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Impedance-based cellular assays for regenerative medicine

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Impedance-based cellular assays for regenerative medicine

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Abstract

Therapies based on regenerative techniques have the potential to radically improve healthcare in the coming years. As a result, there is an emerging need for non-destructive and label-free technologies to assess the quality of engineered tissues and cell-based products prior to their use in the clinic. In parallel, the emerging regenerative medicine industry that aim to produce stem cells and their progeny on a large scale will benefit from moving away from existing destructive biochemical assays towards data-driven automation and control at the industrial scale.

Impedance-based cellular assays (IBCA) have emerged as an alternative approach to study stem cell properties and cumulative studies, reviewed here, showed their potential to monitor stem cell renewal, differentiation and maturation. They offer a novel method to non-destructively assess and quality control stem cell cultures. In addition, when combined with in vitro disease models they provide complementary insights as label-free phenotypic assays. IBCA provide quantitative and very sensitive results that can easily automated and upscaled in multi-well format. When facing the emerging challenge of real-time monitoring of 3D cell culture dielectric spectroscopy and electrical impedance tomography represent viable alternatives to 2D impedance sensing.

1 Introduction

Regenerative medicine aims to re-establish normal function of cells, tissues or organs by cell therapy, tissue engineering or by stimulating endogenous repair. Therapies based on regenerative techniques have the potential to radically improve healthcare in the coming years. A successful translation to the clinical setting will be facilitated by the production of renewable and tuneable tissues. These tissues could also address a need in developing stable and robust *in-vitro* assays for pharmacological investigations.

1.1) An emerging need for non-destructive label-free approaches in tissue engineering and regenerative medicine

There is an emerging need for non-destructive and label-free technologies to assess the quality of engineered tissue and cell-based products prior to their use in the clinic. Clinicians must be able to assess that the right cells in the right state are being transplanted without interfering with their therapeutic potential, and integrity of a sample. In parallel, the emerging

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regenerative medicine industry that develop cell-based products for cell therapy will benefit from moving away from existing destructive biochemical assays to assess on-line the quality of their production.

The state of the art in imaging tissue engineering and regenerative medicine is based on a combination of different techniques, the majority of which are destructive end-point tests, such as histology, scanning electron microscopy (SEM), fluorescence microscopy immunohistochemistry, and other biochemical assays. They require the use of staining agents and sample processing which should ideally be avoided to limit safety issues.

In parallel to their exploitation for clinical translation, stem cell technologies have triggered a step-change in the development of human *in vitro* disease models. Animal-based disease models have been widely used for many years. However, many diseases are species-specific and animal models cannot fully reflect the human behaviour in these cases(1). Hence, primary human cells are still the preferred cell type for physiologically relevant disease models. However, primary cells are limited, expensive and difficult to obtain and culture (2). Stem-cell based disease models address several of these issues and are becoming an essential tool for investigating underlying mechanisms of diseases, and a vital platform for drug development and novel therapies (3).

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1.2. Impedance-based cellular assays as nondestructive label-free approaches.

This review is focused on the recent emergence of Impedance-Based Cellular Assays (IBCA) in the field of tissue engineering and regenerative medicine. We define broadly IBCA as a range of methods using microelectrodes to measure the impedance of biological systems to gain information on the cellular behaviour of adherent cell cultures, cell suspensions and 3D tissue models. Cell sorting and manipulation based on their dielectric properties as in dielectrophoresis and impedance flow cytometry is out of scope for this review.

IBCAs have emerged as an alternative approach to study stem cell properties and cumulative studies, reviewed here, showed their potential to monitor stem cell renewal, differentiation and maturation. They offer a novel method to non-destructively assess and quality-control stem cell cultures. In addition, when combined with in vitro disease models they provide complementary insights by allowing real-time monitoring of cell viability, measurement of cell-substrate and cell-cell adhesion parameters. IBCAs provide quantitative and very sensitive results that can easily automated and up-scaled in multi-well formats.

2. An overview of Impedance technology

Here we provide a brief overview of various techniques used in biology to measure impedance. Recent reviews have covered some aspects in more details (4, 5).

2.1 Passive electrical behaviour of cells

The Coulter counter is likely the most established impedance-based instrument in biology(6). It measures the resistance – the ratio of measured voltage to input current between two electrodes – of biological cells flowing in narrow channels, to count cell and deduce cell volume.

Another impedance based assay conventionally used in biology laboratories is the measurement of Trans-Epithelial Electrical Resistance (TEER) with chopstick electrodes, or epithelial Voltohmmeter, to estimate cell monolayer integrity (7). The TEER across an

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epithelium or endothelium correlates with tight-junctions formation between neighbouring cells.

The passive electrical behaviour of cells, as opposed to active electrophysiological potential, is mainly due to the presence of an insulating bi-lipid membrane that separates two ion-rich media. It creates an interfacial polarisation under an applied field(8, 9). This results in cells being of an insulative nature at low frequency. Consequently, the impedance increases with increasing number of cells in a system. This response has been exploited to monitor yeast density in the brewing process (10) or biomass in large bioreactor(11-13).

Similarly, when cells are cultured directly on microelectrode systems (Fig1), for improved sensitivity, the impedance increases with cell coverage (14). In addition any changes in cell shape(15), spreading on surface(16), and membrane integrity(17), results in a change of single cell dielectric properties, and of the measured impedance.(16) Then, when live cells form layers or more complex tissues, the measured impedance depends on cell-substrate adhesion and cell-cell junction or barrier function(18) (19). Finally, collective cellular micromotion correlated to cell metabolism can be quantified with impedance sensing by analysing the fluctuations generated the time-course impedance(20-22).

2.2 Electrical Impedance Spectroscopy (EIS)

EIS has been applied to biological tissues as early as 1925 by Fricke and Morse(23). When impedance of tissue is measured with increasing frequencies, a decrease by successive plateau is observed. There are four main transitions to lower plateaus(24, 25), i.e. dielectric dispersions. The Beta-dispersion related to interfacial polarization across the cellular plasma membrane is the most informative when studying cell behaviour and occurs at the kHz range. Dielectric parameters of cell suspensions can be then retrieved by fitting the frequency-response to effective medium approximations (EMA) such as the Maxwell-Wagner-Hanai and are very well described in(9). However, the cell volume fraction should be sufficiently large to stand out from the highly conductive media contribution. A more detailed analysis is beyond the scope of this review and can be found here(4). In general, the impedance is acquired through the use of a four-electrode system to compensate for the double layer effect at low frequency, i.e. the accumulation of charged ions on the measuring electrodes. As mentioned above, EIS was proved particularly useful to measure the biomass in bioreactors.

Impedance flow cytometry: With the development of microelectrodes and microfluidic the field as branched out into impedance flow cytometry where single cells flow in microchannels surrounded by micro-electrodes. It has been showed to discriminate cells based on both cell size and intracellular dielectric properties (26, 27).

2.3. Electric-cell substrate impedance sensing

A major drawback of EIS is the overwhelming contribution of the cell medium to the impedance spectra. It can entirely mask the cell contribution for low cell to media volume ratio. In a pioneering work, Giaever and Keese(18, 28, 29) have been able to free themselves from the contribution of the medium – improving considerably the sensitivity and the ease of interpretation. Their technology, Electric Cell-substrate Impedance Sensing (ECIS)(15) is a real-time, label-free monitoring technology in which a small non-invasive a.c. current (~1 μA) is applied through gold microelectrodes. ECIS originally used a two-electrode set-up with a very small sensing electrode, when compared to the counter electrode, onto which adherent cells are directly grown, providing very high sensitivity.

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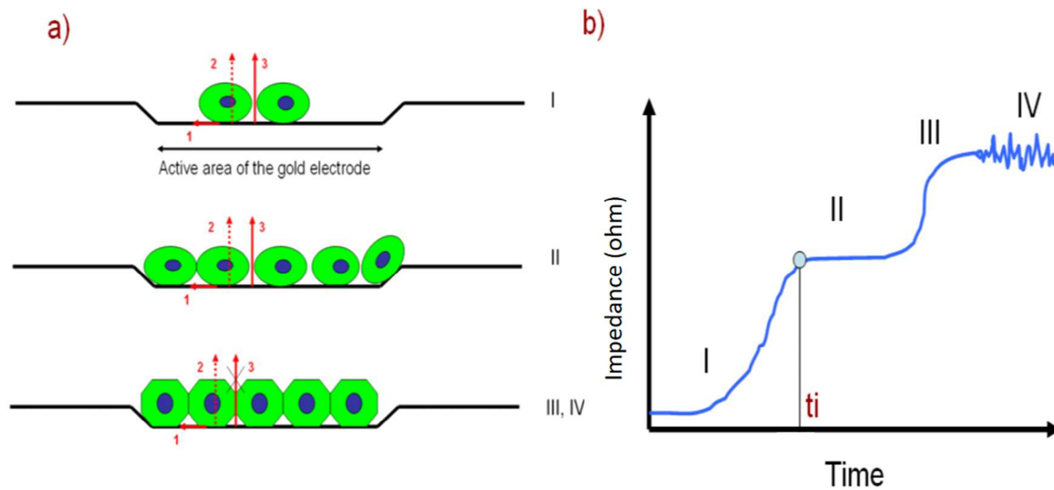

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Figure1: a) Schematic showing cells growing on microelectrode with corresponding stages in impedance (b). (I) proliferation stage (II), confluency (III), optional formation of barrier function and (IV) cell metabolic activity.

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When cells are cultured on top of the microelectrodes (Fig1), they alter the current pathways due to the insulating properties of the cell plasma membranes. The measured impedance increases with cell growth until it reaches a plateau as the cells form a confluent monolayer on top of the electrodes. At low frequencies, the current is forced to flow under and in-between neighbouring cells and the measured impedance in this case is directly related to the properties of cell-substrate adhesion and cell-cell tight junctions. Only at high frequencies, can the current capacitively couple through the plasma membrane and the impedance can give an insight into the integrity and the dielectric properties of the cell membrane (16, 30).

Since this pioneering work, impedance sensing has been extensively used for a wide range of biological assays including cell proliferation (16, 31-33), cytotoxiciy(34-38), wound healing (39-41), cell signalling(42-44) cell invasion(45) and blood-brain barrier permeability studies(19).

2.4 Electrical impedance tomography for cellular assays

Electrical impedance tomography (EIT) was first developed in 1978 (46), mainly focused on clinical applications such as thorax imaging (47), brain function monitoring (48) and breast cancer screening (49, 50). EIT reconstructs the conductivity images of an object based on the voltages and the currents at the surface of the object (51).

The EIT measurement system is mainly comprised of three parts: a current source to stimulate the AC current into the subject; the multiplexer array for switching the current source and the data acquisition unit. (51). The first EIT system for medical research, the Sheffield Mk1, was developed in 1987 (52). Now, the development of micro technologies allowed the application of EIT at the cellular scale. One of the first EIT sensor for in vitro assay was developed by Linderholm *et al* (53). It was exemplified by studying cellular migration of Human epithelial stem cells (YF 29). A sensor for single organism, Physarum

polycephalum, a slime mold growing on agar gel, was also demonstrated by Sun et al(54). These two studies paved the way for further research in the field of IBCA based on EIT.

3. Current progress of IBCA in tissue engineering and regenerative medicine

3.1. Real-time label-free monitoring of cell differentiation

3.1.1 Adult stem cells

Differentiation of stem cells is associated with a change in cell morphology, proliferation capacity and dielectric properties. Impedance sensing can detect these changes, defining distinctive impedance profiles for different differentiation paths.Cho and Thielecke(55) were the first to use impedance spectroscopy to study and characterize the growth of human mesenchymal stem cells (hMSCs). Then, an increase in resistance measurements associated with the differentiation of human mesenchymal stem cells (hMSCs) towards osteoblasts(56), using a planar 1mm diameter platinum electrode-based chip was reported.

In parallel, independent studies have explored the ability to differentiate cell lineages arising from adult stem cell sources with impedance sensing (57, 58). In our group, Adipose-derived stem cells (ADSCs) were differentiated into osteoblasts and adipocytes and monitored throughout their differentiation (Fig2) (58). We reported an increase in the impedance measurements with the osteo-induced ADSCs that was also accompanied by an increase in tightness of the cell-cell junction. On the other hand, the adipo-induced cells showed a drop in the impedance measurements and looseness in cell-cell junctions. A variation in the cell membrane capacitance between undifferentiated stem cell, osteo-induced and adipo-induced was also measured and pointed out as an early (<4days) marker of stem cell fate. Similar changes in dielectric properties accompanying stem cell differentiation towards adipocytes was also reported by Lee et al.(59) and Fu et al.(60), which was related to lipid vacuoles accumulation.

In a similar work, Angstmann et al. (57) used two commercial systems ECIS and xCELLigence to examine differences in early phases of MSCs differentiation towards adipocytes and osteoblasts, while Kramer et al. have used xCELLigence to study adipogenesis of the preadipocytes 3T3-L1 cells(61). In agreement with the previous studies, osteo-induced cells showed an increase in impedance when compared to un-differentiated ASCs; while adipo-induced cells had a marked decrease. Interestingly they showed that impedance sensing can distinguish differentiation potential between low (p6) and high (p12) passage; paving the way to more recent studies looking at donor variability (62). Striking differences were found in the impedance profile when comparing hASCs isolated from different donors, opening the door to predict osteogenic potential with impedance sensing with direct applications/implication for their translational potential.

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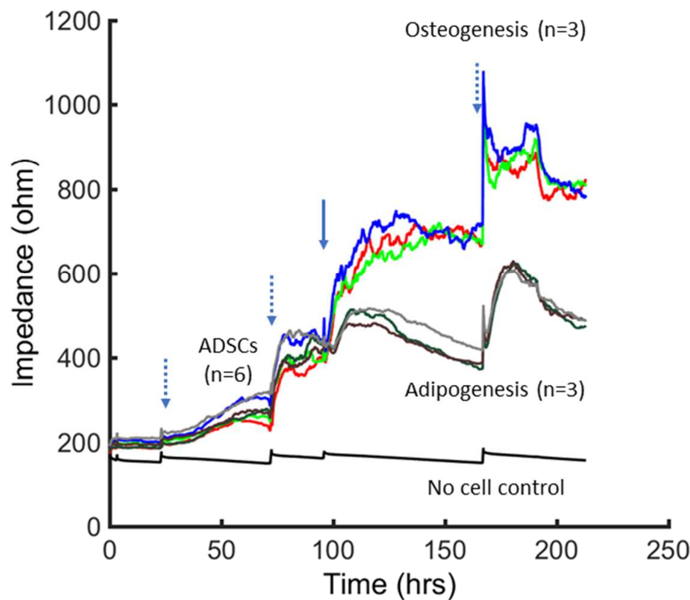

Figure2: Representative time-course impedance for ADSCs into two distinct lineages, i.e. osteogenesis (n=3) and adipogenesis (n=3), and a no cell control recorded with ECIS. Dotted arrows point at feeding time, plain arrow at induction time.

Effect of coatings, collagen I, collagen IV, fibronectin and laminin on cell differentiation were also investigated(57, 63).Authors showed increase of impedance profile for osteo-lineage differentiated on collagen I and IV and less pronounced drop in impedance for adipogenesis. These findings correlated well with previous observation of increase osteogenesis and decrease adipogenesis on collagen coating. Other studies used the xCelligence system in the context of regenerative medicine as a quantitative measurement of cell adhesion, e.g. for human endometrial MSCs(63), and proliferation of adipose-derived mesenchymal stem cells from ovariectomized mice(64), vascular smooth muscle cells derived from skin-derived precursors human Wharton's Jelly Stem Cells(65), and to assess bone marrow derived stem cells in the context of good manufacturing practice(93). Reitinger et al.(66) used a RFID-based sensor platform to monitor impedance wirelessly and in a reusable manner. They found similar impedance profile when differentiating adipose-derived stem cells to osteoblasts and adipocytes.

The effect of neural differentiation media on MSCs growth and differentiation have also been investigated with ECIS(67). The authors reported a slower increase in impedance, due to lower cell number and not morphological changes, for the differentiated cells that corresponded to the arrest of the cell cycle induced by the differentiation.

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3.1.2 Pluripotent stem cells

Impedance sensing with microelectrodes is not always well suited for monitoring embryonic stem cell or hIPS renewal. They grow in patchy compact cell colonies and expand, and that may not be uniformly sampled by the microelectrodes. Similarly, the use of a feeder layer is clearly not compatible with impedance sensing, although this can be avoided. Recent advances in media and substrate formulation has allowed embryonic cells growth in single layer. And our group has been first to monitor mouse embryonic stem cell renewal on ECIS(68). Impedance sensing was useful to demonstrate in quantifiable way that the Leukaemia Inhibitory Factor (LIF), a soluble growth factor necessary to the maintenance of pluripotency could be replaced with LIF encapsulated in hydrogel-based liposomal system or in Poly(lactide-co-glycolic acid) polyester nanoparticles without being detrimental to cell growth.

The human cell line NT2 D1, an embryonic carcinoma cell line, was cultured on ECIS multiwell arrays and differentiated by retinoic acid (RA) into the neural lineage(69). This study showed that both the impedance and the slope of the impedance increased in a dose-dependent manner with RA addition. The efficiency(state) of differentiation was then quantified by qRT-PCR expression as a decrease in stem cell factors OCT4 and Nanog and an increase in differentiation markers HOXA1 and SNAP 25; and was found correlated in a dose-dependent manner to RA addition. A panel of differentiation inducing drug was tested, and the authors were able to analyse both the differentiation and the cytotoxicity of the candidates This study paved the way to use impedance sensing as a screening platform to study molecules that induce differentiation in a quantitative way.

Induced pluripotent stem cells (IPSCs) self-renewal and differentiation to the mesendodermal and ectodermal lineage were recorded with ad hoc device combining impedance sensing and a quartz microbalance(70). Impedance was measured between 0.1 Hz and 10kHz up to 96h, and an equivalent circuit model, taking in account the gel layer was used to derive the resistance and capacitance of the cell layer. The authors clearly showed distinct impedance time course for each lineage that significantly correlated with morphological changes.

Neural stem cells were showed to have differences in time-course capacitance when cultured on interdigitated capacitance sensors and cultured with different conditions(71). Proliferation and differentiation into either neuronal or astroglial cells could be differentiated in real-time. However, neural network formation and the full maturation process was only reported with a biochip layout optimized for monitoring in real-time neural pluripotent stem cell differentiation by Seidel et al. (72). The potential of their platform, amenable to a 96 well plate format, for quality control in industrial processes was clearly demonstrated using the gamma secretase inhibitor DAPT to modulate the differentiation process. The same group developed a 384 multi-well microelectrode array version(73) by solving a key challenge in multiplexing counter electrodes; opening the door to automated screening for pharmaceutical investigations. This was exemplified by establishing the dose-and time-dependent therapeutic effects of a kinase inhibitor (SRN-003-556) on a Sh-SY5SY tauopathy model

3.2 Impedance-based cell phenotypic assays for stem-cell based in vitro diseases models

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Generally, label-free assays are promising tools for drug discovery (74-76). They monitor drug-cell interactions in real-time and in living cells. They are particularly well suited to assess drug polypharmacology as they are not based on a particular molecular marker(76). These assays can quantify whole cell integrated responses which encompass the full complexity of drug-target interactions. This convoluted time-course signal can then be deciphered to determine the mechanism of action of drugs by acquiring the profile of individual compound from a library. The most prominent label-free approach to assess drug polypharmacology is currently the optical measurement of dynamic mass redistribution with nanogratings. Now, impedance sensing is rapidly emerging as an alternative methods(44). They are both sensitive to cell density, cell-substrate adhesion and morphological changes.

Abassi et al. (77) led the way in 2012 by demonstrating an impedance sensing system that monitored dynamically the beating periodicity of stem-cell derived cardiomyocytes. They obtained a dose-response profile for over 60 compounds, assessing simultaneously and noninvasively the periodicity of beating, contractility and overall viability of stem cell derived cardiomyocytes. Similalry Guo et al.(78) used the same system, to assess on IPSC-derived cardiomyocytes 28 compounds with known cardiac effects. These two studies based on the equipment commercialised by Roche, and now by ACEA, paved the way for an host of research studies combining impedance sensing and stem-cell based cardiomyocytes (Fig3) which were reviewed by Peters et al. (79). Indeed, impedance sensing at high acquisition frequency landed itself very well to the field, yielding quantitative parameters without any post-processing steps.

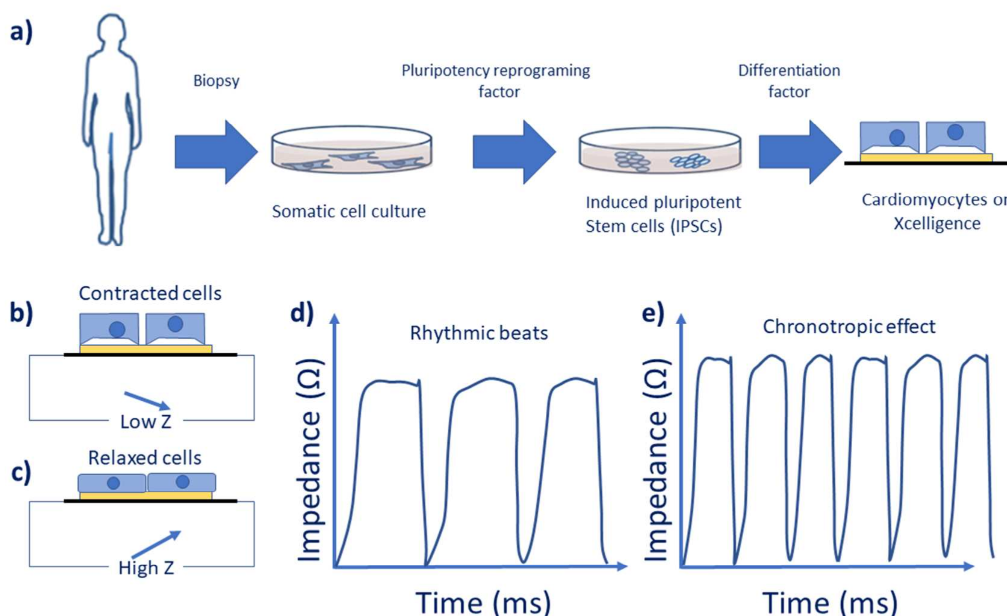

Figure3: (a) IPSCs generated from a patient can be differentiated into cardiomyocytes directly on top of gold microelectrodes. The spontaneous beating, an alternation of contracted (b) and relaxed states (c) results in cycles in the impedance (d). Changes in amplitude and frequency of the cycles can easily quantified, e.g. for a compound inducing chronotropic effect (e).

IPS-derived Retinal Pigment Epithelium (IPS-RPE) cells generated from a patient with an inherited macular degeneration and from an unaffected sibling were cultured on ECIS(80). RPE maturation was monitored in real-time for >25 days before a reproducible and spatially

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controlled RPE layer damage was induced by elevated current pulse to mimic cell loss in AMD disease. Migration rates between the two cell lines were then studied, and this showed the potential of the platform to quantitatively assess patient-specific RPE cell repair, and to screen therapeutic compounds.

4. Conclusion and perspectives

4.1 The challenge of monitoring 3D cultures

Increasingly, cells are cultured in 3D environments where they exhibit cellular physiological functions closer to *in vivo(81, 82).* The regenerative field is increasingly investigating cells in 3D cultures such organoids, multicellular spheroids, artificial and organotypic tissues. They offer better in vitro models to improve our understanding of cell biology and are regarded as a step-change in drug discovery. They are also developed as potential candidates for replacement and repair therapies.

As pointed out earlier, the state of the art in imaging tissue engineering and regenerative medicine is mostly based on destructive end-point tests. Optical technologies are progressing rapidly to fill the gap(83) but generally require extra computer-intensive steps to retrieve quantitative parameters.

In this context IBCAs present themselves as a viable complementary technique to assess cell behaviour in 3D cell culture. Although only few applications have been demonstrated so far, monitoring 3D cell culture is the main perspective for the IBCA field.

There are some technical challenges to translate directly impedance sensing to 3D cell monitoring as the microelectrode needs to be in contact with the sample. Whereas the application of impedance spectroscopy and EIT have been more straightforward.

Arrays of microcavities were designed by Robitzki group to host 3D cardiomyocytes cluster derived from embryonic stem cells and assess them in real-time with EIS and field potential measurements (84). Both Chronotropic and action potential duration prolongation effects were detected with this system. Considering the current trend in the regenerative field this platform could have a great potential to assess stem-cell based organoids and spheroids for pharmaceutical investigations.

Cell proliferation and differentiation have been investigated with EIS with a dielectric probe that limited the use of low frequency field(85-87) but was able to monitor large scale sample. The sensitivity was increased by Daoud et al. by using ad-hoc macrochambers lined up with parallel plate platinum electrodes; they enabled impedance measurements in the beta-dispersion frequency range. The epithelial differentiation processes of Madin–Darby canine kidney cells to hollow cyst-like structures was captured with this technique(88). The cells were embedded in to collagen gels and hosted into 3D printed Poly (DL-lactide-co-glycolide) acid (PLGA) scaffolds demonstrating the potential of IS to monitor non-destructively tissue formation in complex tissue engineered products. Recently vertically aligned pairs of microelectrodes were used to monitor MSC migration in alginate gels. This technology was also demonstrated to measure cell proliferation and death(89).

Large production of stem cells at an industrial and clinical scale will likely lead to the use of bioreactors (90). EIS is already used in bioreactors (11-13) and will therefore represent a viable strategy to monitor stem cell growth and differentiation state when culturing stem cell progeny. Mesenchymal stem cell expansion on microcarriers cultured in bioreactors was monitored successfully with EIS(91).

Although, impedance 3D imaging could lend itself very well to monitoring 3D cell culture, only few applications have been reported so far. Yang *et al*. carried out 3D impedance

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imaging of breast cancer cell spheroid based on 3D-Laplacian and sparsity joint regularization algorithm (92). Overall, EIT is a relatively novel impedance-based measurement technique which has the potential to monitor in real-time tissue engineering products and in vitro disease models with a high temporal resolution. However further work, in both sensor and algorithm design, needs to be conducted to increase its sensitivity.

4.2. Conclusion

IBCA have firmly established themselves in the field of biology as complementary assays providing time course quantitative values of cell adhesion, cell-cell junctions and proliferation. With the recent demonstration of their ability to monitor stem cell differentiation (Supplementary Table 1), and as label-free phenotyping assays, IBCAs are becoming attractive for regenerative medicine applications. They could be integrated online to provide data-driven operation and quality-control of large scale culture, and in bench-top equipment to assess the quality of cell therapies products before their use in patient.

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1. Tiscornia G, Vivas EL, Belmonte JCI. Diseases in a dish: modeling human genetic disorders using induced pluripotent cells. Nature Medicine. 2011;17(12):1570-6.
2. Borooah S, Phillips MJ, Bilican B, Wright AF, Wilmut I, Chandran S, et al. Using human induced pluripotent stem cells to treat retinal disease. Progress in Retinal and Eye Research. 2013;37:163-81.
3. Sterneckert JL, Reinhardt P, Scholer HR. Investigating human disease using stem cell models. Nature Reviews Genetics. 2014;15(9):625-39.
4. Heileman K, Daoud J, Tabrizian M. Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis. Biosensors & Bioelectronics. 2013;49:348-59.
5. Xu YC, Xie XW, Duan Y, Wang L, Cheng Z, Cheng J. A review of impedance measurements of whole cells. Biosensors & Bioelectronics. 2016;77:824-36.
6. Coulter WH. High speed automatic blood cell counter and cell size analyzer. Proceedings of the National Electronics Conference, 1956. 1956;12:1034.
7. Benson K, Cramer S, Galla H-J. Impedance-based cell monitoring: barrier properties and beyond. Fluids and Barriers of the CNS. 2013;10(1):5.
8. Asami K. Characterization of heterogeneous systems by dielectric spectroscopy. Progress in Polymer Science. 2002;27(8):1617-59.
9. Asami K. Characterization of biological cells by dielectric spectroscopy. Journal of Non-Crystalline Solids. 2002;305(1-3):268-77.
10. Austin GD, Watson RWJ, Damore T. STUDIES OF ONLINE VIABLE YEAST BIOMASS WITH A CAPACITANCE BIOMASS MONITOR. Biotechnology and Bioengineering. 1994;43(4):337-41.
11. Ducommun P, Kadouri A, von Stockar U, Marison IW. On-line determination of animal cell concentration in two industrial high-density culture processes by dielectric spectroscopy. Biotechnology and Bioengineering. 2002;77(3):316-23.
12. Carvell JP, Dowd JE. On-line measurements and control of viable cell density in cell culture manufacturing processes using radio-frequency impedance. Cytotechnology. 2006;50(1-3):35-48.
13. Justice C, Brix A, Freimark D, Kraume M, Pfromm P, Eichenmueller B, et al. Process control in cell culture technology using dielectric spectroscopy. Biotechnology Advances. 2011;29(4):391-401.
14. Giaever I, Keese C. Monitoring fibroblast behavior in tissue culture with an applied electric field. Proc Natl Acad Sci U S A. 1984;81(12):3761-4.
15. Giaever I, Keese C. A morphological biosensor for mammalian cells. Nature. 1993;366(6455):591-2.
16. Wegener J, Keese CR, Giaever I. Electric cell-substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces. Experimental Cell Research. 2000;259(1):158-66.

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17. Wegener J, Keese CR, Giaever I. Recovery of Adherent Cells after In Situ Electroporation Monitored Electrically. BioTechniques. 2002;33:348-57.
18. Tiruppathi C, Malik A, Del Vecchio P, Keese C, Giaever I. Electrical method for detection of endothelial cell shape change in real time: assessment of endothelial barrier function. Proc Natl Acad Sci U S A. 1992;89(17):7919-23.
19. Wegener J, Seebach J. Experimental tools to monitor the dynamics of endothelial barrier function: a survey of in vitro approaches. Cell and tissue research. 2014;355:485-514.
20. Lovelady DC, Richmond TC, Maggi AN, Lo CM, Rabson DA. Distinguishing cancerous from noncancerous cells through analysis of electrical noise. Physical Review E. 2007;76(4).
21. Keese CR, Lo CM, Giaever I. THE RELATIONSHIP BETWEEN MAMMALIAN-CELL METABOLISM AND MOTION MEASURED ELECTRICALLY. Biophysical Journal. 1994;66(2):A412-A.
22. Lo CM, Keese CR, Giaever I. Monitoring motion of confluent cells in tissue culture. Exp Cell Res. 1993;204:102-9.
23. Fricke H, Morse S. The electric resistance and capacity of blood for frequencies between 800 and 4 1/2 million cycles. Journal of General Physiology. 1925;9(2):153-67.
24. Foster KR, Schwan HP. Dielectric properties of tissues and biological materials: a critical review. Critical Reviews in Biomedical Engineering. 1989;17(1):25-104.
25. Schwan HP. Electrical properties of tissue and cell suspensions. Lawrence JH, Tobias, C.A., editor. New York: Acad. Press; 1957. 147-209 p.
26. Holmes D, Pettigrew D, Reccius CH, Gwyer JD, van Berkel C, Holloway J, et al. Leukocyte analysis and differentiation using high speed microfluidic single cell impedance cytometry. Lab on a Chip. 2009;9(20).
27. Morgan H, Sun T, Holmes D, Gawad S, Green NG. Single cell dielectric spectroscopy. Journal of Physics D-Applied Physics. 2007;40(1):61-70.
28. Giaever I, Keese CR. Micromotion of mammalian cells measured electrically. proc Natl acad Sci USA. 1991;88:7896-900.
29. Giaever I, Keese CR. Monitoring Fibroblast Behaviour in Tissue Culture with an Applied Electric Field. Proceedings of the National Academy of Sciences of the United States of America. 1984;81(12):3761-4.
30. Keese C, Giaever I. A biosensor that monitors cell morphology with electrical fields. IEEE Eng Med Bio. 1994;13:402-8.
31. Lo CM, Keese CR, Giaever I. Impedance analysis of MDCK cells measured by electric cell-substrate impedance sensing. Biophysical Journal. 1995;69(6):2800-7.
32. Zhang Y, Wang L, Zhu J, Hu Y, Xing W, Cheng J. Real-time monitoring of extracellular matrix-mediated PC12 cell attachment and proliferation using an electronic biosensing device. Biotechnology Letters. 2012;34(2):397-404.
33. Rahman ARA, Register J, Vuppala G, Bhansali S. Cell culture monitoring by impedance mapping using a multielectrode scanning impedance spectroscopy system (CellMap). Physiological Measurement. 2008;29(6):S227-S39.
34. Lovelady DC, Friedman J, Patel S, Rabson DA, Lo CM. Detecting effects of low levels of cytochalasin B in 3T3 fibroblast cultures by analysis of electrical noise obtained from cellular micromotion. Biosensors & Bioelectronics. 2009;24(7):2250-4.
35. Atienzar FA, Gerets H, Tilmant K, Toussaint G, Dhalluin S. Evaluation of impedance-based label-free technology as a tool for pharmacology and toxicology investigations. Biosensors. 2013;3:132-56.
36. Opp D, Wafula B, Lim J, Huang E, Lo JC, Lo CM. Use of electric cell-substrate impedance sensing to assess in vitro cytotoxicity. Biosensors & Bioelectronics. 2009;24(8):2625-9.
37. Xiao CD, Lachance B, Sunahara G, Luong JHT. Assessment of cytotoxicity using electric cell-substrate impedance sensing: Concentration and time response function approach. Analytical Chemistry. 2002;74(22):5748-53.

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38. Xiao C, Luong JHT. Assessment of cytotoxicity by emerging impedance spectroscopy. Toxicology and Applied Pharmacology. 2005;206(2):102-12.
39. Keese CR, Wegener J, Walker SR, Giaever I. Electrical Wound-Healing Assay for cells in vitro. PNAS. 2004;101(6):1554-9.
40. Jiang WG, Lin Y, Patel G, Harding KG. Expression of WAVEs, the WASP (Wiskott-Aldrich syndrome protein) family of verprolin homologous proteins in human wound tissues and the biological influence on human keratinocytes Jiang et al. WAVEs in wound repair. Wound Repair & Regeneration. 2010;18(6):594-604.
41. Liu CL, Tam JCW, Sanders AJ, Jiang DG, Ko CH, Fung KP, et al. Electric Cell-Substrate Impedance Sensing as a Screening Tool for Wound Healing Agents. Electric Cell-Substrate Impedance Sensing and Cancer Metastasis: Springer; 2012. p. 203-16.
42. Han J, Liu G, Profirovic J, Niu J, Voyno-Yasenetskaya T. Zyxin is involved in thrombin signaling *via* interaction with PAR-1 receptor. FASEB. 2009;23:4193-206.
43. Yu N, Atienza JM, Bernard J, Blanc S, Zhu J, Wang X, et al. Real-Time Monitoring of Morphological Changes in Living Cells by Electronic Cell Sensor Arrays: An Approach To Study G Protein-Coupled Receptors. Analytical Chemistry. 2006;78:35-43.
44. Lieb S, Michaelis S, Plank N, Bernhardt G, Buschauer A, Wegener J. Label-free analysis of GPCR-stimulation: The critical impact of cell adhesion. Pharmacological Research. 2016;108:65-74.
45. Keese CR, Bhawe K, Wegener J, Giaever I. Real-Time Impedance Assay to Follow the Invasive Activities of Metastatic Cells in Culture. BioTechniques. 2002;33:842-50.
46. Henderson RP, Webster JG. An impedance camera for spatially specific measurements of the thorax. IEEE Transactions on Biomedical Engineering. 1978(3):250-4.
47. Kang SI, Khambampati AK, Jeon MH, Kim BS, Kim KY. A sub-domain based regularization method with prior information for human thorax imaging using electrical impedance tomography. Measurement Science and Technology. 2016;27(2):025703.
48. Aristovich KY, Packham BC, Koo H, dos Santos GS, McEvoy A, Holder DS. Imaging fast electrical activity in the brain with electrical impedance tomography. NeuroImage. 2016;124:204-13.
49. Lee E, Ts M-E, Seo JK, Woo EJ. Breast EIT using a new projected image reconstruction method with multi-frequency measurements. Physiological measurement. 2012;33(5):751.
50. Cherepenin V, Karpov A, Korjenevsky A, Kornienko V, Mazaletskaya A, Mazourov D, et al. A 3D electrical impedance tomography (EIT) system for breast cancer detection. Physiological measurement. 2001;22(1):9.
51. Harikumar R, Prabu R, Raghavan S. Electrical Impedance Tomography (EIT) and Its Medical Applications: A Review. International Journal of Soft Computing Engineering. 2013;3(4):2231-307.
52. Brown B, Seagar A. The Sheffield data collection system. Clinical Physics and Physiological Measurement. 1987;8(4A):91.
53. Linderholm P, Braschler T, Vannod J, Barrandon Y, Brouard M, Renaud P. Two-dimensional impedance imaging of cell migration and epithelial stratification. Lab on a Chip. 2006;6(9):1155-62.
54. Sun T, Tsuda S, Zauner K-P, Morgan H. On-chip electrical impedance tomography for imaging biological cells. Biosensors and Bioelectronics. 2010;25(5):1109-15.
55. Cho S, Thielecke H. Electrical characterization of human mesenchymal stem cell growth on microelectrode. Microelectronic Engineering. 2008;85:1272-4.
56. Hildebrandt C, Büth H, Cho S, Impidjati, Thielecke H. Detection of the osteogenic differentiation of mesenchymal stem cells in 2D and 3D cultures by electrochemical impedance spectroscopy. Journal of Biotechnology. 2010;148:83-90.
57. Angstmann M, Brinkmann I, Bieback K, Breitkreutz D, Maercker C. Monitoring human mesenchymal stromal cell differentiation by electrochemical impedance sensing. Cytotherapy . 2011;13:1074-89.
58. Bagnaninchi PO, Drummond N. Real-time label-free monitoring of adipose-derived stem cell differentiation with electric cell-substrate impedance sensing. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(16):6462-7.

For Review Only

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59. Lee R, Jung I, Park M, Ha H, Yoo KH. Real-time monitoring of adipocyte differentiation using a capacitance sensor array. Lab on a Chip. 2013;13:3410-6.
60. Fu Y, Li R, Zhong J, Fu N, Wei X, Cun X, et al. Adipogenic differentiation potential of adipose-derived mesenchymal stem cells from ovariectomized mice. Cell Proliferation. 2014;47(6):604-14.
61. Kramer AH, Joos-Vandewalle J, Edkins AL, Frost CL, Prinsloo E. Real-time monitoring of 3T3-L1 preadipocyte differentiation using a commercially available electric cell-substrate impedance sensor system. Biochemical and Biophysical Research Communications. 2014;443:1245-50.
62. Nordberg RC, Zhang JL, Griffith EH, Frank MW, Starly B, Loboa EG. Electrical Cell-Substrate Impedance Spectroscopy Can Monitor Age-Grouped Human Adipose Stem Cell Variability During Osteogenic Differentiation. Stem Cells Translational Medicine. 2017;6(2):502-11.
63. Rajaraman G, White J, Tan KS, Ulrich D, Rosamilia A, Werkmeister J, et al. Optimization and Scale-up Culture of Human Endometrial Multipotent Mesenchymal Stromal Cells: Potential for Clinical Application. Tissue Engineering Part C-Methods. 2013;19(1).
64. Fu Y, Li R, Zhong J, Fu N, Wei X, Cun X, et al. Adipogenic differentiation potential of adipose-derived mesenchymal stem cells from ovariectomized mice. Cell Prolif. 2014;47(6):604-14.
65. Garzon I, Perez-Kohler B, Garrido-Gomez J, Carriel V, Nieto-Aguilar R, Martin-Piedra MA, et al. Evaluation of the Cell Viability of Human Wharton's Jelly Stem Cells for Use in Cell Therapy. Tissue Engineering Part C-Methods. 2012;18(6):408-19.
66. Reitinger S, Wissenwasser J, Kapferer W, Heer R, Lepperdinger G. Electric impedance sensing in cell-substrates for rapid and selective multipotential differentiation capacity monitoring of human mesenchymal stem cells. Biosensors & Bioelectronics. 2012;34(1):63-9.
67. Park HE, Kim D, Koh HS, Cho S, Jung-Suk Sung, Kim1 aJY. Real-Time Monitoring of Neural Differentiation of Human Mesenchymal Stem Cells by Electric Cell-Substrate Impedance Sensing. Journal of Biomedicine and Biotechnology. 2011:1-8.
68. Corradetti B, Freile P, Pells S, Bagnaninchi P, Park J, Fahmy TM, et al. Paracrine signalling events in embryonic stem cell renewal mediated by affinity targeted nanoparticles. Biomaterials. 2012;33(28):6634-43.
69. Oz S, Maercker C, Breiling A. Embryonic Carcinoma Cells Show Specific Dielectric Resistance Profiles during Induced Differentiation. Plos One. 2013;8(3).
70. Low K, Wong LY, Maldonado M, Manjunath C, Horner CB, Perez M, et al. Physico-electrochemical Characterization of Pluripotent Stem Cells during Self-Renewal or Differentiation by a Multi-modal Monitoring System. Stem Cell Reports. 2017;8(5):1329-39.
71. Lee R, Kim IS, Han N, Yun S, Park KI, Yoo KH. Real-Time Discrimination between Proliferation and Neuronal and Astroglial Differentiation of Human Neural Stem Cells. Scientific Reports. 2014;4.
72. Seidel D, Obendorf J, Englich B, Jahnke HG, Semkova V, Haupt S, et al. Impedimetric real-time monitoring of neural pluripotent stem cell differentiation process on microelectrode arrays. Biosensors & Bioelectronics. 2016;86:277-86.
73. Jahnke HG, Krinke D, Seidel D, Lilienthal K, Schmidt S, Azendorf R, et al. A novel 384-multiwell microelectrode array for the impedimetric monitoring of Tau protein induced neurodegenerative processes. Biosensors & Bioelectronics. 2017;88:78-84.
74. Fang Y. Label-Free Cell Phenotypic Drug Discovery. Combinatorial Chemistry & High Throughput Screening. 2014;17(7):566-78.
75. Fang Y. Troubleshooting and deconvoluting label-free cell phenotypic assays in drug discovery. Journal of Pharmacological and Toxicological Methods. 2013;67(2):69-81.
76. Wang JX, Zhang XL, Fang Y, Liang XM. Label-Free Cell Phenotypic Assays for Assessing Drug Polypharmacology. Current Pharmaceutical Design. 2016;22(21):3190-200.
77. Abassi YA, Xi BA, Li N, Ouyang W, Seiler A, Watzele M, et al. Dynamic monitoring of beating periodicity of stem cell-derived cardiomyocytes as a predictive tool for preclinical safety assessment. British Journal of Pharmacology. 2012;165(5):1424-41.

For Review Only

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78. Guo L, Abrams RMC, Babiarz JE, Cohen JD, Kameoka S, Sanders MJ, et al. Estimating the Risk of Drug-Induced Proarrhythmia Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes. Toxicological Sciences. 2011;123(1):281-9.
79. Peters MF, Lamore SD, Guo L, Scott CW, Kolaja KL. Human Stem Cell-Derived Cardiomyocytes in Cellular Impedance Assays: Bringing Cardiotoxicity Screening to the Front Line. Cardiovascular Toxicology. 2015;15(2):127-39.
80. Gamal W, Borooah S, Smith S, Underwood I, Srsen V, Chandran S, et al. Real-time quantitative monitoring of hiPSC-based model of macular degeneration on Electric Cell-Substrate Impedance Sensing microelectrodes. Biosensors and Bioelectronics. 2015;71:445-55.
81. Pampaloni F, Reynaud EG, Stelzer EHK. The third dimension bridges the gap between cell culture and live tissue. Nature Reviews Molecular Cell Biology. 2007;8:839-45.
82. Keller PJ, Pampaloni F, Stelzer EHK. Life sciences require the third dimension. Current Opinion in Cell Biology. 2006;18(1):117-24.
83. Leahy M, Thompson K, Zafar H, Alexandrov S, Foley M, O'Flatharta C, et al. Functional imaging for regenerative medicine. Stem Cell Research & Therapy. 2016;7.
84. Jahnke HG, Steel D, Fleischer S, Seidel D, Kurz R, Vinz S, et al. A Novel 3D Label-Free Monitoring System of hES-Derived Cardiomyocyte Clusters: A Step Forward to In Vitro Cardiotoxicity Testing. Plos One. 2013;8(7).
85. Dziong D, Bagnaninchi PO, Kearney RE, Tabrizian M. Nondestructive online in vitro monitoring of pre-osteoblast cell proliferation within microporous polymer scaffolds. Ieee Transactions on Nanobioscience. 2007;6(3):249-58.
86. Bagnaninchi PO, Dikeakos M, Veres T, Tabrizian M. Complex permittivity measurement as a new noninvasive tool for monitoring In vitro tissue engineering and cell signature through the detection of cell proliferation, differentiation, and pretissue formation. Ieee Transactions on Nanobioscience. 2004;3(4):243-50.
87. Bagnaninchi PO, Dikeakos M, Veres T, Tabrizian M. Towards on-line monitoring of cell growth in microporous scaffolds: Utilization and interpretation of complex permittivity measurements. Biotechnology and Bioengineering. 2003;84(3):343-50.
88. Daoud J, Heileman K, Shapka S, Rosenberg L, Tabrizian M. Dielectric spectroscopy for monitoring human pancreatic islet differentiation within cell-seeded scaffolds in a perfusion bioreactor system. Analyst. 2015;140(18):6295-305.
89. Lee SM, Han N, Lee R, Choi IH, Park YB, Shin JS, et al. Real-time monitoring of 3D cell culture using a 3D capacitance biosensor. Biosensors & Bioelectronics. 2016;77:56-61.
90. Kropp C, Massai D, Zweigerdt R. Progress and challenges in large-scale expansion of human pluripotent stem cells. Process Biochemistry. 2017;59:244-54.
91. Justice C, Leber J, Freimark D, Grace P, Kraume M, Czermak P. Online- and offline-monitoring of stem cell expansion on microcarrier. Cytotechnology. 2011;63(4):325-35.
92. Yang Y, Jia J, Smith S, Jamil N, Gamal W, Bagnaninchi P. A Miniature Electrical Impedance Tomography Sensor and 3D Image Reconstruction for Cell Imaging. IEEE Sensors Journal. 2016;PP(99):1-.

93. Pham P , et al. (2014). Good manufacturing practice-compliant isolation and culture of human umbilical cord blood-derived mesenchymal stem cells. Journal of translational medicine. 12. 56. 10.1186/1479-5876-12-56.

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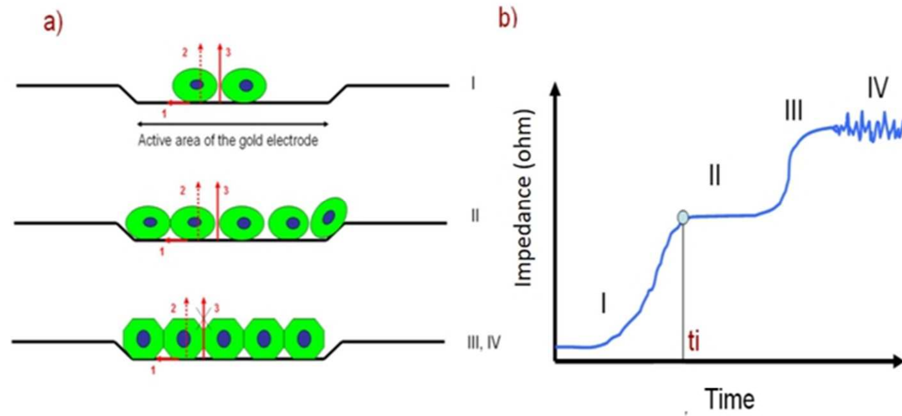

Figure1: a) Schematic showing cells growing on microelectrode with corresponding stages in impedance (b). (I) proliferation stage (II) confluency (III) Optional formation of barrier function and (IV) cell metabolic activity.

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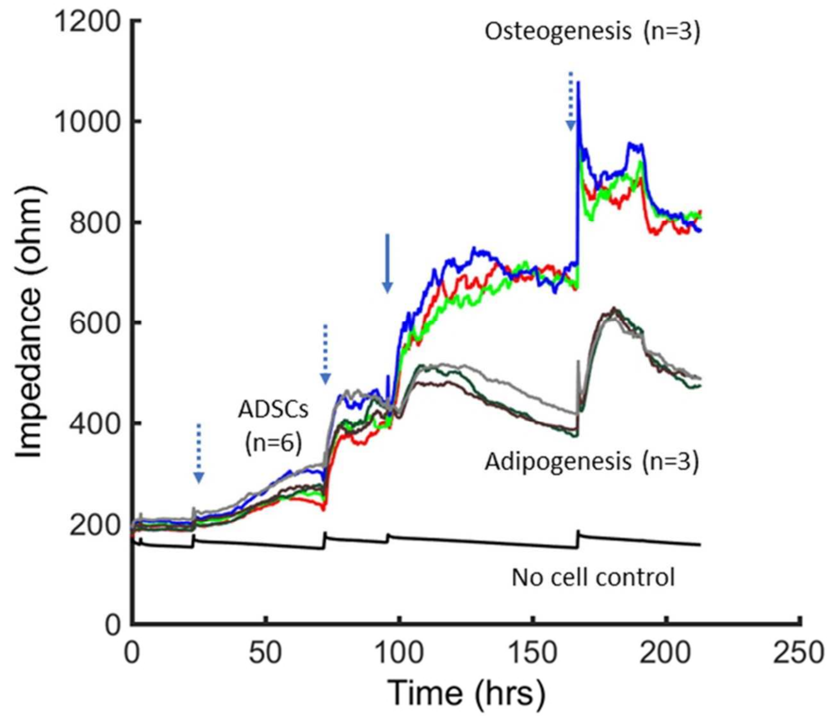

Figure2: Representative time-course impedance for ASCs differentiation into two distinct lineages, i.e. osteogenesis (n=3) and adipogenesis (n=3), and a no cell control recorded with ECIS. Dotted arrows point at feeding time, plain arrow at induction time.

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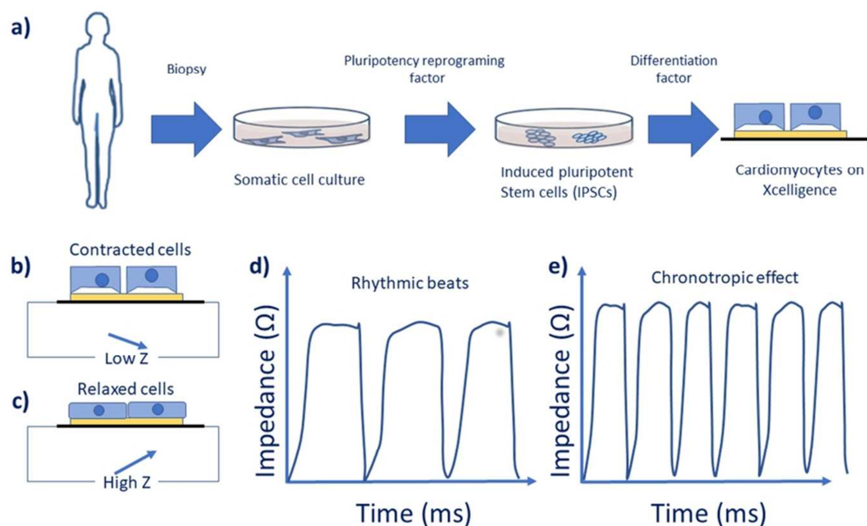

Figure3: (a) IPSCs genererated from a patient can be differentiated into cardiomyocytes directly on top of gold microelectrodes. The spontaneous beating, an alternance of contracted (b) and relaxed states (c) results in cycles in the impedance. Changes in amplitude and frequency of the cycles can easily quantified, e.g. for a compound inducing chronotropic effect (e).

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