Microfluidic Concentration Gradient Generator Chips for Applications in Cellular and Organismal Biology

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Abstract

In this article, we review the design and function of microfluidic chips purposed as fluidic concentration gradient generators for different experiments in chemistry and biology. The microfluidic chips are designed using CAD tools and fabricated using methods of soft lithography and etching using SU-8 as the master mold and polydimethylsiloxane as the microfluidic chip material. The most common design of concentration gradient chips are Christmas-tree design structures which have evolved to speed up the fabrication process and address any issues with fluid flow. A number of experiments in bioengineering have been conducted using these concentration gradient generators, including tests using model organisms such as C. elegans, nematodes, zebrafish, bacteria and different types of cells for cancer screening and toxicological screening. The toxic effects of chemicals on the model organisms have been studied with simultaneous imaging and video recording of cellular health outcomes or behavioral changes indicative of mortality. The concentration gradient chip designs have been improved over the past years to make them more user-friendly and low-cost with a wider range of applications in bioengineering. New technologies, such as 3D printing, has further incorporated automation and design flexibility in the fabrication of microchips for generating concentration gradients. Moving forward, the value of concentration gradient chips lies in its ability to conduct many tests in parallel that help to better predict the clinical outcomes. For this, the concentration gradient chips need to have design considerations that closely mimic the natural biological systems and hierarchical topologies seen in cells, tissues, and organs.

Introduction

With advancements in food processing and industrialization, there has been an increased awareness about the harmful effects of toxins in our air, food and water, with more need for measuring the toxicological levels of various ingredients in our food processes, chemicals, and manufacturing products [1-7]. New applications in microfluidics leverage cells and cellular systems, including organ-on-chip systems, to test the toxicity levels of chemicals over long periods of time. The dose response curves are typically obtained over a series of concentrations of a certain chemical or drug, which in turn provides the critical concentration where 50% of the

cells are damaged. In vivo experiments are discouraged until later experiments, and the in vitro experiments are promoted that help to design and test the initial studies at a reduced cost and improved yield [5-10]. Moreover, there is lesser use of animals in such experiments and a more targeted use of small, model organisms for toxicological studies so as to save the lives of animals involved in biological research [10-17].

Many of these experiments are designed in miniaturized lab-on-chip systems where the entire experiment can be conducted automatically and at a small scale [12-20]. Microfluidics has greatly helped to further the research and development of technologies that save the lives of animals and promote the use of small, model organisms for such studies. Today, CAD software tools are available to design and draw the 3D structure of proposed microfluidic chips with great accuracy and precision [18-28]. The typical soft lithography process uses a master mold fabricated in SU-8 and used to create replicates in PDMS material. This process has not changed over several decades because of its simplicity and ease-of-use, while providing reproducible results. There have been different silicon-based materials tested to replace the SU-8 or PDMS but has not yielded better results for mass adoption [20-32].

Concentration Gradient Chip Designs

The concentration gradient microfluidic chip is designed to create a fluid flow utilizing mixing of different fluids to create a concentration gradient of fluids that could be used to test the toxicity of cells or cellular systems [2-17]. The mixing of fluids to create the concentration gradients can be done by creating meandering structures where the fluids mix by convection. Alternatively, fluids can be mixed by exerting pressure or filter at the microfluidic level to the fluid flow. The concentration gradient can be utilized to test a number of biological experiments related to cell growth, cell migration, cell adhesion, behavioral assays or toxicity assessments of cells and cellular systems [21-35]. Concentration gradient microdevices offer better precision and control over fluid flow and mixing, faster results in smaller scale, better spatial and time-resolved throughput, customization of inputs, and control and automation over the modular components. The concentration gradient chips can be constructed in 3D manner to enable seamless integration with the 3D architecture of cells and tissues in the in vitro setups [21-25]. Furthermore, different concentration gradients and drug combinations can be tested in parallel and simultaneous manner on multiple cell culture platforms to mimic the internal body structure and accelerate the rate of data production from experiments. This aspect of high-throughput data collection through experimental parallelization is an attractive attribute of microfluidic concentration gradients, particularly with the rise of new diseases and new disease symptoms that need faster screening of multiple targets [32-40].

Simulation of Concentration Gradient Chips

To design microfluidic chips as concentration gradient generators, the steps involve understanding the general and specific requirements of the experiment which will inform about the inputs, process, and outputs [28-38]. Simulation tools such as COMSOL can be used to design the concentration gradient generators. Concentration gradient chips can use fluid flow with splitting and mixing of fluids in separate, serpentine channels, depending on the height and width of the individual channels [35-41]. Automation in fluid flow and mixing is critical for ease of use of these concentration gradient chips. Other physical forces such as centrifugal forces, centripetal forces, surface tension, etc. can be used for mixing fluids to create concentration gradients. Concentration gradients can be produced by fluid forces of convection or diffusion [32-40]. The convection method is simple and easier to implement as it depends on the shear stress and the fluid flow field. The diffusion method of producing concentration gradients depends on the diffusion of reagents or samples at the interface of two fluid channels. The most common concentration gradient design is the Christmas tree design because of its simplicity and specific concentration gradients derived from simple calculations [24-32]. The Y-channel of concentration gradient chips and its modified versions provide a simple, well-defined way to expose cell cultures to concentration gradients of chemicals and observe the changes in cell growth and reproducibility. Concentration gradient chips can also be produced by membranes and droplets where the physical dimensions control the mixing rates and definitions of the concentration levels [11-24].

The concentration gradients can be studied by simulation tools and validated by experiments. COMSOL is the best known software for simulating fluid flow in microchips [1-9]. The software provides a quick overview of the system design and fabrication protocol, which can further be modified depending on the user needs and definitions. Fluorescent dyes have been used to validate the concentration gradients in microchannels where the imaging has been done using fluorescent microscopes. Here, the level of fluorescence intensity is correlated to the level of sample concentration at that specific location. Fluorescence imaging can be done with high temporal and spatial resolution to quantify the level of sample or chemicals needed at a certain time and a specific location in the chip [11-23].

Drug Screening in Concentration Gradient Chips

One application of concentration generators is to test the toxicity or antibiotic resistance of chemicals [11-29]. One frequently used antibiotic is Ampicillin and the other is Tetracycline; both of which have been used in several microfluidic experiments. Other chemicals used are kanamycin and amoxicillin. Drug screening is an important application for concentration gradient microchips where researchers want to quickly assess the range of drug chemicals needed to kill the bacterial population while producing less damaging effects on the host [23-30]. Several articles use ampicillin or tetracycline as the common drugs. A higher dose of drugs is not often preferred here because of the risks of overdose and drug resistance. An optimal dose of

drugs can be found using the concentration gradient chips through a proper selection of individual chemicals and mixing formulation.

Micro-organisms under study

The common organisms used in toxicity analysis are E. coli, zebrafish, and C. elegans nematodes [9-17]. The drug flow rates and exposure rate of chemicals and pollutants for culture of these organisms and co-culture are dependent on the experimental goals. Cell cultures from animals and humans have been used to assess cell viability using drugs in a dose dependent manner [18-29]. Studies have also used cell cultures in microfluidics to assess the exposure effects, time of exposure, and possible chemotherapy directions using a single or multitude of chemicals.

Microfluidic devices show results that mirror the results from traditional experiments. Microfluidic experiments also mirror the in vivo experiments, especially in toxicity studies where we want to study how toxins affect the organisms under test [11-19]. Many different concentrations can be tested in a single, one platform chip with the use of concentration gradient generators. The input fluids can be mixed in defined ratios to create new cocktails of existing drugs or chemicals which can be exposed to the microorganisms for specific time periods of hours or minutes [21-29]. The small size of the microfluidic chips make it easier to conduct the concentration gradient generations in a smaller space, requiring less reagents, and producing result sin a faster time. The microchips can be fabricated easily using standard SU-8 or PDMS fabrication technology, along with combinations of new materials for fluid flow control, such as hydrogels, paper, or plastic [30-41]. These materials are often chosen for their gas permeability, biocompatibility, thermal stability, transparency, and non-toxicity to test chemicals. Three dimensional printing of biomaterials has been also proven as an emerging technology of interest where automation and design flexibility are both attractive traits. Microchips allow us to create two dimensional and three dimensional cell cultures that mimic the natural processes in the body very closely so that their results are more credible and trustworthy for future clinical studies. The lower cost of microchips and faster reaction time are also beneficial traits of microfluidics compared to agarose plate experiments. The flexibility in chip design, fabrication, and testing of biological samples is an added advantage for microfluidic technologies [11-18].

Applications of Concentration Gradient Chips

The use of concentration gradient chips provides a reliable way to test for toxins, drugs, pollutants, and lethality of chemicals such as lead and mercury [5-10]. Concentration gradient generators require a small amount of reagents or buffer solutions for multiplexing the target chemicals at a certain dose level. The transparent microchips also provide a nice way to image the cellular growth, behavior, phenotype, and reproduction of organisms or cell cultures when exposed to the chemicals in the concentration generator chips that successfully employ automated fluid flow and integration of various modules for conducting several test runs.

One emerging area of use for concentration gradient chips is the testing of environmental pollutants, such as metals (lead, mercury, cadmium, arsenic) and contaminants that have significant impact on human health and the natural ecology [2-9]. Synthetic biology has helped to create microorganisms (such as microalgae) that have the ability to detoxify the environment, remove pollutants, and clean the contaminated fluids in industrial wastes. These microorganisms need to be characterized for their efficiency against different doses of chemicals and pollutants. Considering the range and variety of tests needed for accurate and detailed characterization of their efficacy, concentration gradient chips are noteworthy in conducting tests on their efficiency in removing and digesting toxins in the environment [7-17].

Challenges and Future Scope

There are certain limitations and challenges of concentration gradient generator chips. One challenge lies in understanding whether the engineering designs and obtained results match the natural phenomena [13-23]. It is difficult to predict which structural designs and topologies work well in replicating or mimicking the natural processes of cells, tissues, and other biological specimens. Also the scale of testing is critical as there is a hierarchy of operations and system performance from cells to tissues and organs within the body. It is difficult to replicate the concentration gradients at the various levels of cellular organization within the human body. Furthermore, the microfluidic chips have concentration gradients that collapse in a matter of few hours, while natural systems may sustain concentration gradients up to several days and weeks. It is difficult to replicate and create concentration gradients that can be sustained over such long time periods. Simulation tools need to be modified to accommodate the time scale of operations while lowering the costs of conducting such simulations [32-40].

Conclusions

This review article centered around one type of microfluidic chips, called the concentration gradient generators. These chips have been used to create defined concentration gradients of specific biological or chemical samples, which may be toxins, pollutants, drugs, or metal ions that may have undesired effects on microorganisms and cell cultures. There are several designs of concentration gradient chips, the most popular being the Christmas Tree shape. This design is simple and easy to implement with well-defined formulae to represent the concentration gradients. Simulation tools, such as COMSOL, have been used to predict the fluid mixing and fluid flow through these microchips. Moving forward, it is desirable to design microchips that closely resemble the natural organization of cells, tissues, and organs within the human body so that the results could help with clinical studies.

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