Hybrid Microfluidic Sensing Platforms forCancer Cell Study: Recent Advances and Future Prospects

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Abstract

The importance of mammalian cell culture is vividly seen in biotechnology, drug screening, and large-scale pharmaceuticals production. There have been numerous efforts in designing an automated cell culture system replicating the natural microenvironment of the cells to improve throughput analysis with reduced process costs. The conventional monolayer cell culture methods are widely used to study the behaviour of various types of cells, including cancer cells. However, various limitations are associated with two-dimensional (2D) cell culturing methods. These limitations include the disturbance of interactions between the cellular and extracellular environments, cell morphology, polarity, and division method changes. Due to these limitations, 3D cell culturing techniques are significant for their resemblance in vivo tissue and cellular interactions. Research groups have employed microfluidic systems with this strategy to develop novel organ-on chips to study cancerous tumor invasion and substitute animal testing.

Furthermore, many efforts have been made by incorporating sensing instruments with these microfluidic systems to monitor cellular/organ activities continuously and quantitively evaluate their behaviour and response to various molecules/drugs. This thesis will comprehensively examine the most recent studies about two different aspects of developing microfluidic and sensing devices by focusing on numerous forms of cancer. These emerging microfluidic/sensing system technologies will play crucial roles in enhancing our understanding of cancer cell behaviours and accelerate the research activities to find the most suitable drugs for cancer treatment.

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This thesis was realized through the encouragement and assistance of unselfish mentors. I want to express my profound gratitude to:

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List of Abbreviations

oPMNs	Oral Polymorphonuclear Neutrophils
PMNs	Polymorphonuclear Neutrophils
WBCs	White blood cells
µm	Micrometre
ml	Millilitres
WHO	World health organization
CBC	Complete blood count
CT	Computerized tomography
MRI	Magnetic resonance imaging
POC	Point of care
ECIS	Electric Cell-substrate Impedance Sensing
ECM	Extracellular matrix
EIS	Electrolyte insulator semiconductors
DNA	Deoxyribonucleic acid
CMOS	Complementary Metal Oxide Semiconductor
CCD	Charge-coupled device
ISFET	Ion-Selective Field-Effect Transistor

pH	Potential of Hydrogen
LAPS	Light-addressable potentiometric sensor
HCG	Human chorionic gonadotropin
LOC	Lab-on-a-chip
OOC	Organ-on-a-chip
BBB	Blood-brain barrier
HUVECs	Human umbilical vein endothelial cells
TAMS	Tumor-associated Macrophages
VEGFα	Vascular endothelial growth factor
SEM	Scanning Electron Microscopy
TCM	Tumor cell-conditioned media
BMTM	Biomimetic Microfluidic Tumor Microenvironment
HBTAE	Human Breast Tumor Associated Endothelial cells
ER	Estrogen receptor
PR	Progesterone receptor
PSA	Prostate-specific antigen
CBI	Cell-based impedance
UWB	Ultrawideband

GMP	Gaussian monocycle pulse
CNS	Central nervous system
NSCLC	Non-small cell lung cancer
LCSC	Lung cancer stem cell
dLCSC	Differentiated lung cancer stem cell
EF	Electric field
PIGF	Placenta growth factor

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List of Definition

Microchannel	Known as a channel in microtechnology with a hydraulic diameter below 1 mm. Microchannels are widely used in heat transfer and fluid control.
Microchambers	A microscale chamber that is used for accurate and precise measurements.
Micropumps	A microscale pump used for accurate and precise measurements.
Microfabricated devices	Devices that are made through process of fabricating miniature structures in micrometre scales and smaller.
Point of care	When clinicians deliver the right health care service or product topatients at the time of care.
Microfluidic devices	These devices including microchannels, microchambers and micropumps are used for precise control, and manipulation of small volume of fluids in the range of micro, nano or even micro litre.
2D cell culture	Cell culture system that cells grow on coated plastic flat dishes, where they adhere and spread.
3D cell culture	Cell culture system that allows grow and spread of cells with surrounding extracellular framework in three dimensions and not in flat monolayer surface.
Bio-fabrication of chitosan membranes	A form of natural polymers that is used for engineering the corneal tissue scaffolds. Other examples of natural polymers are Gelatin, hyaluronic acid, and cellulose.

Cell-based biosensors	Genetically engineered biosensors that are constructed to detect a response of a single cell with high sensitivity in a cost-effective manner.
Electrochemical sensor	Sensors that convert the electrochemical information associated with reactions, such as the reaction between an electrode and analyte, into a qualitative or quantitative signal.
Alternating current (I)	An electric current which reverses direction and continuously changes its magnitude with time in contrast to direct current flowing in one direction.
Potential (V)	Voltage, also known as electric potential difference, is the external work required to bring a charge in an electric field from one location to another.
Impedance (Z)	Amount of opposition faced by direct or alternating current whenit passes through a conductor system.
Impedance Sensor	Sensor that its measuring principle relies on impedance measurement.
Electrolyte insulator semiconductors (EIS)	A device for pH measurements, which operates just like Metal- Oxide-Semiconductor capacitor but in order to apply voltage instead of having the metal electrode, an electrolyte solution and areference electrode are used.
Capacitor	A device consisting of one or more pairs of conductors that is usedto store an electric charge.

Metal-oxide-semiconductor (MOS) capacitor	A capacitor made of a semiconductor substrate, an insulator film,such. as SiO2, and a metal electrode called a gate.
EIS system	An electrochemical technique used to measure the impedance of asystem in dependency to the AC potentials frequency.
Complimentary metal-oxide-semiconductor (CMOS)	A combined circuit design on a printed circuit board (PCB) that uses semiconductor technology. CMOS is used in microprocessors, image sensors, static RAM, and microcontrollers.
Chemiluminescent imaging	An imaging technique where light is produced without heat from a chemical reaction. This imaging methodology does not use excitation or emission filters; therefore, light is captured directly by charge-coupled device camera.
CCD (charge-coupled device)	An integrated circuit imprinted onto a silicon surface forming pixels known as light sensitive elements.
CMOS image sensor	An electronic chip that converts photons to electrons for digital processing and creating images in digital cameras, digital video cameras.
Ion-Selective Field-Effect Transistor (ISFET)	Type of field-effect transistor used for measuring ion concentrations; As H+, see pH scale changes, the current throughthe transistor will change as well accordingly.
Light-addressable potentiometric sensor (LAPS)	A semiconductor-based chemical sensor, in which a measurementsite on the sensing surface is light (e.g. LEDs).

Pyrex substrate	Pyrex is a line of clear, low-thermal-expansion glass substrate.
Lab on a chip	A device that can apply one or several laboratory functions on a single circuit with only millimeters in size for high-throughput screening.
Organ on a chip	A multi-channel 3-D microfluidic cell culture chip capable of simulating activities, mechanics and physiological response of entire organ systems. This microfluidic cell culture is known as a type of artificial organ.
PDMS	Known as dimethylpolysiloxane or dimethicone which belong to silicones and is the most widely used silicon-based organic polymer due to its flexibility.
Physiological electric field (EF)	Physiological sensing method that allows computers to detect andevaluate objects in their vicinity.
Microelectrode arrays	Are devices that contain multiple plates in which neural signals, are attained. This device serves as interfaces that connect cells to electronic circuitry.
Electrochemical impedimetric	Electrochemical Impedimetric biosensor is composed of immobilized biological recognition elements onto an electrode surface. Monitoring the targeted analyte is made through measuring the output of an electrical impedance signal that is proportional to analyte activity.

Photolithography	Lithography that uses plates that are made photographically. Photolithography is used for detailed patterns. In this method, a pattern can be imprinted through exposure of a light sensitive polymer.
Pericytes	Cells along the walls of capillaries and post-capillary venules. In the CNS, these cells are important for maintenance of the blood– brain barrier, regulation of immune cell entry to (CNS) and controlof brain blood flow.
Astrocyte	Specialized glial cells that contiguously tile the entire (CNS) and are also known collectively as astroglia. Their main characteristicis their star-shape. They perform many functions, including biochemical support of endothelial cells and provision of nutrientsto the nervous tissue.
Tumor-associated Macrophages	Key cells that generate an immunosuppressive tumor microenvironment (TME) by producing chemokines, cytokines, and growth factors.
Interstitial fluidic	Fluid that is in between cell-cell space. This fluid is composed of water, coenzymes, amino acids, fatty acids, sugars, hormones, andneurotransmitters.
Invasive ductal carcinoma	Also known as infiltrating ductal carcinoma, is a form of cancer that begins by growing in a milk duct and invades the fatty tissue of the breast duct.
Invasive lobular carcinoma	Also called infiltrating lobular carcinoma and is the second most common type of breast cancer. ILC begins in the wall of lobule and spreads beyond it.

Human chorionic gonadotropin (HCG)	A hormone for the maternal recognition of pregnancy that is produced by trophoblast cells.
Extracellular matrix (ECM)	ECM is the non-cellular component within all tissues and organs. ECM is an extensive molecule network composed of three major components known as protein, glycosaminoglycan, and glycoconjugate.

I. Introduction

Cell cultures are widely performed by using Petri dishes, flasks and microtiter plates. However, interactions in the cell environment that can determine cell functionalities and phenotype cannot be replicated or controlled in these traditional methods [1]. Moreover, living cells are microscale, and microfabricated devices made in microscale can more precisely control the culture environment [2]. Due to this reason, microfluidic technology for cell-based assays has the potential to increase the biological relevance of cell models besides increasing the throughput of conventional methods [3.] The current human biology investigation and development of novel therapeutic drugs has heavily relied on two-dimensional (2D) monolayer cell culture systems. However, as stated previously, 2D cell culture cannot accurately replicate the structure, function, physiology of living tissues, and highly complex three-dimensional (3D) environment presented in vivo.

Microfluidics can provide this micro-scale structure with properly controlled parameters with the ability to mimic the in vivo environment of cells. The combination of microfluidic with 3D cell culture is potentially one of the most important discoveries for in vivo-like tissue-based applications [4]. One of the substantial shortfalls of conventional constant culture media is their inability in producing a concertation gradient. This shortfall is significantly seen in cell-based drug screening that rely on concentration gradients and gradient generator [5]. Conventional testing platform for drug concentration gradient is done by multi-well plate cell culture platform that consumes a long time with tedious processing, large reagent consumption and low diagnostic reliability [6]. Concentration gradient is one of the determining factors of cellular inflammation [7], wound healing [8], cell differentiation and growth [9] and cancer metastasis [10]. Furthermore, microscopes are used conventionally for tracking single cells, but monitoring multiple cells using a single microscope cannot be possible. For this reason, other sensing strategies should be developed.

There are various forms of microfluidic gradient generators, and those can be listed as convection mixing-based gradient generators [11], laminar flow diffusion-based gradient generators[12], static diffusion-based gradient generator [13] and geometric metering mixing-based gradient generator [14]. One of the most popular forms of gradient generators is the gradient

generator ofcompositions [15]. Numerous advantages associated with a gradient of compositions in solutions arecell growth and differentiation [16,] axon guidance [17], neutrophil chemotaxis [18], cell migration [19,] cancer chemotaxis [20], bacteria growth and chemotaxis [21], cytotoxicity [22], optimization of reactionconditions [23] and bio-fabrication of chitosan membranes [24]. The usage of microfluidics in cancer studies have been significantly influential since microfluidics can provide nutrients and dissolvedgasses and apply stimuli such as chemical gradients, spatial homogeneity, time-dependent biochemical stimulations, and substrate mechanical properties. The microfluidic-based cell culturerequires a new sensing modality instead of microscopic methods. Combining new sensing methodsand microfluidic devices can offer several advantages, such as monitoring several fluidic chamberssimultaneously.

For this reason, novel sensing methods should be developed for cell monitoring in microfluidic channels. To date, various sensors have been widely used for monitoring cell adhesion, detachment, death and response to osmotic stress [25,26,27,28]. Studying cancerous cell properties using advanced nanotechnological sensing methods can add much informative biophysical information to our existing knowledge about these cells that can substantially improve our knowledge about metastasis [29].

According to data, one in every two Canadians will develop cancer, and one out of every four cancer patients die, making cancer one of the leading causes of death in Canada. In 2019, around 220'400 Canadians were diagnosed with cancer, with 82'100 death reports [1]. To lower the casualties' physicians, more data about cancer and behavioural changes in the body can detect cancer in its early stages. A list of ten specific cancer cell hallmarks can identify a cancer cell [2]. These hallmarks can prevent immune destruction, invasion and metastasis, induced angiogenesis, proliferative signalling causing growth suppressors [3].

The first step for cancer diagnosis consists of a few steps, including physical examination to look for abnormalities such as skin colour change or enlargement of an organ. The second step comes with running laboratory tests, including blood and urine tests. For this aim, a complete blood count (CBC) is performed to measure the number of blood cells, followed by cytogenetic analysis, which closely measures changes in shape and number of patient's white blood cells (WBCs) [4]. Tumor marker testing is another method used to identify tumor cells from normal cells

by recognizing chemicals utilized from abnormal cells. Circulating tumor cell test is another test performed to detect cells that have moved from their original cancer site into the bloodstream. Blood protein testing and bone marrow biopsy are two strategies to confirm a suspected diagnosis[5]. Eventually, a biopsy is recommended to collect cells from the tissue or tumor for a closer examination based on their physical features. The third step is followed by the clinical evaluations composed of imaging tests such as computerized tomography (CT) scan, magnetic resonance imaging (MRI), ultrasound, X-ray, and bone scan. Since cancer cells are not uniformed in shape, it is easy to differentiate them from normal cells [6].

Although the methods mentioned above have saved numerous lives, they suffer from some limitations for cancer diagnosis. As an illustration, tumor markers can also be produced by normal, noncancerous cells, along with the risk of false-positive or false-negative test results [36]. Also, screening tests are not meant to prevent disease, and these tests cannot influence whether someone will get that disease or not [7]. In general, tumor cells are formed under selective pressure, nutrient availability, fluctuating oxygen level, and low pH and tissue perfusion [8]. This complex environment is usually not reproduced in standard in vitro culture conditions that provide excessive nutrient and growth factors. Various research groups have utilized three-dimensional (3D) cell culture; however, 3D cell culturing comes with some limitations, such as transportation of oxygen and nutrients. Some types of microscopic analysis can be complex due to the larger size of 3D cell culturing [9]. To face this challenge, efforts have been made using membrane-based reactors, perfusion reactors, and stirred-suspension-culture reactors. On top of that, there is the challenge of cell and tissue imaging as their complexity increases. The viability of encapsulated cells, analysis of entrapped cells, and sampling are the other challenges of existing 3D models [10]. Microfluidics is valuable alternatives that are fast in development. Microfluidics is essential in cancer studies due to their high sensitivity, low cost, high throughput, and enhanced Spatio-temporal control of physics, biology, chemistry and physiology at cellular level [42]. Furthermore, microfluidic-based platforms are portable and can be efficiently designed for point-of-care diagnostics.[11]

Microfluidics is appropriate alternatives which are essential in cancer studies due to their high sensitivity, low cost, high throughput, high-resolution spectroscopies, real-time imaging [41], and enhanced Spatio-temporal control of physics, biology, chemistry and physiology at cellular level [42]. Furthermore, microfluidic-based platforms are portable and can be efficiently designed for

point-of-care diagnostics.[42] Unlike conventional cell culture methods, a microfluidic cell culture system can deliver continuous nutrient supply, waste removal, and liquid handling systems.1 Moreover, cell-based biosensors can provide us with new horizons for POC medical diagnostics by implementing recognition elements such as mammalian cells in microfluidic devices [43]. Electrochemical sensor platforms have been developed as powerful tools for label-free detection of infection-related biomarkers due to their ability to provide increased sensitivity, fast response times, simplicity of operation, lower cost device miniaturization, disposable, and flexible electrochemical devices [44].

The main goal of this thesis is to shed more light on the interesting research field of different non-invasive cell culturing sensing methods that use microfluidics to study cancer migration. Chapter 1, which is the introduction goes over the main advantage of microfluidics over conventional cell culture methods. Chapter 2 highlights the importance of different microfluidics and sensing devices that are being widely used in cell culturing. Chapter 3 emphasizes the usage of microfluidics in the cancer research field. Also, this chapter goes through several related pieces of works of literature. Furthermore, the main clinical applications of microfluidics are discussed in deep depth in chapter 4. Then, chapter 5 wraps up this thesis by giving a summary of the content of this thesis. It also tackles the future directions that should be taken in this field of research.

II. Microfluidics and Sensing Devices for cellular/organmonitoring

In this subsection, we discuss the about various examples of biosensors used instead of conventional microscope in microfluidics and sensing devices for cellular and organ monitoring along with in this field.

2.1. Sensing Devices used in Microfluidics

In this subsection, we discuss microfluidics and sensing devices for cellular and organ monitoring,along with various examples of biosensors used in this field.

2.1.1. Electric Cell–Substrate Impedance Sensing (ECIS)

ECIS (Electric Cell-substrate Impedance Sensing) is a real-time, label-free, impedance-based method to study the activities of cells grown, such as morphological changes and cell locomotion (See Fig. 1). At the bottom of the ECIS, a small alternating current (I) is applied across the electrode pattern, which results in a potential (V) across the electrodes measured by the ECIS instrument. As illustrated in Fig. 1 the impedance (Z) is measured by Ohm's law Z = V/I. When cells are added to the ECIS Arrays and attach to the electrodes, they act as insulators resulting in increasement in the impedance. As cells cover the electrodes by growing or changing their function, the current impeded by several cells covering the electrode, the changes in cell morphology, and the nature of the cell attachment will change, altering the impedance and the data generated impedance versus time [45]. ECIS can monitor a group of cells, specifically in providing us with kinetic information about cell migration and invasion process in the 3D extracellular matrix. ECIS studies have significantly influenced cell morphology studies [46], and this is due to the progress in microfabrication technology and electrode designing [47,48,49]. Interaction of mammalian cell and extracellular matrix (ECM) proteins can help study tumor metastasis, woundhealing, and cell migration.

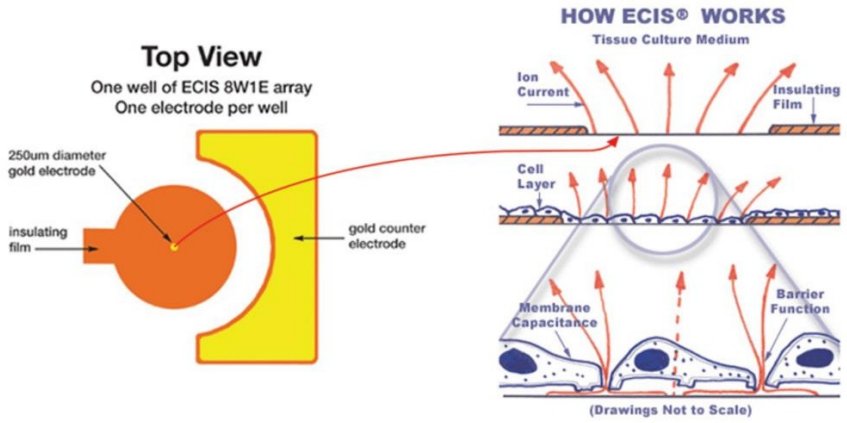

Figure 1. Representation of ECIS sensor. As cells attach on the surface of the electrodes, they act as insulators resulting in increasing the impedance. At the bottom of the ECIS an alternating current (I) is applied which results in a potential (V).

2.1.2. Electrolyte Insulator Semiconductor (EIS)

Electrolyte insulator semiconductors (EIS) can help us detect biological and chemical processes using charge coupling to allow the electrical recording of biomolecular activities [52]. As represented in Fig. 2 the EIS sensor consists of a semiconductor substrate (p-type silicon in this example) that is separated from the solution with a thin (10–100 nm) gate insulator layer/ layers and a rear-side contact layer (e.g., Al in this example). The gate insulator is designed in a way that no current passes through it [55]. As illustrated in Fig.2 sensor could have different pH-/ion-sensing, semiconductor substrate and a gate insulator. The EIS device's operating system is similar to a metal-oxide-semiconductor (MOS) capacitor due to the electrolyte solution [50]. EIS sensors operate so that a gate voltage (VG) is applied between the reference electrode and the rear-side contact to regulate the capacitance; a small alternating voltage (~10–50 mV) is superimposed to measure the capacitance of the structure. For a proper measurement, the reference electrode should provide a stable potential independent of the pH value of the solution [51]. Based on the properties of the EIS system along with other transistors, the electrolyte-semiconductor interface is utilized to establish proper biosensors [53,54]. EIS sensors can be used explicitly for monitoring chemical and biological contaminations, gene expression analysis, and molecular interactions. With all that being said, there is still a problem with this method, as it cannot detect large ions since large ions cannot approach the surface where the chemical reaction can occur [55].

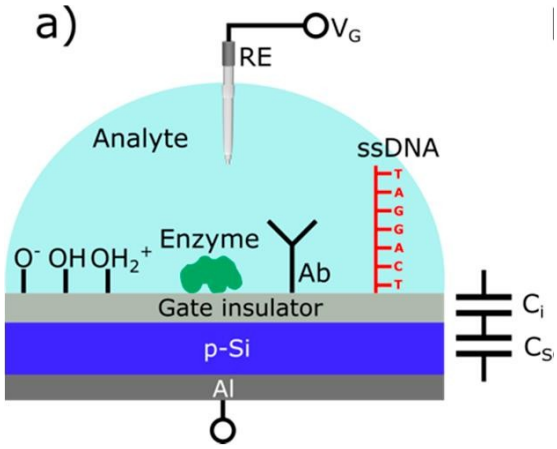

Figure 2. Representation of electrolyte-insulator-semiconductor (EIS) sensor with different pH-/ion-sensing, enzyme, antibody, and DNA receptor functionalities. RE: reference electrode, VG: gate voltage, Ab: antibody, DNA: deoxyribonucleic acid, Ci: gate-insulator capacitance, CSC: space-charge capacitance, ssDNA: single-stranded. [1]

2.1.3. Complementary Metal Oxide Semiconductor (CMOS)

CMOS is another novel hybrid platform used to study drug cytotoxicity and cellular growth monitoring; the CMOS sensor has sufficient sensitivity for chemiluminescent imaging of single cells [56]. As illustrated in Fig. 3 CMOS-based cell sensors can be developed to perform cell sizing, colorimetric cellular biochemical measurements, and real-time measurements. Image sensors in digital cameras and mobile phones mostly use either the CCD (charge-coupled device) or CMOS technology. Similar to CCDs, CMOS sensors are digital semiconductor image sensors that convert light into electrical signals. CMOS sensors contain rows of photodiodes coupled with individual amplifiers to amplify the electric signal from the photodiodes. This technology can provide applications not just for environmental and process monitoring or food safety testing purposes but also in medical diagnostics [99], overlapping with point-of-care diagnostics. CMOS image sensor technology shows appreciable progress in improving the image quality to achieve high resolution, depth-profiling images. The concept tried to get introduced here for cell sensing to draw attention to its potential in fulfilling the needs of novel testing schemes. However, there may be a lack of demonstrations, which can easily be justified by the underdeveloped nature of collaborated actions for bringing the concept into reality. So, this hurdle will be overcome since it is just a matter of time.

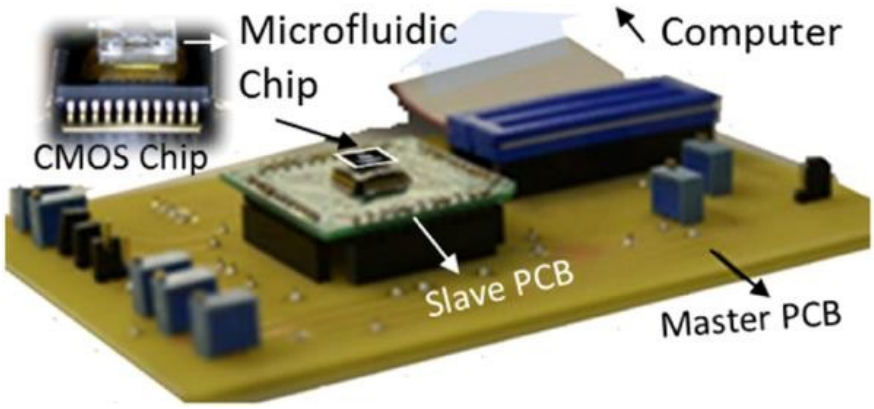

Figure 3. Representation of electrical and microfluidic packaging of CMOS platform. This chip will get connected to a computer for further analysis [152].

2.1.4. Ion-Selective Field-Effect Transistor (ISFET)

Another sensing technology is the Ion-Selective Field-Effect Transistor (ISFET), developed from the cell-semiconductor hybrid biosensor system [57]. An ISFET is a field-effect transistor usedfor measuring ion concentrations in solution; as the ion concentration, such as H+, changes, the current through the transistor will change accordingly [58]. Initially, this method was used as a pH sensor. Therefore, the platform's sensitivity is directly determined by the change in flat band potential per pH change in the solution. Consequently, the platform's sensitivity is determined bythe number of binding sites located on the insulator's surface [59]. As illustrated in Fig. 4 in a typical FET system, sensing elements are attached to the sensing channels (semiconductor path), which are connected to source (S) and drain (D) electrodes for capturing targets. Usually, a bias potentialis applied to the third electrode (gate). There are two types of FET systems, n-type in which electrons are the primary charge carriers and p-type, in which holes are the primary charge carriers [60]. FET biosensors are potentially one of the most promising technologies that can assist the scientist in the development of label-free and sensitive analyte detectors for cancer diagnostics.

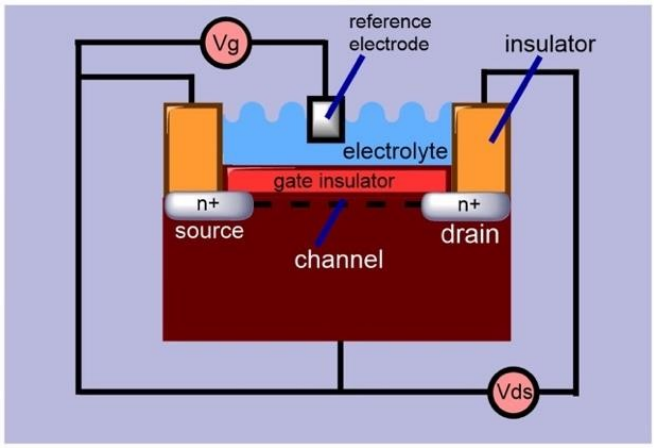

Figure 4. The schematic view of an ISFET sensor. The two electrodes used in a FET system are source and drain which the electron flow takes place in a channel between the drain and source. The gate potential controls the flow of current between the two electrodes.

2.1.5. (Light-addressable potentiometric sensor) LAPS

Light-addressable potentiometric sensor (LAPS) is one of the latest developments in the family of field-effect-based sensor devices. As demonstrated in Fig. 5, in LAPS sensor voltage is applied across the LAPS structure to induce a space-charge region. A modulated focused light beam lightens a certain region of the LAPS structure from below and the photo current will get stored by a trans-impedance amplifier. This sensor can measure the electrolyte-transducer interface's surface potential with a lateral resolution; Therefore, the surface potential is directly dependent on the chemical interaction between the transducer and the electrolyte solution. LAPS has utilised an integrated taste sensor with artificial lipid membranes as the ion-sensitive material [61]. LAPS has received the most attention among biosensors based on photovoltage techniques due to its excellent sensitivity, stability, and high signal-to-noise ratio [62]. Using LAPS, the response of cells to chemical substances is studied and monitored by acidifying living cells [63] and changes in the concentration of inorganic ions [64]. LAPS was first introduced by Hafeman et al. in 1988 as a measurement device for biological applications such as phospholipid bilayer membrane-based LAPS, enzyme-based (urase) microchamber-LAPS device, and sandwich immunoassay for human chorionic gonadotropin (HCG) [65]. Moreover, LAPS has an excellent opportunity to become one of the biomedical research tools for drug testing. Even though this detection device can be very beneficial, it still faces some limitations, such as the inability to detect small biomolecular resulting in its lower accuracy than traditional antibody or antigen detecting methods [66].

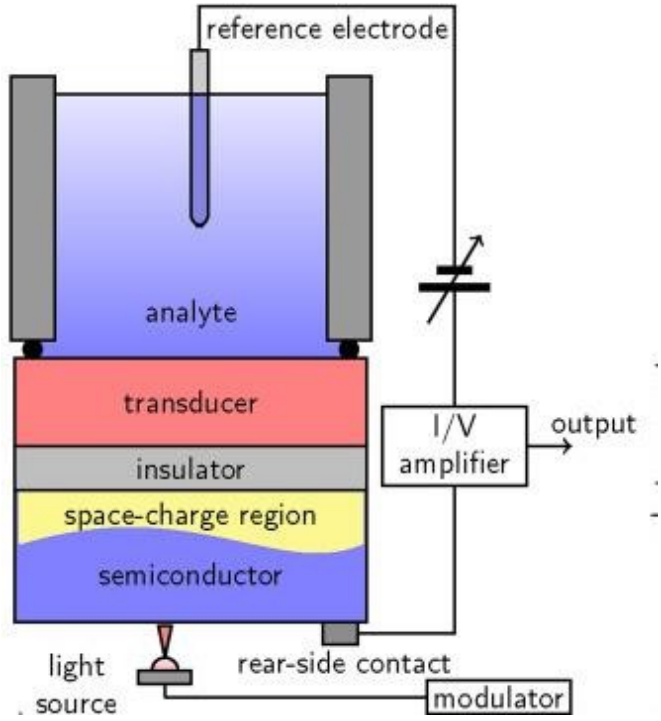

Figure 5. Illustrative of a LAPS sensor set-up. A bias voltage is applied across the LAPS structure to induce a space-charge region. A modulated focused light beam illuminates a certain region of the LAPS structure from below. The generated photo current will get recorded by a trans-impedance amplifier.

2.1.6. Sensing Arrays

This section looks closely into microscopic images of the sensing mentioned above devices being used by a couple of research groups that have used any of the above devices as their sensor tool. The main goal of this section is to give a more accurate representation of biological sensors. For instance, Otto and collogues used ISFET biosensor as their sensing method for multiparametric testing system (see Fig. 6). Their research group grew tumor cells directly on glass- or silicon-based electronic sensor chips. Extracellular pH and pO2 changes, that each reflected metabolic activities, morphological properties and changes in impedance, were monitored. This experiment took place by using colon cancer cells, LS174T cell line derived from a liver metastasis of a colon carcinoma, were exposed to cytochalasin B, chloro acetaldehyde, or doxorubicin. As illustrated in (Fig. 6) in picture (a) cell culture area on glass chip that has used is composed of not on ISFET

sensor but also IDES, temperature, O2 sensors. In section (b) of (See Fig.6) illustration of an experimental setup with glass chip is presented. Results showed that modulations in impedance correlates with morphological changes in which these changes are not observed in drug-resistant cells. This result highlighting the sensitivity advantage of this micro physiological monitoring as a versatile tool for chemosensitivity testing of tumor cells [289]. Additionally, Nguyen et al. successfully assessed long term attachment, adhesion and spreading of MCF-7 breast cancer cells. As demonstrated in Fig.7, an impedance cell-based biosensor was designed on a microelectrode surface. The main focus of this study was to study the response of breast cancer cells to the anti-cancer drug Cisplatin treatment. The circuit model and the impedance spectra both indicated that the electrical parameters in the circuit could determine the cellular activities such as cell adhesion, attachment and spreading. This biosensor can open up a wide range of applications in cell biology for anti-cancer drugs discovery [290].

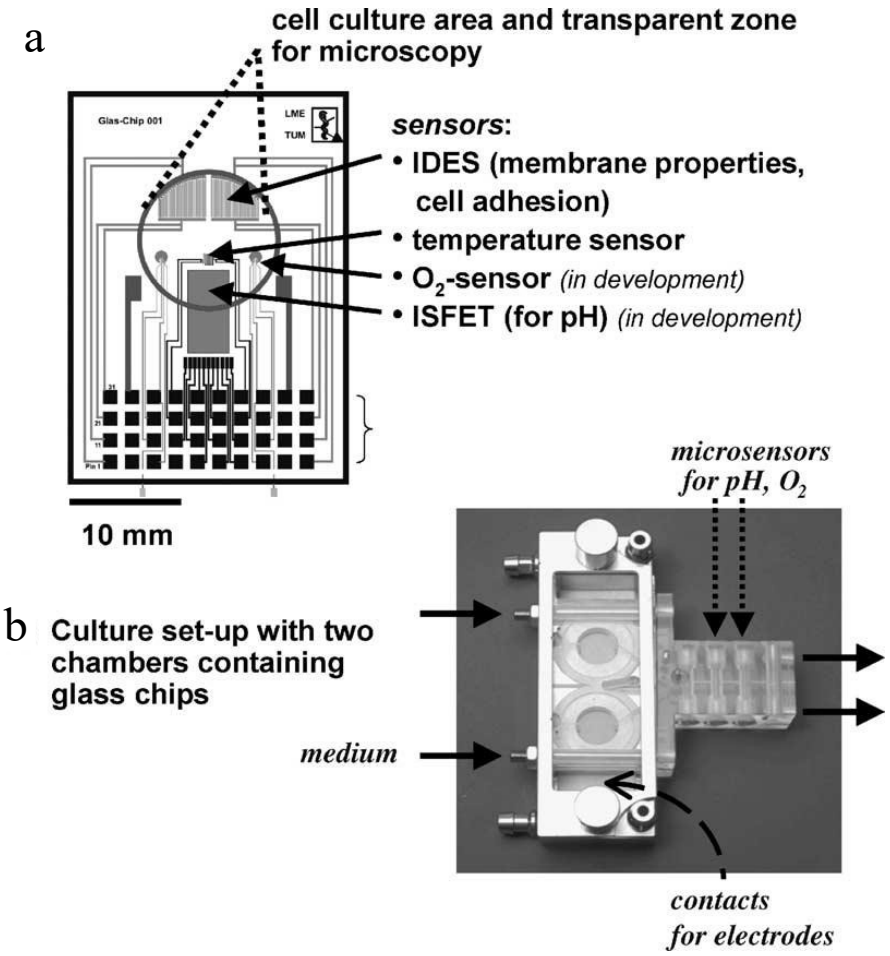

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Figure 6. Graphic representation of cell culture setup. (a) Schematic view of a glass chip. (b) Illustration of an experimental setup with two chambers that each contains a glass chip. Image of tubing that provides and removes the culture medium to the chambers and the electrical connections are indicated schematically.[289]

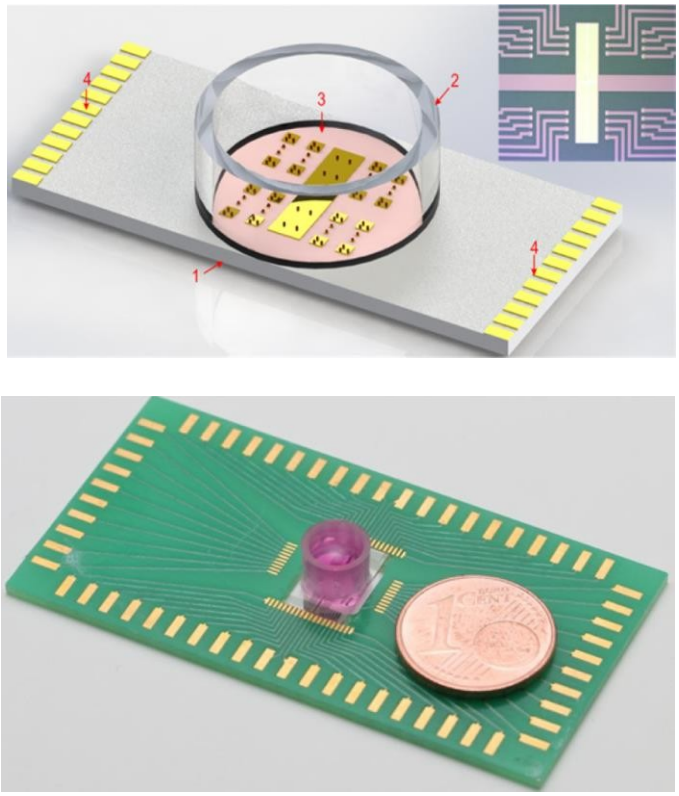

Figure 7. Pictorial representation of micrograph setup. (a) Illustration of the MEAs micrograph along with its main four parts: (4) Bonding pads; (3) MEAs (1) Pyrexsubstrate; (2) Small well. (b) Image of a chip with cell culture medium inside the well.[290]

2.2. Microfluidics in Cancer Studies

In this section, we discuss the main microfluidic used for cellular/organ monitoring. Among various life science applications, we focus on cancer cell studies.

2.2.1. Lab on a chip (LOC)

Studying cancer cell migration is also one of the most modern microfluidic devices. Withinthe last couple of years, there have been various attempts in using 3D cell culturing and microfluidics to study the migration of cancer cells and tumor invasion. In the last 40 years,

the United States has invested more than 200 billion dollars solely on cancer research studies, resulting in a 5% decrease in the total death rate. This slow number is the main reason for the insufficient understanding of cancer cell migration and invasion mechanisms [13]. Finding a high throughput cell culture assay to illustrate cell migration, specifically cancer cell migration, is a popular study field. Within the last couple of years, many papers have tried to replicate a 3D microenvironment that can help cancer cells grow and form the proper communication between themselves. One of the new solutions in tissue microengineering is micro bioanalytical Lab-on-a-chip (LOC) systems for cell culture. The microsystems allow obtaining conditions that simulate the flow of physiologicalfluids in the body [57].

Over the last years, there have been efforts to substitute animal testing with clinical trials on-chip. One of the new solutions in tissue microengineering is micro bioanalytical Lab-on-a-chip (LOC) systems for cell culture. Lab-on-a-chip biosensors are known to have a fast and sensitive detection system that can be used to detect pathogens in packaging/processing facilities, farms, and delivery/distribution systems at a commercial level [58]. Lab-on-a-Chip (LOC) based devices can perform multiple laboratory functions on only a single chip in size of a few square millimetres [59]. This platform provides miniaturized, automated and integrated chemical and biological analysis [60]. Generally speaking, LOC is used to integrate several analyses done in a laboratory, such as DNA sequencing [61], measuring disease biomarkers in blood [62] and biochemical detection into one single chip. LOC is used to quantify parameters with your body by using fluidics, cells, and tissues as the input [144]. On the other hand, an organ-on-a-chip is a microsystem used to mimic the humanbody environment by replicating the functionality of the organ [65].

2.2.2. Organ on a chip (OOC)

An organ-on-a-chip is a microfluidic cell culture device created by microchip manufacturing methods [19]. OOAC is a biomimetic system that can mimic the environment of an organ, with the ability to regulate critical parameters, including concentration gradients, shear force, and tissue–organ interactions [76]. OOAC also enable high-resolution, real-time imaging and in vitro analysis of biochemical, genetic, and metabolic activities of living cells in a functional tissue and organ context [78]. This technology has great potential to expand our knowledge in tissue development, organ physiology, disease etiology and drug development [19]. Successful examples

of OOC are stem cell-based brain on chips [77], liver on chips [78], vasculature on chips [79], bone-Marrow on chips [80], heart on chips [81], BBB on chips [82], tumor on chips [83], skin-on chips [84] and finally lung on chips [85,86]. As illustrated in Fig. 8 an alveolar–capillary barrier was produced on PDMS membranes. This device is capable of mimicking respiratory motion through a vacuum; pictures (b-d) illustrated the process followed inhalation as the diaphragm contracts and picture (e) represents an actual image of the device. In OOC technology, organoids can be replaced by research studies culturing many cells to a much smaller cell count.

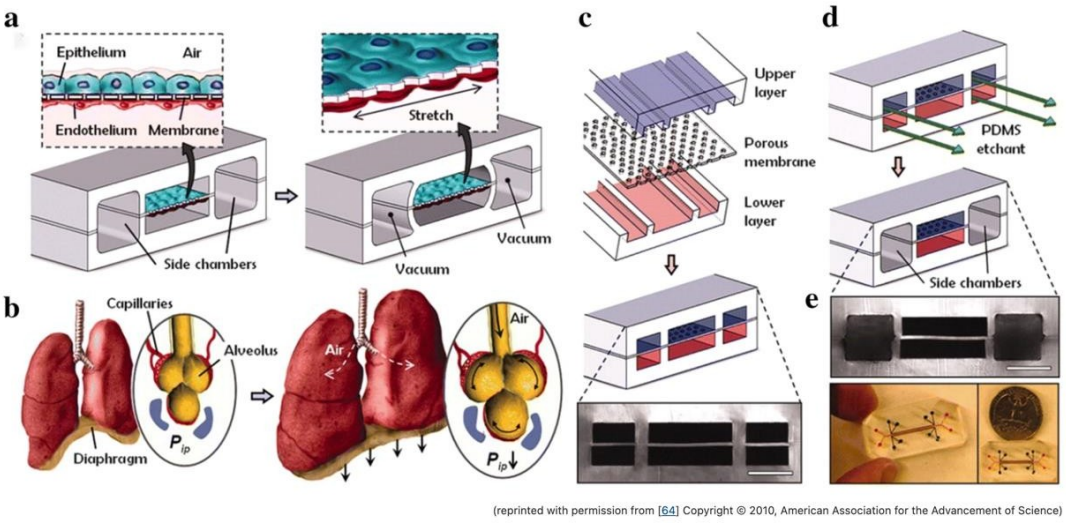

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Figure 8. Pictographic representation of PDMS based alveolar–capillary barrier microchannel device (Lung on a system). (a) An alveolar–capillary barrier was produced on PDMS membranes coated with ECM by using spaced PDMS microchannels. The represented device can reproduce respiratory motion through a vacuum; Which leads into alveolar–capillary barrier formation and mechanical stretching; (b) Following inhalation, the diaphragm contracts, resulting in reducing the pleura pressure; Resulting in stretching of the alveolar–capillary interface due to alveoli tension; (c) Illustration of device development: first a porous membrane between the upper and lower channels bound irreversibly; (d) PDMS moved through the side of the channels and then removed after vacuum pressure. (e) Actual images of the device.

2.3. Summary

This chapter has looked into various microfluidic biosensors used instead of microscopic imaging for more precise cell monitoring that can be advantageous in cancer research studies. Some of these biosensors are EIS, ECIS, ISFET, CMOS and LAP. Each of these sensors is

beneficial for the detection of cell activity like proliferation, migration and apoptosis. Electrolyte insulator semiconductors (EIS) can monitor cells using charge coupling to record electrical biomolecular activities [52]. ECIS (Electric Cell-substrate Impedance Sensing) can monitor a group of cells by providing kinetic information about cell migration and invasion process in the 3D extracellular matrix. Complementary Metal Oxide Semiconductor (CMOS) can monitor cells by its rows of photodiodes coupled with individual amplifiers to amplify the electric signal from the photodiodes. A light-addressable potentiometric sensor (LAPS) monitors the response of cells to chemical substances and monitored by acidifying living cells [63] and changes in the concentration of inorganic ions [64]. Ion-Selective Field-Effect Transistor (ISFET) can monitor cell activity by measuring ion concentrations in solution, such as changes in H+, that results in current changes through the transistor [58].

Furthermore, two forms of microfluidics in cancer studies named Lab-on-a-Chip (LOC) and Organ-on-a-chip (OOC) were mentioned. Lab-on-a-chip biosensors are known to have a fast and sensitive detection system on only a single chip in size of a few square millimetres [59] that can be used to detect pathogens in miniaturized and automated approach [60]. An organ-on-a-chip is another form of microfluidic cell culture device that can mimic the environment of an organ, with the ability to regulate critical parameters, including concentration gradients, shear force, and tissue–organ interactions [76], which results in high-resolution, real-time imaging and in vitro analysis of biochemical activities of living cells in an organ [78]. Both of these microfluidic biosensor platforms can assist researchers in anti-cancer drug development. There have been various attempts in using 3D cell culturing and microfluidics to study the migration of cancer cells; however, as mentioned previously, 3D cell culturing would not present an accurate cell microenvironment which explains the poor outcome of the research studies in this field, resulting in a 5% decrease in the total death rate caused by cancer. This poor outcome highlights the urgency in finding more high throughput sensing techniques.

III. Applications of Microfluidics in Cancer studies

This chapter discusses various examples of microfluidics used instead of conventional cell culturing for cancer cell monitoring deliberately in breast, brain, and lung cancer.

3.1. Breast Cancer

Breast cancer is defined as a cancerous disease in which cells in the breast grow out of control. There are different kinds of breast cancer, and it can begin in different parts of the breast. The kind of breast cancer is dependent on which area in the breast cells turn into cancer. Breast cancer can spread outside the breast through blood vessels and lymph vessels. When breast cancer spreads to other parts of the body, it is said to have metastasized. As illustrated in Fig. 9 breast is made up of three main parts: lobules, ducts, and connective tissue and usually, most breast cancers begin in the ducts or lobules. The most common kinds of breast cancer are invasive ductal carcinoma and invasive lobular carcinoma. [87]

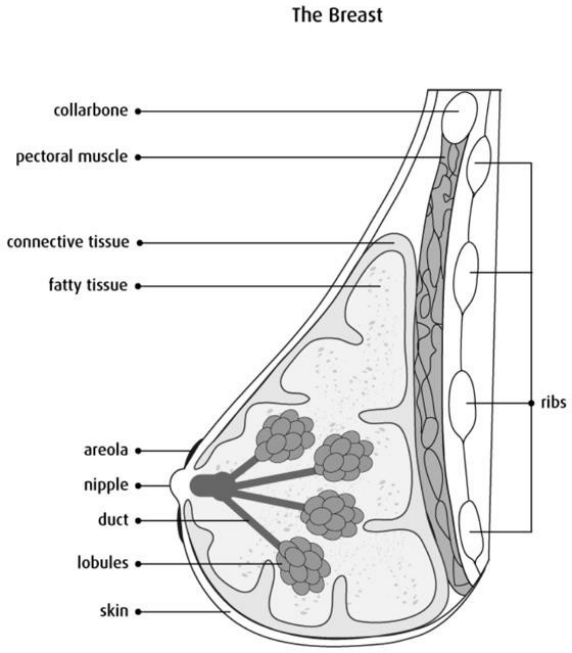


Figure 9. Illustration of the breast physiology. This picture gives a visual representation of possible at-risk areas of the breast that cancer tumor can get formed at. There are physical

symptoms that can help in early detection of breast cancer such as changes over the breast skin, change of colour in nipples and overall appearance of breast.

Microfluidic technology has become a powerful tool for cell-based research due to its advantage in controlled alteration of the cell-cell microenvironment by providing continuous perfusion or creating chemical gradients. Furthermore, microfluidics allows us to study low numbers of cells or single cells in high resolution and real-time. At the same time, microfluidics offers efficient high throughput experimentation with reduced consumption of reagents and contamination risk. [89] In the following section, we will be discussing some of the challenges with the 2D cell culturing system that been nearly solved with the help of microfluidics.

3.1.1. Production of microscale environment

Controlling the parameters related to the tumor microenvironment can significantly assist scientists in observing cellular responses and interactions in real-time. Essentially, controlling the microenvironment of a cancer cell can be useful for rapid screening of cancer drug therapeutics and studies in cell-cell/cell-drug carrier interaction. Tissue-engineered tumor models can act as a bridge to fill in the gap between in vitro cultures and animal models towards a better understanding of tumor biology. Villasante et al. developed a pre-vascularized cell sheets by co-culturing the NB cell line (MYCN+ cell line) and human umbilical vein endothelial cells (HUVECs), as a pre-clinical platform for anti-cancer drug screening illustrated in (Fig. 10a). Neuroblastoma is a vascularized pediatric tumor derived from neural crest stem cells that express markers, such as SOX2 and NANOG. Neuroblastoma (NB), that arises from adrenal medulla is a heterogeneous vascularized tumor from undifferentiated neural crest cells. NB is the most common solid tumor frequently diagnosed in first years of life. SOX2 and NANOG are embryonic stem cell regulators that maintain the stem-like phenotype in cancer cells. In addition, high levels of both genes would result in drug resistance and reformation of tumors. Furthermore, with the usage of microfluidic system they also studied the effect of Isotretinoin (INN) on tumor vasculature and stem-like cells.

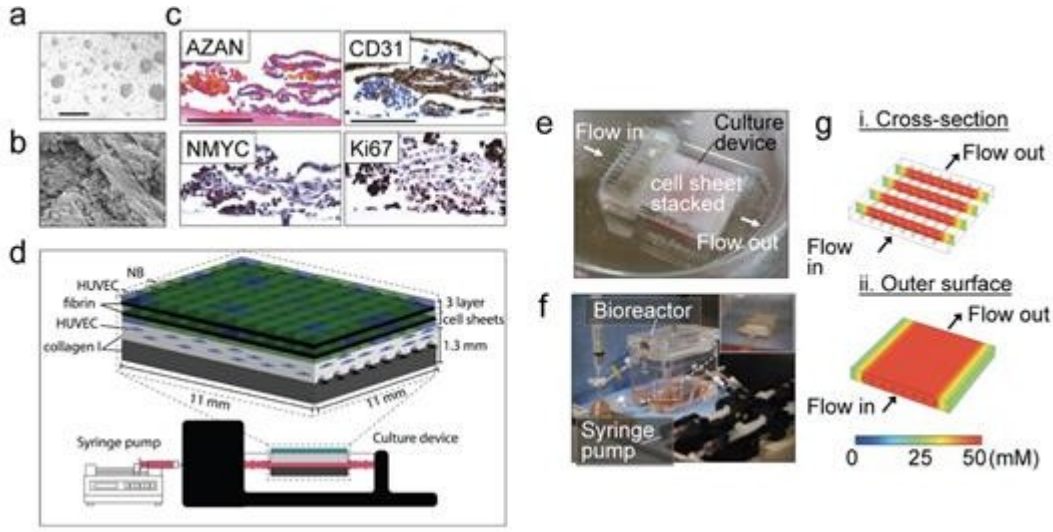
It is believed that high doses of INN could induce cell differentiation, cell growth arrest, and inhibition of angiogenesis in vitro. Angiogenesis has a role in the regulation of neuroblastoma growth. With the usage of tissue engineered model they were able to highlight the

role of SOX2 as a potential therapeutic target in neuroblastoma, based on its role in developing resistance to isotretinoin treatment. Investigating tumor microenvironment by using microfluidics has given scientists the ability to study various biochemicals that are involved in tumor progression. Two important biochemical and biomechanical cues in tumor microenvironment that play essential roles in tumor progression are tumor-associated macrophages (TAMs) and interstitial flow (IF). Moreover, macrophages had been reported to enhance both speed and persistence of cancer cell migration. However, their combined effects on tumor cell migration remains largely unknown.

In order to study the crosstalk between macrophages and endothelial cells and the combined effects of IF and macrophages in tumor microenvironment Song et al., developed a microfluidic-based 3D breast cancer model. This model was composed by co-culturing tumor aggregates, macrophages, monocytes and endothelial cells in the presence of IF, generated across the central microchannels, through hydrostatic pressure gradient. To investigate the abilities of different phenotypes of breast cancer cells differentiate U937 monocytes cells into TAMs, U937 cells were co-cultured with normal breast cells MCF10A, two typical epithelial-like cancer cells (MCF7 and T47D) and two mesenchymal-like breast cancer cells (MDA-MB-231 and BT549) separately in the adjacent microchannels for two days [191].When co-culture of U937 with human umbilical vein endothelial cells (HUVECs) or MDA-MB-231 cells and tri-culture of U937 with HUVECs and MDA-MB-231 cells were looked into, they found that mesenchymal-like MDA-MB-231 aggregates activated the monocytes to TAM-like phenotype macrophages. Results also demonstrated that MDA-MB-231 cells could directly activate U937 monocytes towards TAM-like phenotype macrophages. Moreover, MDA-MB-231 and interstitial fluidic (IF) promote the vascular sprouting by vascular endothelial growth factor (VEGFα) signal and tumor cell invasion of breast cancer cells.

This study has a leading insight, revealing the importance of macrophage-tumor communication's physiological and pathological mechanisms [90,191]. Moreover, the established platform can present a more mimetic 3D breast cancer model with potentiality for drug screening. Furthermore, as seen in (Fig. 10b) Breast cancer cells were observed that by slightly controlling the microenvironment of cells in a real-time manner, both U937 co-culture with human umbilical vein endothelial cells (HUVECs) and co-culture of MDA-MB-231 breast cancer cell line with U937 and HUVECs (See Fig. 10c). Additionally, schematic illustration of

engineered tissue model, (e) representation of culture device. (f) Tissue- engineered tumor model. (g) Computer simulation of concentration profiles is presented in (Figs. 10d-10e.)



As mentioned previously, microenvironment of cell provides numerous information about cancer cell invasion. Another research group has designed a network of vessels cultured with endothelial cells to assess vascular components and to better understand the complex tumor microenvironment. Tang and colleagues utilized a new 3D biomimetic microfluidic tumor microenvironment (BMTM). This novel (BMTM) platform was a co-culture of human breast cancer cells and human breast tumor associated endothelial cells (HBTAEC). (See Fig. 11a) composed of endothelial cells and the tumor. BMTM, BMTM with (HBTAE) and BMTM with MDA-MB-231 is represented in (See Figs.11b to 11d) respectfully. Furthermore, as illustrated by confocal imaging (See Figure 11f to 11i), represents HBTAE cultured in vascular compartment under flow. This platform consisted of a vascular compartment combined with a network of vessels cultured by endothelial cells, which assisted in a total lumen under shear flow in communication with tumor cells. This feature can essentially mimic the link between solid tumor cells and their microenvironment. According to fluorescence microscopy results, endothelial cells permeability significantly increases in the presence of tumor cell-conditioned media (TCM) or metastatic breast cancer tumor cells. This new platform can be used for high throughput, and rapid screening of cell-cell interactions and cell-drug interaction studies [92].

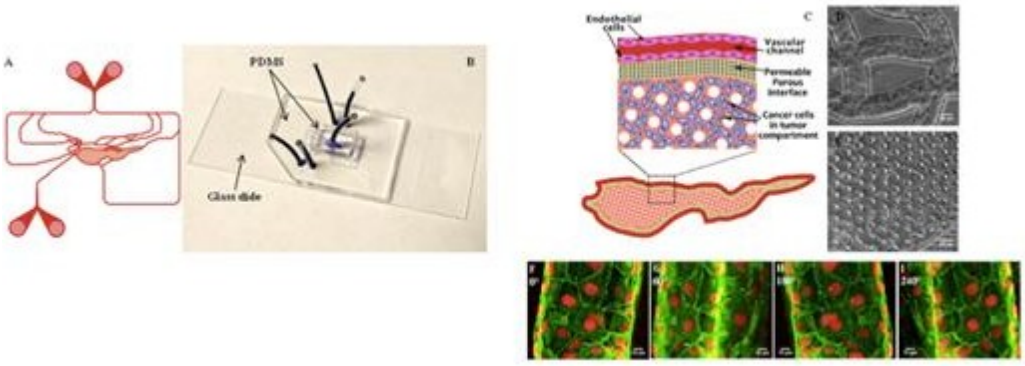


Figure 11. Pictorial image of (BMTM) in different microenvironment under confocal microscopy. PDMS based alveolar–capillary barrier microchannel device. (a) Schematic representation of Biomimetic Microfluidic Tumor Microenvironment (BMTM) with an illustration of vascular compartment, vascular-tumor compartment interface and tumor compartment. (b) Representation of BMTM. (c) Representation of BMTM with Human Breast Tumor Associated Endothelial cells (HBTAE). (d) Representation of BMTM with MDA-MB-231 cells in tumor compartment. (f-i) HBTAE cultured in vascular compartment under flow, forming a complete lumen as illustrated by confocal imaging. HBTAE cultured in BMTM and stained with f-actin (green) and Draq (red) stain. Cell culture was maintained for 4 days under flow of 0. [92]

Toh et al. have developed a microfluidic-based-culture chip that stimulated cancer cell migration and its ability to invade across the basement membrane. This microfluidic chip engineered in a 3D microenvironment was designed to elaborate the metastasis of breast cancer (MX1), across a 3D tumor model. In order to investigate the migration of these cancer cells, chemo-attractants were used to stimulate motility across the membrane. It was shown that their platform could monitor cell migration in real-time, and that can be used for anti-cancer drug screening [100].With high resolution multi-dimensional platform they compared the invasiveness of MX-1 cells in the microfluidic model in comparison to conventional Boyden chambers. The invading MX-1 cells in the microfluidic model exhibited both amoeboid-like motility, where the cells changed direction rapidly with amorphous cell morphology and mesenchymal-like motility, where membrane protrusions is formed at the leading edge of cells making them an elongated form. It is important to mention that the amoeboid mode of cancer cell motility is only observed in animal or 3D in vitro models, which is different from mesenchymal motility observed in 2D cell culture. According to results the majority of the metastatic MX-1 cells were highly motile when compared to non-metastatic breast cancer cell lines, such as MCF7. This microfluidic cancer migration model was able to clearly differentiate hallmark events during cancer intravasation such as reduced cell adhesions, increased cell motility, and disruption of the ECM. This microfluidic model can be used for screening anti-metastatic drugs that specifically can target a process of intravasation.

3.1.2. High sensitivity and high throughput screening

The ability to analyze small volumes like single-cell analysis and multiplexed analysis has given microfluidics its high-speed and high-throughput screening applications. Due to this reason, many studies have used microfluidics for cell culturing. Mohanty Et al. investigated the fabrication and usage of FET biosensors for early detection of breast cancer [93]. The primary assessment of classifying tumors in patients is by evaluating estrogen receptor (ER), progesterone receptor (PR), and Her2/neu. Moreover, it has been suggested that each patient's tumor is associated with a unique "fingerprint" [94]. Traditional medical treatment protocols cannot correctly distinguish these specific fingerprints and may not be effective for a particular patient [98,99]. Specific biomarkers presented in each fingerprint may have a prognostic value that can estimate the response to a specific treatment or survival to specific metastasized breast cancer [95,96,97]. Breast cancer biomarkers are important determinant of diagnosis, progression, and therapy decision-making. An example of these cancer biomarkers could be the CA15.3 epithelial marker highly overexpressed in breast, ovarian cancers compared with the normal cells. And the CA15.3 levels rise, is an indicator of progression. The fundamental advantage of this label-free device is its high detection sensitivity, diagnosis and prognosis of breast cancer disease.

Lu et al. proposed a two-channelPDMS microfluidic integrated CMOS-compatible silicon nanowire (SiNW) field-effect transistor arrays for label-free and highly sensitive electrical detection of cancer biomarkers. The integrated CMOS sensor showed high sensitivity of cytokeratin 19 fragments (CYFRA21-1), soluble (CK19) fragment, proven to be the most sensitive in the diagnosis and prognostic of NSCLC, and prostate- specific antigen (PSA), a glycoprotein, secreted by the prostate gland and used as a tumor marker for screening of early prostate cancer. This microfluidic device can be used in identifying clinical samples for early diagnosis of cancer was demonstrated by analyzing biomarkers in the SiNW-FET device opens great opportunities for a point-of-care test (POCT) for early diagnosis of cancer [291]. This study highlighted the importance of microfluidics in cancer cell diagnosis by proposing a two-channel PDMS microfluidic with label-free and ultrasensitive electrical detection of cancer biomarkers.

Correspondingly, Jia et al. used LAPS as a primary method to detect mammary adenocarcinoma cell (MDA-MB-231) cancer cells and their markers (See Fig. 12). According to the obtained results, their device had more capability to detect cancer cells than cancer biomarkers. LAPS has many benefits over the conventional methods due to its cheaper cost, high

throughput analysis and adaptability to the testing environment. [86] Traditional antibodies are costly and difficult to get however, preserve phage probe overcome these shortcomings, due to their high yield capacity and low cost. In this study the phage probe is used on the LAPS for labeling free and detection of cancer cell directly. This phage-LAPS differs from traditional antibodies or antigens probes on bio- LAPS and the phage-chips in terms of detection and surface modification. In previous cell-LAPS models or so-called cell-based sensors, cells are used to form the variations that can get detected by LAPS. However, in this study authors introduced the cell-detecting sensor that were able to effectively detect the protein human phosphatase of regenerating liver-3 (hPRL-3), and the mammary adenocarcinoma cell, MDAMB231. This study has highlighted the usage of phage-LAPS as high throughput, label-free and real-time platform for detection of cancer cells.

Stupin D.D used Fourier-EIS and AF-EIS for detecting the presence of HeLa cells on the electrode surface. Estimating the viability of HeLa cells shown that for the cell-substrates and the large electrodes, both EIS techniques can distinguish living and dead cells, however in the case of the single-cell standing on the small micro-scale electrode, only AF-EIS yields interpretable results [126]. Cell-based impedance spectroscopy (CBI) is a powerful tool that uses the principles of electrochemical impedance spectroscopy (EIS) by measuring changes in electrical impedance relative to a voltage applied to a cell layer. CBI provides a promising platform for detecting several cell properties, including the adhesion, motility, proliferation, viability, and metabolism of cell culture. CBI is rapidly becoming an established approach to non-destructively evaluate and perform the quality control of cell cultures with quantitative and sensitive data easily adapted for single-cell analysis. Since heterogeneity exists even within the smallest populations of cells, single-cell studies have been essential to provide more profound observation of the molecular machinery of individual cells in terms of biophysically, biochemically, and functionally aspects. The idea of EIS usage for studying single cells was recently reported [117].

Song et al. presented an ultrawideband (UWB) radar-based breast cancer detection system composed of complementary metal-oxide-semiconductor integrated circuits. This system includes Gaussian monocycle pulse (GMP) generation circuits, switching (SW) matrix circuits, equivalent-time sampling circuits, and a compact UWB antenna array. During the detection process, a GMP signal is generated and transmitted. The GMP signal is sent to the transmitter antenna by the SW matrix module, and the receiver antennas capture the reflected signal. During

the next step, which is the retrieval process, the equivalent-time sampling circuits retrieve the reflected GMP signal and then respond to the adhesion of MDA-MB-231 breast cancer cells. Finally, in the last step of the procedure, a breast image will be reconstructed by using a confocal algorithm, and a 1-cm cancer target in the breast phantom was detected. [86,292]

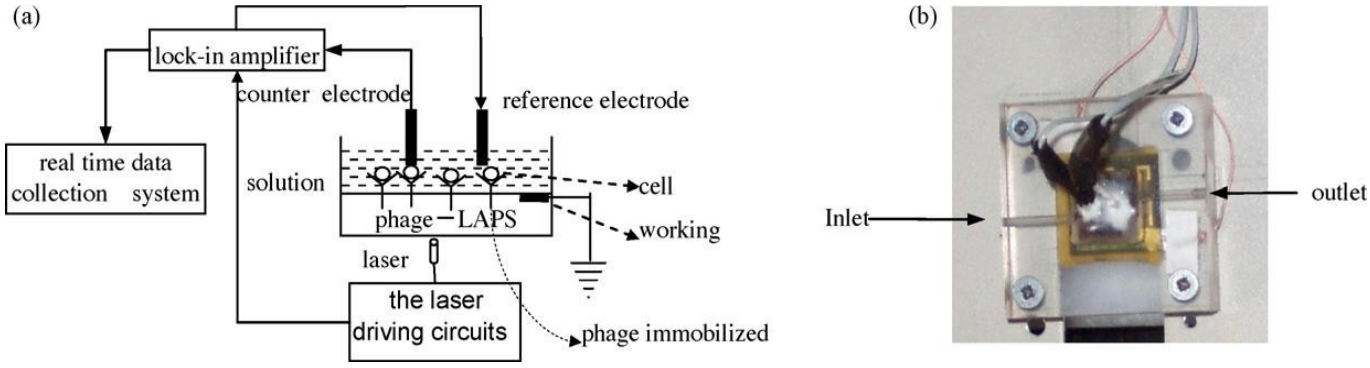


Figure 12. Diagram of LAPS biosensor with inlet and outlet. (a) Schematic illustration of LAPS testing system with real time data collection system. As shown in figure there are two sets of electrodes: counter and reference electrode. The back of the sensor is brightened by the infrared light source. The front side of LAPS is in contact with the solution, when the cell is captured on this surface, and as a result the effective gate voltage will be changed. (b) Representation of the phage-LAPS package with inlet and outlet.[86]

3.2. Brain Cancer

Brain cancer either can arise from brain cells, termed primary brain cancer, or arise from other parts of the body metastasize to the brain, termed metastatic or secondary brain tumors.[101] Stages of brain cancers indicate how aggressive the cancer is by demonstrating the extent of the spread of cancer.[102] Brain tumor interferes with brain functions such as muscle control, sensation, memory, and other normal body functions. As demonstrated in Fig. 13 the highest percentage of all brain tumours that originate in the central nervous system (CNS) are benign growths. However, in the cerebral and the frontal lobe, most malignant primary brain tumours develop.[103]

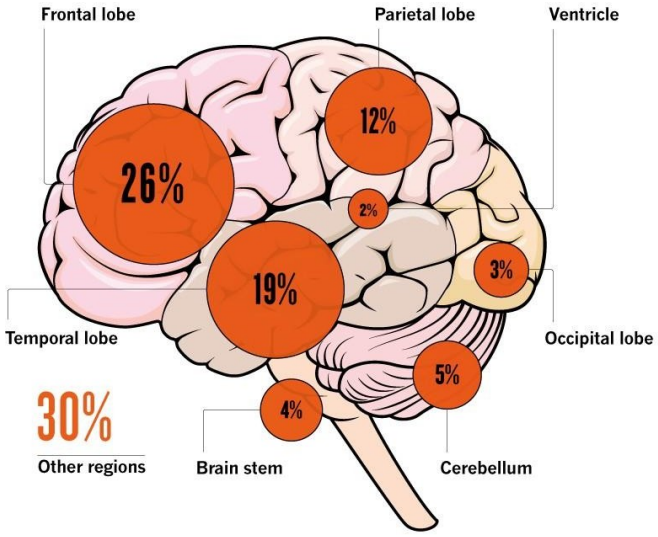


Figure 13. Breakdown of epidemiology of brain cancer and brain physiology. As represented, brain is composed of four lobes and the highest percentage of brain tumor occurs mostly in the frontal lobe.[104]

3.2.1. Stimulation of Blood Brain Barrier (BBB)

One of the most important fields of study that Organon chips can use is studying the function of the BBB. The purpose of the blood-brain barrier is to protect against circulating toxins or pathogens that could cause brain infections while at the same time allowing vital nutrients to reach the brain. Another protective element is the blood-brain barrier; this is a barrier between the brain's blood vessels and the cells and other components that make up brain tissue.[105] In brain cancer cells, studying anticancer drugs' effect depends on the passage of treatment from the blood-brain barrier as the blood-brain barrier plays a crucial protective role. One of the most important fields of study that Organon chips can use is studying the function of the Brain Blood Barrier (BBB). In the conventional method, BBB is studied in transwell, in which it cannot answer several complicated questions. Scientists hope to study this complicated mechanism through microfluidic devices in which physiologically relevant blood pressure, intracranial pressure, and flows can be applied [106].

Herland and colleagues designed a three-dimensional (3D) model of the human BBB within a microfluidic chip by creating a cylindrical collagen gel containing a central hollow lumen

inside a microchannel as illustrated in Fig. 14, culturing primary human brain microvascular endothelial, human brain microvascular endothelial cells (hBMVECs), pericytes and astrocytes cells on the gel's inner surface, and flowing medium through the lumen. The BBB is formed by the continuous brain microvascular endothelium, pericytes that tightly encircle the endothelium in underlying basement membrane, and astrocytes in the surrounding tissue. Together, these cells maintain a highly selective permeability barrier between the blood and the brain compartments. More importantly, the pericytes and astrocytes send cues that are required for normal function of the brain. Astrocytes also have been shown to be involved in innate immunity, and when activated, mediate both innate and adaptive immune responses. Pericytes have likewise been demonstrated to release pro-inflammatory cytokines.

This human 3D BBB-on-a-chip displayed barrier permeability comparable to in vitro BBB models created with non-human cells. In this study, they studied the neurovascular inflammation by measuring cytokine release by adding tumor necrosis factor-alpha (TNF-α) as an inflammatory stimulus and analyzing the presence of astrocytes and pericytes independently in response to inflammation. TNF-α is a pro-inflammatory cytokine involved in various inflammatory diseases of the central nervous system. Levels of granulocyte colony stimulating factor (G-CSF), interleukin-6 (IL-6), interleukin-8 (IL-8) responses detected in the 3D BBB chip were significantly more remarkable when the same cells were co-cultured. Moreover, according to gathered results, these responses play essential roles in neuroprotection and neuro-activation in vivo. Levels of these responses detected in the 3D BBB chip were significantly greater than when the same cells were co-cultured in static Transwell plates. Quantitative comparisons also showed that secretion levels of G-CSF, IL-6 and IL-8 were significantly higher in the microfluidic BBB chip compared to static Transwell cultures. These findings suggest that the 3D microfluidic BBB is suitable for studying the vascular component of neuroinflammation [109].

Ayuso et al. presented a new microfluidic model of GBM that mimics the dynamics of pseudopalisade formation by modelling in vivo nutrient and oxygen gradients during tumor formation. To do this, they used U-251 MG cells inside a collagen hydrogel in a custom-designed microfluidic device. By controlling the medium flow through lateral microchannels, they could mimic the environment associated with this disease. Using this new system, they showed that nutrient and oxygen starvation results in a migratory process leading to pseudopalisade generation in vitro [108]. As proposed by Wong et al., cell migration and proliferation levels can

predict patient-specific clinical outcomes. They were able to demonstrate this funding by using a microfluidic assay to quantify cell migration and proliferation. Furthermore, they were able to test the proliferative ability of primary glioblastoma cells by identifying the protein biomarker Ki-67 as cells were passing through microfluidic channels, mimicking the white-matter tracts in the brain [107].

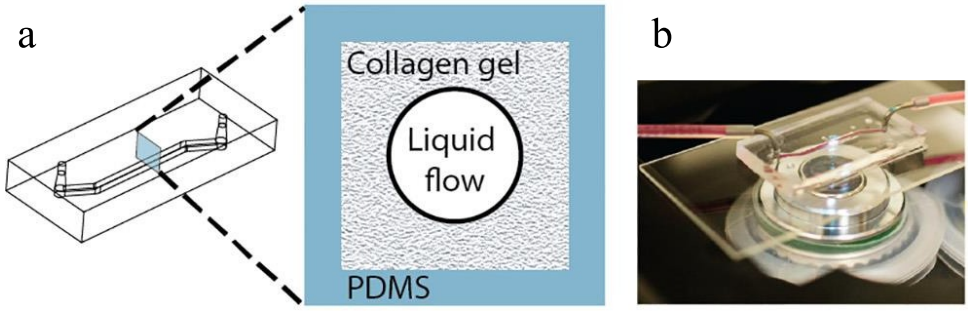


Figure 14. Pictographic representation of PDMS based 3D BBB chip. a) (Left) Schematic illustration of the PDMS structure used for 3D BBB chip generation. (Right) cross-sectional illustration of through the chip demonstrates the PDMS channel composed of the collagen gel made with a central lumen and viscous fingering. b) Image of the 3D BBB chip under an inverted microscope. [109]

3.3. Lung Cancer

Lungs are composed of two sponge-like organs. The right lung is composed of three sections, termed lobes, and the left lung is composed of two lobes. A thin covering protects the lungs called the pleura. As you breathe, air passes through the lungs through the trachea, and the trachea divides into tubes called bronchi. As bronchi enter the lungs, it divides into smaller branches called bronchioles.[4] When cancer starts in lung cells, it is called primary lung cancer [5]. As illustrated in the Fig.15, squamous carcinoma cell (SCC) is a form of non-small-cell lung carcinoma (NSCLC). NSCLC originates from the bronchi and is associated with tobacco smoking. About 80% to 85% of lung cancers are non-small cell lung cancer (NSCLC). This type of cancer has a slower growth rate and spreads to other parts of the body slower than small cell lung cancer. The main subtypes of NSCLC are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

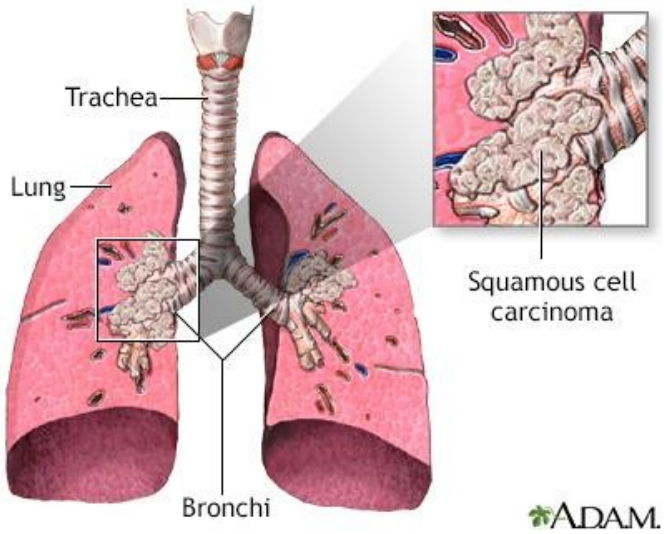


Figure 15. Illustration of squamous carcinoma cell formed in the lung. Squamous-cell carcinoma (SCC) of the lung is a form of non-small-cell lung carcinoma (NSCLC). NSCLC is one of the most common types of lung cancer after lung adenocarcinoma. NSCLC originates a form of non-small-cell lung carcinoma (NSCLC). NSCLC is one of the most common types of lung cancer after lung adenocarcinoma. NSCLC originates from the bronchi. Furthermore, squamous cell carcinoma is associated with tobacco smoking.[113]

3.3.1. Production of gradient

Microfluidic technology has been widely applied to form concentration gradient, stimulation of the tumor cell metastasis, and screening of the anti-tumour drug. In particular, the construction of a "tumor on a chip" based on a drug-concentration gradient generator has dramatically expanded worldwide and has been widely accepted by pharmaceutical companies as a tool for drug development. These technologies could rapidly form a drug gradient and precisely monitor the cell physiological process in real-time. To mimic the osmolarity gradient formed in real-time, Zou and colleagues integrated a novel electro-osmotic microfluidic system capable of applying controlled osmolarity gradients to lung cancer stem cell (LCSC) and differentiated LCSC (dLCSC) cancer cells in microchannels to study gradient-induced chemotaxis in real-time (Fig. 16). As demonstrated in Fig. 16, the constant flow perfusion was controlled by a digital syringe pump that kept the concentration in two main channels stable, such that different gradients in the five connecting parallel channels were generated. To compare the results fluorescence microscope combined with a digital camera and scanning electron microscopy (SEM) was used. This novel

platform can be used to study cellular microenvironments and cancer cell metastasis [114]. The microfluidic analysis showed that LCSC and dLCSC from the exact origin behaved differently with the same external stimuli, highlighting the importance of cancer cell heterogeneity. Furthermore, it was suggested that the β-catenin dependent Wnt signalling pathway regulates this different response. The ability to analyze single-cell chemotaxis under controlled spatial conditions can provide us with a novel analytical platform to study the cellular microenvironments and cancer cell metastasis[251].

Similarly, Li et al. developed a PDMS based microfluidic device to study electrotaxis under physiological electric field (EF) by generating different intensities of (EF) into a single channel to study the electrotactic behavior of cells. Studies has suggested that electrotactic migration of cancer cells play crucial role in directing the metastasis of various tumors, such as breast, prostate, and lung cancer. It was observed that lung adenocarcinomas, H1975, will go under cathodal migration as cells' orientation changes. For colony formation assay and transcriptional analysis of genes associated with migration, high and low electrotactic cells were collected separately. To explore the molecular mechanisms underlying the different electrotaxis responses, they examined transcriptional levels of different cell regulator such as regulators of actin dynamics (Rock1, RhoA), regulators of cell motility and survival (MEK1, MEK2, EKR1, ERK2), regulators of cell growth and metabolism (PI3K, PTEN), and migration-related receptor tyrosine kinase (EGFR). Furthermore, the trajectory of cell migration was tracked using cell center as the tracking point. The trajectory speed and migration speed along channel were calculated and reflected as cell motility and electrotaxis. By using microfluidics Herland and colleges were able to find new discoveries. For instance, they illustrated that H1975 cells' motility and electrotactic responses of MDA-MB-231 and A549 cell lines are directed by EGFR expression in the absence of EF stimulation. On the other hand, in the presence of EF cells' motility is controlled by the expression of PTEN. Additionally, they demonstrated that up-regulation of RhoA would be seen in high motility cells regardless of EF intensity [116]. Moreover, results suggested that endogenous electrical field could be considered as one of the regulatory factors for metastasis in lung cancer cells and by analyzing the morphology change of cells during EF stimulation, they demonstrated that the cytoskeleton and motility-related proteins will get rearranged in the cancer cells.

3.3.2. Ability to mimic cell migration

The ability of cells to migrate is fundamental to many physiological processes, such as tissue repair and regeneration, protective immunity, and embryogenesis. Cell migration is defined by cell adhesion to substrates, directional guidance cues, and extracellular signaling molecules. One of the most critical challenges that microfluidics have solved is its ability to stimulate cell migration. By using microfluidics and applying electric cell-substrate impedance sensing (ECIS) Jiang et al. used Human lung cancer cells, SKMES1 and A549, to investigate cell migration, in vitro invasion, and cell-matrix adhesion. Furthermore, in vivo growth of lung cancer was tested using a vivo tumor model. Recently, there have been early clinical reports that a Chinese medicinal formula, known as YangZheng XiaoJi (YZXJ), has an effect in increasing the survival rate in cancer patients. The herbal medicinal formula is contained of 16 ingredients. By using microfluidics, they pursued to investigate the effects of YangZheng XiaoJi on the migration, of lung cancer cells, in stimulation of HGF. In the major forms of solid tumour types, HGF and its receptor, cMET, are over-expressed. Hepatocyte growth factor (HGF) is a cytokine that in normal physiology, is involved in tissue regeneration. However, in cancerous physiology this cytokine acts as a powerful angiogenic factor. Jiang and colleagues illustrated that YangZheng XiaoJi is able to directly inhibit angiogenesis and migration of cancer cells. Consequently, Lung cancer cells increased their migration in response to HGF and reduced their migration speed and, essentially, the rate of tumor growth in response to YZXJ. It was further revealed that YZXJ significantly reduced the HGF receptor's phosphorylation in lung tumors in vitro and in vivo.

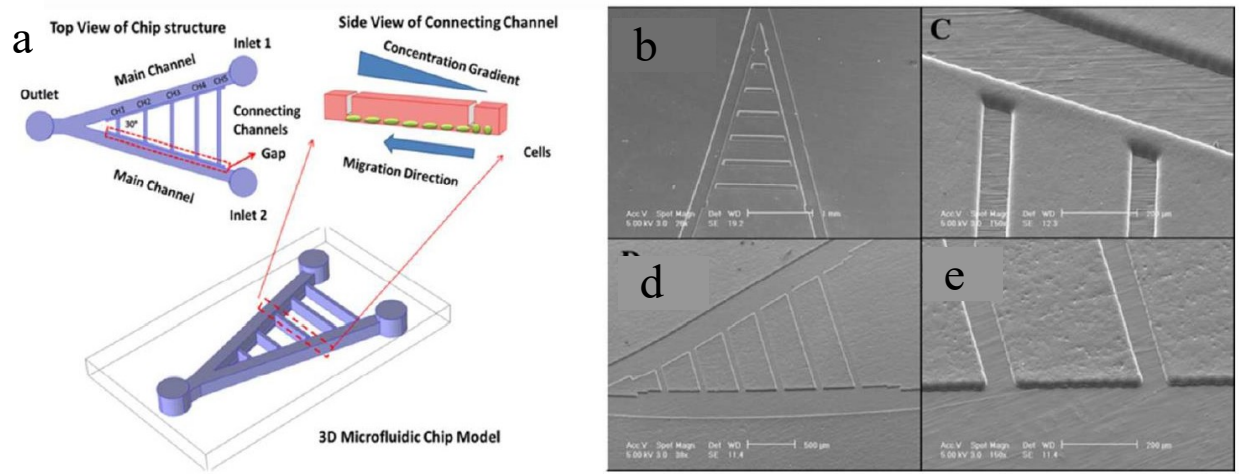


Figure 16. Visual representation of PDMS based microfluidic chip under SEM microscopy. a) The microfluidic chip consists of two main channels forming a V-shaped structure with five parallel connecting channels. Two gaps between the main channel and connecting channels enable the trapping of suspension cells at the entrance of the connecting channels, and cells can undergo migration after adhesion. Inlet 1 is for cell loading and medium perfusion, and inlet 2 is for perfusion. Cell chemotaxis is induced through continuous gradients that are generated in connecting channels. b-e). The SEM imaging of the PDMS layers of the microfluidic chip. That is composed of two PDMS replicas bonded face-to-face.[114]

Chen et al. studied the biological function of endogenous PIGF and migration of human non-small lung cancer (A549) cell lines by using the ECIS system (see Fig. 17) [121,122]. As illustrated in fig.17, for generation of multiple electric fields, current was induced to the chip through the agar salt bridges. Moreover, as described by authors suspension cells were trapped only at one side of the migration channels. Cells were migrated under EF stimulation and were moved through the gap and the parallel channels. Placenta growth factor (PIGF) is a member of the VEGF family, and due to their impact on angiogenesis, it is hypothesized that PIGF causes the aggregation of tumors, and this regulation is dependent on ROCK-1. PlGF activates the VEGF receptor, resulting in amplifying VEGF derived angiogenesis. PlGF protein level is significantly higher in many tumours, such as breast cancer, lung cancer. By using microfluidics, this study has highlighted the importance of PIGF as a potential therapeutic target in lung cancer [123].

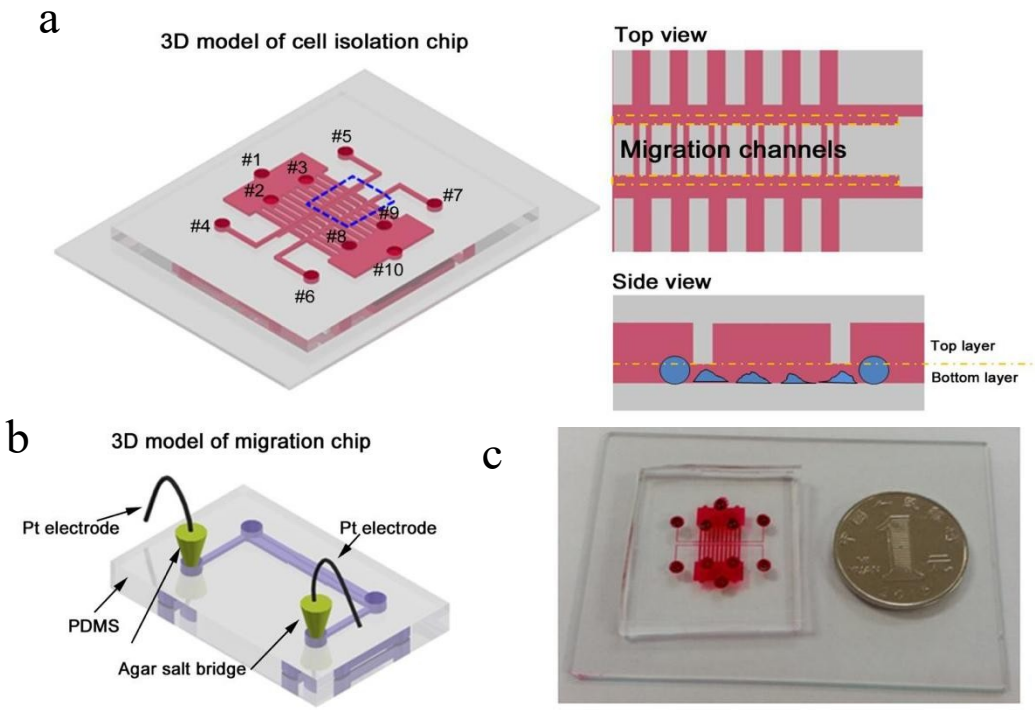


Figure 17. Pictographic demonstration of 3D based migration channels. (a) Schematic illustration of top view of the microfluidic chip. 3D cell isolation chip. The blue dotted area the chip represents, 20 parallel migration channels which were blocked by two gaps. As illustrated more closely, suspension cells were trapped into a line at one side of the migration channels. After the cells migrated under EF stimulation, the cells moved through the gap and the parallel channels moving downstream. (b) The current was induced to the chip through the agar salt bridges for multiple electric fields generation. (c) Image of the entire microfluidics system. [121]

In another study, the usage of a microfluidic device composed of collagen-Matrigel hydrogels was highlighted. This study looked into the migration of lung cancer cells, H1299 lung adenocarcinoma cancer cells, under different cancer invasion environments. Using a microfluidic device, they were able to characterize the morphology of hydrogels and obtain more data by using quantitative imagining analysis. This study investigated the plasticity of lung cancer cell migration as it turns from mesenchymal into lobopodian in collagen-Matrigel matrices. Subsequently, their data highlighted the role of Matrigel as a biphasic in which in low concentration, Matrigel facilities migration and low concentration slow down the migration. Lastly, they demonstrated the role of antibody-based integrin blockage in changing the migration phenotype from mesenchymal to amoeboid. In conclusion, they illustrated the usage of microfluidics in studying lung cancer migration under different microenvironments [115].

3.3.3. High diagnostic reliability

As mentioned previously, microfluidics is contained channels and chambers with dimensions of 1 mm or less. The fluid flow is laminar at these levels, allowing the control of transport and mixing of molecules. Microfluidics has solved the challenge regarding controlling the flow behaviour of small volumes of fluids. Precise manipulation of molecular interactions in microfluidics allows susceptible and rapid processing of samples, and usage of biosensors in microfluidics would enhance the monitoring scale. Tanaka and co-workers [262] performed chemiluminescent imaging of HeLa cells with the CMOS sensor, along with cell staining. Living and dead cells were successfully distinguished by white- and blue-coloured images using trypan blue staining of HeLa cells [262]. The aim was to develop a miniature cytometer for high throughput cell profiling.

Along with the same concept, Tran et al. highlighted the importance of microfluidics by investigating the interaction between cancer cells and stromal cells. Their research focused on determining cell aggregation progress by using an on-chip co-culture model for human lung carcinoma cells (A549) and human lung epithelial cells (MRC-5) in both standard and treatment conditions by highlighting the usage of electric ECIS. They used a co-culture device consisting of two fluidic chambers in parallel that each was separated by a 100 μm fence for cell patterning. Microelectrode arrays were installed at various distances separated from the confrontation line to measure electrochemical impedimetric sensing assessment of cell-cell influence. They were evaluating the impedance signal responses that represented cell condition and behaviour. Using the ECIS sensing method, the impact of specific distances that lead to different influences of fibroblast cells on cancer cells was defined. Moreover, they removed the fence that allowed cell-to-cell interaction to occur, and impedance signal responses were evaluated [119]. As seen in Fig.18, microelectrode array is composed of two sections the working electrode, and the common counter electrode, both fabricated on glass slides by the photolithography process. As described in in the paper, the sensing platform was separated into two areas, one for the microenvironment agents and one for cancer cells. One specific aspect of this device is the usage of its hydrodynamic narrowing to trap single cells to obtain the impedance spectrum by using EIS and discrete impedance data points as the cells pass through IFC [119,120].

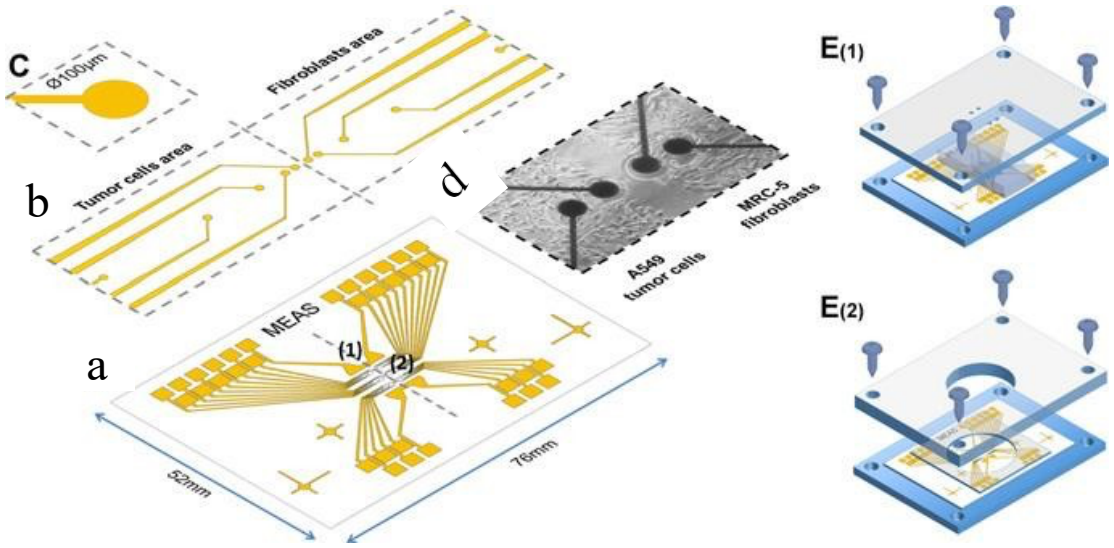


Figure 18. Graphic illustration of microelectrode array in dual chamber PDMS based microfluidic system. (a) Microelectrode arrays composed of two sections, 1: working electrode, 2: common counter electrode, fabricated on glass slides by the photolithography process. (b) The sensing platform was separated into two areas, one for the microenvironment agents and one for cancer cells. (c) Representation of a single electrode in 100µm diameter. (d) An image of the chip after the co-culturing of two different cell types on both sides. Illustration of co-culture patterning process by using a dual-chamber mold. (E1) At first, the cell chip was placed on the fixture, and then the dual-chamber mold was fixed at its location. (E2) After attachment of the cells to the chip surface, the dual-chamber mold was replaced by a well-type open reservoir and a PDMS bed to prevent leaking of solution. [119]

Feng et al. [118] have also developed a microfluidic system, a combination of electric impedance flow cytometry (IFC) and EIS, to differentiate three different cancer cell lines, including HeLa, A549, HepG2, and LAC (human lung adenocarcinoma). They tested the performance of IFC and EIS individually and in the combination setup, which showed the system with the combination of IFC and EIS had higher efficiency than each technique separately. The authors showed the ability of IFC to classify HeLa, A549, and HepG2 compared to microbeads [118]. Prendecka et al. aimed to show the usage of (ECIS) technique in studying cancer metastasis and its usage in detecting and quantifying recording morphology changes in the subnanometer to micrometre range. Intensive research to explain the relationship between electrical changes in cells or on their surface and cells survival. As mentioned previously, the electric parameters measured by the ECIS system are impedance, resistance and capacitance, which can be used to examine cell transformation, migration and invasion and cell proliferation. In order to attain two-dimensional (2D) imaging of mammalian cells by using a CMOS sensor at zero distance between the cell and

sensor surface. Stern and co-workers [233] fabricated silicon nanowires with a CMOS-compatible technology and performed label-free immunodetection of mouse immunoglobulins G and A, as well as real-time monitoring of cellular immune response. Cellular immune response was analyzed through T-lymphocyte activation. CMOS can be used as an intelligent and label-free cellular analysis for high throughput cell-based drug testing [152]. This paper has looked into tracking the response of Hek293 and H1299 lung cancer cell lines to Geneticin selective antibodies using CMOS capacitive sensor Array. Their main objective was to integrate a low complexity post-CMOS fabrication procedure for microfluidic packaging and CMOS biocompatibility purposes[152].

3.4. Summary

This chapter looked into various advantages of microfluidics, specifically in detecting various cancer cells such as breast, brain and lung cancer. This chapter critically analyzed and reviewed several research papers that have shed light on the usage of microfluidics as a tool for high sensitivity and high throughput screening, high diagnostics reliability, capable of mimicking cell migration and of BBB microenvironment. Table1 demonstrates a summary of all the microfluidic deceives and cancer cell types that have been mentioned in this chapter. As seen in this table, most reviewed papers have used expensive microscopic methods, while only some have used low-cost biosensors such as CMOS. Moreover, this table can suggest a couple of points. One is that usage of biosensors as a sensing method should increase due to the advantages promised by these sensors compared to conventional microscopic imaging.

Another point to mention is that a vast number of anti-cancer drug industries rely on research in this field. Furthermore, the microscopic imaging technique that research groups widely use has shown its disadvantages in terms of accuracy in test results compared to electrochemical biosensors. Therefore, we can suggest that more accurate sensing techniques should be used in cancer research studies for more advantageous outcomes

Table 1. Representation of detailed summary comparison of different biosensors advantages and their application in studying various cancer types.

Ref	Type of cancer	Microfluidic device	Detection method
191	BCC[1]	uF[2] 3D design	SEM uS[3]
92	BCC	3D biomimetic uF	Fluorescence uS
91	BCC	PDMS based microfluidic	CMOS BioS[4]
86	BCC	uF 3D design	LAPS BioS
109	hBMVEC[5]	PDMS based uF BBB chip	Inverted uS
90	HUVECs[6], BCC	uF 3D design	Immunofluorescence uS
251	dLCSC)	Electro-osmotic uF system	Digital camera and SEM uS
116	H1975	PDMS based uF	EIS BioS
121	A549	uF 3D design	ECIS BioS
115	H1299	uF composed of collagen-Matrigel hydrogels	SEM uS
262	HeLa cell	uF 3D design	CMOS BioS
119	A549[9]	uF3D design	ECIS BioS
118	HeLa, A549, and LAC	uF 3D design	EIS BioS
195	H1299[10]	uF design	CMOS BioS

[1]BCC: Breast Cancer cell, [2]uF:Mirofluidics, [3]uS:Microscopy,[4]BioS, [5]hBMVECs:Human brain microvascular endothelial cells, [6]HUVECs: human umbilical vein endothelial cells,[7]dLCSC : Differentiated lung cancer stem cell, [8]LAC: human lung adenocarcinoma, [9]A549: Human lung carcinoma cells: [10]H1299: lung adenocarcinoma cancer cells

IV. Conclusion

The main goal of this thesis was to shed light on a different form of cancer cells along with new platforms that are currently used to provide an effective and efficient technique for the early detection of cancer. There have been numerous efforts in designing an automated cell culture system replicating the cells' natural microenvironment to improve throughput analysis with reduced process costs. The conventional method used to study cell's behaviour is in monolayer (2D) cell culture. Due to the importance of 3D cell culturing to resemble in vivo tissue and cellular interactions, research groups have employed microfluidic systems with this strategy to study tumor invasion cancer cells migration. These emerging microfluidic/sensing system technologies will play crucial roles in enhancing our understanding of cancer cell behaviours and accelerating the research activities to find the most suitable drugs for cancer treatment. As mentioned in the previous section, new platforms that use sensors for non-invasive monitoring are being substituted with conventional methods.

Due to easy accessibility, low cost, and small microfluidics, there is a potential that microfluidics completely replaces conventional screening assays in the future. Microdevices can assist in the detection, diagnosis, and mechanistic research of cancer cells, and they have presented a great stride in creating a system to study cancer cell growth, cell cycle, and cell apoptosis. The usage of microfluidics in this field of study has been significantly influential since microfluidics can provide nutrients and dissolved gasses and apply stimuli such as chemical gradients, spatial homogeneity, and time-dependent biochemical stimulations, and substrate mechanical properties. Microfluidics can enable us to provide personalized medicine that can establish a much better response to cancer therapy. Modern medicine's central idea is to provide a patient-centred response that requires more sophisticated measurements and is distinct from the conventional model of "one-size-fits-all." The papers mentioned in this review can identify the importance of a high throughput device that focuses on non-destructive and label-free platforms.

Other novel strategies such as organ-on chips have also been helpful in cancer cell studies to substitute animal testing. The core technological goal is to develop high-throughput platforms consisted of microfluidic devices and measurement sensing instruments for studying cancer cells

and quantitively evaluating their behaviour. According to all of the listed devices, there is

still insufficient data to reproduce the optimal microenvironment for cells to grow and migrate. It is understood that even with the most advanced 3D organ-on-chips devices, cells do not react to drugs the same way as they do in the human body.

From the collective knowledge of the listed papers, we can conclude that there should be a change of paradigm, and we should start looking for other sensing devices; For instance, as a starter, we could substitute detecting method from microscopes to biosensors which can detect early stages of behavioural changes in cancer cells. This platform has evolved within the last couple of years; however, there are still questions that need to be answered for cell migration; as mentioned previously, there are still gaps between goal sensors. In conclusion, it can be stated that there is still numerous information required to fully understand and reproduce cancer cell's migration in microfluidic systems for early detection of metastatic cells.

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