3D cell culture monitoring: opportunities and challenges for impedance spectroscopy

Sally McArthur[1], Sorel De Leon[1], and Aleta Pupovac[1]

[1]Swinburne University of Technology - Hawthorn Campus

April 28, 2020

Abstract

3D cell culture has developed rapidly over the past 5-10 years with the goal of better replicating human physiology and tissue complexity in the laboratory. Quantifying cellular responses is fundamental in understanding how cells and tissues respond during their growth cycle and in response to external stimuli. There is a need to develop and validate tools that can give insight into cell number, viability and distribution in real-time, non-destructively and without the use of stains or other labelling processes. Impedance spectroscopy can address all of these challenges and is currently used both commercially and in academic laboratories to measure cellular processes in 2D cell culture systems. However, its use in 3D cultures is not straight forward due to the complexity of the electrical circuit model of 3D tissues. In addition, there are challenges in the design and integration of electrodes within 3D cell culture systems. Researchers have used a range of strategies to implement impedance spectroscopy in 3D systems. This review examines electrode design, integration and outcomes of a range of impedance spectroscopy studies and multi-parametric systems relevant to 3D cell cultures. While these systems provide whole culture data, impedance tomography approaches have shown how this technique can be used to achieve spatial resolution. This review demonstrates how impedance spectroscopy and tomography can be used to provide real-time sensing in 3D cell cultures, but challenges remain in integrating electrodes without affecting cell culture functionality. If these challenges can be addressed and more realistic electrical models for 3D tissues developed, the implementation of impedance-based systems will be able to provide real-time, quantitative tracking of 3D cell culture systems.

Posted on Authorea 19 Nov 2019 | CC BY 4.0 | https://doi.org/10.22541/au.157419361.11339080 | This a preprint and has not been peer reviewed. Data may be preliminary.

1. Introduction

As researchers have attempted to better replicate in vivo cell biology using in vitro systems, 3D cell culture systems have developed rapidly.(Baker & Chen, 2012; Edmondson, Broglie, Adcock, & Yang, 2014) One of the key goals of 3D cell cultures is to reproduce the spatial organization and function of cells in the human body. There are a variety of formats including spheroids, organoids and engineered tissues. In 2D a cell becomes polarized as surface integrins enable it to attach to the substrate. In 3D, the cell surface integrins are distributed more randomly as the cell attaches to the scaffold/matrix in multiple directions. The physiological differences induced by the different attachment modes and the resulting cell/media interactions mean that cellular functions such as proliferation, differentiation, survival and mechanical signaling vary between 2D and 3D cell culture. The details of these variations have been reviewed extensively,(Duval et al., 2017) and many studies have shown variations in metabolism between 2D and 3D systems(Brajša, Trzun, Zlatar, & Jelić, 2016; Verjans, Doijen, Luyten, Landuyt, & Schoofs, 2017) leading to a focus of these systems for early stage drug testing and disease progression.

As experiments migrate from 2D to 3D models, traditional monitoring techniques need to evolve to accommodate the needs of researchers and the specific questions being asked of the 3D models. Currently, it may take up to 30 days to grow a fully differentiated and mature 3D tissue such as skin.(Carlson, Alt-Holland, Egles, & Garlick, 2008; V. Lee et al., 2014) During this time and after it has matured, researchers need to ensure the tissue has grown correctly and is biologically sound for experimental use. However, at this point

in time, there are few convenient, bench-top platforms to monitor this process without physically disrupting the model. In 2D culture, bright field microscopy is often used in real-time to monitor cell growth, but this is generally not possible with non-transparent 3D tissues. To date, the most common techniques used to visualize and validate 3D models are end-point histology and fluorescence microscopy.(Dmitriev, 2017) Even though these techniques are gold standards, they are time consuming and in most cases requires the destruction of the sample for sectioning and staining. While live cell approaches abound, reporter constructs and stains have a limited lifespan and are not targeted to 3D cultures.(Walker-Daniels, 2012) Introducing new standardized methodologies to monitor 3D cultures in real-time would save time, reducing research cost and providing a quality assurance pathway for manufacturing.

Cell visualization is the most intuitive way of identifying cell health, but a range of other methods have been developed to indirectly assess cells in culture. Physical and chemical probes that measure the levels of oxygen, glucose, CO2 and pH of cell culture media can determine cell metabolism and health status.(Bavli et al., 2016; Hossein Mahfouzi, Amoabediny, Doryab, Hamid Safiabadi-Tali, & Ghanei, 2018; Jenkins, Dmitriev, Morten, McDermott, & Papkovsky, 2015; Shaibani, Etayash, Naicker, Kaur, & Thundat, 2016; Weltin et al., 2014; Weyand et al., 2015) Traditional techniques that measure parameters such as glucose and oxygen concentrations take advantage of ion permeable membranes and selective enzymes to determine concentration levels.(Oliver, Toumazou, Cass, & Johnston, 2009) However, a broad range of transducers can also be used to indirectly measure these parameters of cell health.(Modena, Chawla, Misun, & Hierlemann, 2018) Field effect transistors (FET) and light-addressable potentiometric sensors (LAPs) have emerged as useful sensors capable of detecting cellular metabolic products and provide effective spatial resolution within 2D cell culture systems.(Dantism, Takenaga, Wagner, Wagner, & Schöning, 2015; Poghossian, Ingebrandt, Offenhäusser, & Schöning, 2009) Most of these culture characteristics are measured in the media downstream of cells and they give real-time information without destruction of the culture or tissue. These sensors are commercially available and are usually incorporated within bioreactor systems that are used to grow and monitor spheroids or cells in suspension over time.(Alexander, Eggert, & Wiest, 2018; Weyand et al., 2015) This approach assumes that the media is reflecting the behavior inside the tissue. With 3D cultures such as spheroids, this approach may become problematic as spheroids display diffusion limits to nutrients leading to cell death at the center of the culture.(Mehta, Hsiao, Ingram, Luker, & Takayama, 2012) Further, co-culture systems can lead to local variations in behavior(Goers, Freemont, & Polizzi, 2014) which are difficult to detect in the whole system by media sampling.

An alternative approach involves the characterization of electrical properties of cells in culture. Trans-epithelial electrical resistance (TEER) measurements are widely used in biology and tissue engineering to determine epithelial and endothelial cell health and membrane integrity. These measurements give an indirect, non-invasive assessment of the permeability of cellular tight junctions and ultimately the barrier function of a cellular monolayer or 3D tissues.(Chen, Einspanier, & Schoen, 2015; Schmitz et al., 2018) There are several variables known to affect the final readout including electrode size, measurement temperature, media formulation, time in culture and cell passage number, making it difficult to standardize measurements.(Elbrecht, Long, & Hickman, 2018; Srinivasan et al., 2015) A detailed review of TEERs and a guide to laboratory standardization is presented by Srinivasan and colleagues.(Srinivasan et al., 2015)

Derived from the same principles as TEER, electrical impedance spectroscopy (EIS), is a well-established tool for monitoring 2D cell cultures. EIS measurements are acquired with electrodes positioned beneath the culture and changes in current/voltage through the cells are measured as a frequency sweep is undertaken. This process allows the electrical resistance and capacitance of cells to be monitored.(Benson, Cramer, & Galla, 2013; Groeber et al., 2015) While TEER measures currents at one frequency, sweeping the frequency allows EIS to obtain more detailed information about cellular health status over time. Currently, there are a range of commercially available TEER and EIS devices used for assessing cell barrier function, integrity and cell proliferation in real time. These include OrganoTEER (MIMETAS, Leiden, The Netherlands), xCELLigence Real Time Cell Analysis (ACEA Biosciences, Inc., CA, USA), EVOM2 (World Precision Instruments, FL, USA) and Millicell-ERS2 (EMD Millipore Corporation Billerica, MA, USA). However, these devices are mainly designed for monitoring adherent cells grown in 2D or transwell based co-culture

Posted on Authorea 19 Nov 2019 | CC BY 4.0 | https://doi.org/10.22541/au.157419361.11339080 | This a preprint and has not been peer reviewed. Data may be preliminary.

systems. Electrodes have also been integrated into a range of different 3D cell culture models and microfluidic platforms for EIS monitoring.(V. F. Curto, Ferro, Mariani, Scavetta, & Owens, 2018; S.-M. Lee et al., 2016; Lei, Liu, & Tsang, 2018a). In particular, cell proliferation within spheroids have been successfully monitored using this technique.(Bu, Diener, Frey, Kim, & Hierlemann, 2016; Lei, Lin, & Tsang, 2017; Lei, Liu, & Tsang, 2018b; Pan et al., 2019)

Additionally, EIS has also been incorporated into 3D culture systems for multi-parametric monitoring including bioelectrical sensing and the detection of cell metabolites. Despite the need for the integration of in-line sensing and optical monitoring tools for real time and rapid biological readouts, little research exists where these have been successfully integrated. This is especially evident with highly modular, sensor-based organ-on-chip systems that are difficult to integrate with current microscopy approaches due to design incompatibilities.(Vincenzo F. Curto et al., 2017; Esch, Ueno, Applegate, & Shuler, 2016; Zhang et al., 2017) Multi-parametric approaches incorporating sensing systems, optical readouts and techniques that can provide better spatial resolution are currently needed. This review focuses on the varied methods that have been used, the challenges and limitations associated with different strategies and the factors that need to still be addressed to enable real time, quantifiable results from these systems.

2. Impedance measurements in 3D cell culture

The implementation of EIS in 2D cultures relies on cells attaching to the electrodes, this does not occur in 3D cultures as they contain matrices such as biopolymers (collagen, fibrin), polymer scaffolds or discrete suspended cell clusters. As a result, there are a number of areas that the EIS system needs to be adapted for 3D applications:

1) Ensuring sensitivity of the electrode array to cells suspended in or attached to the matrix/scaffold by ensuring there is an even electric field through the whole system.

2) Adaptable to a range of different 3D cell culture formats – systems are often designed to address a specific biological research question or tissue function and so their architecture varies.

3) Ensuring that electrodes do not influence cell responses or change the mechanical environment in 3D cell culture systems.

The design and integration of electrodes within culture systems appears to be central to the effective monitoring of cells within scaffolds and hydrogels. It has been demonstrated that the electrical properties of cells can be monitored when they are immersed within a hydrogel rather than directly attached to a planar electrode.(S.-M. Lee et al., 2016) Lee et al. incorporated a matrix of vertical electrodes **(Figure 1a)** that were able to monitor the migration, proliferation and apoptosis of cells through a gel matrix in 3D. In this system, cells were grown in an alginate hydrogel scaffold and vertical parallel electrodes were used to measure the capacitance of the system. An increase in capacitance was shown to be proportional to an increase in seeded cell density within the hydrogel **(Figure 1b and Figure 1c)** , showing that by measuring capacitance of the system it is possible to monitor cell number. To monitor cell proliferation and migration, one frequency (1 kHz) was used to obtain the capacitance of the system rather than the whole impedance spectrum. The chosen frequency coincides with the frequency typically used to monitor cells in 2D,(Benson et al., 2013; Srinivasan et al., 2015) but the study did not explore how cell death or migration affects the whole spectrum or whether other changes in the spectrum could be correlated to these cellular processes.

In many instances, cells are grown in a 3D spheroidal structure in which the cells clump together and proliferate. These models are used extensively in cancer research to understand drug treatments for tumors. Lei et al. used in-plane electrodes to monitor the viability of spheroids developed in an agarose hydrogel using the hanging drop technique **(Figure 2a)** .(Lei et al., 2018) Changes in the impedance magnitude was successfully correlated with different cell numbers seeded in the hydrogel **(Figure 2b)** . It is interesting to note here that while the authors chose the standard 1 kHz as the optimal sensing frequency, as was seen in the earlier study, there were larger variations in the impedance magnitude at lower frequencies. These variations were accounted for by the authors as being related to the increases in the noise as the frequency

Posted on Authorea 19 Nov 2019 | CC BY 4.0 | https://doi.org/10.22541/au.157419361.11339080 | This a preprint and has not been peer reviewed. Data may be preliminary.

is reduced, due to noise being proportional to 1/f.

The position of the electrodes within 3D culture systems (parallel vs in-plane) has also been shown to affect detection outcomes.(Y. Xu et al., 2015) Pan et al. found that in-plane electrodes were not able to significantly detect the proliferation of spheroids in a matrigel, whereas parallel electrodes successfully monitored their proliferation over time **(Figure 3a and Figure 3c)** . This data corresponds with the theory that parallel electrodes can achieve more sensitive detection due to a more uniform electric field.(Y. Xu et al., 2015) Nevertheless, this challenges the results from the earlier studies from Lei et al. using in-plane electrodes. Factors influencing these differences may be gel thickness and electrode design. When in-plane electrodes are used in a 3D system, the applied current can only penetrate a small volume and distance, whereas parallel electrodes force the applied current to go across the entire sample. In this instance it is to be expected that more signal can be detected and signal to noise increases as the current penetrates a larger volume of the 3D culture.

While the integration of standard metal electrodes into 3D culture is challenging due to the stiffness of the electrode materials and the need to connect them back to the outside world, one group has been exploring the application of conductive polymers as both the cell scaffold and the electrodes.(Khan et al., 2019) As the cells adhere to this conductive scaffold, its impedance changes enabling cell adhesion to be detected**(Figure 4a and Figure 4c)** .(Del Agua et al., 2018) Interestingly, the optimal sensing frequency found using a poly(3,4-ethyenedioythiophene): xanthan scaffold was between 0.1 and 100 Hz **(Figure 4b)** . As mentioned in the previous example, noise varies with 1/f, so at lower frequencies we would expect that noise would increase significantly. The potentiostat used for the impedance measurements may be able to compensate, however, little information is given by the authors of this study to determine if this approach has been implemented. Acquisition rate may also be problematic at low frequencies, though in the case of cell monitoring this may not be an issue since these are long-term cultures of days and weeks rather than minutes and hours.

In 2D, it is well established that low frequency signals are dominated by the double layer capacitance of the electrodes.(Benson et al., 2013) Nevertheless, in this study the electrode properties change as the cells are growing within the conductive hydrogel and is consistent with other studies using conductive scaffolds.(Inal et al., 2017) Further, modifying the properties of the 3D scaffolds (physical and chemical properties) should be done with caution as scaffold properties can have a big influence on cell behavior and function (Bahcecioglu, Hasirci, & Hasirci, 2019; Caliari & Burdick, 2016; Sridharan, Ryan, Kearney, Kelly, & O'Brien, 2019) and these influences need to be understood and cross-validated with other characterization tools prior to implementation. This suggests that the nature of the scaffold, its potential use as a working electrode and the nature of the 3D model (spheroids or tissue) plays a significant role in the final outcome of the measurements and as such models are needed to compare results with theoretical systems.

Several equivalent circuits have been designed to model and quantify the electrical properties of cells grown in 2D systems. **Figure 5**shows the equivalent circuit generally used to describe live cells in a monolayer.(Benson et al., 2013) This circuit takes into account the resistance of the solution or electrolyte (phosphate buffered saline (PBS) or cell media) Rmedium, the resistance and capacitance of the cell monolayer that contributes to the total spectrum RTEER and Ccl, respectively, and the capacitance of the electrodes and electrode-medium interphase CEl.(Benson et al., 2013) The electrical circuit and electrode setup for 2D adherent cells are well developed in EIS and comprehensive reviews of impedance measurements of cells are available and demonstrated.(Benson et al., 2013; Canali et al., 2016; Elbrecht et al., 2018; Srinivasan et al., 2015) In undertaking this review we could find only one study that has directly compared equivalent circuits for 2D and 3D systems.(Pan et al., 2019) They demonstrated that a matrigel matrix resistance component was in parallel with the electrical components representing cell impedance (Figure 3b). This in contrast to a typical 2D equivalent circuit which shows that the media component is in series with the components representing cell impedance (Figure 5). This is explained by looking at the fundamental differences in cell interactions occurring in the two different models. In 2D, the cells reside below the media (series circuit) while in 3D, the cells reside within the scaffold, which resembles more of a parallel circuit. Furthermore, Pan and colleagues paired simulations derived from their 3D equivalent circuit with their experimental data using epithelial cells

Posted on Authorea 19 Nov 2019 | CC BY 4.0 | https://doi.org/10.22541/au.157419361.11339080 | This a preprint and has not been peer reviewed. Data may be preliminary.

and found that the presence of cells in 3D decreased the overall impedance of the system. This was due to an increase in gel conductivity after the addition of cells, which was attributed to the presence of gap junctions between cells that allowed electrical connection between neighboring cell membranes.(Pan et al., 2019) However, other studies with similar cell types have shown the opposite effect, with the introduction of cells increasing the overall impedance of 3D systems.(Inal et al., 2017; S.-M. Lee et al., 2016; Lei et al., 2018)

Groeber et al. are amongst the only published groups using the whole EIS spectrum and information provided by an equivalent circuit to monitor tissue growth in a co-culture system. They used parallel electrodes to measure the impedance of an artificially grown epidermis at different levels of maturity as shown in **Figure 6b** .(Groeber et al., 2015) They developed an equivalent circuit in which there are a series of connections of n parallel resistor-capacitor circuits which correlated with a different maturity phase of the artificially grown epidermis (see**Figure 6a**). The data obtained was validated against an isolated human epidermis showing that the artificially grown epidermis at days 9-12 had the same electrical characteristics as the human epidermis. In the study, 1kHz was found to be an optimal sensing frequency, suggesting that it may well be the nature of the conductive scaffold that reduced the frequency in the study by Agua et al. To measure the impedance of the tissue, Groeber et al. grew the tissue in a transwell and transferred the transwell to an impedance platform every time a measurement was required. It is possible to use ideas from the literature to envisage how electrodes could be inserted into the transwell to monitor the system in situ or within a microfluidic device as undertaken by Sriram et al.(Sriram et al., 2018) A natural extension of this approach would be to design electrodes, so they are small enough to contact only one layer of tissue construct. This could enable the extraction of information of cells within a select region and monitoring of the individual features as the tissue grows. While Groeber and colleagues monitored growth and differentiation of epidermal cells, electrodes placed at different layers of a full thickness skin equivalent may allow for multicellular monitoring as well as the potential monitoring of skin processes such as wound healing and barrier function. Currently, skin tissue maturity is assessed by histology and immunostaining at different time points, so a layer-by-layer electrode setup which monitors tissue impedance as it grows could allow researchers to track growth and cellular processes quickly and without destroying the model.

3. Multi-parametric monitoring of 3D cultures

Impedance changes are a compilation of multiple processes occurring in cell culture systems. In the case of 2D cultures, significant impedance changes have been successfully correlated with cell proliferation and death. However, in 3D cultures, cellular processes are more complex, thus impedance changes may be influenced by different factors such as matrix degradation, cell spreading, migration or proliferation. This raises the need for a secondary or multi-parametric approach to further understand, monitor and validate 3D culture systems. In-line monitoring systems coupled with impedance can lead to better real-time and multi-parametric biological understanding of complex 3D systems. Zhang et al. developed a multisensor-integrated organ-on-chip platform that contains two organ modules, one physical/chemical sensing module and one bioelectrochemical sensing module, all connected via a perfused system.(Zhang et al., 2017) The physical/chemical sensing system consisted of optical pH and oxygen sensors. Additionally, gold electrodes functionalized with desired antibodies for specific detection of molecules in the media could be incorporated into the bioelectrochemical sensing module. The EIS changed upon binding of the target molecule and detection of different concentrations of the analyte/biomarker was possible. They found that the amount of biomarker that could be captured by the functionalized electrode surface was proportional to the concentration in the solution, with detection levels of 0.1 to 100 ng/ml, similar to many commercial ELISA systems. Thus, biologically relevant concentrations were detected. Even though this is a complex system, it contains fundamental parameters for real-time monitoring of organoids. However, this system is highly modular and specific to the microfluidics system they utilize. This makes it harder to standardize and integrate these systems with current tissue culture consumables and microscopes. Nevertheless, to date, this is the most complete multi-parametric monitoring system for organoids.

Others have also used a multi-parametric monitoring approach coupled with electrical measurements to

Posted on Authorea 19 Nov 2019 | CC BY 4.0 | https://doi.org/10.22541/au.157419361.11339080 | This a preprint and has not been peer reviewed. Data may be preliminary.

monitor 3D cell cultures. Misun and colleagues correlated amperometric (current) changes to glucose and lactate concentrations in spheroids, giving real-time information on the metabolic state of these microtissues. For the detection of glucose and lactose in these spheroids, a microfluidic polydimethylsiloxane (PDMS) chip with an enzyme-based hydrogel coated onto sensing electrodes was proposed.(Misun, Hierlemann, & Frey, 2018) However, the sensor chip and well was limited in its use as it is specifically designed for spheroid growth via the hanging-drop culture technique. The sensor also depends on the effective release of glucose from the spheroid to the media. Another approach to monitor glucose concentrations and cell proliferation was shown by Curto et al. They developed an in vitro cell monitoring platform, which monitored the impedance changes of tissue by means of an organic electrochemical transistor (OECT) at the bottom of the well.(Vincenzo F. Curto et al., 2017) Glucose was also monitored with an OECT-based glucose biosensor, but the glucose biosensor was not in situ. Media was collected at the outlet of the perfused system and later measured with the sensor. This multi parameter system allowed the monitoring of kidney cell metabolic state via the glucose sensor as well as changes in resistance and capacitance in real-time while the kidney cell epithelium recovered from an electrical wound.

All of the above systems allow for real-time monitoring of the metabolic activity of cells through measuring glucose, pH and oxygen levels in the media. Even though they are sensitive, they depend on the efficient release of the molecule of interest into the media, which can be limited in a 3D cell culture system. The lack of spatial resolution displayed by most of the monitoring techniques within these devices remains a drawback. Correlating metabolite levels in media with a technique that gives information about spatial resolution would allow for more specialized biological information to be gained from the monitoring system.

Posted on Authorea 19 Nov 2019 | CC BY 4.0 | https://doi.org/10.22541/au.157419361.11339080 | This a preprint and has not been peer reviewed. Data may be preliminary.

4. 3D impedance tomography

In 3D cell culture, obtaining spatial resolution is of interest as it allows researchers to determine the location of cells over time, monitor the specific cell health and helps to highlight differences in cell health and function in different areas of the tissue. Electrical impedance tomography (EIT) is a relatively new technique that has emerged as a potential solution to provide better spatial resolution within 3D tissue cultures. However, most recent efforts in EIT have been focused on its use in vivo and as a medical diagnostic, where electrodes are placed on the body to obtain an image and health status of an organ of interest.(Fernandez-Corazza et al., 2018; Schwartz, Chauhan, & Sadleir, 2018; Sun, Yue, Hao, Cui, & Wang, 2019)

Similar to the implementation of EIS, implementing EIT involves the application of a small current to a sample, such as the human body, tissue model or inanimate objects with different conductive properties (generally used for prototyping), and the voltage is measured in order the obtain the electrical properties of the sample.(Chitturi & Nagi, 2017) Different electrode designs, current injection strategies and image reconstruction algorithms have been recently reviewed by Chitturi and colleagues.(Chitturi & Nagi, 2017) In general, the set up involves a matrix of electrodes that is placed around the sample, a current is injected through a pair of electrodes while the voltage changes are measured through a different pair of electrodes. The values obtained are then used to calculate the resistivity of the sample and reconstruct the image using the chosen algorithm.

The successful use of EIT as a medical diagnostic and its ability to obtain in vivo information, suggests that it may be an effective monitoring technique for future 3D cell cultures. Researchers have demonstrated the measurement of 3D tissue impedance and subsequently reconstruction of the respective images using EIT.(Jamil et al., 2016; Wu, Yang, Bagnaninchi, & Jia, 2017, 2018; Wu, Zhou, Yang, Jia, & Bagnaninchi, 2018; Yang et al., 2017; Yin, Wu, Jia, & Yang, 2018; Yin, Yang, Jia, & Tan, 2017) For monitoring 3D cell aggregates or spheroids, two electrode distribution designs were implemented. In one study, circular electrodes were radially distributed at the bottom of a well, in which case current was injected through a pair of electrodes and voltage was measured at the adjacent pair, while the reference electrode was connected to ground **(Figure 7a)**. In another study, current was applied to rectangular electrodes placed at the boundary of the well and voltage was measured as shown in **Figure 7b**. Using both electrodes designs the authors were able monitor the presence and growth of spheroids(Yin et al., 2018) as well as spheroid disintegration upon the addition of Triton-X 100 **(Figure 7c)**. However, the resolution of the output is not yet at the cellular

level as shown by the heat maps in Figure 7c. Nevertheless, compared to EIS, additional information can be gathered using EIT including the position and potentially the size of spheroids and tissues in culture systems. Given the issue of contraction in some collagen-based 3D models, as well as the presence of contraction in wound healing models,(Lotz et al., 2017) a potential application for EIT could be to monitor contraction over time or after wounding in these models. Others have designed similar set ups to those demonstrated in Figure 7, with the eventual aim of monitoring cell cultures, but have not yet tested it using cells.(Z. Xu et al., 2018)

Likewise, a more complex setup of electrodes was designed with the intention of monitoring 3D cultures using EIT.(E. J. Lee et al., 2014) The quality of the designed device **(Figure 8)** was assessed using performance metrics such as crosstalk, amplitude stability error, total harmonic distortion and signal to noise ratio. While this system was not tested using cells, the high quantity of electrodes positioned all over the well (Figure 8) could potentially increase the final image resolution and subsequently biological output, as more data points may be obtained from the well. Conversely, having electrodes in such close proximity may also increase the crosstalk and artefacts such as stray capacitance. A fine balance between the number of electrodes and electrode design is needed to obtain optimal resolution in the presence of 3D cell cultures.

The above studies have demonstrated a prospective use for EIT in 3D cell culture monitoring, however current applications do not offer a clear advantage over EIS. EIS is comparatively simpler and may be more cost effective in obtaining the electrical properties of a biological 3D sample. While EIT may be an attractive solution for better spatial resolution in the monitoring of 3D cultures, the current sensitivity obtained can only give qualitative information at the tissue level as opposed to information at the cellular level. Achieving resolution at the cellular level will give a better indication of what is occurring as cell cultures grow over time, after treatments and within different regions of the 3D culture.

5. Future directions

3D culture is expanding across research and industrial laboratories, as it offers the possibility of more physiologically relevant models for drug testing and replication of diseased tissues. Therefore, there is a need to characterize and standardize monitoring techniques across different laboratories. This will increase the scope of 3D culture as well as ease its implementation.(Verjans et al., 2017) Further it will allow researchers to select matured tissues that are physiologically relevant in their contexts and allow them to perform experiments in a more controlled environment where variability within cultures can be monitored. However, a lack of monitoring techniques represents a limitation for further growth. Developing monitoring techniques targeted for 3D cultures will allow for easier implementation and standardization of these cultures. The development of reproducible, scalable and validated 3D cell culture systems that can handle a diverse range of cell types and co-cultures whilst providing real-time feedback on culture health will improve our fundamental understanding of biology. The ability to monitor the cultures as they grow and assess their viability prior to testing them with an external stimulus will accelerate the rate of discovery.

Multiple approaches to integrating electrodes into 3D cell culture systems have enabled the monitoring of cell health over time. To date, researchers have developed in situ sensors using EIS to monitor cell proliferation and viability. Furthermore, they have created devices with functionalized electrode surfaces to enable the measurement of the metabolic activity of 3D cell cultures. However, each of these devices are highly customized for the specific application and 3D culture technique, making standardization difficult. In future, EIT may provide better spatial resolution, but the currently achieved resolution remains low and the data gathered can also be obtained with EIS, which utilizes simpler hardware and data analysis. An ideal monitoring system for 3D cell culture would be able to monitor multicellular models in situ and in real-time. Having an ideal spatial resolution at the cellular level (˜50 µm) will allow monitoring of specific cells in a multicellular construct and their state within the system (live, death differentiated). Impedance alone can achieve real-time monitoring of cell growth and proliferation, however, it should be complemented and correlated with measurements from the monitoring of other cell functions such as metabolic activity

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

which can be detected downstream in the media using functionalized surfaces as transducers or sensors of interest.

Acknowledgements

The authors gratefully acknowledge funding from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Probing Biosystems Future Science Platform, CSIRO Research+ Science Leader Scheme and Swinburne University of Technology for the PhD stipend and scholarship for SDL.

References

Alexander, F., Eggert, S., & Wiest, J. (2018). A novel lab-on-a-chip platform for spheroid metabolism monitoring. *Cytotechnology* ,*70* (1), 375–386. https://doi.org/10.1007/s10616-017-0152-x

Bahcecioglu, G., Hasirci, N., & Hasirci, V. (2019). Cell behavior on the alginate-coated PLLA/PLGA scaffolds. *International Journal of Biological Macromolecules* , *124* , 444–450. https://doi.org/10.1016/J.IJBIOMAC.2018.11.169

Baker, B. M., & Chen, C. S. (2012). Deconstructing the third dimension-how 3D culture microenvironments alter cellular cues.*Journal of Cell Science* , *125* , 3015–3024. https://doi.org/10.1242/jcs.079509

Bavli, D., Prill, S., Ezra, E., Levy, G., Cohen, M., Vinken, M., ... Nahmias, Y. (2016). Real-time monitoring of metabolic function in liver-on-chip microdevices tracks the dynamics of mitochondrial dysfunction. *Proceedings of the National Academy of Sciences of the United States of America* , *113* (16), E2231-40. https://doi.org/10.1073/pnas.1522556113

Benson, K., Cramer, S., & Galla, H.-J. (2013). Impedance-based cell monitoring: barrier properties and beyond. *Fluids and Barriers of the CNS* , *10* (1), 5. https://doi.org/10.1186/2045-8118-10-5

Brajša, K., Trzun, M., Zlatar, I., & Jelić, D. (2016). Three-dimensional cell cultures as a new tool in drug discovery.*Periodicum Biologorum* , *118* (1), 59–65. https://doi.org/10.18054/pb.2016.118.1.3940

Bu, S. C., Diener, L., Frey, O., Kim, J.-Y., & Hierlemann, A. (2016). Automated, Multiplexed Electrical Impedance Spectroscopy Platform for Continuous Monitoring of Microtissue Spheroids. https://doi.org/10.1021/acs.analchem

Caliari, S. R., & Burdick, J. A. (2016). A Practical Guide to Hydrogels for Cell Culture Introduction: why hydrogels for cell culture? *Nat Methods* , *13* (5), 405–414. https://doi.org/10.1038/nmeth.3839

Canali, C., Heiskanen, A., Martinsen, Ø. G., Mohanty, S., Dufva, M., Wolff, A., & Emnéus, J. (2016). Impedance-Based Monitoring for Tissue Engineering Applications. *IFMBE Proceedings* ,*54* (September), 48–51. https://doi.org/10.1007/978-981-287-928-8

Carlson, M. W., Alt-Holland, A., Egles, C., & Garlick, J. A. (2008). Three-Dimensional Tissue Models of Normal and Diseased Skin.*Current Protocols in Cell Biology* , *41* (1), 19.9.1-19.9.17. https://doi.org/10.1002/0471143030.cb1909s4

Chen, S., Einspanier, R., & Schoen, J. (2015). Transepithelial electrical resistance (TEER): a functional parameter to monitor the quality of oviduct epithelial cells cultured on filter supports.*Histochemistry and Cell Biology* , *144* (5), 509–515. https://doi.org/10.1007/s00418-015-1351-1

Chitturi, V., & Nagi, F. (2017). Spatial resolution in electrical impedance tomography: A topical review. *Journal of Electrical Bioimpedance* , *8* (1), 66. https://doi.org/10.5617/jeb.3350

Curto, V. F., Ferro, M. P., Mariani, F., Scavetta, E., & Owens, R. M. (2018). A planar impedance sensor for 3D spheroids. *Lab on a Chip* , *18* (6), 933–943. https://doi.org/10.1039/c8lc00067k

Curto, Vincenzo F., Marchiori, B., Hama, A., Pappa, A.-M., Ferro, M. P., Braendlein, M., ... Owens, R. M. (2017). Organic transistor platform with integrated microfluidics for in-line multi-parametric in vitro cell monitoring. *Microsystems & Nanoengineering* , *3* , 17028. https://doi.org/10.1038/micronano.2017.28

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

Dantism, S., Takenaga, S., Wagner, P., Wagner, T., & Schöning, M. J. (2015). Light-addressable Potentiometric Sensor (LAPS) Combined with Multi-chamber Structures to Investigate the Metabolic Activity of Cells.*Procedia Engineering* , *120* , 384–387. https://doi.org/10.1016/J.PROENG.2015.08.647

Del Agua, I., Marina, S., Pitsalidis, C., Mantione, D., Ferro, M., Iandolo, D., … Mecerreyes, D. (2018). Conducting Polymer Scaffolds Based on Poly(3,4-ethylenedioxythiophene) and Xanthan Gum for Live-Cell Monitoring. https://doi.org/10.1021/acsomega.8b00458

Dmitriev, R. I. (2017). *Multi-Parametric Live Cell Microscopy of 3D Tissue Models* (Vol. 1035). https://doi.org/10.1007/978-3-319-67358-5

Duval, K., Grover, H., Han, L.-H., Mou, Y., Pegoraro, A. F., Fredberg, J., … Han, L.-H. (2017). Modeling Physiological Events in 2D vs. 3D Cell Culture. https://doi.org/10.1152/physiol.00036.2016

Edmondson, R., Broglie, J. J., Adcock, A. F., & Yang, L. (2014). Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay and Drug Development Technologies* , *12* (4), 207–218. https://doi.org/10.1089/adt.2014.573

Elbrecht, D. H., Long, C. J., & Hickman, J. J. (2018). Transepithelial/endothelial Electrical Resistance (TEER) theory and applications for microfluidic body-on-a-chip devices. *Journal of Rare Diseases Research & Treatment* , *1* (3), 46–52. https://doi.org/10.29245/2572-9411/2016/3.1026

Esch, M. B., Ueno, H., Applegate, D. R., & Shuler, M. L. (2016). Modular, pumpless body-on-a-chip platform for the co-culture of GI tract epithelium and 3D primary liver tissue. *Lab on a Chip* ,*16* (14), 2719–2729. https://doi.org/10.1039/c6lc00461j

Fernandez-Corazza, M., Turovets, S., Luu, P., Price, N., Muravchik, C. H., & Tucker, D. (2018). Skull modeling effects in conductivity estimates using parametric electrical impedance tomography. *IEEE Transactions on Biomedical Engineering* , *65* (8), 1785–1797. https://doi.org/10.1109/TBME.2017.2777143

Goers, L., Freemont, P., & Polizzi, K. M. (2014). Co-culture systems and technologies: taking synthetic biology to the next level.*Journal of the Royal Society, Interface* , *11* (96). https://doi.org/10.1098/rsif.2014.0065

Groeber, F., Engelhardt, L., Egger, S., Werthmann, H., Monaghan, M., Walles, H., & Hansmann, J. (2015). Impedance Spectroscopy for the Non-Destructive Evaluation of in Vitro Epidermal Models.*Pharmaceutical Research* . https://doi.org/10.1007/s11095-014-1580-3

Hossein Mahfouzi, S., Amoabediny, G., Doryab, A., Hamid Safiabadi-Tali, S., & Ghanei, M. (2018). Noninvasive Real-Time Assessment of Cell Viability in a Three-Dimensional Tissue. *Termis. Tissue Engineering & Regenerative Medicine Internal Society* , *24* (4). https://doi.org/10.1089/ten.tec.2017.0371

Inal, S., Hama, A., Ferro, M., Pitsalidis, C., Oziat, J., Iandolo, D., … Owens, R. M. (2017). Conducting Polymer Scaffolds for Hosting and Monitoring 3D Cell Culture. *Advanced Biosystems* , *1* (6), 1700052. https://doi.org/10.1002/adbi.201700052

Jamil, N., Smith, S., Yang, Y., Jia, J., Bagnaninchi, P., & Gonzalez-Fernandez, E. (2016). Design and fabrication of microelectrodes for electrical impedance tomography of cell spheroids. In *2016 IEEE EMBS Conference on Biomedical Engineering and Sciences (IECBES)*(pp. 426–431). IEEE. https://doi.org/10.1109/IECBES.2016.7843486

Jenkins, J., Dmitriev, R. I., Morten, K., McDermott, K. W., & Papkovsky, D. B. (2015). Oxygen-sensing scaffolds for 3-dimensional cell and tissue culture. *Acta Biomaterialia* , *16* , 126–135. https://doi.org/10.1016/J.ACTBIO.2015.01.0

Khan, M., Cantù, E., Tonello, S., Serpelloni, M., Lopomo, N., & Sardini, E. (2019). A Review on Biomaterials for 3D Conductive Scaffolds for Stimulating and Monitoring Cellular Activities. *Applied Sciences* , *9* (5), 961. https://doi.org/10.3390/app9050961

Lee, E. J., Wi, H., McEwan, A. L., Farooq, A., Sohal, H., Woo, E. J., … Oh, T. I. (2014). Design of a microscopic electrical impedance tomography system for 3D continuous non-destructive monitoring of tissue culture. *BioMedical Engineering OnLine* , *13* . https://doi.org/10.1186/1475-925X-13-142

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

Lee, S.-M., Han, N., Lee, R., Choi, I.-H., Park, Y.-B., Shin, J.-S., & Yoo, K.-H. (2016). Real-time monitoring of 3D cell culture using a 3D capacitance biosensor. *Biosensors and Bioelectronics* , *77* , 56–61. https://doi.org/10.1016/j.bios.2015.09.005

Lee, V., Singh, G., Trasatti, J. P., Bjornsson, C., Xu, X., Tran, T. N., ... Karande, P. (2014). Design and fabrication of human skin by three-dimensional bioprinting. *Tissue Engineering. Part C, Methods* , *20* (6), 473–484. https://doi.org/10.1089/ten.TEC.2013.0335

Lei, K. F., Lin, B.-Y., & Tsang, N.-M. (2017). Real-time and label-free impedimetric analysis of the formation and drug testing of tumor spheroids formed via the liquid overlay technique. *RSC Adv.* ,*7* (23), 13939–13946. https://doi.org/10.1039/C7RA00209B

Lei, K. F., Liu, T. K., & Tsang, N. M. (2018b). Towards a high throughput impedimetric screening of chemosensitivity of cancer cells suspended in hydrogel and cultured in a paper substrate.*Biosensors and Bioelectronics* , *100* (259), 355–360. https://doi.org/10.1016/j.bios.2017.09.029

Lotz, C., Schmid, F. F., Oechsle, E., Monaghan, M. G., Walles, H., & Groeber-Becker, F. (2017). Cross-linked Collagen Hydrogel Matrix Resisting Contraction to Facilitate Full-Thickness Skin Equivalents.*ACS Applied Materials and Interfaces* , *9* (24), 20417–20425. https://doi.org/10.1021/acsami.7b04017

Mehta, G., Hsiao, A. Y., Ingram, M., Luker, G. D., & Takayama, S. (2012). Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. *Journal of Controlled Release : Official Journal of the Controlled Release Society* ,*164* (2), 192–204. https://doi.org/10.1016/j.jconrel.2012.04.045

Misun, P. M., Hierlemann, A., & Frey, O. (2018). Miniature Fluidic Microtissue Culturing Device for Rapid Biological Detection (pp. 207–225). Springer, Cham. https://doi.org/10.1007/978-3-319-64747-0_8

Modena, M. M., Chawla, K., Misun, P. M., & Hierlemann, A. (2018). Smart Cell-Culture Systems: Integration of Sensors and Actuators into Microphysiological Systems. *ACS Chemical Biology* , acschembio.7b01029. https://doi.org/10.1021/acschembio.7b01029

Oliver, N. S., Toumazou, C., Cass, A. E. G., & Johnston, D. G. (2009). Glucose sensors: a review of current and emerging technology.*Diabetic Medicine* , *26* (3), 197–210. https://doi.org/10.1111/j.1464-5491.2008.02642.x

Pan, Y., Hu, N., Wei, X., Gong, L., Zhang, B., Wan, H., & Wang, P. (2019). 3D cell-based biosensor for cell viability and drug assessment by 3D electric cell/matrigel-substrate impedance sensing.*Biosensors and Bioelectronics* , *130* , 344–351. https://doi.org/10.1016/j.bios.2018.09.046

Poghossian, A., Ingebrandt, S., Offenhausser, A., & Schoning, M. J. (2009). Field-effect devices for detecting cellular signals.*Seminars in Cell & Developmental Biology* , *20* (1), 41–48. https://doi.org/10.1016/J.SEMCDB.2009.01.014

Schmitz, T., Schweinlin, M., Kollhoff, R. T., Engelhardt, L., Lotz, C., Groeber-Becker, F., ... Hansmann, J. (2018). Nanostructured TiN-Coated Electrodes for High-Sensitivity Noninvasive Characterization of in Vitro Tissue Models. *ACS Applied Nano Materials* ,*1* (5), 2284–2293. https://doi.org/10.1021/acsanm.8b00345

Schwartz, B. L., Chauhan, M., & Sadleir, R. J. (2018). Analytic modeling of conductively anisotropic neural tissue. *Journal of Applied Physics* , *124* (6), 064701. https://doi.org/10.1063/1.5036659

Shaibani, P. M., Etayash, H., Naicker, S., Kaur, K., & Thundat, T. (2016). Metabolic Study of Cancer Cells Using a pH Sensitive Hydrogel Nanofiber Light Addressable Potentiometric Sensor. https://doi.org/10.1021/acssensors.6b0063

Sridharan, R., Ryan, E. J., Kearney, C. J., Kelly, D. J., & O'Brien, F. J. (2019). Macrophage Polarization in Response to Collagen Scaffold Stiffness Is Dependent on Cross-Linking Agent Used To Modulate the Stiffness. *ACS Biomaterials Science & Engineering* , *5* (2), 544–552. https://doi.org/10.1021/acsbiomaterials.8b00910

Srinivasan, B., Kolli, A. R., Esch, M. B., Abaci, H. E., Shuler, M. L., & Hickman, J. J. (2015). TEER measurement techniques for in vitro barrier model systems. *Journal of Laboratory Automation* ,*20* (2), 107–126. https://doi.org/10.1177/2211068214561025

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

Sriram, G., Alberti, M., Dancik, Y., Wu, B., Wu, R., Feng, Z., ... Wang, Z. (2018). Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. *Materials Today* ,*21* (4), 326–340. https://doi.org/10.1016/J.MATTOD.2017.11.002

Sun, B., Yue, S., Hao, Z., Cui, Z., & Wang, H. (2019). An Improved Tikhonov Regularization Method for Lung Cancer Monitoring Using Electrical Impedance Tomography. *IEEE Sensors Journal* , 1–1. https://doi.org/10.1109/JSE

Verjans, E.-T., Doijen, J., Luyten, W., Landuyt, B., & Schoofs, L. (2017). Three-dimensional cell culture models for anticancer drug screening: Worth the effort? *Journal of Cellular Physiology* ,*233* (4), 2993–3003. https://doi.org/10.1002/jcp.26052

Walker-Daniels, J. (2012). Live Cell Imaging Methods Review. *Materials and Methods* , *2* . https://doi.org/10.13070/mm.en.2.

Weltin, A., Slotwinski, K., Kieninger, J., Moser, I., Jobst, G., Wego, M., ... Urban, G. A. (2014). Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem. *Lab Chip* , *14* (1), 138–146. https://doi.org/10.1039/C3LC50759A

Weyand, B., Nohre, M., Schmalzlin, E., Stolz, M., Israelowitz, M., Gille, C., ... Vogt, P. M. (2015). Noninvasive Oxygen Monitoring in Three-Dimensional Tissue Cultures Under Static and Dynamic Culture Conditions. *BioResearch Open Access* , *4* (1), 266–277. https://doi.org/10.1089/biores.2015.0004

Wu, H., Yang, Y., Bagnaninchi, P. O., & Jia, J. (2017). Imaging cell-drug response in 3D bioscaffolds by electrical impedance tomography. In *2017 IEEE International Conference on Imaging Systems and Techniques (IST)* (pp. 1–5). IEEE. https://doi.org/10.1109/IST.2017.8261511

Wu, H., Yang, Y., Bagnaninchi, P. O., & Jia, J. (2018). Electrical impedance tomography for real-time and label-free cellular viability assays of 3D tumour spheroids. *The Analyst* , *143* (17), 4189–4198. https://doi.org/10.1039/C8AN00729B

Wu, H., Zhou, W., Yang, Y., Jia, J., & Bagnaninchi, P. (2018). Exploring the potential of electrical impedance tomography for tissue engineering applications. *Materials* , *11* (6), 1–11. https://doi.org/10.3390/ma11060930

Xu, Y., Xie, X., Duan, Y., Wang, L., Cheng, Z., & Cheng, J. (2015). A review of impedance measurements of whole cells. *Biosensors and Bioelectronics* , *77* , 824–836. https://doi.org/10.1016/J.BIOS.2015.10.027

Xu, Z., Yao, J., Wang, Z., Liu, Y., Wang, H., Chen, B., & Wu, H. (2018). Development of a Portable Electrical Impedance Tomography System for Biomedical Applications. *IEEE Sensors Journal* , *18* (19), 8117–8124. https://doi.org/10.1109/JSEN.2018.2864539

Yang, Y., Jia, J., Smith, S., Jamil, N., Gamal, W., & Bagnaninchi, P. O. (2017). A miniature electrical impedance tomography sensor and 3-D Image Reconstruction for Cell Imaging. *IEEE Sensors Journal* ,*17* (2), 514–523. https://doi.org/10.1109/JSEN.2016.2631263

Yin, X., Wu, H., Jia, J., & Yang, Y. (2018). A Micro EIT Sensor for Real-Time and Non-Destructive 3-D Cultivated Cell Imaging. *IEEE Sensors Journal* , *18* (13), 5402–5412. https://doi.org/10.1109/JSEN.2018.2834509

Yin, X., Yang, Y., Jia, J., & Tan, C. (2017). 3D image reconstruction on a miniature planar EIT sensor using sparsity with median filter. In*2017 IEEE SENSORS* (pp. 1–3). IEEE. https://doi.org/10.1109/ICSENS.2017.8234213

Zhang, Y. S., Aleman, J., Shin, S. R., Kilic, T., Kim, D., Mousavi Shaegh, S. A., ... Khademhosseini, A. (2017). Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. *Proceedings of the National Academy of Sciences of the United States of America* ,*114* (12), E2293–E2302. https://doi.org/10.1073/pnas.1612906114

Figure legends

Figure 1. (a) Vertical electrodes to measure the capacitance of cells in a hydrogel with vertical resolution. (b) Real-time capacitance of hydrogels seeded with different cell concentrations. (c) Fluorescent optical

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

images of hydrogels containing different cell densities. (scale bar: 100 μm). Reproduced with permission.(S.-M. Lee et al., 2016) Copyright 2015, Elsevier B.V.

Figure 2. (a) Horizontal electrodes for measuring cells in hydrogel using the hanging-drop technique. (b) Correlation between cell number and impedance magnitude measured at discrete frequencies (500, 1000 and 5000 Hz). Reproduced with permission.(Lei et al., 2018) Copyright 2017, Elsevier B.V.

Figure 3. (a) Schematic of the parallel electrodes for the impedance biosensor. (b) Equivalent circuit of cells in 3D within the matrigel. (c) Measurement of cells in 3D culture using the parallel electrodes (3D ECMIS). Reproduced with permission.(Pan et al., 2019) Copyright 2018, Elsevier B.V.

Figure 4. (a) Schematic of the poly(3,4-ethylenedioxythiophene) xanthan gum (PEDOT:xanthan) electroactive scaffold used to host and monitor cells. (b) Impedance spectrum of the scaffold with no cells (blue), after cell growth within the scaffold over 7 days (red) and PEDOT:xanthan gum scaffold after 7 days of incubation (37 °C, 5% CO2) in culture media without cells (dashed lines). Reproduced with permission.(Del Agua et al., 2018) Copyright 2018, American Chemical Society. (c) Typical impedance spectrum of 2D cells grown on electrodes. Reproduced with permission.(Del Agua et al., 2018) Copyright 2015, Elsevier B.V.

Figure 5. Equivalent circuit and typical impedance spectrum of cells in 2D. Adapted with permission.(Benson et al., 2013) Copyright 2013, BioMed Central Ltd.

Figure 6. Parallel impedance electrodes to measure the maturity of a reconstructed human epidermis (RHE). (a) Detailed view of one measurement chamber with a RHE in a transwell insert and equivalent circuit. (b) Bode plots of impedance spectra from RHE at different time points. Reproduced with permission.(Groeber et al., 2015) Copyright Groeber et al., 2014.

Figure 7. (a) Radially distributed circular electrodes (adapted from (Yin et al., 2018)) (b) Rectangular electrodes at the boundary of the well were used to monitor spheroid and (c) reconstructed images for the spheroid samples in 2% Triton X-100 solution (1-2) and HG culture medium (3-4). Reproduced with permission.(Wu, Yang, et al., 2018; Wu, Zhou, et al., 2018) Copyright Wu et al., 2018.

Figure 8. The electrode design shows the placement of current injecting electrodes on parallel walls and the placement of voltage sensing electrodes on the bottom surface as well as the two parallel walls. Reproduced with permission.(E. J. Lee et al., 2014) Copyright Lee et al.; licensee BioMed Central Ltd. 2014.

Figures

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

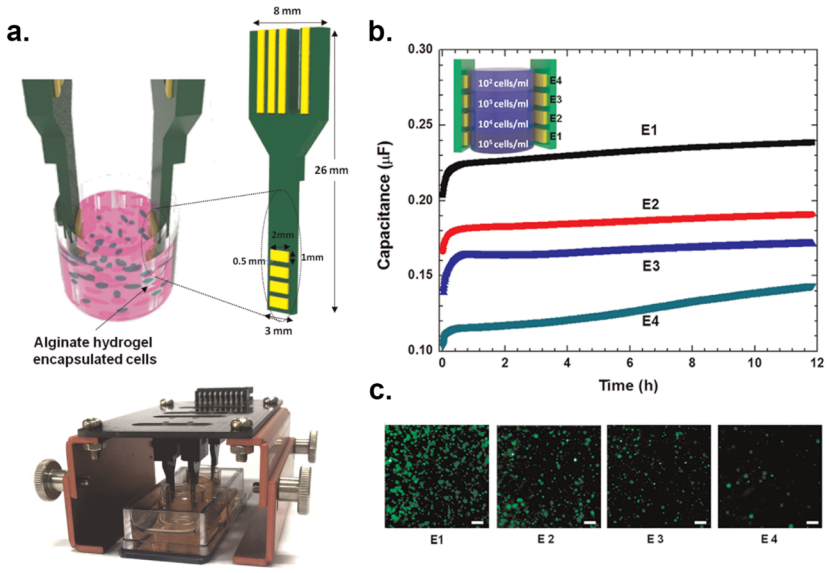

Figure 1.

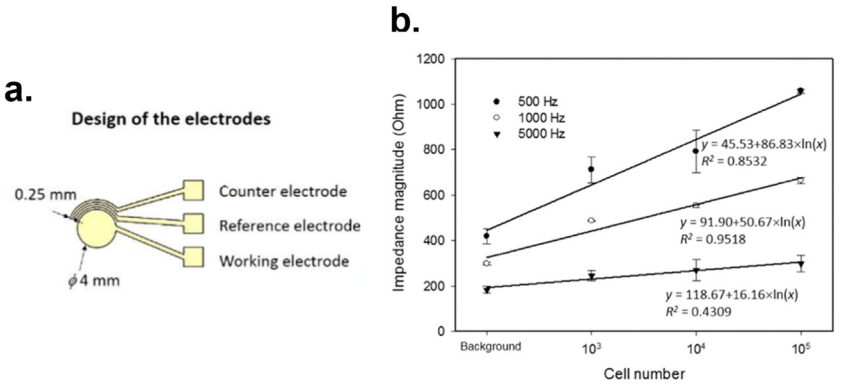

Figure 2.

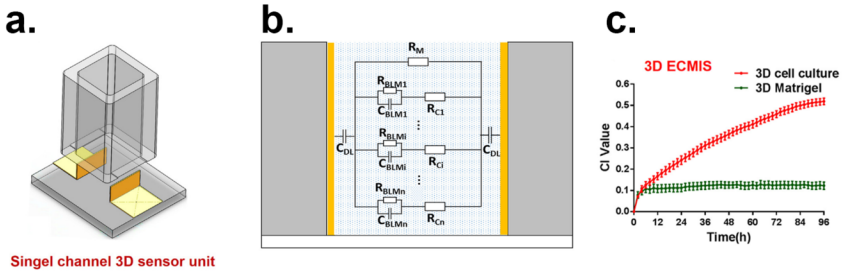

Figure 3.

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

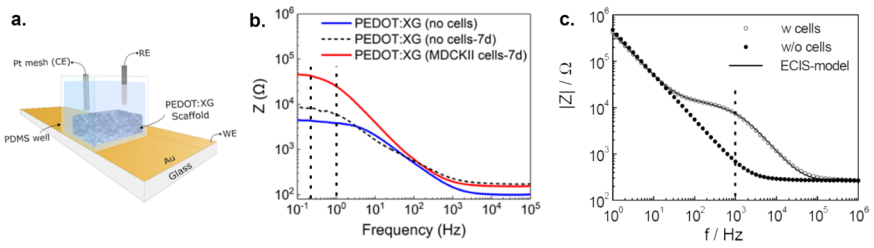

Figure 4.

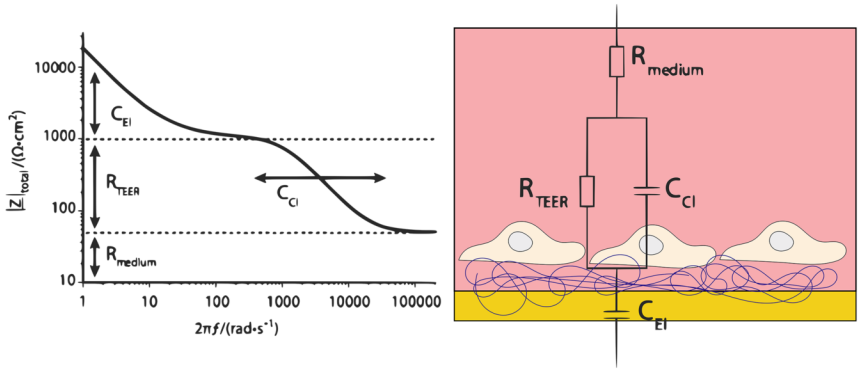

Figure 5.

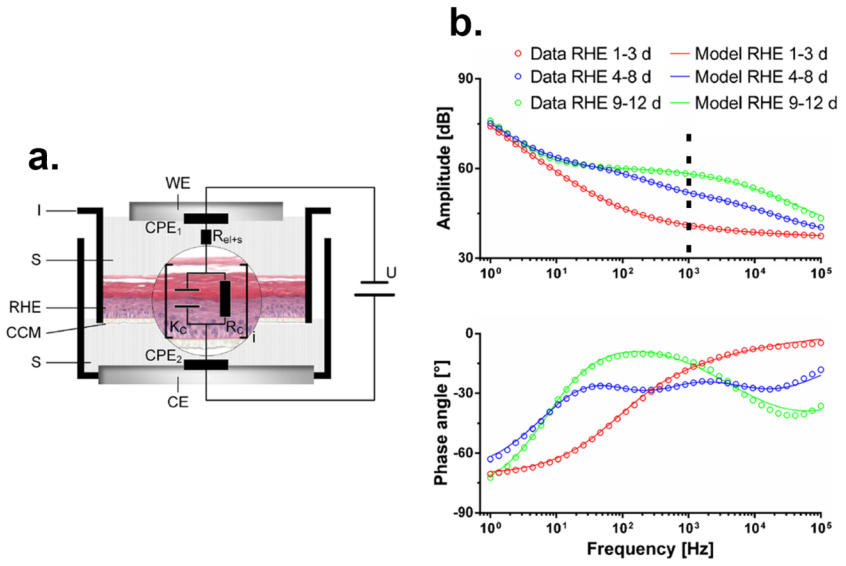

Figure 6.

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.

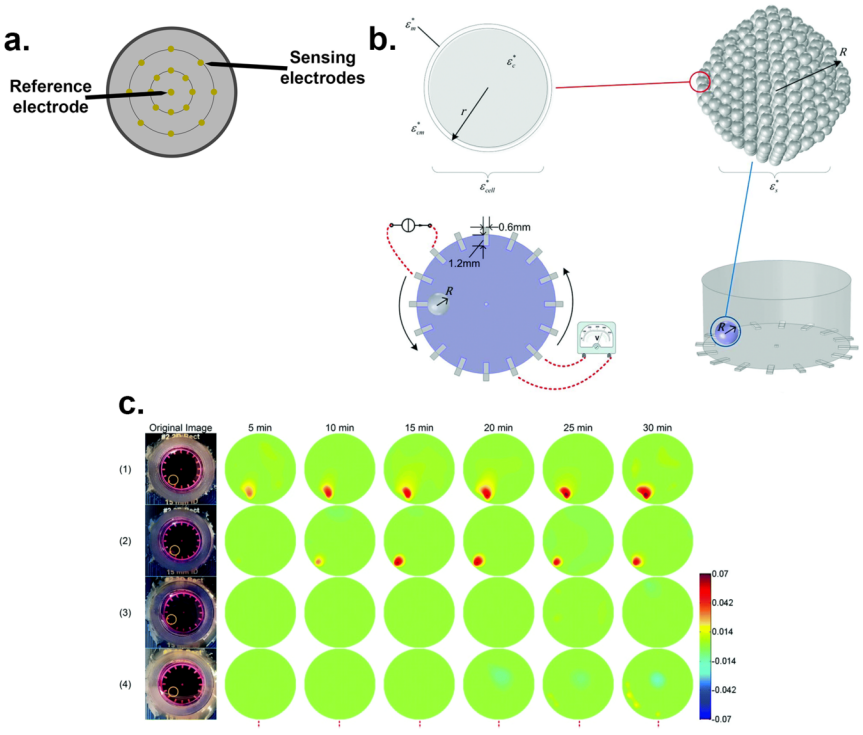

Figure 7.

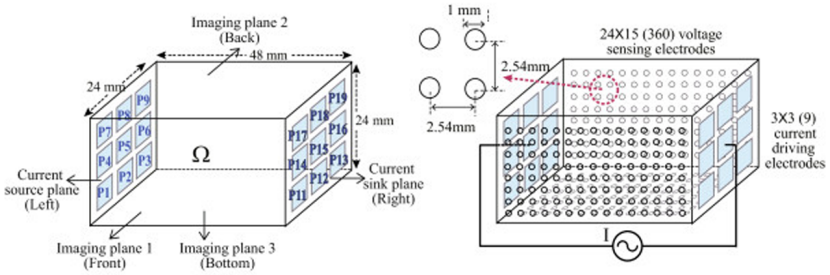

Figure 8.

Posted on Authorea 19 Nov 2019 — CC BY 4.0 — https://doi.org/10.22541/au.157419361.11339080 — This a preprint and has not been peer reviewed. Data may be preliminary.