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Review Article

THE FUTURE AND PROSPECTS OF BIO-CHIPS

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ABSTRACT

Nanotechnology deals with manipulation of matter at atomic and molecular level. A general description for nanotechnology is provided by National Nanotechnology Initiative, that defines nanotechnology as the manipulation of matter with at least one dimension is around 1-100 nanometer. The major application of modern nanotechnology in future would be creation of biological chips which include organ-on-chip, human-on-chip, and lab-on-chip. The world needs easy, readily accessible health care for which one of the solutions would be lab-on-chip. A lab-on-chip is a device that consists of several laboratory functions on single chip of only few millimeters to centimeter in size. Apart from health care a major problem to be addressed is the drug development for out raging diseases where many drugs fail on human trial, for such problems the boon given by nanotechnology is the organ-on-chip and human-on-chip. These chips are multichannel 3D micro fluidic cell culture chips which mimics the organs environment and its interaction within the cells. This would help us to understand the human physiology with respect to each organ thus avoiding the testing of new drugs on animals and the drugs toxicity tests on them. Although the concept is still in infancy stage many initiative are taken to improve this technology as this could replace the already existing traditional technology that is time and cost consuming.

Keywords: lab-on-chip, body-on-chip, organ-on-chip.

INTRODUCTION

Nanotechnology is the new growing area of science that is to take over the world in future.

Richard Feynman's talk at an American Physical Society meeting at Caltech on December 29, 1959, "There is plenty of room at the bottom" is most often considered as the origin of nanotechnology. After which the term Nanotechnology was first time used by Norio Taniguchi of the Tokyo University of Science in 1974. Later there were many who popularized this area of science of which K.Eric Drexler stands out in developing and popularizing the new field.

The prefix nano is taken from a Greek word which means 'dwarf'. Technically nano means anything that measure in 10 or one billionth of something.

Nanotechnology is defined as the manipulation of matter at atomic and molecular level; where at least on dimension of matter is in nanoscale ranging from 1-100nm. It can also be defined as a technology for designing, characterizing and producing any device or systems by controlling the shape and size at nanoscale.

With the tremendous growth of this field of science, the applications of this is also growing at a fast pace. There are several applications of nanotechnology amongst which the modern 4th generation nanotechnology would deal with is the organ-on-chip, human-on-chip, lab-on-chip. These applications which would be revolutionizing the nanotechnology are still at infancy stage.

Organ-On-Chip

Organ-on-chip as the word itself suggests that the entire organ is being mimicked on a chip. The organ-on-chip is defined as a multi-channeled 3 dimension micro fluidic cell culture chip.

This is a transition from the traditional two dimensional cell culturing which include the growth of the cells on the surface in a monolayer, which has major disadvantages i.e. the absence of cell to cell interactions which is the key component in any drug testing procedures. To overcome these lacunas and to ease the drug testing procedure one of the major step in the application of nanotechnology would be Organ-on-chip.

The organ-on-chip would permit use to study the human physiology with respect to each organ; enable the development of novel in vitro disease models and would potentially serve as replacements for animals used in drug development and toxin testing.

Fabrication of the chip

The construction of the base i.e. the chip includes the nano fabrication techniques. First is the hard lithography i.e. here photolithography is involved in obtaining the desired pattern.

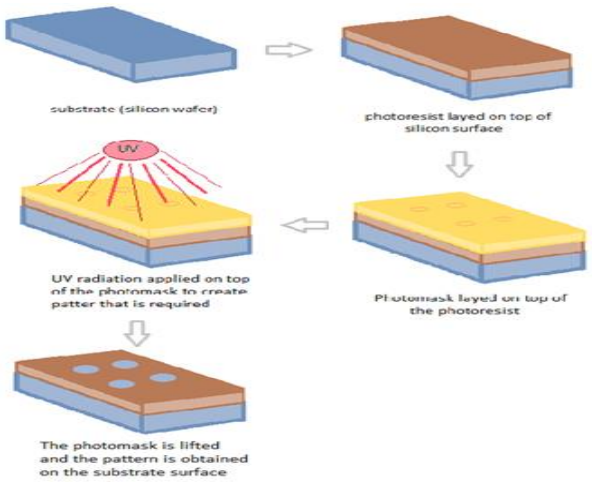

Fig.1: hard lithography: creation of pattern for the stamp

The substrate on which the required pattern is to be obtained is selected and a layer of photomask followed by photoresist and then UV radiation of desired wavelength is applied over the surface.

The soft lithography i.e. the micro contact printing includes the creation of stamp where on the previously created pattern on silicon wafer is taken and PDMS (Polydimethylsiloxane) is poured and allowed to set. Instead of the resin i.e. PDMS other resins can be used such as flurosilicon. This is the stamp on which the ink is coated as shown in the fig.2. Where the stamp thus created is lifted out and is dipped in medium containing ethanol and ODT (octadecanethiol). The ink molecules i.e. ODT molecules then diffuse onto the stamp.The inked stamp is brought in contact with the substrate for a certain length of time, allowing ink molecules to transfer onto the

substrate surface. The stamp is removed, leaving the desired single-molecule thick pattern on the substrate. PDMS has several unique properties that make it a perfect choice for the fabrication of micro devices for the culture of cells and tissues because of high gas permeability, optical transparency and high flexibility [2].

Further the micro fluidics system is employed for the interaction of the cells within the organ.

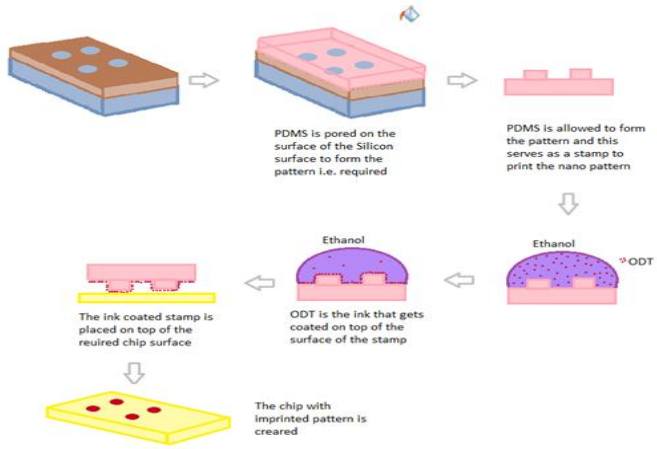

Fig.2: Soft lithography: creation of stamp

There are different organs that is mimicked on chip, the most common once include kidney, liver, lung, heart-on-chip.

Kidney-on-chip

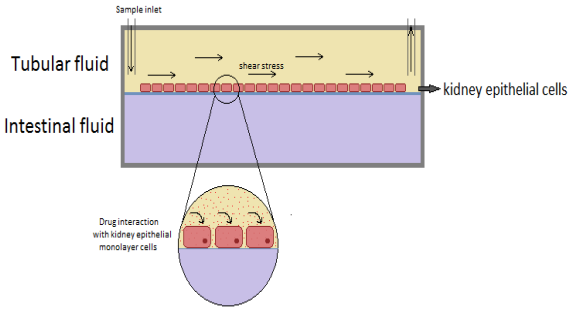

Fig.3: Kidney epithelial cells grow on a support between fluids resembling blood and the primary urine generated

The word kidney-on-chip suggests us that the kidney is mimicked on a chip. Here the renal cells or the nephrons are mimicked on the chip and this is used for checking the toxicity of drug and its screening. This model also helps us to know more about the filtration, reabsorption of the necessary molecules from the drug[4].

Nephron is the basic unit of kidney and mainly consists of glomerulus which acts as a filtering unit which helps in filtering unwanted toxic particle from the required molecules and helps in throwing out these unwanted molecules.

Nephron's glomerulus, proximal convoluted tubule and loop of Henle are mimicked on the chip. Here in this microchip generally a fabricated layers and have the living kidney epithelial cells. The epithelial cells layered on the surface are generally a monolayer for better interaction with the sample. Surrounding these cells is the tubular fluid generally blood and other layer would be the primary urine that if formed after the epithelial cells filter the fluid. Here the upper layer is the blood and the lower layer indicates the inner tubular layer that mainly consists of the primary urine.

This would work were same way as the normal kidney epithelial cells. The sample generally any drug is injected through the inlet port as shown in the fig.3 were the sample injected would create the shear stress of about 0.2 dyne cm^{-2} that is similar to that of the living kidney tubules surrounding [3] as it flows on these cells and the drug that is passing over the epithelial cells interact with the cells. At the outlet, the sample is collected and analyzed for toxicity this would help us to better understand about the filtration pattern and absorption behavior.

B. Liver on chip

Liver is considered to be one of the versatile organ performing thousands of functions which includes detoxification, protein synthesis, hormone production, glycogen storage, etc.

This liver on chip mimics the liver on a micro device. Here in this 3d microenvironment, the cellular interactions and signaling mechanisms are present and hence this liver-on-chip is of great use to the pharmaceutical sector where the metabolism and the toxicity levels of drug, studying the pathology of chronic liver disease and other infections can be clearly studied.

Liver mainly is made of hepatocytes. The liver is said to be detoxifier system in human body. These hepatocytes are the serum protein synthesizer and know this and to better understand this synthesis liver on chip can be used.

Apart from synthesis of protein pattern these cells also perform functions such as removal of toxic components from blood i.e. the poisonous ingestions. Hence is very important as a model for drug formulation where to formulate a drug there are binders used for the stability of drug and to decide the binders that has to be employed majority of time is lost to find one such binder that would be easily eliminated and being nontoxic. Here in this situation liver-on-chip can be employed.

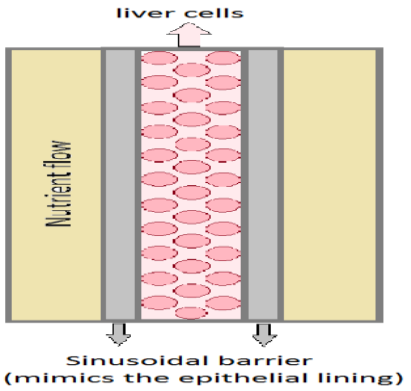

Fig.5: liver on chip

Here the micro device mimics the sinusoid of the liver where the device mainly consists of the cells in the central layer and the nutrient flow in the outer layer. The two layers are separated by the micro fluidic barrier i.e. the sinusoidal barrier [5]. Such a device is used in studying the interaction patterns and cell to cell interactions.

C. Lung-on-chip

Lung is the respiratory organ of an organism. It mainly consists of alveoli which is the functional unit of lung. For easy and efficient delivery of drug, oral forms are considered most effective. Hence oral drug to be tested needs models on which they are tested initially before human trials; best solution for this problem is lung-on-chip.

Lung-on-chip is the micro replica of the lung on a micro chip. This is used for nanotoxicology studies of various nanoparticles which are introduced into the air channels [6].

This micro device mainly consists of the alveolar and the capillary interface. To mimic the breathing pattern there are 2 chambers at the side through which air is pumped in at certain required pressure, continuous increase and decrease of the flow is done in order to accomplish the inhalation and exhalation pattern. This

device mainly consists of the central chamber where a layer of very thin flexible PDMS is used [7].Here in this chamber co-culturing human alveolar epithelial cells and blood vessel wall cells on the opposite sides is done.

The membrane stretches and relaxes according to the flow of air. The culture medium is pumped through the lower micro channel to mimic the blood flow and the sample is injected on the top layer which interacts with the alveolar epithelial cells.

Lung-on-chip is used to study the airway closure and opening, lung inflammation, airway protein synthesis, pulmonary absorption.

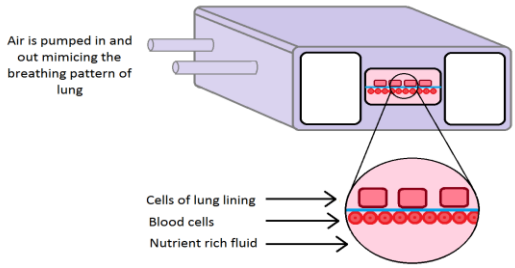

Fig.7: lung-on-chip

Heart -On -A- Chip

Heart –on –a-chip is developed to mimic the contractility and electrophysiological response of heart in invitro condition. Micro fluidics has already used to invitro experiments on cardiomyocytes which generates the electrical impulse that controls the heart rate.

The device is created using small thin strips of tissue made from heart muscle cells that are connected to electrodes to stimulate contraction. Observing the contraction response of the tissue allows scientists to study the effect of physiological factors or test drugs for cardiotoxicity. Replicating segments of heart tissue helps to rapidly measure the contraction data at the tissue level [8].

Heart –on-a- chip substrate is fabricated by using following method. An edge of a glass plate is covered with a tape such that to obtain the substrate's desired shape. A spin coat layer of PNIPA (Poly (*N*-isopropylacrylamide)) is then applied. Once it dissolute, the tape is removed. This will results a self standing body of PNIPA. The final step involves the spin coating of protective surface of PDMS over the cover slip and curing.

Once the substrate is ready the cardiac muscle monolayer is engineered on the substrate of PDMS. A micro contact printing technique is used to form a fibronectin "brick wall" pattern on the PDMS surface. This fibronectin pattern oriented the myocytes to generate an anisotropic monolayer.

After the cutting of the thin films into two rows with rectangular teeth, the whole device is placed in a bath; electrodes will stimulate the contraction of myocytes via a field stimulation which results in the curving of the strip/teeth in the MTF (muscular thin films). The correlation between tissue stress and the radius of curvature of MTF strips during the contraction cycle helps for quantification of stress, electrophysiology and cellular architecture.

HUMAN-ON-A-CHIP

The human on a chip focuses on in vitro human organ constructs (for heart, liver, lung and the circulatory system) in communication with each other. The aim is to assess effectiveness and toxicity of drugs in a way that relevant to humans and their ability to process these drugs [9].

In some cases animal testing cannot mimic the human response. For example, Asthma, is a uniquely a human disease. So human on a chip is made from human cells.

Biomimetic micro system representing different organs can be integrated into a single microdevice and linked by a micro fluidic circulatory system in a physiologically relevant manner. This is used to model a complex, dynamic process of drug adsorption, distribution, metabolism and excretion and also evaluate drug efficacy and toxicity.

The following diagram shows the integrated system of micro engineered organ mimics (lung, heart, liver, and kidney). This system is used to study the absorption of inhaled aerosol drugs from the lung to microcirculation, as well as to measure their cardiotoxicity (e.g. changes in heart contractility or conduction), transport and clearance in the kidney, metabolism in the liver. Drug substances also can be introduced into the gut compartment to investigate interplay between orally administrated drugs and molecular transporters and metabolizing enzymes expressed in various organs.

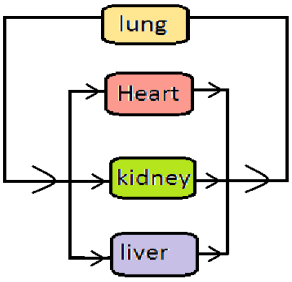

Fig.8: general representation of human on chip where several organs like lung, heart, kidney, liver are integrated on a single chip.

Lab-On-Chip

A lab-on-a-chip (LOC) is a miniaturized, large scale integration of one or more laboratory assays. They are sometimes referred to as µTAS i.e. Micro Total Analysis Systems. This is because they are capable of carrying of several functions using a very small volume of sample (few microlitres). This is a technology that merges Microfluidic technologies with biochemical protocol for creating portable systems, increasing high throughput testing and to save labour. Clinically, LOC's can serve as Point Of Care diagnostic tools. LOC's are fabricated in similar ways as Organs-on-chips using lithography and etching techniques. Many LOCs are available for various biochemical and microbiological studies. Following are few of them

Detection of DNA

Various DNA detection techniques are available in Microfluidic devices including PCR, Fluorescent based detection, Southern blotting etc. The most commonly used is the PCR based detection and amplification of DNA. These devices not only serve as a tool for DNA detection, it can also be used for amplification of DNA. When combined with suitable technologies, quantification is also possible. DNA arrays or microarrays are one of the earliest LOCs used at lab scale for detection of DNA.

Fluorescence based detection

This is done by fluorescently labeling a specific probe of DNA that is immobilized on a substratum. This substratum is fabricated using soft lithography. One such LOC [10] substratum harbors BSA (bovine Serum Albumin) which is linked to Neutravidin or Streptavidin molecule. The probe carrying the label also has a Biotin tag. This biotin tag helps the fixation of the probe to the Streptavidin or Neutravidin molecule. Probes such as molecular beacons, which exists as hairpin at room temperature can be used. On hybridization with its complimentary strand of DNA, the beacon linearizes and emits fluorescence which can be detected using suitable equipments.

FISH-on-a-chip

FISH (Fluorescence *in situ* Hybridization) is a cytological technique used to study chromosomal changes occurring in a cell. Based on the stage at which the cells are studies, they are classified as metaphase FISH and interphase FISH. Microfluidic FISH-on-a-chip is a recent introduction, which is advantageous due to small sample

requirement, easy to control and also due to the reduction in reagents used. FISH can be effectively used in the diagnosis of genetic disorders such as Alzheimer's disease, Huntington's disease, autosomal dominant Parkinson's disease, and charcot-marie-tooth disease [15]. FISH on chip can also be used to identify species by using specific probe labeling. This may help in the identification of contaminants in several industries.

PCR on a chip

PCR on a chip is advantageous over the standard PCR in many ways. These include, time reduction, requirements of small volumes of DNA sample and low energy requirement as in heating is easier. Real time PCR employing light based heating can be fabricated on Microfluidic chips which require nanolitre of sample only. [17]

A typical Reverse Tanscriptase PCR on a chip consists of 3 main chambers- RT chamber, PCR chamber and Detection chamber. [11] The RT chamber consists of all the requirements for a reverse transcription reaction including the primers and Reverse Transcriptase. The cDNA obtained here is biotinylated using a biotin labeled primer for further detection techniques. In the PCR chamber, the biotinylated cDNA undergoes amplification to give rise to several strands of dsDNA each carrying the biotin label. The amplified DNA then enters the detection chamber where the amplified DNA is quantified using chemiluminescence assay.

This LOC has been successfully used in the detection of HIV (Human Immuno Deficiency Virus) for the early diagnosis of Acquired Immuno Deficiency Syndrome (AIDS) [11].

Cells on chip

Microfabricated devices can make cell culture, a more controlled practice which may not be easily achieved by standard tissue culture practices. This can be extended to Organ-in-chip, has several potential applications in drug testing, etc. Cell culture techniques combined with selection and biochemical assay provide a platform for highly controlled handling of cell culture without being prone to contamination. This also opens up scope for automation technologies as this kind of control and detection requires precision.

A typical cell-on-chip would include a growth chamber, a chamber for cell selection, cell lysis chamber and biochemical assay chamber.[13] The growth chamber contains small volumes of solid or liquid growth media with essential nutrients. A chamber for cell selection facilitates the culturing of a specific cell line only. An imaging chamber can be integrated to view and image the cells at the micro scale. Also, a cell lysis chamber can be added to study the components of the cell. Cell lysis can be achieved by electrical cell lysis devices using microelectrodes. The cell lysates can be quantified for protein, nucleic acids and other molecules.

Another more advanced cell-on-chip technology employs a continuous supply of growth medium to microbioreactors that are fabricated onto the chip [14].

Detection of proteins

Protein detection using chips that are similar to protein arrays, can serve as a promising technology for expression profiling [19]. It can be used for both qualitatively and quantitatively identifying the protein. Some common methods of detection of protein on Microfluidic devices are discussed subsequently.

Immunoassay on a chip

Protein detection using Immunoassays is a quantitative technique for analysis of proteins in a sample. These involve on one or more antibodies (Abs) or antigens (Ags) that are immobilized or are freely available. The assays rely on a specific Antigen-Antibody reaction.

One such Immunoassay on chip involves two Abs- Magnetic Ab and the other a reporter Ab [18]. The channel in the device employs a magnet for retaining the complex formed [20]. The analyte containing the protein to be quantified is injected in the channels. The protein complexes with both the Abs, forming a sandwich-like complex. This complex binds to the magnet present in the channel. The reporter antibody helps in the detection of the complex for quantification.

Immunoassays can also be performed using quantum dot microspheres [19] in combination with antibodies that are specific to the proteins that need to detected. The advantage of using Quantum dots is that there is increased light scattering and reduction in emission which facilitated the detection process.

Micro western blotting (μWB)

Traditional Western blot can detect a single protein using milli amounts of sample. μWB is capable of detecting multiple proteins using a nano amount of sample. Integrating microfluidics with traditional western blotting can be done by incorporating molecular weight markers, control samples and the various antibodies required for the detection of the different proteins [21].

One such μWB involves three steps- Protein sizing, blotting on membrane and probing [22]. Protein sizing was done using photoactive gel with tunable porosity (PACTgel) on microchannels. Photopatterning is required for control of the PAGE process and light responsiveness is required for transfer onto a membrane during blotting. Immobilizing onto membrane was done using UV light, i.e. transfer of protein bands occur due to photocapture. Probing is done by previously electrophoresing the different probes along a microchannel and then incubated with the membrane immobilized with the sample. This technique is highly sensitive and gives precise identification and quantification.

Chromatography-on-a-chip

Chromatography is a need of the hour separation technique for separation of molecules. It is being widely used for separation and purification of proteins. An on-chip liquid chromatographic separation in a droplet based Microfluidic system which is capable of handling picoliters of sample [23].

Another such chromatography-on-chip uses sol-gel based stationery phase in an optical fibre channel fabricated using photolithography [24]. The optical fibre facilitates online detection of the process using UV absorbance.

Microscope on a chip

A microscope on a chip works on optofluidic microscopy in which there is absence of lens [25]. The sensor used in these microscopes are Complementary metal oxide semiconductors (CMOS) based sensors [26]. These sensors initially create a series of low resolution images. The low resolution is due to the low pixel size of the CMOS sensor. These series of low resolution images are then processed using an algorithm to obtain a high resolution image.

Microscope on a chip is an easy way to image cells for online control and management of cell cultures on chips. A cell on chip accompanied by a microscope on chip can serve as a tool for maintenance and easy imaging of cells to study interactions of e cells with other molecules.

Fluorescent microscopy is one the widely used microscopic techniques for analysis of various substances. Such a microscopy can be fabricated easily on a chip. This can be done by using optical fibres and the detection can be done by using total internal reflection (TIR) [28]. Again CMOS sensors are used for the detection of the fluorescence [27] of the moving cells in the channel.

Clinical diagnosis on a chip

Diagnostic chips serve as point of care technologies for detecting and monitoring levels of oxygen, glucose, and lactate in blood samples [29]. Oxygen is measured by creating a constant oxygen gradient between the electrodes making the current depend directly on the oxygen concentration. Glucose measurement is done by converting it to gluconic acid and hydrogen peroxide in the presence on glucose oxidase. The electrons generated by the reduction of hydrogen peroxide are detected to give the glucose concentration. Similar

detection for lactate is done using the enzyme lactate oxidase.

Complex diagnostics can be employed by integrating PCR and imaging onto the chip for detection of genetic disorders [11, 15].

CONCLUSION

Bio-chips serve can serve as an integral part of a lab. Organ on chips serve as disease models for studying the drug-body interaction. Lab-on-chips serve as point of care tools for analysis of biological samples. Bio-chips are indeed the future of nanotechnology. Although they were invented and studied since a decade, they are yet to be commercialized. Commercialization involves several challenges such as ethical issues, scale up issues, cost issues and precision management. But, when commercialized, these can make lab protocols easier.

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