Accepted Manuscript

Whole Cell Analysis Ranging from Intercellular Assay to Organ on a Chip

Lin Tong, Maedeh Mozneb, Elisa Bravo, Victoria Ferrando, Chen-Zhong Li

PII: S0165-9936(19)30202-X

DOI: https://doi.org/10.1016/j.trac.2019.05.021

Reference: TRAC 15519

To appear in: *Trends in Analytical Chemistry*

Received Date: 1 April 2019

Revised Date: 15 May 2019

Accepted Date: 15 May 2019

Please cite this article as: L. Tong, M. Mozneb, E. Bravo, V. Ferrando, C.-Z. Li, Whole Cell Analysis Ranging from Intercellular Assay to Organ on a Chip, *Trends in Analytical Chemistry*, https://doi.org/10.1016/j.trac.2019.05.021.

Whole Cell Analysis Ranging from Intercellular Assay to Organ on a Chip

Lin Tong, Maedeh Mozneb, Elisa Bravo, Victoria Ferrando, Chen-Zhong Li *

Nanobioengineering/Bioelectronics Lab, Department of Biomedical Engineering, Florida International University, 10555 West Flagler Street, Miami, Florida 33174, USA

* Corresponding author Email: licz@fiu.edu

Abstract:

Current treatments and diagnostic devices will be efficient and successful given that the knowledge of cell and tissue is well known. To understand the sub-micron to macro world that is contained in a cell or tissue on a functional and structural level, a comprehensive method is necessary for not only measurement of physiological parameters but also of its molecular interactions, protein bindings and membrane changes that happen during a natural or stimulus induced phenomena. Several groups have introduced techniques in several areas for measuring of the smallest scale of a biological system, the cell, to organ on chip devices that replicate a small portion of a full organ. In this review, we have categorized those methods based on scale and application and reviewed several views that'll give an overall understanding of current available techniques for such application.

Key words: Cell electronics, Cellular probes, Organ on a Chip, single cell analysis, Intercellular analysis, Nanotoxicity, Drug screening, Diagnostics

Introduction

For design of diagnostic devices, drug screening modalities and therapeutic wearable and implantable devices, a well-established knowledge of cellular functions as a single entity and as a whole in a 3D tissue, is highly essential. Being a complex biological component, cells have micro and macro scale physiological cascades that lead to functionality or abnormality of their corresponding organ. These cascades of events should be studied and well-understood not only in a single cell level, but also in monolayers and 3D constructs of cells. Knowledge acquired from this will give more input in designing accurate and effective drug delivery systems, stimulating devices and therapeutics that could contribute an enormous amount to our current healthcare industry.

Each targeted phenomenon should be studied from inside of a single cell (intracellular activities), a cell as a whole, to cells communicating in a monolayer and 3D structures. Cellular biology can be categorized into four different areas of proteomics, metabolomics, transcriptomics and genomics. Each of them used to have their own conventional method for analysis and monitoring. 2-dimensional electrophoresis (2DE) was used as a method for monitoring proteomics [1][2]. Metabolomics have been studied using gas chromatography and mass

spectrometry with several clinical applications, such as providing a better understanding of cancers [3][4]. For functional genomics and transcriptomics analysis techniques like Next Generation Sequencing (NGS), Giemsa Banding Technique (GTG banding), northern blot and qPCR have been used [5][6][7][8]. However, due to the enormous amount of information that had been compiling in the past century of the cellular biology, a need for a higher throughput techniques and methodologies arose to be able to analyze several different targets of cellular biology at the same time. Information received from metabolomics needed to be linked to proteomics and genomics for better understanding of the pathways, and that could not have been possible with single-targeted conventional methods. Researchers over the past few decades have put a great amount of effort to develop techniques and devices, besides the traditional methods such as spectroscopy and chromatography, for measurement of such actions within cells. With the favorable option going towards Microelectromechanical Systems (MEMS) based devices and photolithography techniques for cell capture and sensing, many other has used microelectrodes and Nanosensors for analysis [9][10][11][12]. A combination of new technologies with the state of the art artificial intelligence and deep learning techniques, is a necessity these days in processing large data received from multi array MEMS based devices for cellular and tissue engineering[13].

In this review, we will cover the most recent studies and novel techniques that has been developed for whole cell analysis from inside of a single cell to 3D tissues. Their shortcomings will be addressed along with their capabilities and we will further discuss the future need for new devices to be developed as a comprehensive model in cell and tissue analysis.

1. Intracellular and intercellular measurement devices

The path for improved diagnostic capabilities and active management of health can be traced to the advancement of intercellular measurement. Used to further our knowledge on the scope of science and medicine, intercellular measurement is the quantification of dynamic changes that occur within and around cells. Research on diseases, such as cancer and Alzheimer's, require cellular kinetics and viability to be studied through measurements taken at the nanometer scale inside of and between cells. Biomarkers like glucose, oxygen, pH, etc. are all measured through the various forms of intracellular study. Microelectrodes and optical Nanosensors are widely used methods of quantifying the number of intracellular markers and determining its effect. Here we'll discuss the most recent investigations on such technology and their specific clinical applications.

1.1. Microelectrodes

Biopotential electrodes are commonly used to measure intracellular marker components. Real- time monitoring, low cost, reproducibility, and fabrication ease are all aspects of electrochemical devices that results in their abundance of use in this field [14]. The average electrochemical cell contains three electrodes: the working, counter, and reference. The entire basis of electrochemistry is to obtain the electric potential that occurs within the system by applying a voltage through the working electrode. The true results of the electric potential are

obtained through subtracting the reference potential by the working electrode potential. In studying cellular actions, microelectrodes are used as working electrodes in an electrochemical system. Microelectrodes can be fabricated using various types of fibers and metals etc. Flexible fiber-based electrodes have become highly favorable due to their high spatial and temporal resolutions, and their mechanical characteristics [15]. Great elasticity and tensile strength, high stiffness and toughness, and good biocompatibility makes carbon fibers highly favorable for use in microelectrodes [15]. Carbon fiber, although low in electrochemical activity, has been widely used due to its ability to be modified. Wang et al. has coated the bare carbon fiber electrode with graphene nanosheets [15]. ZnO based Nanosensors have also gained a great amount of attention due to its high sensitivity to chemical species and large surface to volume ratios [14]. Biocompatibility, non-toxicity, and formation of various shapes has led ZnO nanostructures to be highly favorable for the modification of electrodes. Glucose, cholesterol, L-lactic acid, uric acid and metal ions, which are all important factors in cellular metabolism, can be measured through ZnO based sensors. For the majority of these systems, enzymes are placed onto ZnO in order to catalyze the redox reaction of the analyte in order to read out the amount of H_2O_2 in the system, which will change the electric potential. For metal ions however, the electrodes modified with ZnO are then coated with an ion selective monolayer thin membrane to carry out the electrochemical detection. One distinction exhibited in carbon fibers compared to ZnO coatings is the need for modification; the electrochemical activity experienced in a bare carbon fiber is lower than what is required for ultrasonic electroanalysis hence modification is necessary [15]. ZnO experiences very high electrochemical sensitivity due to its small dimension, large surface contact, and its chemical and biological nature, therefore there is no need for modification. ZnO also is fabricated quite easily, is biocompatible, and can be formed into various different shapes, which makes it favorable for electrode development [14]. Some of the drawbacks to this method is the damage to the cell and protection of the sensing device from interference.

1.2. Probes encapsulated by biologically localized embedding (PEBBLE)

Optical Nanosensors are devices that can measure specific chemical or biological cellular components and respond to external stimuli using an optical signal [16][17]. They can be a good alternative to microelectrodes due to their non-invasive interaction with cells, long lasting response and higher sensitivity and selectivity in some cases. Probes encapsulated by biologically localized embedding (PEBBLE) are used solely for intercellular measurement. Examples of these would be pH sensors, glucose sensors, oxygen consumption sensors, and much more. PEBBLEs are different from that of labelled nanoparticles and electrodes due to their sensor protection from interfering species and the cellular environment from toxins. Various methods are employed to deliver these Nanosensors into biological cells. The most common method being the gene gun, in which Nanosensors are dried onto a plastic disk and covered with another disk then to be released into various cells through pressurized helium. Generic design of optical Nanosensors contains an inert matrix, where the sensing component and reference component are kept. PEBBLEs are differentiated through the methods in which measurements are taken and how they specifically are set up. PEBBLEs have been a promising intracellular analysis technique of

metabolites. Kopelman et al., a leading group in fabrication and application of PEBBLEs, used their designed sensors to monitor oxygen, pH (6.0-7.0), Potassium, Calcium and sodium concentration variations within a cell, all in millimolar detection limits [16][18][19]. Glucose sensing analysis and techniques have also been furthered with the use of a polyacrylamide Nanosensor containing glucose oxidase, an enzyme, an oxygen sensitive fluorescent dye, and an oxygen insensitive dye. This specific technique allows for the ease of creating ratiometric measurements of glucose concentrations [20].

1.3. Fluorescent Nanosensors

Fluorescence based Nanosensors are the most widely used form of optical Nano sensing. The fundamental components of fluorescent Nanosensors are: the analyte sensitive fluorophore, the insensitive reference fluorophore, and the nontoxic matrix. Polyacrylamide and silica sol-gel are two forms of inert matrix that shield the sensing elements of the sensor from external interference [14][21][22]. Fluorescent Nanosensors are then calibrated to correctly sense the analyte. Through in-situ calibration, cells are immersed into buffer solution with ionophores thereby equilibrating the pH and giving a more substantial calibration for the sensor. The extraction of data of this method is done through image analysis. Fluorescent images are taken of the cells, which are then analyzed through custom software. Data is presented in the form of a histogram or colormap.

1.4. Liquid polymer ion selective Nano optodes

Certain analytes are non-measurable with available fluorophores, hence another method for optical sensing is utilized. Based on ion selective optode theory, the adjacent optically visible agent acts as indicator while the optically silent ionophore receives the signal from the analyte of interest [15]. Brasuel M. and group showcased this form of optical Nanosensors with decyl methacrylate and poly(ethylene glycol) (PEG) as the inert matrix in order to measure potassium concentration [16]. Due to its complex nature and cumbersome process to design the sensor, not many groups have approached the application of such technique. Though it could be highly useful in cases of important markers like cardiac troponin T (cTnT), for stroke.

1.5. Alternative Miscellaneous Methods

Most of the intracellular measurement falls into the two forms of sensing explained above, but there are various other methods employed to quantify other aspects of cellular function such as biophysical interactions, like contractile forces within cells during vasoconstriction. Fluorescent probes, fluorescent labelled atoms, and optical analysis of cellular forces also include their findings into the advancement of intercellular measurement [23][24]. Force probing, studied with Silicon Nanowires (SiNWs) can greatly lend to the research of cellular behavior [17]. Deformation of kinked SiNWs indicates the force load in the system, this measurement is directly proportional to the diameter of the SiNW. Optical electron microscope transform is used to determine the diameter of SiNW and hence find the force load of system.

All abovementioned techniques and methods are important for analysis of cellular metabolism, proliferation and genesis in a Micro and Nano level. The information acquired from

such investigations can lead to development of devices for a higher throughput, more automated and faster analysis of single cells' biological phenomenon, which will be discussed further in the next section.

2. Single cell analysis devices

Recent studies have shown that individual cell behaves differently in cell populations and nanotoxicity is also more complexity in studying of cell populations. Single cell analysis, on the other hand, can provide information without the interference from cell populations. Therefore, single cell analysis can help us simplify the research subject and understand the fundamental principles, especially for neural cell, cancer cell and stem cell. However, classic analysis methodologies such as high-performance liquid chromatography with electrochemical detection (HPLC-ED), gas-chromatography–mass spectroscopy (GC–MS), immunohistochemical methods, can only provide an average performance or resultant from outcome of cells population [20]. Some behavior can be obscured in multiple cells analysis approach. In order to conduct disease diagnostic, treatment evaluation, drug screening, or nanotoxicity analysis, new approaches are highly needed for single cell analysis [25][26][27]. Single cell analysis is more sensitive and its response to a lower amount of sample can be easily observed with shorter period of reaction time. Our lab has been heavily involved with innovation of several novel approaches for single cell analysis, which will be discussed shortly.

2.1. Needle microelectrodes

As we discussed earlier, microelectrodes are important tools for monitoring below nanomolar level concentration of material, based on a variation of electrical and electrochemical techniques. Needle shaped microelectrodes have the potential of entering the cell's membrane without puncturing the cell for vesicle release, due to a very small tip size (1μm). However, they can also be used in close proximity of a single cell for monitoring vesicular release without entering the cytosol. A needle shape non-invasive carbon fiber microelectrode was developed for single cell real-time monitoring by Dr. Li's lab [20]. The carbon fiber diameter was 5 μm, connected with an insulated copper wire by a silver paste, then sealed with borosilicate glass capillary. (Figure 1 (A)) The needle microelectrodes were capable to monitor cell exosomes such as 8-OHdG and electrochemical signal was recorded in real-time. The device had been applied for nicotine influence for human alveolar epithelial cells. Tan et al. had also reported an application of a needle microelectrode made by tungsten with a tip size around 1μm, which can be manipulated to surrounding of a single HepG2 cell (cancer cell) for monitoring the morphological and structure changes in real time without affecting any surrounding cells [28].

The advantages of needle microelectrodes for single cell monitoring are rapidly real-time response, high sensitivity and high signal to noise ratio (SNR) [29]. The miniaturization of microneedle tip allows a micron or sub-micron sized sensing area and ultimately can approach single biomolecule size, which could result a high detection sensitivity. Meanwhile, Small sensing area of microneedle tip can focus on target single cell and be less sensitive to disturbance

in surrounding or environment. It even allows to observe and focus on a specifically targeted cell. However, needle microelectrodes measurement needs an assistant of precise manipulation of robotic arms, which require a sophisticated trained person to handle the equipment and increased the application limitation [25]. In order to solve this limitation, a highly automatic lab on a chip device was developed.

2.2. Cell on a chip (CoC) device

One key technology in single cell analysis is cell sorting. Exciting methods includes acoustic, magnetic, optical, mechanical or electrical. However, acoustic, magnetic and optical cell sorting needs additional labeling with antibody conjugated Nanoparticles. Mechanical cell sorting needs precisely manipulate the cells and cells can be affected by significant shear force. In order to solve the issue, our group developed Cell on a Chip (CoC) device.

CoC is a chip microelectrode-based MEMS device for single cell isolation and Nanotoxicity analysis. A positive-dielectrophoretic (pDEP) methods was integrated with CoC device giving a fast and robust method to do cell sorting. The advantages are label free, shear stress free and high efficiency [25][30]. The fabrication process is detailed in Figure 1 (B) [25]. Briefly, 1) 25 nm of Cr and 250nm of Au was deposited on cleaned glass wafer as electrode layer. 2) Electrode pattern was created using lithography process and wet chemical etching. 3) Negative photoresist Su-8 2025 was spin coated to achieve a 25 μm thickness capturing well layer. 4) Capturing well pattern was created using lithography process and SU-8 developer. The microchip has a total number of 8 microwells (2 by 4). The diameter of each microwell is 20 μm and with gold micro electrode chip at the bottom. 5) The microchip is assembled with a top ITO electrode, which is modified with a positively charged self-assembled monolayer in order to attract normally negatively charged cell membrane. 6) Microfluidic device was packaged into a plastic leaded chip carrier (PLCC) for robust and easy signal connections [25]. The novel platform could trap single cell within 30s and record the electrochemical signal allowed drug screening, cytotoxicity analysis and nanotoxicity analysis. Shah et al. had also successfully demonstrated that a concentration of silver nanoparticles higher than 10 μg/mL will affect cell PC12 cell neuronal exocytosis function by the devised CoC device [31].

As we mentioned earlier, single cell analysis can help us understand the basic principle of cell behaviors in a rapidly real-time level. However, an isolated cell without cell interaction tend to die easily and it cannot well mimic a real living environment as cell population. On top of these, our group designed a new generation CoC device which combined single cell analysis and multiple cells analysis. As shown in Figure 1 (C), the new generation of CoC device contains 16 microwells for single cell analysis and a large well for small population of cells analysis, which can provide a better comparison and evaluation in real-time. The nanotoxicity of CuO nano particles and TiO_2 nano particles were successfully analyzed at both single cell level and small population of cells by using multi-functional CoC device [32].

Several other cell on chip devices exist, confirming the capability of currently available science and technology for fabrication of novel devices for automated cellular analysis. Same platforms could be made with electrochemical/optical sensing, sampling ports and in conjugation with natural forces of electromagnetism or gravity, they could be a great contribution to today's knowledge on cellular biology.

2.3. In situ novel nanodevices for cancer cell detection

Cancer therapy is one of the worldwide challenging topics. MicroRNAs (miRNAs) has been discovered for cancer cells regulation, which could be used for cancer diagnostic and therapy. The difficulty to use miRNA as biomarker is the low amount in a single cell. In collaboration with Zhu's lab, we developed a novel nanodevices used for amplification of miRNAs in situ so that it could be used for cancer diagnostic, risk assessment and drug deliveries [33]. The mechanism of nanodevices is shown in figure 1 (D). Briefly, DNAzymes were split into multi-component nucleic acid enzymes (MNAzymes). MNAzymes then were assembled on the surface of mesoporous silica-coated gold nanorods. Gold nanorods were used for heat generator with the excitation of NIR light, which can be controlled at 45°C to catalyze the miRNA duplication and generating continuous fluorescence. The ratio of up-regulated miRNAs (miR-21) to down-regulated miRNA (miR-145) were used for cancer risk assessment. Drugs were released when triggered by miRNA. The nanodevices were verified with a promising result for HeLa, HL-60 cancer cell and NIH 3T3 control cells.

Understanding the biology of a single cell is important in the grasp of each cell's function and structure, which comes most important today in the case of tissue engineering and stem cell tissue fabrications. However, to go from a single cell to tissue, there are several steps that take place. Cellular communication is highly important in transferring the biology from one single cell to the other for genies and proliferation of cells, possible mutations and signal conduction (in case of cardiac and neuronal cells). Understanding the communication of cells in a 2-dimensional and 3-dimensional structure are the further steps that needs to be taken before application of such knowledge in tissue engineering.

3. Two-dimensional cell monolayer measurement devices

The majority of in vivo experiments with adherent cells are performed in two-dimensional (2D) cultures, where the cells are grown coating the surface of the chip previously treated for cell binding. This process varies depending on the cell type, growing directly on the surface, secreting extracellular matrix (ECM) to facilitate adhesion, or requiring an ECM pre-coating of the desired surface. Biosensors which integrate whole cells have an advantage over previous biosensors as they have the ability of providing feedback on the total physiological effect of external factors being tested [34]. Important advancements have been made over the last decade on the application of 'cells on chips'. Much of this work falls under a 'lab-on-a-chip' framework which aims to create microsystems incorporating independent steps of an assay into a single system [35][36]. The compact design and low power consumption, in addition to its rapid and

reproducible measurements makes them great candidates for point-of-care devices. These novel technologies will compete with laboratory-scale instruments in the analysis of intricate biological processes since they eliminate labor-intensive and possibly error-prone laboratory handling.

3.1. Cell monolayer on a chip

Hondrouliet et al. successfully engineered an array-formatted whole cell based electrical impedance sensing system (EIS) which measures the resistance produced by the cell monolayer over an electrode and the resistance following nanoparticle exposure, monitoring the variation in the cell density or cell morphology. This technology provides a compact structure, simplicity in use and the capacity of measurement of multiple samples concurrently, key factors in monitoring cytotoxicity [34]. Regarding in vivo biological applications of external bodies such as chips, sensors or drug delivery systems, cytotoxicity is a major factor that needs to be accounted for prior to their employment [30].

Pratikkumar et al., utilized the EIS method to test the cytotoxicity of MoS2 nanosheets. The results of this study showed the 2D nanosheets synthesized had a negligible cytotoxicity/nanotoxicity. This decreased cytotoxicity of MoS2 nanosheets can be directly correlated to the fact that the edges of these sheets are not sharp, thus, they were unable to penetrate the cell membrane to cause stress or cell death. Tus, this material, with its semiconducting nature, large area synthesis, electronic properties and a layered structure similar to graphene, is superior for use in biomedical applications over many of its counterparts [30]. Similarly, graphene, a commonly used material in biomedical application was also assessed in terms of cytotoxicity in order to ensure biocompatibility. The in vitro testing with astrocytes and epithelial cells provided resistance measurements which reflected little toxicity towards the model, proving graphene to be a viable vehicle for drug delivery amongst others [37]. Additionally, the kinetic effects of gold nanoparticles (AuNPs; 10, 100 nm), silver nanoparticles (AgNPs; 10, 100 nm), single walled carbon nanotubes (SWCNTs; cut, uncut) and cadmium oxide (CdO) when in contact with CCL-153 and RTgill-W1 cells were assessed using an array-formatted whole cell based EIS [34].

Electrotherapy has become a promising innovative treatment. The basis of this new science is the difference in electrical properties between normal cells and cancerous cells, found to be more electronegative. Studies have shown in the presence of an applied electric field, gold nanoparticles (AuNPs) could alter the distribution of the field in the cell's cytoplasm leading to an inward net electric force which could change the function of the cell [38].

As we mentioned in the previous section, cancer affects millions of people worldwide and efforts to eradicate this disease have led to treatments with much potential. However, an important limitation of these treatments is the side effects experienced due to the use of toxic chemicals and possible immunogenic responses in the host tissue. In order to decrease or eliminate all side effects, cancer electrotherapy has become a hopeful innovative treatment. Hondroulis et al, utilized EIS to measure cell viability following the application of an electric

field with the incorporation of HER2 antibody functionalized gold nanoparticles (HER2-AuNPs) to determine the changes in proliferation rates. The charged nanoparticle was directly bound to the HER2 receptors found abundantly in the cell membrane of cancerous cells, altering the zeta potential value of the cell [39]. This method aims to target and eradicate cancerous cells without affecting any of the surrounding normal proliferating cells [38][40]. In order to differentiate cancer cells from normal cells, Haitham el al., examined the over-sialylation of cell surface glycans in a sialic acid fed-batch culture system while lacking the proper nutrients and employing sialic acid binding lectins in order to detect differences in the cell surface. Sialylation, in addition to increased branching of N-linked glycans, are seen in cancer progression, thus, this method has the potential to be used for cancer diagnosis and prognosis [41].

3.2. SPR for cell monolayer analysis

Surface Plasmon Resonance (SPR) technology is a label-free optical method, highly sensitive to refractive index changes of the medium flowing over the sensor chip. It takes use of a noble metal like gold as a sensor and by shining a polarized light on a point, it triggers the resonance of surface plasmons, which in turn produces an evanescent wave that dissipates award from the surface perpendicularly and an electromagnetic wave which propagates along the surface. Any mass changes in the evanescent field (~200 nm) which could be due to biding of molecules like antigen-antibodies, will affect the refractive index change which will result in a shift in the refracted light. The angle of the refracted light corresponds to the concentration and kinetics of association and dissociation of the target analyte. The technology has been used recently by some groups for monitoring cellular responses to target proteins or detection of their vesicle release. Liu et al., integrated a mini culture system onto an SPR system [42]. SP biosensors measure the refractive index changes at the interface between two media with constant opposite charges such as a metal and a dielectric. This simple, real-time and highly sensitive new technology allows for an in vivo assessment of the drug functionality, making this bio-mimic platform ideal for future studies on signaling pathways, antineoplastic drug development and drug function evaluation [43]. Cancer consists of the abnormal growth of cells, often causing a tumor [44] . When a tumor becomes larger than a millimeter, it will be deprived of oxygen if new vasculature is not provided. Angiogenesis is the process of growing new blood vessels from existing ones, this process is commonly seen at tumor site. It is mediated by the vascular endothelial growth factor (VEGF) which binds to vascular endothelial growth factor receptor (VEGFR) leading to neovasculature [45]. In order to test the effectiveness of drug therapies based on antibodies to regulate the VEGF−VEGFR angiogenic switch, Liu et al. integrated a mini culture system onto a Surface Plasmon Resonance (SPR) system. This method was utilized to provide real time measurements of bevacizumab drug e□cacy to the VEGF− VEGFR angiogenic switch on living SKOV-3 cells [42].

3.3. Microimmunoelectrode (MIE) Device

A novel microimmunoelectrode (MIE) using amperometry, an electrochemical technique to measure oxidation, was used in conjunction with antibodies, which provide specificity for the target and influence MIE longevity, to make the chip-based sensors. These sensors we able to

quantify a wide range of concentrations, take longitudinal measurement and be directly placed in the brain [46].

Progression of Alzheimer's disease has been found to be directly correlated to the accumulation levels of β-amyloid (Aβ) in the brain [47]. Studies suggest this accumulation comes from a defect in Aβ elimination. A novel microimmunoelectrode (MIE) to measure rapid changes in the brain interstitial fluid (ISF) Aβ metabolism in vivo was examined by Yuede et al. This amperometric, antibody-based electrode, at 0.65 V induces oxidation of single tyrosine (Tyr) residue found at position 10 of human Aβ. This oxidation releases electrons which are then detected as current by the carbon fibers. Since Tyr is present in many proteins in the extracellular space, the anti-body anti-Aβ is crucial to ensure specificity of the electrode [46].

The scope of this paper doesn't allow us for more examples of modern fabricated devices used for analysis of a monolayer of cells, however several other researches exist in the field which can be categorized based on the titles mentioned in this section. Current ongoing studies are targeted to developing a comprehensive model for metabolic analysis of cells for multiple analytes and from several perspectives (functional and structural), in response to drugs and with the use of several disease models. This will help lots in the drug development industry as well as providing a better understanding of complicated diseases such as Alzheimer's disease.

4. Three-dimensional tissue and organ on a chip

Fabrication of 3D tissue has been a challenge for over a couple of decades. There are several approaches on how to fabricate and improve the long lasting of a 3D tissue. There are many parameters to be controlled; adequate cell–cell and cell–matrix contact, crucial for signal transduction and cellular communication to regulate fundamental cellular processes such as growth, proliferation, differentiation, migration and programmed cell death, are to name a few. Methods are generally categorized into top down and bottom up approaches. In a top down approach, the fundamental constructs of the final desired tissue are broken down to its elements, consisting of scaffolds and hydrogels as supporting material. Afterwards, cells are cultured within the biocompatible 3D structure and allowed for growth and proliferation until a confluent structure in 3D is acquired. However, a top-down approach has the limitation of overlapping high proliferating cells and their ECM expression on the scaffold and counteracting the effect to hold them in place. Hence, the bottom up approach has gained a lot of attention recently. In a bottom up approach, first the cells are layered or patterned within a dish and then scaffolds are added to shape them and support them into a final construct. Based on the method of fabricating 3D tissue, one can decide on how to use a sensing mechanism for analysis of health or drug testing. Making tissue into spheroids is another 3D fabrication method that has been favorable in many labs due to their perfect replica of the in-vivo environment.

Quantitative and real time analysis of 3D tissue constructs based on information received from 2D, is highly essential in understanding how the in vivo environment behaves. Cellular

communication, metabolic distribution, action potential propagation, cellular morphology, growth and proliferation, etc. all differ in 3D vs. 2D constructs. Also, for drug screening, which is one of the major applications of in vitro studies, to estimate the realistic effect of drug in body and get a comprehensive understanding of what the drug does to each element, a 3D model is highly superior compared to flat monolayers of cells [48]. Thus, it is very important that a sensing mechanism is in place to establish such a knowledge on a scaled-up 3D tissue. Microfluidic devices have been a favorable option for 3D tissue sensing platform fabrication as several manipulations, cell sorting and sensing modalities, vasculature embedding techniques with channels etc. are feasible within a microfluidic design.

4.1. Contact-Based Sensing Devices

It is a known fact that although the contact-based devices with 3D tissue, are to disrupt the original tissue environment and decrease stability over a long-term study, though they open more options in terms of possible sensing mechanisms. Zhang et al. developed a novel blood brain barrier (BBB) replica using an astrocyte-based ECM as the connection between astrocytes and endothelial cells and tested the effect of nanodrugs passing through BBB [49]. Tests included Trans-Endothelial Electrical Resistance (TEER), transport studies, using calcein, caffeine and doxorubicin solutions, electron microscopy studies for morphology analysis and measurement of tight junctions, western blotting, and immunofluorescence analysis, which all became possible as this was a contact-based technique. Interestingly, paper has also been used as a microfluidic device for construction and sensing of 3D tissue, to avoid cumbersome procedures associated with cleanroom for MEMS devices. Boyce et al. used a compact device made of several layers, with vertical flow, including an oxygen sensing film to monitor oxygen distribution in 3D cells [50]. A wax printer was used to print each layer, cells were seeded, and the complete setup was screwed together using a stainless-steel platform. Another try was given by Kenney et al, using paper as scaffolds for 3D cell seeding and pH-sensing optodes, that gave a high spatial and temporal resolution map of pH gradient in breast adenocarcinoma cells [51].

Using electrochemistry and MEMS technology, a novel device with 400 amperometric sensor electrodes was developed by Li et al. to monitor dopamine release and response to drug from neuronal PC12 spheroids [48]. They used the concept of electrochemical imaging to quantify the amount of dopamine released from PC12 cells in a spheroid construct under a high concentration of K^+ media. Another interesting report was given by Mostafalu et al. in 2016, in which they used nanomaterial infused conductive thread as an interconnected microfluidic system and physiochemical sensors with biological tissue, for measurement of strain, temperature, pH and glucose markers in 3D tissue [52]. As threads are biocompatible and cost effective, once modified with advance engineering techniques to carry fluids selectively and sense at the same time, they can be used as an interesting integrated sensing platform within 3D tissue. Figure 2 summarizes these devices as mentioned in a chronological order.

4.2. Non-Contact Sensing Devices

Analysis of cells and tissue in 3D doesn't only depend on devices to be in contact with

tissue's surface, it also could be done non-invasively without disrupting tissue, like Nanosensors of optical or electrochemical nature or a combination of devices and spheroids.

Spheroids, as mentioned earlier are a good replication of in-vivo environment and the sensing platform for them should be label free and noninvasive, so it doesn't disrupt the natural environment of tissue. Many groups have used MEMS technology as a safe yet multifaceted option for monitoring their 3D tissue. Anderegg et al. fabricated a microelectrode array device using MEMS technology for monitoring health of tissue and its expressions in real-time [53]. Their reusable device, designed for harsh incubator environments and high tolerance for ethanol and acidic conditions, encompassed cavities with sensors embedded for spheroid entrapment and empty cavities that were used as a reference. They used an impedance-based technique along with fluorescent and bright field microcopy for recording of extracellular field potentials of cardiomyocyte spheroid and effect of drug exposure. Another group developed a device that allowed the presence of hang drops (spheroid making technique) within their electrochemical sensing elements, to monitor the amount of lactate produced from their human colon derived carcinoma (HCT%-116) spheroids [54]. The real-time readout enables the acquisition of on-line information on the metabolic activity of 3D microtissue structures in microfluidic networks for a wide range of applications. Though spheroids are more convenient to make, their size control has not yet been established. Therefore Zhang et al. developed a device that could make a ratio between a spheroids size and its physiological read-outs using a sorting device for trapping different size neuronal spheroids [55]. They also tried to improve cellular communication between neuronal spheroids using embedded channels connecting their trapping wells. In a previous study they had already improved the construct of wells by using PLLA which helped the cells get attached not only within the floors of device but also to climb up and communicate through the wells [56]. Another group measured the oxygen in the inlet and outlet of a bioreactor that bear the tissue in the middle and found a correlation between the oxygen drop and number of cells that compose the 3D tissue construct [57]. One smart move is to input the sensing elements within the scaffolding of the 3D tissue and monitor the intrinsic response of scaffolds using an optical or electrochemical method. Dai et al. tried to upgrade their scaffolds by adding chemicals to improve it for accepting additional functional groups which opens the rout to adding fluorescently tagged sensing proteins or electrochemically bound enzymes for monitoring tissue health [58].

Several studies have also shown the use of optical Nanosensors for non-invasive and real time monitoring of physiological properties of 3D tissue [59]–[65]. The concept involves the encapsulation of a target sensitive dye (oxygen, pH, biomarker) within a diffusion-optimized shell for that specific target and delivering to either inter or intracellular space. Müller et al. for example, used RL100 and F13, the positively charged polymeric nanoparticles encapsulated with hydrophobic Pt (II) porphyrins sensitive to K^+, to compare the efficiency of them entering cells and monitoring dynamics of potassium ion in 3D tissue [59]. Figure 3 summarizes some of the abovementioned devices.

Conclusion and Future Perspectives

Electrochemical Nanosensors and optical Nanosensors are able to greatly advance the field of intracellular measurement. Their use has been directly mentioned in this review for some analytes, however with further research on the detection of H_2O_2 in cells through microelectrode sensing, diagnostics in cancer can be greatly advanced, as understanding H_2O_2 concentration variations within the cell cytosol as well as intracellularly is highly important in cell's function and life span. [66]. Simple intracellular oxygen sensing with fluorescence lifetime imaging microscopy (FLIM) can aid in the understanding of mitochondrial and metabolic functioning within the cell [67]. The future of intercellular measurements will be highly based upon the correction and modification of methods already used and the creation of new forms of measurement. For the methods currently in use today, delivery mechanisms and calibration will be further evolved and corrected.

Single cell analysis is more sensitive to a lower amount of sample test within a shorter period of reaction time. 2D cell monolayer devices allow to analyze cell population reactions and behaviors with a lower cost. A combination of single cell and 2D CoC is preferred for its multi-functional advantage. Besides, with the fast response advantage of CoC devices for single cell and small cell population, it can potentially be used in clinical treatment study, such as a tailor study for cancer therapy. It can also be applied in clinical disease diagnosis, such as single cell-based mRNA, protein biomarker or exosome analysis.

With all the cell culture and 3D fabrications going towards stem cell derived cultures and their maturation properties, it is more important to combine all the above-mentioned techniques, along with the right scaffolding to serve such goal. However, understanding the 3D culture and its behaviors highly depends on a comprehensive analysis of the tissue and not only monitoring its quality and health in one or two aspects. There still is a need for development of a sensing technique that can analyze tissue in all its aspects in real time and correspond its abnormalities to the correct biological reason. Organ-on-chip technology is a method that serves such need, given that the sensing mechanisms for it is well and accurately developed. Taking the advantage of integrating electrical, electrochemical, mechanical and optical sensors along with a tissue sample within an organ-on-chip device, is highly favorable due to its fast response, atomized structure, lower sample quantity and less need for labeling and tagging procedures. Combination of such devices with deep learning and artificial intelligence for improving the culture environment for tissue (ex. A closed-loop feedback system that increases the mechanical strain given to mature cardiac cells, based on their contraction force acquired using sensors), will advance the future of drug discovery to a very high extend. The development of such devices with high accuracy and good replication of the original organ, could possibly be an addition to preclinical and clinical

trials in drug development industry, pending the Food and Drug Administration's (FDA) approval.

Acknowledgement

This work was supported by the Engineering Research Centers Program of the National Science Foundation under NSF Cooperative Agreement No. EEC-1647837 and Agreement No. EEC-1648451. Dr. Li thanks the NSF Independent Research Development Program to support his research during his service at NSF.

Reference:

[1] Véronique Santoni, Sylvie Kieffer, Dominique Desclaux, Florence Masson, and Thierry Rabilloud, "Membrane proteomics: Use of additive main effects with multiplicative interaction model to classify plasma membrane proteins according to their solubility and electrophoretic properties," *Electrophoresis*, vol. 21, no. 16, pp. 3329–3344, 2000.

[2] Angelika Görg, Walter Weiss, and Michael J. Dunn, "Current two-dimensional electrophoresis technology for proteomics," *Proteomics*, vol. 4, no. 12, pp. 3665–3685, 2004.

[3] S. C. Gates and C. C. Sweeley, "Quantitative metabolic profiling based on gas chromatography," *Clin. Chem.*, vol. 24, no. 10, pp. 1663–1673, 1978.

[4] Michael D. Williams, Raymond Reeves, Linda S. Resar, and Herbert H. Hill, "Metabolomics of colorectal cancer: Past and current analytical platforms," *Anal. Bioanal. Chem.*, vol. 405, no. 15, pp. 5013–5030, 2013.

[5] Jay Shendure and Hanlee Ji, "Next-generation DNA sequencing.," *Nat. Biotechnol.*, vol. 26, no. 10, pp. 1135–45, 2008.

[6] Lisbeth Barkholt, Egbert Flory, Veronika Jekerle, Sophie Lucas-Samuel, Peter Ahnert, Louise Bisset, Dirk Büscher, Willem Fibbe, Arnaud Foussat, Marcel Kwa, Olivier Lantz, Romaldas MačIulaitis, Tiina Palomäki, Christian K. Schneider, Luc Sensebé, Gérard Tachdjian, Karin Tarte, Lucie Tosca, and Paula Salmikangas, "Risk of tumorigenicity in mesenchymal stromal cell-based therapies - Bridging scientific observations and regulatory viewpoints," *Cytotherapy*, vol. 15, no. 7, pp. 753–759, 2013.

[7] R. R. Scherberger, H. Kaess, and S. Brückner, "Studies on the action of an anticholinergic agent in combination with a tranquilizer on gastric juice secretion in man," *Arzneimittelforschung.*, vol. 25, no. 9, pp. 1460–3, 1975.

[8] Gurman S. Pall and Andrew J. Hamilton, "Improved northern blot method for enhanced

detection of small RNA," *Nat. Protoc.*, vol. 3, no. 6, pp. 1077–1084, 2008.

[9] Enrique Azuaje-Hualde, Maite García-Hernando, Jaione Etxebarria-Elezgarai, Marian M. De Pancorbo, Fernando Benito-Lopez, and Lourdes Basabe-Desmonts, "Microtechnologies for cell microenvironment control and monitoring," *Micromachines*, vol. 8, no. 6, 2017.

[10] Parisutham Vinuselvi, Seongyong Park, Minseok Kim, Jung Min Park, Taesung Kim, and Sung Kuk Lee, "Microfluidic technologies for synthetic biology," *Int. J. Mol. Sci.*, vol. 12, no. 6, pp. 3576–3593, 2011.

[11] Feifei Tan, Tianbao Wang, Haishi Wang, and Yuzheng Zheng, "Microfluidic techniques for tumor cell detection," *Electrophoresis*, vol. 40, no. 8, pp. 1230–1244, 2019.

[12] Tim Stuart and Rahul Satija, "Integrative single-cell analysis," *Nat. Rev. Genet.*, vol. 20, no. 5, pp. 257–272, 2019.

[13] Miroslava Cuperlovic-Culf, "Machine learning methods for analysis of metabolic data and metabolic pathway modeling," *Metabolites*, vol. 8, no. 1, 2018.

[14] Arpan S. Desai, Veeren M. Chauhan, Angus P. R. Johnston, Tim Esler, and Jonathan W. Aylott, "Fluorescent nanosensors for intracellular measurements: Synthesis, characterization, calibration, and measurement," *Front. Physiol.*, vol. 4 JAN, no. January, pp. 1–15, 2014.

[15] Jonathan W. Aylott, "Optical nanosensors - An enabling technology for intracellular measurements," *Analyst*, vol. 128, no. 4, pp. 309–312, 2003.

[16] M. Brasuel, R. Kopelman, T. J. Miller, R. Tjalkens, and M. A. Philbert, "Fluorescent nanosensors for intracellular chemical analysis: Decyl methacrylate liquid polymer matrix and ion-exchange-based potassium PEBBLE sensors with real-time application to viable rat C6 glioma cells," *Anal. Chem.*, vol. 73, no. 10, pp. 2221–2228, 2001.

[17] John F. Zimmerman, Graeme F. Murray, and Bozhi Tian, "Optical Determination of Silicon Nanowire Diameters for Intracellular Applications," *J. Phys. Chem. C*, vol. 119, no. 52, pp. 29105–29115, 2015.

[18] Wenjing Lu, Yifang Gao, Yuan Jiao, Shaomin Shuang, Chenzhong Li, and Chuan Dong, "Carbon nano-dots as a fluorescent and colorimetric dual-readout probe for the detection of arginine and Cu2+ and its logic gate operation," *Nanoscale*, vol. 9, no. 32, pp. 11545–11552, 2017.

[19] Wenjing Lu, Yifang Gao, Yuan Jiao, Shaomin Shuang, Chenzhong Li, and Chuan Dong, "Carbon nano-dots as a fluorescent and colorimetric dual-readout probe for the detection

of arginine and Cu 2+ and its logic gate operation," *Nanoscale*, vol. 9, no. 32, pp. 11545–11552, 2017.

[20] Shradha Prabhulkar and Chen Zhong Li, "Assessment of oxidative DNA damage and repair at single cellular level via real-time monitoring of 8-OHdG biomarker," *Biosens. Bioelectron.*, vol. 26, no. 4, pp. 1743–1749, 2010.

[21] Li Meng, Aihua Jiang, Rui Chen, Chen zhong Li, Liming Wang, Ying Qu, Peng Wang, Yuliang Zhao, and Chunying Chen, "Inhibitory effects of multiwall carbon nanotubes with high iron impurity on viability and neuronal differentiation in cultured PC12 cells," *Toxicology*, vol. 313, no. 1, pp. 49–58, 2013.

[22] Li Meng, Aihua Jiang, Rui Chen, Chen zhong Li, Liming Wang, Ying Qu, Peng Wang, Yuliang Zhao, and Chunying Chen, "Inhibitory effects of multiwall carbon nanotubes with high iron impurity on viability and neuronal differentiation in cultured PC12 cells," *Toxicology*, vol. 313, no. 1, pp. 49–58, 2013.

[23] Lingjie Hou, Xiangyu Kong, Yishou Wang, Jianbin Chao, Chenzhong Li, Chuan Dong, Yu Wang, and Shaomin Shuang, "An anthraquinone-based highly selective colorimetric and fluorometric sensor for sequential detection of Cu 2+ and S 2- with intracellular application," *J. Mater. Chem. B*, vol. 5, no. 45, pp. 8957–8966, 2017.

[24] Xia Li, Juan Song, Qingwang Xue, Haiyan Zhao, Min Liu, Baoli Chen, Yun Liu, Wei Jiang, and Chen Zhong Li, "Sensitive and selective detection of the p53 gene based on a triple-helix magnetic probe coupled to a fluorescent liposome hybridization assembly: Via rolling circle amplification," *Analyst*, vol. 142, no. 19, pp. 3598–3604, 2017.

[25] Pratikkumar Shah, Xuena Zhu, Chunying Chen, Ye Hu, and Chen Zhong Li, "Lab-on-chip device for single cell trapping and analysis," *Biomed. Microdevices*, vol. 16, no. 1, pp. 35–41, 2014.

[26] Fang Fang Cheng, Wei Chen, Li Hui Hu, Gang Chen, Hai Tao Miao, Chenzhong Li, and Jun Jie Zhu, "Highly dispersible PEGylated graphene/Au composites as gene delivery vector and potential cancer therapeutic agent," *J. Mater. Chem. B*, vol. 1, no. 38, pp. 4956–4962, 2013.

[27] Pratikkumar Shah, Ajeet Kaushik, Xuena Zhu, Chengxiao Zhang, and Chen Zhong Li, "Chip based single cell analysis for nanotoxicity assessment," *Analyst*, vol. 139, no. 9, pp. 2088–2098, 2014.

[28] Xiao Tan, Shasha Zhao, Qian Lei, Xinpei Lu, Guangyuan He, and Kostya Ostrikov, "Single-cell-precision microplasma-induced cancer cell apoptosis," *PLoS One*, vol. 9, no. 6, pp. 2–11, 2014.

[29] Rahim Esfandyarpour, Hesaam Esfandyarpour, Mehdi Javanmard, James S. Harris, and Ronald W. Davis, "Microneedle biosensor: A method for direct label-free real time protein detection," *Sensors Actuators, B Chem.*, vol. 177, pp. 848–855, 2013.

[30] Pratikkumar Shah, Tharangattu N. Narayanan, Chen Zhong Li, and Subbiah Alwarappan, "Probing the biocompatibility of MoS2 nanosheets by cytotoxicity assay and electrical impedance spectroscopy," *Nanotechnology*, vol. 26, no. 31, 2015.

[31] Pratikkumar Shah, Qiaoli Yue, Xuena Zhu, Fangcheng Xu, Hui Sheng Wang, and Chen Zhong Li, "PC12 cell integrated biosensing neuron devices for evaluating neuronal exocytosis function upon silver nanoparticles exposure," *Sci. China Chem.*, vol. 58, no. 10, pp. 1600–1604, 2015.

[32] Pratikkumar Shah, Xuena Zhu, Xueji Zhang, Jin He, and Chen Zhong Li, "Microelectromechanical System-Based Sensing Arrays for Comparative in Vitro Nanotoxicity Assessment at Single Cell and Small Cell-Population Using Electrochemical Impedance Spectroscopy," *ACS Appl. Mater. Interfaces*, vol. 8, no. 9, pp. 5804–5812, 2016.

[33] Qianhao Min, Jiangning Chen, Zhimei He, Penghui Zhang, Xuena Zhu, Chen Wang, Chen-Zhong Li, Jun-Jie Zhu, and Jingjing Zhao, "In Situ Amplification of Intracellular MicroRNA with MNAzyme Nanodevices for Multiplexed Imaging, Logic Operation, and Controlled Drug Release ," *ACS Nano*, vol. 9, no. 1, pp. 789–798, 2014.

[34] Evangelia Hondroulis, Chang Liu, and Chen Zhong Li, "Whole cell based electrical impedance sensing approach for a rapid nanotoxicity assay," *Nanotechnology*, vol. 21, no. 31, 2010.

[35] Janelle R. Anderson, Daniel T. Chiu, Rebecca J. Jackman, Oksana Cherniavskaya, J. Cooper McDonald, Hongkai Wu, Sue H. Whitesides, and George M. Whitesides, "Microsystem Technology in Chemistry and Life Sciences," *J. J. Vac. Sci. Technol. B*, 1997.

[36] Petra S. Dittrich and Andreas Manz, "Lab-on-a-chip: Microfluidics in drug discovery," *Nature Reviews Drug Discovery*. 2006.

[37] Evangelia Hondroulis, Zhiqi Zhang, Chunying Chen, and Chen Zhong Li, "Impedance Based Nanotoxicity Assessment of Graphene Nanomaterials at the Cellular and Tissue Level," *Anal. Lett.*, vol. 45, no. 2–3, pp. 272–282, 2012.

[38] Evangelia Hondroulis, Rui Zhang, Chengxiao Zhang, Chunying Chen, Kosuke Ino, Tomokazu Matsue, and Chen Zhong Li, "Immuno nanoparticles integrated electrical control of targeted cancer cell development using whole cell bioelectronic device,"

Theranostics, vol. 4, no. 9, pp. 919–930, 2014.

[39] Yu Zhang, Mo Yang, Ji Ho Park, Jennifer Singelyn, Huiqing Ma, Michael J. Sailor, Erkki Ruoslahti, Mihrimah Ozkan, and Cengiz Ozkan, "A surface-charge study on cellular-uptake behavior of F3- peptide-conjugated iron oxide nanoparticles," *Small*, 2009.

[40] Evangelia Hondroulis, Steven J. Melnick, Xueji Zhang, Ze Zhi Wu, and Chen Zhong Li, "Electrical field manipulation of cancer cell behavior monitored by whole cell biosensing device," *Biomed. Microdevices*, vol. 15, no. 4, pp. 657–663, 2013.

[41] Haitham A. Badr, Abdelaleim I. Elsayed, Hafiz Ahmed, Miriam V. Dwek, Chen Zhong Li, and Leyla B. Djansugurova, "Preferential lectin binding of cancer cells upon sialic acid treatment under nutrient deprivation," *Appl. Biochem. Biotechnol.*, vol. 171, no. 4, pp. 963–974, 2013.

[42] Haitham A. Badr, Rui Zhang, Hongyun Liu, Chen-Zhong Li, Chang Liu, Subbiah Alwarappan, and Jun-Jie Zhu, "Live Cell Integrated Surface Plasmon Resonance Biosensing Approach to Mimic the Regulation of Angiogenic Switch upon Anti-Cancer Drug Exposure," *Anal. Chem.*, vol. 86, no. 15, pp. 7305–7310, 2014.

[43] Chang Liu, Tingjun Lei, Kosuke Ino, Tomokazu Matsue, Nongjian Tao, and Chen Zhong Li, "Real-time monitoring biomarker expression of carcinoma cells by surface plasmon resonance biosensors," *Chem. Commun.*, vol. 48, no. 84, pp. 10389–10391, 2012.

[44] Qing R. Yu, "Stem cells and cancer stem cells," *Journal of Clinical Rehabilitative Tissue Engineering Research*. 2007.

[45] Jan Novotný and Michal Zikán, "Tumor angiogenesis," *Klinicka Farmakologie a Farmacie*. 2010.

[46] Jane C. Hettinger, Clare E. Wallace, Jessica L. Restivo, Todd A. Davis, Margaret R. Hayne, Chen-zhong Li, John R. Cirrito, Katherine L. Young, Carla M. Yuede, Guojun Bu, and Hyo Lee, "Rapid in vivo measurement of β-amyloid reveals biphasic clearance kinetics in an Alzheimer's mouse model," *J. Exp. Med.*, vol. 213, no. 5, pp. 677–685, 2016.

[47] Erik S. Musiek and David M. Holtzman, "Three dimensions of the amyloid hypothesis: Time, space and 'wingmen,'" *Nature Neuroscience*. 2015.

[48] Chen-Zhong Li, Masahki Matsudaira, Yusuke Kanno, Kosuke Ino, Kumi Y. Inoue, Hiroya Abe, Hitoshi Shiku, Atsushi Suda, Ryota Kunikata, Yasufumi Takahashi, and Tomokazu Matsue, "Electrochemical Imaging of Dopamine Release from Three-Dimensional-Cultured PC12 Cells Using Large-Scale Integration-Based Amperometric Sensors," *Anal.*

Chem., vol. 87, no. 12, pp. 6364–6370, 2015.

[49] Zhiqi Zhang, Anthony J. McGoron, Eric T. Crumpler, and Chen Zhong Li, "Co-culture based blood-brain barrier in vitro model, a tissue engineering approach using immortalized cell lines for drug transport study," *Appl. Biochem. Biotechnol.*, vol. 163, no. 2, pp. 278–295, 2011.

[50] Matthew W. Boyce, Rachael M. Kenney, Andrew S. Truong, and Matthew R. Lockett, "Quantifying oxygen in paper-based cell cultures with luminescent thin film sensors," *Anal. Bioanal. Chem.*, vol. 408, no. 11, pp. 2985–2992, 2015.

[51] Rachael M. Kenney, Matthew W. Boyce, Nathan A. Whitman, Brenden P. Kromhout, and Matthew R. Lockett, "A pH-Sensing Optode for Mapping Spatiotemporal Gradients in 3D Paper-Based Cell Cultures," *Anal. Chem.*, vol. 90, no. 3, pp. 2376–2383, 2018.

[52] Pooria Mostafalu, Mohsen Akbari, Kyle A. Alberti, Qiaobing Xu, Ali Khademhosseini, and Sameer R. Sonkusale, "A toolkit of thread-based microfluidics, sensors, and electronics for 3D tissue embedding for medical diagnostics," *Microsystems Nanoeng.*, vol. 2, no. March, 2016.

[53] Ulf Anderegg, Daniel Kloß, Randy Kurz, Jan C. Simon, Andrée Rothermel, Andrea A. Robitzki, Heinz-Georg Jahnke, and Michael Fischer, "Microcavity array (MCA)-based biosensor chip for functional drug screening of 3D tissue models," *Biosens. Bioelectron.*, vol. 23, no. 10, pp. 1473–1480, 2008.

[54] Oliver Frey, Patrick M. Misun, Jörg Rothe, and Andreas Hierlemann, "Real-time in-situ lactate monitoring in 3D multi-cellular spheroid cultures by using enzyme-based biosensors in hanging drop networks," *Procedia Eng.*, vol. 87, pp. 96–99, 2014.

[55] Li-Guang Zhang, William S. Kisaalita, Dong-Huo Zhong, Ze-Zhi Wu, Yiguo Zhang, and Chen-Zhong Li, "A microwell pattern for C17.2 cell aggregate formation with concave cylindrical surface induced cell peeling," *Biomaterials*, vol. 35, no. 35, pp. 9423–9437, 2014.

[56] Zhang, Chenzhong Li, Ze-Zhi Wu, Luo, An, Kisaalita, Liao, Wang, Zhong, Jin, and Huang, "Responsiveness of voltage-gated calcium channels in SH-SY5Y human neuroblastoma cells on quasi-three-dimensional micropatterns formed with poly (l-lactic acid)," *Int. J. Nanomedicine*, p. 93, 2013.

[57] p. Nancy, S. Shilpita, MA. Sen, and S. Swathi, "Explant culture: a simple, reproducible, efficient and economic technique for isolation of mesenchymal stromal cells from human adipose tissue and lipoaspirate," *J. Tissue Eng. Regen. Med.*, vol. 4, no. 7, pp. 524–531, 2012.

[58] Xizi Dai, Yen Chih Huang, Jared Leichner, Madhvan Nair, Wei Chiang Lin, and Chen Zhong Li, "Peptide modified polymer poly (glycerol- dodecanedioate co-fumarate) for efficient control of motor neuron differentiation," *Biomed. Mater.*, vol. 10, no. 6, 2015.

[59] Ruslan I. Dmitriev, Reha S. Erzurumlu, Bernhard J. Müller, Alexander V. Zhdanov, Haijiang Zhang, Ingo Klimant, Vassiliy Tsytsarev, Yu Chen, Tara Foley, Qinggong Tang, Irina A. Okkelman, Dmitri B. Papkovsky, Sergey M. Borisov, and Claudio Toncelli, "Nanoparticle-Based Fluoroionophore for Analysis of Potassium Ion Dynamics in 3D Tissue Models and In Vivo," *Adv. Funct. Mater.*, vol. 28, no. 9, p. 1704598, 2018.

[60] Sisi Lu, Wei Xu, Jinliang Zhang, Yiying Chen, Lei Xie, Qiuhong Yao, Yaqi Jiang, Yiru Wang, and Xi Chen, "Facile synthesis of a ratiometric oxygen nanosensor for cellular imaging," *Biosens. Bioelectron.*, vol. 86, pp. 176–184, 2016.

[61] Xianchuang Zheng, Huang Tang, Chen Xie, Jialiang Zhang, Wei Wu, and Xiqun Jiang, "Tracking Cancer Metastasis InVivo by Using an Iridium-Based Hypoxia-Activated Optical Oxygen Nanosensor," *Angew. Chemie - Int. Ed.*, vol. 54, no. 28, pp. 8094–8099, 2015.

[62] G. Zhang, M. McShane, and C. Robinson, "Quenching Properties of a Self-Referenced Fluorescence Oxygen Nanosensor under a Wide-Field Intrinsic Optical Signal Imaging System," *Conf Proc IEEE Eng Med Biol Soc*, vol. 2, pp. 1438–1441, 2005.

[63] Kevin J. Cash and Heather A. Clark, "Phosphorescent nanosensors for in vivo tracking of histamine levels," *Anal. Chem.*, vol. 85, no. 13, pp. 6312–6318, 2013.

[64] Yong Eun Lee Koo, Youfu Cao, Raoul Kopelman, Sang Man Koo, Murphy Brasuel, and Martin A. Philbert, "Real-Time Measurements of Dissolved Oxygen Inside Live Cells by Organically Modified Silicate Fluorescent Nanosensors," *Anal. Chem.*, vol. 76, no. 9, pp. 2498–2505, 2004.

[65] Pick Chung Lau, Robert A. Norwood, Masud Mansuripur, and Nasser Peyghambarian, "An effective and simple oxygen nanosensor made from MPA-capped water soluble CdTe nanocrystals," *Nanotechnology*, vol. 24, no. 1, 2013.

[66] Shuai Wang, Zheye Zhang, Anshun Zhao, Lu Wang, Yue Dong, Kai Chi, Hao Yuan, Fei Xiao, Jinghua Ren, and Yan Zhang, "PtAu alloy nanoflowers on 3D porous ionic liquid functionalized graphene-wrapped activated carbon fiber as a flexible microelectrode for near-cell detection of cancer," *NPG Asia Mater.*, vol. 8, no. 12, pp. e337–e337, 2016.

[67] Dhruv Sud and Mary-Ann Mycek, "Calibration and validation of an optical sensor for intracellular oxygen measurements," *J. Biomed. Opt.*, vol. 14, no. 2, p. 20506, 2009.

Figures

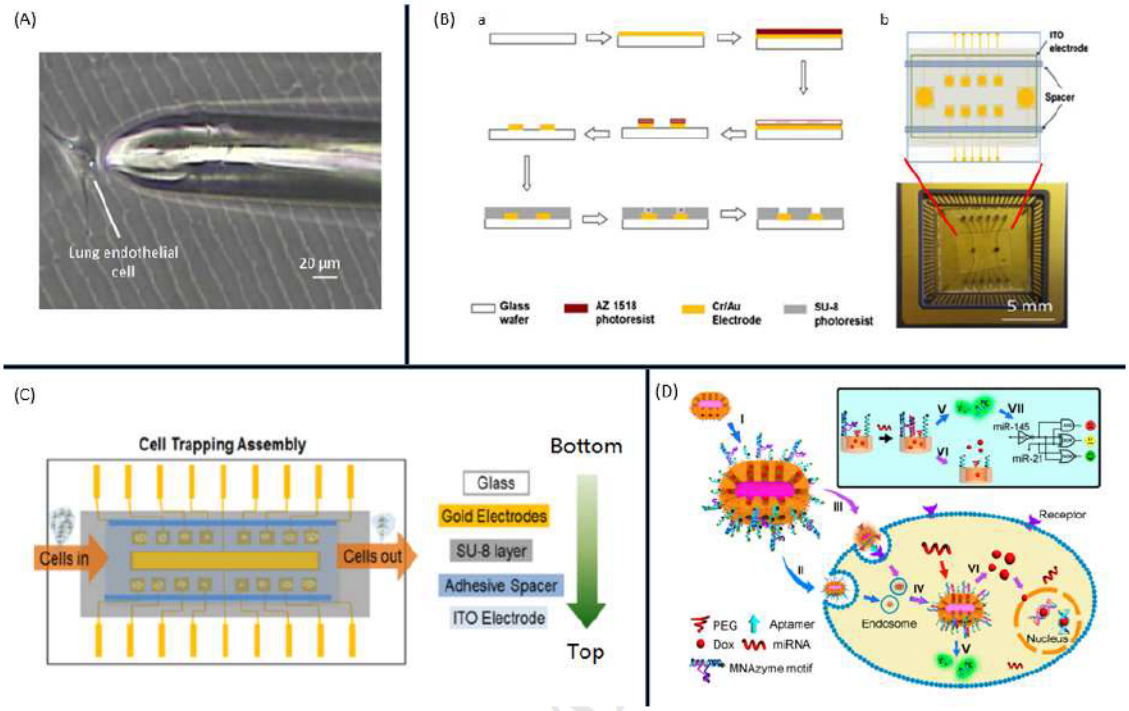

Figure 1. (A) Needle microelectrode device. (B) MEMS Cell on Chip device. (C) Multi-function CoC. (D) MNAzyme-based nanodevices for cancer cell diagnostic and therapy. I, preparation of MMSGR nanode- vices; II, clathrin-mediated endocytosis of MMSGR probes; III, receptor-mediated endocytosis via aptamer; IV, endosome escape; V, intracellular miRNA imaging; VI, miRNA-triggered Dox release; VII, logic operation for cancer risk assessment.

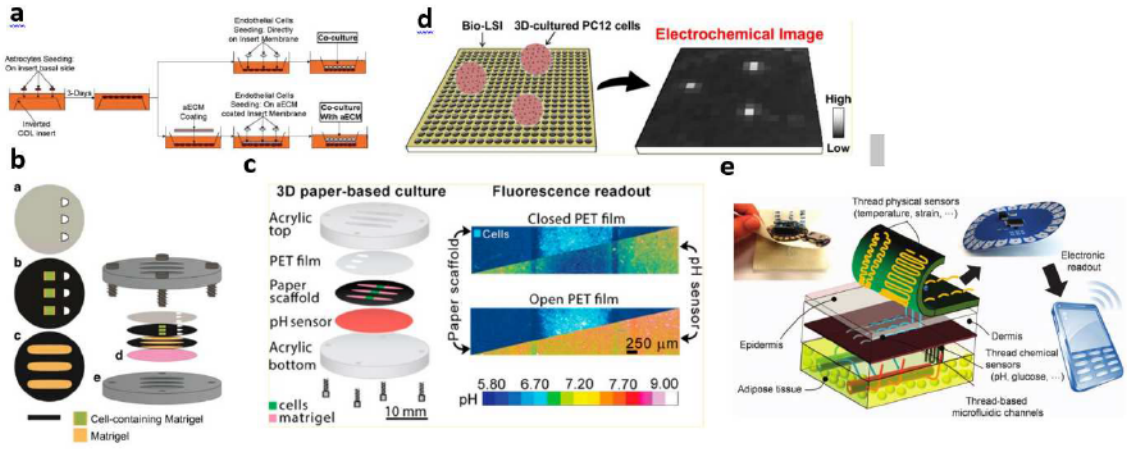

Figure 2. Contact-based devices used for measuring physiological properties of 3d constructed tissue.

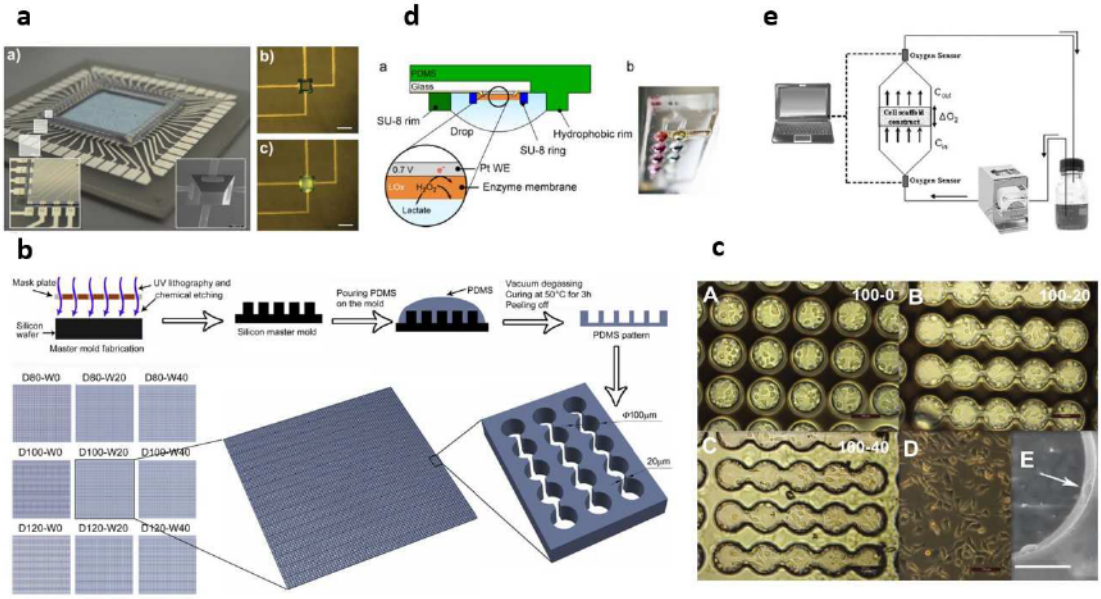

Figure 3. Non-Contact based Devices for measurement of 3D tissue characteristics

Here, we covered the most recent studies and novel techniques that has been developed for whole cell analysis from inside of a single cell to 3D tissues. Their shortcomings were addressed along with their capabilities and we further discussed the future need for new devices to be developed as a comprehensive model in cell and tissue analysis. The main body was categorized as:

- **Intracellular and intercellular**
- **Single cell**
- **Two-dimensional cell monolayer**
- **Three-dimensional tissue sensing**
- **Paper based point of care testing**