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Application of Micro-Fluidics in Biological World

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Abstract:
Micro-fluidics is new, evolving, multi-disciplinary field that works with handling of fluids at very small volumes (micro to nanoscale). Microfluidics has already been used in other fields of sciences and now progressing towards various domains of biological sciences to make fundamental changes. The uniqueness of using fluid at such small scale suggests uncountable benefits in research of biological sciences including, instantaneous processing of sample, assay's miniaturization and expeditious detection. This review discusses the micro-fluidic technology applications in few of the major research discipline of biological sciences including diagnostics, neurosciences, environment, forensics, pharmaceutical industry, toxicology etc. It also indicates some limitations that must be envisioned for its wide use in future.

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Introduction

Bio-microfluidics is an emerging technology that involves the use of biomimetic (biologically inspired designs) and biopolymers (biologically derived materials) to microfluidics devices.

Microfluidics involves the engineering and kinetics of the fluids restricted on the channels having very small scale dimensions (micrometer to nanometer) that aids in the investigation of biological systems ranging from molecules, cells, tissues to multicellular organism (Aryasomayajula et al,. 2017).

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The most important and crucial part of this technique is to uniquely confine the fluid on sub-micrometer and micrometer scale with low Reynold number (<1) which gives the fluid laminar flow without any chaos (turbulence flow).
The microchannel allow different fluids to flow in parallel direction and mix through the diffusion process at liquid layer's interface and allows prediction of transport molecules. The major property of fluids in microchannel that enables fluids to flow in parallel direction is its high Peclet number (ratio between advective and diffusive transport rate) (Khoshmanesh et al., 2017).

Microfluidic technology has many uses in biological research by allowing rapid processing and reducing several experimental techniques and provides strict control of the reagents. More benefits of this technology include:
a) Use of small volume of reagents (nano or microliters), which is of significance when reagents are expensive
b) Simple and easy methods of fabrication for microfluidic devices
c) A number of analytical functions can be implemented on a single device i.e. lab-on-a-chip
d) Integration of other systems with the microfluidic device can replace labour intensive steps into automation

These characteristics of microfluidics permit numerous distinctive functions for integrated and exceptionally compact platforms with application in microbiology, proteomics, genomics, medicine, drug delivery and many others.

Origin, Advancement and recent developments

In the last six decades, biological researchers have exploited, measured and also tried to mimic the biology with the use of microscale physics as well as chemistry (Duncombe, Tentori & Herr, 2015). The physical phenomenon of microfluidics was used by the researchers long ago before the 'microfluidics' term was coined (fig1). During that period, seminal microfluidic techniques were raising the resolution and speed of powerful analytical tools for biological component analysis. For instance, the flow cytometer and the coulter counter has transformed highly efficient single cell analysis (Lanier, 2014) and the acceleration in the Human Genome Project was strikingly because of the small-bore capillary tubes for DNA sequencing (Mathies, 2018). In 1990, some significant developments in fabrication, permitted the transition of pulled glass capillaries to the micro-fabricated micro-channels made up of polymer or glass depends upon the nature of the experiment.

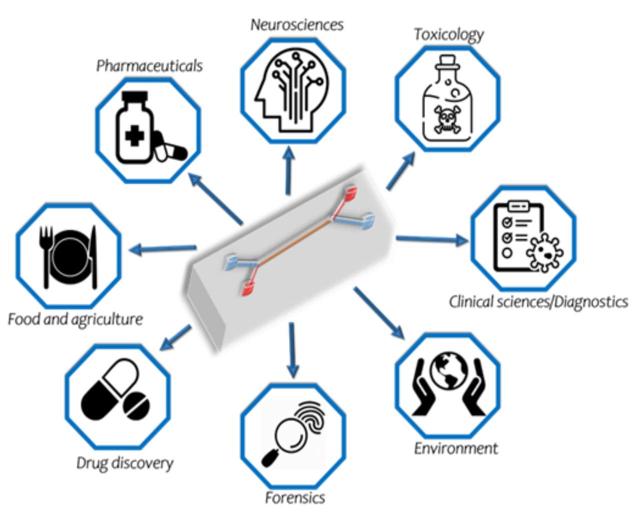

Figure 1: Major applications of Microfluidic-Chip device in Biological World

The biological research (to detect molecules and cells) was stirred by the introduction of micro-fabrication techniques using micro-scale tools that were intended for semi-conductor industry to detect/track electrons.

The maturation of nano- and micro-fabrication technology leads to the development of microfluidic tools also, which are explicitly aimed for biological investigations. As for example, a technique which was developed for the micro device prototyping (soft lithography) eradicates the necessity for protracted processes of fabrication in clean room facility for semiconductor, in this manner it creates the feasible and rapid devices in biological laboratories (Gale et al., 2018: Mukherjee et al., 2019).

The research in the microfluidics instigated as the chromatography technique develops. HPLC (High-Pressure Liquid Chromatography) and gas phased chromatography allowed the analytes detection with the use of very small volume of samples. Then in 1980s, the IBM Company applied the inkjet printers made up of micro-valves and micro-pumps to control the fluids of minute volume (dos Santos Rosa &Seabra, 2019). The unprecedented role was played in 1990s by the Department of Defense, United States in the improvement of this technology by developing a tool for the detection of biological and chemical threat based on microfluidic systems (Venkatesan, Jerald, Asokan & Prabakaran, 2020). The rapid evolution of molecular biology and genomics requiring higher efficiency analytical methods possessing greater sensitivity was the main impetus for greater than before development of microfluidics technology.

A classic microfluidic device usually comprises of a micro-channel, micro-valves, micro-pumps and some inlets for injecting reagents and fluids for employment of fluid inside the chip, a coupled- detection system (for analytical studies) and outlets for removal of fluids (Li, 2016). Initially, silicon was mostly used to fabricate the microfluidic devices, in the meantime the lithographic technology for patterned silicon wafers had been previously developed by the electronic industry. PolyMethylMethAcrylate (PMMA) and glass because of their transparency offers the integrated ability to Optical Detection System that's why they were used as alternative to silicon (Matellan & Armando, 2018; Su, Cook, Fang & Tentzeris, 2016). Although, the cumbersome step was to pattern the microstructures on PMMA and glass as they are brittle in nature. Not so long ago, PolyDiMethylSiloxane (PDMS) which is a polymer has materialized as a substrate of an option for the application in various fields including biological samples owing to its several beneficial characteristics like; stability under storage conditions, elastic property, low cost, low toxicity, optical transparency and fast processing (Teo et al., 2016; Zhu, Handschuh-Wang & Zhou, 2017). Other major advantage of PDMS based devices is that it can be made in ordinary laboratory conditions as contrasted with clean room conditions whose access and maintenance is expensive (de Camargo et al., 2016).

From cells to clinics, Micro-fluidics application in biological and biomedical analysis

The technique of microfluidics has been widely implemented in cellular biology (microbial cell to human cell). In the field of microbiology, the extensively researched area is the prevention of biofilm formation, in which bacteria attaches to the surface and colonizes over it. The bacteria forms biofilm as a protective shield against the antimicrobial agent or immune system of the host. Bacterial biofilms on the implant devices and prosthetics causes severe chronic infections (Koo et al., 2017). For this, to monitor and control the environment of biofilms elicited by Candida albicans (causes candidiasis), a microfluidic device was developed. This microfluidic device had sensors (dielectric) intended to detect the real-time effect of Candida albicans biofilms when subjected to different antimicrobial concentrations and shear stresses. The results from the experiment with microfluidic device suggested that there were significant changes in the patterns of biofilm when shear stress was increased while the behavior of the dynamic biofilm changes as the Amphotericin B (antibiotic) was added (Gulati et al., 2017).

The systems in Flow cytometer has been reduced through the use of micro-fabrication and micro fluidic technique. Flow cytometric tool has been applied in various cell biological and microbiological studies to identify the individual cell in a mixed cellular sample based on various parameters, including granularity, fluorescent markers and cellular size. Flow cytometer uses three systems, fluidics, electronics and optics to obtain information regarding quantitative as well as qualitative properties of individual cell in a population (Adan et al., 2017). In comparison with conventional techniques for single cell analysis for example, micromanipulation on agar and FACS (Fluorescent Activated Cell Sorting), microfluidics offers real time analysis for monitoring single cell (Liu et al., 2019).

Another microfluidic device was utilized in the analysis of aging process of individual yeast cells by examining the real time gene expression levels of hsp104 and ras2. In yeast, hsp104 is involved in the heat-shock induced extension in life while ras2 also extends the life span when it is over-expressed and reduced lifespan when under-expressed. For this study, yeast cells were 'trapped' in a device in a specific manner that single cell expression can be monitored easily with fluorescent microscopy when Yellow Fluorescent Protein was attached with ras2 and Green Fluorescent Protein with hsp104. The expression of these two genes were constantly observed for many hours and through this, researchers were able to explain inter- cell variation in gene expression. At the present time, microfluidics technology has also been implemented extensively in explaining other arenas of cellular biology such as, separation of non-motile and motile sperms, cell adhesion properties and cytoskeletal elements (Mahesh & Vaidya, 2017).

To analyze the physiological processes, the tool which have been utilized is in vitro cultures. However in vitro culture models remain unsuccessful for tissue-tissue interactions, mechanical and spatio- temporal gradient micro-environment (for instance shear stresses) of an organ (Bhatia & Ingber, 2014). In place of cell cultures, animal models are also not preferable for organ culture experiments on account of its expensiveness, time consuming, ethical and legal concerns. A microfluidic device 'Organ-on-Chips' was designed on microfluidic principles. It in reality mimics the normal biological processes of a living organism and applies processes that are prerequisite for the research experiment. The microfluidic devices uses polymers that are able to produce conditions of biomimetic systems and imitate the critical organ's function. To mimic the lung function on a chip, a breakthrough device was developed (Zhou et al., 2016). In this micro-device, to mimic alveolus, on thin PDMS membrane, pulmonary capillary endothelial cells were cultured one side and human alveolar epithelial cells on the other side.

The device contains hollow side chambers for providing multiple necessary compounds including, culture medium and vacuum pumps. Related organs-on-chip have also been developed for other organs studies for its function, including, human osteoblast, human heart, proximal tubule of kidney and gastrointestinal tract (Ashammakhi, Elkhammas & Hasan, 2019). These micro-devices might also be utilized in the visualization of responses of multiple cells at different time by high definition microscopic imaging.

The other main application of 'Organ-on-chip' device is in clinics for therapy development (Caballero et al., 2017). It successfully analyzes the toxic effects of chemicals, drugs and airborne particles and mimicked the inflammatory responses of human against different pathogens. Recent studies also shows the improvement of personalized medicine; with respect to drug dosages and its combination, side effects and its efficacy based on microfluidic technology (Ahmed et al., 2014).

Application/uses in various areas

Food and agriculture

Food is a hetero-genous matrix and requires devices with ultra-sensitivity and very low detection limit for contaminants, toxins, pathogens, antibiotics and others. The peculiar features including, large surface-to-volume ratios, capillary and surface tension effects at the micro-meter level and laminar flow in the microfluidic devices facilitates more competent strategies for complex samples analysis (Mastiani, 2019).

The largest threat to public health and financial constraint for food industries, society and individuals as well with cases approximately 76 million of illness because of food each year in United States. Particularly, Listeria monocytogenes, Salmonella Typhimurium and Escherichia coli O157:H7 are the three most pathogenic species among others and they are responsible for causing most of the foodborne illness outbreaks (Heredia & García, 2018). Because of their continuous persistence in food, dictated the constant need for less expensive, easier and faster analytical systems which is capable of detecting viable pathogen in samples of complex food.

Novel micro-fluidic devices were designed by using droplet method, for detecting the foodborne pathogens. For routine analysis of food safety, this technique can be used with no trouble. A digital PCR with integrated droplet microfluidic techniques was developed employing a chip of OSP (mineral Oil Saturated PDMS) for detecting Listeria monocytogenes and Escherichia coli O157:H7 (Bian et al., 2015).

In comparison with real-time quantitative PCR method, this novel OSP chip based on dual droplet and digital PCR method possesses more sensitivity up to the single molecule level resolution with devoid of any interference between other assays. One of the proposed applicability of this method has been assessed in the sample of drinking water which is artificially contaminated. Within 2 hours it detects both pathogenic bacterial species up till 10 CFU/mL (Bian et al., 2015).

μPAD (Paper-based Analytical Devices) has been created by combining colorimetric method with it, to analyze the ready-to-eat meat for three most pathogenic foodborne bacteria (Salmonella Typhimurium, E. coli O157:H7 and E. coli O157:H7). Like OSP chip based on dual droplet and digital PCR method, this μPAD device can also detect <10 CFU/ml bacteria with in less than 12 hours (Jokerst et al., 2012).

Furthermore, a device was made for rapid detection of bacterial cells such as E.coli in water. This device uses basic microfluidic cytometric technique (Duarte et al., 2019). This technique was combined with fluorescent staining, to count accurately the number of various bacterial cells in purified household drinking water and natural mineral water and it accurately measured in 15 mins. At the present time, a Lab-on-a-Chip combined with ELISA technique is a proven device with first-rate sensitivity and nearly real-time, for the detection of the foodborne pathogen. A research study conducted by Guan et al. in which they suggest the detection of E. coli O157:H7 by using bioluminescence assay via anti-E.coli. This study was able to detect very less amount of bacteria, 3.2101 CFU/mL within 20 mins (Tahira et al., 2017).

Moreover, for the detection of pathogenic bacterial cells in beef samples, a flow cell using microfluidic technique was built. It was embedded with gold interdigitated array containing integrated microelectrodes along with magnetic nanoparticle conjugated antibody (Jamshaid et al., 2016). Up till now, this is obstructive, label- free as well as novel biosensor for the measurement of bacterial cells impedance and it also don't use antibodies or redox probe on the electrode's surface. This biosensor when tested, was capable of detecting 1.2103 E. coli O157:H7 cells in around 35mins in beef samples.

GMOs, Genetically Modified Organisms are widely used in the production of food and as a result of it labelling regulations have been established by the European Union and some other countries too. Specific proteins of GMOs, RNA and DNA detection is required for the verification of correct labelling (Lievens & Petrillo 2017). Nowadays, Digital Polymerase Chain Reaction (PCR) along with microfluidics integrated is used far and wide for the detection of GMOs. For instance, a compacted PCR microfluidics system attached with continuous flow was designed to identify soybeans which are genetically modified (Xu, 2018). In recent times, Bio-Rad QX 200, a multiplex assays built upon instrument of Droplet Digital PCR have been developed. This system, Bio-Rad QX 200 for the first time shows the comprehensive evaluation of numerous factors (Dobnik et al., 2016). Researchers employed the two of the four-plex assays to quantify eight different targets in DNA, out of which one was maize endogen while other seven were maize events those were genetically modified. In the intervening time, a software was developed to rapidly analyze the four-plex results.

Environment

The devices based on microfluidic technique, possess channels for fluids and reduced analytical equipment are nowadays popping up as a portable devices that are perfect for on the spot sampling. The difficulties by using laboratory based analysis in particular with respect to quality change and prolonged time for preparation of samples before proceeding the experiment were somewhat resolved by the development of portable biological and electrochemical sensors especially for environmental pollutants samples. However, sensors of Lab-on-Chip have integrated several processes in a single system (Cleary, Maher & Diamond, 2013). There are several advantages offered by microfluidic systems of miniaturized features, such as on the spot characterization, demand of reagents, less requirement of samples (Abolhasani, Günther & Kumacheva, 2014).

This handheld micro-device can also have a supplementary section for pretreatment on the chip, some channels in which samples can flow and an advanced detector to be coupled with an analytical unit. Recently, researchers have used miniaturization of analytical units including, field-able and portable mass spectrometry and fluorescent detectors containing micro-machines with micro-lenses. These advanced micro-devices eliminates the requirement of fully equipped laboratory by enabling instantaneous detection (Snyder et al., 2016).

Use of Microfluidics for Environmental Remediation

During this time of growing economies, the hazardous by-products which are excessively discharged from activities of industries and because of this earth is in danger. Industrialization and urbanization that grows for the appeal of clean resources, the handling of capacious waste that is disposed in the atmosphere or in to oceans, becomes extremely challenging. The cost effectiveness, efficiency and effluent limit regulators are some of the limitations of traditional technologies for clean-up and hence new technology is in the request.

The interest in photocatalytic treatment for water has remain on top for last two decades in research for various disciplines of science (Cates, 2017). By using visible light, the organic pollutants are decomposed into non-toxic products, for instance, water and carbon dioxide are the process of photo-catalysis (Wang et al., 2014). A number of micro-fluidic reactors have been testified and utilized in treatment of water through photo-catalysis, particularly for the treatment of organic dyes in waste water (Yew et al., 2019).

Forensics

In forensic laboratory, the process for DNA extraction and its analysis takes about 2 to 3 days and that delay results in an unimportant and less effective contribution to the criminal investigation carried out by police officers (Mapes, Kloosterman & de Poot, 2015). An important application of microfluidics is the development of Solid Phase Extraction method (SPE). This device has silica the efficiently binds DNA to it (Reinholt & Baeumner, 2014). Nucleic acids (RNA & DNA) purification by Solid Phase Extraction method comprised of three steps, loading of DNA, washing and elution. In this method, very small amount of silica resins (nano-grams) are sufficient to desorb and adsorb nucleic acids in the range of pico-grams to nano-grams. Researchers also showed that acidic silica resins when fully hydrated, provides the finest solid phase for binding of nucleic acids and this process is time efficient also, that takes maximum 10 mins (Katevatis, 2016).

Another hurdle in forensic testing is the differential extraction on- chip for analysis of DNA. For the evidence of sexual assault, differential extraction is important, which separated female and male DNA fractions (Klein & Buoncristiani, 2017). Using micro-fluidics, researchers in 2006 published an article on DNA extraction and cell lysis of sperm cells on the chip. It was based on SPE containing micro-channels which were partly packed with mixture of beads or sol-gel. Because of this device, profiling of Short-Tandem Repeats (STR) was made possible (Bruijns et al., 2016). With the integration of acoustics to the chip for differential extraction a device was prepared. It take around 14 mins to purify high fractions of male and female's nucleic acids. By means of the ultra-sound, this advanced device could trap sperm cells particularly, from the sample containing epithelial cells of female (Clark et al., 2019).

Drug discovery

The research by using micro-fluidics for the drug discovery and its development has been accelerated and many tools has emerged in this domain by miniaturization of assays and high performance screening of potential drugs. The process of drug discovery involves selection of target, lead optimization and identification, and pre-clinical studies. The primary step is to identify the target that is a main point where drug molecule will act. The recent advances in micro-fluidic technology helps in the characterization of protein targets as well as quantification of single cells proteins. Researchers have devised a microfluidic chips which are proficient in separating proteins by means of Isoelectric Focusing, Capillary Zone Electrophoresis and Micellar Electro-kinetic Chromatography (MEKC) in not more than 20 mins (Fanali, D'Orazio & Fanali, 2020).

Protein crystallization is the most common hurdle to study the study the interaction between drug and target. In recent times, automatic screening systems using microfluidics have been designed which are very useful in crystallization of various proteins especially those of therapeutic importance for example, glucose isomerase, bovine liver catalase, thaumatin and other membranous proteins. Another recent article determines the interaction of warfarin (anti-coagulant drug) and Human Serum Albumin (HSA) with the help of droplet microfluidics. For this, magnetic beads containing distinct droplets of fluids was made and through magnetic bar the droplets were detached from solution that contains free drug. This allows researchers to calculate the affinity constant for the target-drug binding (Nys & Fillet, 2018).

Clinical sciences/diagnostics

Among a lot of other micro-fluidics applications, POC testing (Point Of Care) which is instantaneous diagnosis in-situ of patient care (Lee & Lee, 2013). For diagnostics, the use of paper based microfluidic devices have radically improved due to its multiple pros including, biocompatibility, widespread availability, cost effectiveness, and capable of transporting liquids through capillary forces without any hindrance. In these micro-fluidic devices, particular patterns are created on the paper by using hydrophobic materials (such as wax) or polymeric coatings. These patterns help to contain the fluid in it. The analyte's quantification is on the basis of electrochemical, chemiluminescent or colorimetric output (Manickam, Nelson & Bhansali, 2016). In the progressively developing market of diagnostic tool, paper based micro-fluidic device has made a great impact. There are wide range of clinical application of paper based microfluidic devices which are commercially used for detecting a number of pathogens (Escherichia coli, plasmodium) and biochemical analytes (troponin-C, Blood glucose, cholesterol). These Paper-based microfluidic devices that produce output on the basis of colorimeter are specifically useful in regions where there is lack of facilities for health care. By capturing colored images from cell phones possibly will play a vital role in disease diagnosis via tele-medicine (Wen et al., 2019).

Immunoassay systems integrated microfluidics have been established for detecting diverse analytes as, C-reactive protein, Escherichia coli, Bacillus globigii and Cholera toxin (Zhu et al., 2019). For immunoassays, fluorescence system is most commonly used for quantification on account of its quick response time, higher sensitivity and the device can easily couple with to fluorescence excitation units.

The PCR based microfluidic chips is all in one chip i.e. it accepts crude sample, target DNA amplification, DNA fragment separation through electrophoresis and identification of targeted DNA. The system nowadays used for detecting Mycobacterium tuberculosis, HIV, Salmonella Typhimurium, Bordetella pertussis, Bacillus anthracis and many more are based on the PCR-based microfluidic devices (Lee et al., 2018).

Toxicology

Almost all of the drugs have side-effects, some of them are severe as hepatotoxicity and nephrotoxicity. Even though the drug's mechanism of action have already been known but there is some other targets also in the human body. During last decade, the technique of microfluidics has shown great potential systems for toxicity and pharmacokinetic studies at the initial phases of the drug development (Zink, Chuah & Ying, 2020). There are two micro-fluidic systems have designed: Cell-on-a-Chip and Organ-on-a-Chip. Cell-on-a-Chip provides the enables to get the information about cytotoxicity which is beneficial in the course of drugs screening (mechanism of action and dose response) on the other hand, Organ-on-a-Chip offers information about toxicology and pharmacokinetics which helps in the final stage for drug development (human and animal trials).

The Cell-on-a-Chip device is comprised of a cell chamber or a cell culture area where monolayers of cells can be cultures and tested easily (Caviglia et al., 2015). The flexibility of microfluidics offers the progress in various complex experiments. For instance, the micro-channel when coupled with gradient marker possibly can act as the easiest system for micro-fluidic cell culture. By exposing cells to different drug concentrations it can offers rapid testing of drug cytotoxicity in a single experiment despite the fact that 24 well plates were utilized for the off-chip experiment reproducibility (Dai, Hamon & Jambovane, 2016).

Micro-fabrication, bio-materials and tissue engineering are combined together to develop a perfect model that imitates the human tissues and organs and it is termed as Organ-on-a-Chip. A number of Organ-on-a-Chip devices have then developed such as, lung, kidney, liver, intestine, central nervous system and bone marrow on a chip. These Organ-on-a-Chip devices can replicate at least three key features of in-vivo organs: 1) functional tissue-tissue interfaces, 2) multifaceted biochemical and mechanical microenvironments which is organ specific and 3) three dimensional microarchitecture (Aref et al., 2013; Bhatia & Ingber, 2014).

A research group presented a Kidney-on-a-Chip, for the analysis of nephrotoxicity caused by Gentamicin. This microfluidic based chip includes two channels which were superimposed over one another but disconnected via a membrane. On the upper layer of the channel, Madin–Darby canine kidney cells were cultured under functional shear-stress environment. This enables the researchers to impressionist the clearance of drug for human bolus injection and relate the differences in constant conventional concentrations and it also showed the low level exposure of cytotoxicity mechanisms over a long run. In this device, another substitute to cells are intact tissues (Kim et al., 2016). These micro-fluidic chips displays the physiological level biochemical components, cell–cell interactions and cell populations.

Neurosciences

Nowadays, the advancement of Micro-fluidics devices is also applied in research for fundamental neurobiology for studying cell to cell interactions, cell to tissue interactions and also in clinics. This technique is an innovative tool which can be used to refine the adjustments in clinics and screening of drugs. For the first time, Compartmentalized Microfluidic Devices CMDs, were designed by using micro-electronic technology for studying the micro-scale level. In neuro-biology, the most critical point is axonal guidance that requires establishing the architecture of complex neurons which can respond to numerous signals in the extracellular micro-environment (Neto et al., 2016). This possibly can direct the correct targets for establishing neural connectivity. Compartmentalized Microfluidic Devices are capable to isolate fractions of axons and control different variables for the growth of axons along with formation and function of synapse (Peyrin et al., 2011).

For the identification of synaptic function, a microfluidic device with 3 compartments was designed by Coquinco and his group in 2014, to analyze in-vitro the synaptic competition in a model. The axons which were instigated from two distinct compartments forms connection to a third central compartment neuronal population. They revealed that the reduced neuronal activity in one of the population can have effect on the axonal growth and formation of synapses of an opposing neural population (Coquinco et al., 2014).

With the help of microfluidic devices, the interaction of a single cell with the different types of cells can be possibly accessible. In 2013, Dinh and team, designed a Compartmentalized Neuronal Array (micro-fluidic device), presents the possibility of patterning the protein integrated in it and arraying HEK293 cells and neurons, this design breaks new ground for many other models of hetero-typic co-cultures and various neuro-biological interactions (Dinh et al., 2013).

The microfluidic technology has enabled researchers to isolate multiple spatially components of neurons and design computational algorithms. These algorithms can analyze cellular mechanisms in an automated and un-biased manner which would other-wise be human erroneous, tedious and time consuming (Neto et al., 2016). The micro-fluidic devices along with computational methods are developed in other corresponding realms. It includes:

1. Control, Processing of signals, analysis of data, viz. specific algorithms for image processing that targets morpho-metric analysis of neurons structure (Park et al., 2014; Li et al., 2014).
2. Using computational fluid dynamics, for the optimization of theoretical models and experimental data (Nguyen et al., 2016).
3. Computerized control systems for managing actuators and sensors with the use of micro-fluidic technique (Kothapalli et al., 2011; Moreno et al., 2015).

Pharmaceuticals

A complex process that the drug experience is: Absorption, Metabolism, Distribution and Excretion. Pharma-co-kinetics have played a significant role in the estimation of drug toxicity as well as its therapeutic effect but the prevailing experiment methods are laborious for the cell's manipulation and combination of drugs. The current technique for drug detection doesn't offer immediate finger-printing of intra-cellular drugs. Microfluidic chip permits co-cultures of different cells and allows complete cell to drug and its metabolites analysis via automated manner (Zhang et al., 2015). The micro-fluidic culture conditions for cultivation of bio-artificial organs offers favorable environment for observing the cyto-protective mechanisms. It may possibly provide significant perception for the use of microfluidic bio-chips as a novel modality in pharmaceutical studies and investigations for predictive toxicity. The microfluidic chip could also exemplify the ability of Metabolomics-On-a-Chip as substitute in- vitro method. This type of micro-fluidic chip facilitated the estimation of drug concerning about the presentation of drug metabolism and basal liver biomarker's production (Jellali et al., 2016). A researcher in his study used this bio-chip for the investigation of seven drugs clearance metabolism (Baudoin et al., 2014).

The micro-fabricated bio-chip created for Caenorhabditis elegans containing four step process of loading the sample for analyzing the drug effect and characterize the drug metabolism (Letizia et al., 2018). An electrochemical biochip was developed which was coupled with Mass Spectrometry or Liquid-Chromatography–Mass-Spectrometry for generation of both phases (I &II) drug metabolites. This screening method shows modification of proteins through reactive metabolites and moreover facilitate in the detection of those metabolites which are short- lived (Van den Brink et al., 2015). An imaging based non-invasive assay (microfluidic bio-chip) was used for the evaluation of the response of drug toxicity. It has a reservoir for culture medium, a pump (syringe), a trap of bubble, oxygenator and also a chip with an imaging system (two photons based) (Yu et al., 2017). It allows two parameters detection on the real-time for analysis of any change in cellular activity after treatment of drug.

In drug-resistant and drug-sensitive breast cancer cells, a droplet based microfluidic device evaluates the changing aspects of drug up-take, drug efflux and drug cytotoxicity (Sarkar et al., 2015). A researcher, Pang in 2016, built a chip with an integrated system of micro-fluidics, to construct the array for single cell and analyze the resistance of various drugs. The principle and design of this device is intended for the individual cell separation on the basis of its deformability and size. This device combines multiple obstacle pattern like micro-valve system and micro-structure matrices. The biochemical heterogenetic effect was compared between normal and individual glioblastoma cells and provides pivotal effects for cells drug resistance (Pang et al., 2016).

Conclusion and future directions

Microfluidics as mentioned above, offers number of advantages in almost every possible field of biological sciences. It led to a shift in pattern to conduct research in biology. Regardless of this, it is considered that microfluidics is in preliminary stages so there are few topics that need consideration. In this day and age, microfluidic devices have restricted its use to limited laboratories because:

1. Limited use of fabrication technology based devices
2. Devices aren't easy-to-use as it uses complex and number of integrated components foe the manipulation of fluid and detection of analyte
3. Its claim to replace "gold standards" for detecting biomolecules is still doubtful and yet its reliability has to be checked.

Academics and some other organizations should work in partnership to solve issues relating to micro-fluidics so that it isn't limited to the purpose of demonstration by laboratories. Therefore, technology of microfluidics should further be developed with the intention to be consistent, cheaper and importantly user- friendly so that everyone can it use it with ease.

Declaration:

Conflict of Interest

I declare that I have no conflict of interest.

Funding

I declare that I have no source of funding

Data

I declare that Data will be available on request.

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