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Microfluidics Technology for Drug Discovery and Development - An Overview

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Abstract: Microfluidic technologies represent an improvement to existing technologies in key areas of drug discovery. Microfluidics involves materials and techniques for controlling the movement of minute quantities of fluids and its ability to miniaturize assays and increase experimental throughput have generated significant interest in the drug discovery and development domain. It affords unique capabilities in sample preparation and separation, combinatorial synthesis and array formation, preclinical testing of drugs in living cells, and incorporating nanotechnology for more functionality. Microfluidics promises to be a better technology both for the production of new chemical entities and for screening them against a range of biological targets. Microfluidic systems provide an ideal medium for nanoparticle production. Scientists are of the opinion that microfluidic technologies are powerful tools for various applications for the drug discovery and development process.
Keywords: Microfluidics; drug discovery; pharmaceuticals; Lab-on-a-chip.

Introduction

A key approach of research in pharmaceutical companies is designed at miniaturizing the assay systems so that they can operate on micron dimension samples to reduce the amount of material required and thus speed up the overall drug discovery process. Microfluidics is a multidisciplinary field intersecting physics, chemistry, engineering, microtechnology and biotechnology, and its importance as an emerging technology is increasing [1-6]. The field of microfluidics continues to grow and it is considered as a toolbox from which researchers can investigate basic questions in their respective fields. The term microfluidics refers to devices, systems, and methods for the manipulation of very small fluid flows (as small as micro-, nano-, pico and even femto litre volumes) [7]. A micrometer is a millionth of a meter. For a sense of scale this is about 100 times smaller than a human hair and a drop of water is approximately 25 microliters. The microfluidic chip offers channels through which fluids can flow, typically under the impetus of an applied electric field. The basic idea of microfluidic biochips is to integrate assay operations such as detection, as well as sample pre-treatment and sample preparation on one chip[8]. Microfluidics is the miniaturization of biological separation and assay techniques so that multiple experiments can be performed in parallel on a small device. In this technology, minute quantities of media, reagents and even nanoparticles are steered through narrow channels on the device where they are delivered, manipulated, and analyzed by such techniques as fluorescence detection. Microfluidics mainly deals with artificial systems, but is present in numerous natural systems inside us, for example, capillary blood vessels transport liquid, and lungs pump air in small alveola. In microfluidics, the differences include faster thermal diffusion, predominantly laminar flow, surface forces responsible for the capillary phenomenon, and an electric double layer. An increasing trend toward novel diagnostic development, particularly point of care, is providing a significant boost toward a greater acceptance of microfluidics methodologies [9]. Nanofluidics deals with the study of the behavior, manipulation, and control of fluids that are confined to structures between 1-100 nm and such fluids exhibit physical behaviors not observed in larger structures, such as those of micrometer dimensions and higher. [10].

Advantages of Microfluidics

Microfluidics technology results in: improved data quality, fewer experiments needed, reduction of reagent consumption, shorter reaction times, better performance, higher throughput due to parallel processes, faster hit-to-lead process, reduction of costs, elimination of the need for highly skilled individuals to perform the analytical steps, and avoidance of storage costs [11,12]. Microfluidics is increasingly being used to scale down and automate laboratory procedures in the fields of pharmaceutical industry and biotechnology [13,14]. Microfludics technologies have been embraced by the pharmaceutical industry because the benefits of miniaturization, integration and automation to research and development [15].

Nanofluidics has the potential for integration into microfluidic systems, i.e. Micro Total Analytical Systems or Lab-on-a-chip structures. Nanofluidics has a significant impact in biotechnology, medicine and clinical diagnostics with the development of lab-on-a-chip devices for PCR and related techniques [16]. Lab-on-a-chip is an advanced technology that integrates a microfluidic system on a microscale chip and it is the miniaturization of a laboratory to a small device which is created by means of channels, mixers, reservoirs, diffusion chambers, integrated electrodes, pumps, valves and more. With lab-on-chip technology, complete laboratories on a square centimeter can be created [17]. Lab-on-a-chip devices are commonly used in biotechnology and in various drug discovery and drug development applications, such as combinational chemistry, high throughput screening, cell manipulation and nanomedicine. Microfluidics technology enables the development of next-generation drug delivery systems. Portable devices, even implantable, are based on lab-on-a-chip technology [18]. Scientists have reviewed microfluidic platforms that enable the miniaturization, integration and automation of biochemical assays [19].

Applications

Microfluidic technologies are emerging as powerful tools for the drug discovery and development processes. Microfluidics has incredible potential in a variety of areas [20-22]. A microfluidics application include DNA analysis methods and have been linked to mass spectrometry and thus enables picomole amounts of peptide to be analyzed within a controlled micro-environment [23]. In pharmaceutical industry; its applications are found in the areas of diagnostics and drug research [24-26]. Researchers have reviewed the advances of microfluidic devices for drug discovery and development and highlighted their applications in different stages of the process, including target selection, lead identification, optimization and preclinical tests, clinical trials, chemical synthesis, formulations studies and product management process. Further, a number of microfluidic technologies can be used to enable High Throughput Screening studies such as multiplexed systems, microwell arrays, plug-based methods and gradient-generating devices [27].

Microfluidic devices based on elastomeric materials such as polydimethylsiloxane (PDMS) are rapidly becoming a ubiquitous platform for applications in pharmaceutical industry and biotechnology [28]. Some advantages of PDMS are that it is very cheap, optically clear and permeable to several substances, including gases. Since air can quickly diffuse out, the latter aspect is very convenient, making it possible to inject fluid into a channel that has no outlet. Elastomeric materials such as PDMS have emerged recently as excellent alternatives to the silicon and glass used in early devices fabricated by microelectromechanical systems processes [29,30]. Recent growth in the field of PDMS microfluidics has far outpaced that in alternative device technologies based on glass and silicon, due in large part to significantly simpler and less expensive fabrication procedures as well as the possibility of easily incorporating integrated mechanical microvalves at extremely high densities [31].

Microfluidic devices provided cheaper and easier screening process than in traditional *in vivo* approaches. The advantage of multi-region devices includes testing of different cell types with various drug combinations leading to an improved understanding of drug effectiveness and its safety profile. PDMS technology can be successfully used for integrating complex preparation protocols of protein samples prior to MS analysis [32].

Microfluidic systems have been adapted and interfaced to most of the common analytical detection techniques, such as electrochemical methods, mass spectrometry and optical methods including absorption, refractive index variation, surface plasmon resonance, chemoluminescence and fluorescence. Microfluidic methods that are valuable for individual steps in the drug discovery process have been reported [33]. The recent advances in microfluidic-based applications in neurobiology, with emphasis on neuron culture, neuron manipulation, neural stem cell differentiation, neuropharmacology, neuroelectrophysiology, and neuron biosensors have been reported [34]. Researchers have designed a microfluidic device that mimics the microenvironment gradients present in tumors [35]. Microfluidics affords unique capabilities in sample preparation and separation, combinatorial synthesis and array formation, and incorporating nanotechnology for more functionality [36]. Microfluidics find applications in drug discovery, drug delivery, *in-vitro* diagnostics, chemical analysis, high-throughput screening, Researchers are of the opinion that microfluidics will enable efficient screening of more drugs in less time and drastically cut down the costs of drug development. Microfluidizer processors are used to create products for numerous applications such as improved bioavailability, stability, uniform particle size reduction, sterile-filtration (< 200 nm) of nanoemulsions, produce several vaccines available including swine flu) on the market , efficiency and target drug delivery in pharmaceutical industry 37. Live cell-based studies using microfluidic devices can be quite useful for observing and analyzing the effect of a drug compound on normal and diseased cells as well as determining optimal dosing. These same microfluidic devices can be used to test the synergistic effect of combinatorial drugs which offer new hope for a number of diseases, where the cost of clinical studies can be prohibitive [38].

Scientists have reviewed recent advances in both arraying strategies based on novel nano/microfluidic devices with high analytical throughput rates [39]. Researchers use microfluidics, a fluorescent reporter and modeling to quantify yeast's response to glucose availability [40]. Microfluidic technology could apply to the study of the nervous system, including architecture for isolation of axons, integrated electrophysiology, patterned physical and chemical substrate cues, and devices for the precisely controlled delivery of possible therapeutic agents such as trophic factors and drugs [41].

A microfluidic device designed and optimized for the multi-step synthesis of Positron Emisssion Tomography probes (radiopharmaceuticals) has been reported. Its PDMS elastomer-based architecture has novel features that facilitate mixing, solvent exchange, product collection and overall synthetic efficiency [42]. Nanofluidic devices have the potential to analyze DNA, proteins, and could separate particles of different sizes from a mixture, and also be useful in the preparation of nanoparticles for gene therapy, drug delivery, and toxicity analysis [43].

Applications of Labs-on-a-Chip like lithium analysis for manic depressive patients have been reported [44]. Scientists have utilized microfluidic reactors to perform highly efficient nanomaterial synthesis [45]. Researchers have reported the design of complex nanochannel structures as small as 60 nm and provided simple design rules for determining the conditions under which nanochannel formation will occur. The applicability of the technique to biomolecular analysis has been demonstrated by showing DNA elongation in a nanochannel and a technique for optofluidic surface enhanced Raman detection of nucleic acids [46].

A microfluidic device made of PDMS addressed key limitations in single-molecule fluorescence experi ments by providing high dye photostability and low sample sticking [47]. Scientists demonstrated that the role of Glucose Regulated Protein-78 (GRP78) played in the chemotherapy resistance to VP-16 in human lung squamous carcinoma cell line SK-MES-1 cell line, suggesting that the integrated microfluidic systems may be a unique approach for characterizing the cellular responses [48]. The applications of microfluidic technology in the area of MALDI-MS and drug discovery were reported [49]. Caliper Life Sciences created its Mobility Shift Assay to interrogate drug able enzymes. It combines the advantages of capillary electrophoresis with microfluidics technology for the direct measurement of substrate and product [50]. Protein microarrays for allergen-specific antibodies detection were integrated in microfluidic chips, with imaging chemilumine scence as the analytical technique [51].Microfluidics-based biochips find applications in clinical diagnosis, deoxyribonucleic acid (DNA) sequencing, and other laboratory procedures involving molecular biology [52]. A programmable microfluidic technology has been developed that addresses the need for flexible, low volume assays in secondary screening and lead optimization [53]. The various approaches employed for performing high-throughput screening experiments on-chip, encompassing biochemical, biophysical, and cell-based assays have been reported [54]. Researchers demonstrated a simple method for self loading and culture of mammalian cells in microfluidic multi-chambers for high throughput screening, using one layer soft lithography with PDMS and thermal bonding on a glass slide [55]. A novel microfluidic device capable of measuring protein concentration in the blood in near real-time has been developed. This device contains a porous membrane separating the blood flow layer from the perfusion flow layer and allows proteins to pass through while excluding interfering cells and platelets [56].Various aspects related to on-chip PCR micro fluidics have been reviewed [57].

An integrated microdevice captured a single cell, transcribed and amplified the mRNA, and quantitatively analyzed the products of interest for the analysis of gene expression in single cells. The key components of the microdevice included integrated nanoliter metering pumps, a 200-nL RT-PCR reactor with a single-cell capture pad, and an affinity capture matrix for the purification and concentration of products that is coupled to a microfabricated capillary electrophoresis separation channel for product analysis. This microdevice was used to measure siRNA knockdown of the GAPDH gene in individual Jurkat cells [58]. Scientists presented an overview of microscale technologies in various tissue engineering applications, such as for fabricating 3D micro fabricated scaffolds, as templates for cell aggregate formation, or for fabricating materials in a spatially regulated manner. In addition, examples of the use of microscale technologies for controlling the cellular microenvironment *in vitro* and for performing high-throughput assays have been reported [59]. It is now possible to track the dynamics of gene networks in single cells under various environmental conditions using microfluidic 'lab-on-a-chip' devices [60]. Using fluorescence microscopy, concentrations of multiple metabolites present within the RBC can also be determined using the microfluidic array [61].

Conclusions and Perspectives

Drug discovery and its development are remarkably dependent on available technologies and microfludics has been the motification for pharmaceutical applications such as drug discovery and drug development. Microfluidics Lab-on-a-chip technology represents a revolution in laboratory experimentations, bringing the potential benefits of miniaturization, integration and automation to pharmaceutical Research & Development. Pharmaceutical industry can greatly benefit from applying new microfluidic assays in various drug development stages, from target screening, lead optimization to absorption distribution metabolism elimination, toxicity studies in preclinical evaluations, diagnostics in clinical trials, drug formulation and manufacturing process optimization. Scientists are of the opinion that microfluidics promises to be a better technology both for the production of new chemical entities and for screening them against a range of biological targets. Moreover, microfluidics offers innovative technological opportunities for obtaining new information about biological systems. The ability to miniaturize assays, increase experimental throughput, and utilization of microfluidic systems to achieve results closer to those expected in *in vivo* studies will continue to generate a significant amount of interest in drug discovery companies. The implementation of extended time imaging along with microfluidics by drug discovery and development companies is a key step on the road to personalized medicine and thus microfluidics has a bright future.

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