Utilizing the cell impedance measurement method in the provided microfluidic chip allows for straightforward detection and recording of cell migration kinetics.

The similarity in impedance values between the reference electrode and the working electrode with MCF-7 suggests that these cells might not respond significantly to the FBS diffusion indicating that the MCF-7 cells may have limited or no migration response to the chemoattractant in the microfluidic device. Conversely, the gradual decrease in impedance values observed for the working electrode with MDA-MB-231 indicates that MDA-MB-231 cells respond actively to the chemoattractant. The decrease in impedance over time suggests that the MDA-MB-231 cells are migrating towards the chemoattractant, showing a chemotactic response. The fact that this response is immediate after the diffusion of FBS implies that the MDA-MB-231 cells detect the presence of the chemoattractant and begin to move towards it promptly.

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The observed difference in impedance values between the reference electrode and the working electrode with different cell lines (MCF-7 and MDA-MB-231) can provide valuable insights into their behavior within our microfluidic device.

- *MTT Assay Results: Enhanced Viability in 3D Microfluidic Device*

In our cell viability experiment, upon removing the final plate from the incubator, we observed distinct and significant color changes, piquing our interest in the cellular responses. To ascertain the metabolic activities of the cells, we transferred the plate to a plate reader, a pivotal step in our quest for precise and data-driven results. Through absorbance measurements of the formazan product shown by the plate reader, a direct consequence of viable cell metabolism of the MTT reagent, we obtained a highly accurate data, allowing us to construct informative graphs that shed light on the intricacies of cellular behavior. These results provide valuable insights into the viability of the cells under investigation and constitute a fundamental basis for further analyses and discussions. The Impedance micro-sensing microfluidic device we used enhanced cell migration, and the cells inside the device were scaffolded with Matrigel, enabling cell-cell interactions, nutrient gradients, and oxygen supply, similar to the native ECM inside the body. Through a comparison of cells grown in the 2D culture method, we observed a better survival rate and growth rate in cells grown in the enhanced microfluidic device. To test our hypothesis, we subjected cells from different backgrounds to the MTT assay. The resulting deeper purple color of the 3D cells served as evidence supporting the idea that a 3D microfluidic device is a more biocompatible technology, holding promise for a better future in medical science.

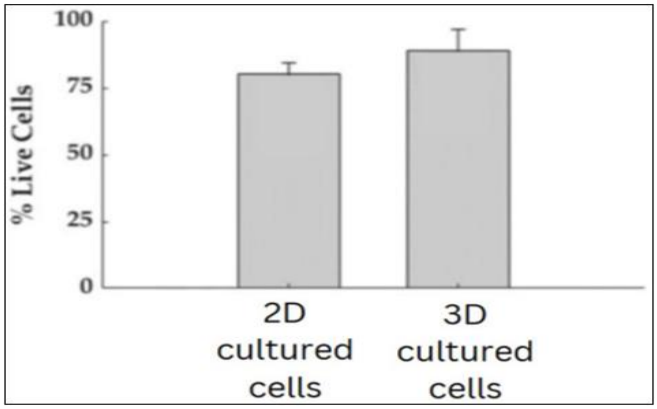

Fig 7 The data reveals the percentage of live cells that were obtained by both 2D and 3D cell cultured cells. The percentage of live cells in 3D was slightly higher than the live cells in 2D cultured cells.

IV. DISCUSSION

Through the results of our characterization experiment we can say that the utilization of 3D in vitro models offers significant advantages over traditional 2D cultures, providing a better representation of complex tissues in the human body and more accurate cellular behavior and responses. In contrast to 2D cultures, which lack the intricate interactions found in native tissues, 3D models allow cells to form complex networks and develop specialized structures, resulting in more accurate and controlled access to essential compounds. This enhanced 3D environment enables better assessment of drug penetration, distribution, and metabolism, leading to more reliable predictions of drug responses in humans.

The microfluidic device featuring integrated Electrical Cell-Substrate Impedance Sensing (ECIS) presented in this study ability the ability to scrutinize the migration of individual cancer cells within 3D matrices. The utilization of impedance spectra, along with an equivalent circuit model, facilitates the identification of specific cellular activities such as adhesion, spreading, and migration at the singular cell level based on electrical parameters. The device also offers a rapid and selective means of detecting the migratory traits of cancer cells in their early developmental stages within a relatively brief time span. As its efficacy is further assessed with diverse breast cancer cell lines, each possessing unique migration abilities, this microfluidic device holds promising potential as an invaluable tool in cancer research, potentially superseding conventional 2D cell cultures.

By offering better tissue architecture recapitulation, improved cell-cell and cell-matrix interactions, and facilitating disease modeling and personalized medicine, 3D models prove to be a superior choice for studying complex biological processes and developing innovative therapeutic approaches. The microfluidic device with integrated ECIS designed in our second experiment represents a remarkable step towards harnessing the benefits of 3D in vitro models and enhancing our understanding of cancer cell migration, with potential implications for cancer research and drug development.

Through our third experiment, we have gained insights that cells thrived more when cultured in 3D compared to 2D. The ECIS-based 3D microfluidic device demonstrated its ability to support cell growth in a more positive manner than the 2D cell culture method. These results lead us to believe that our device holds significant potential as a replacement for 2D cell cultures. The advantages offered by the 3D microfluidic device, such as enhanced cell-cell interactions, the ability to mimic tissue architecture, and better representation of complex cellular behavior, make it a promising tool for advancing research in various fields. By providing a more accurate representation of cellular behavior and responses in its microenvironment, this device could contribute to the development of innovative therapeutic approaches and drug screening for future research.

V. CONCLUSIONS

Here in this paper, we presented a series of experiments to showcase the distinctive characteristics between 2D and 3D cell culture as well as a practical approach for designing an innovative assay that integrates the hydrogel/tissue culture capabilities and micro-sensing technology. Further, we showcase how viable cells are in our designed device to further support our hypothesis. By integrating the hydrogel-based microfluidic device with the suggested sensor, cells can effectively interact with the microelectrode array for sequential 2D or 3D cell culture and impedance measurement without the need for physical connections. Therefore, tumor cells can advantageously be cultured and grown inside an improved microfluidic device. On account of the enhanced migration from micro-sensing device and the close resemblance to native ECM of the hydrogel, statistics of the MTT assay would likely depict the superior viability of 3D culture. The experiments seemingly point out the clear advantages of using 3D culture models over 2D models. However, there may be some limiting factors; for example, the cost of setting up and maintaining a 3D model can be very expensive compared to 2D models. In addition, 3D models are way more complex than traditional 2D models making it a challenge for research institutes to adopt this technology. Also, proper operation and handling of 3D cultures require technical experts having specialized skills. The results analyzed in this study have been limited on testing hydrogel-based cellular matrixes. Additional studies are needed using other ECMs including spheroids, organoids, etc. as well as micro-sensing devices such as fluorescence microscopy to make further assessments of the device and presented technology. Although it requires further validation and study, it can be the first steps towards revolutionizing the landscape of cancer care. It is the foundation of decoding the tumor cell behavior which can deem to forge a ground-breaking path for personalized treatment strategies that overcome therapy evasion. Despite the number of disadvantages 3D cell cultures possess, it has a bright future to look ahead to as it has proven that 3D cell cultures are valuable for studying cell migration and invasion, critical processes in cancer metastasis and tissue repair and our design of ECIS integrated microfluidic device is just another step-forward.

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➢ *Footnotes*

- *Author Contributions*

Rumman Jan designed the microfluidic device along with leading the experimental protocols and did the editing of the final manuscript; Min Thant Naung & Maneesh Reddy worked on performing MTT assays on cultured cells and did data analysis; Pratyush Amatya did literature reviews while also working on characterization experiments on 2D and 3D cell cultures.

- *Competing Financial Interests*

The authors declare no competing financial interests.

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