Microfluidics engineering: Recent trends, valorization, and applications – A Review

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Abstract

In recent years, microfluidics engineering has gained increasing research interests in assimilation and designing novel constructs for various application in bio- and non-bio sectors of the modern world. Soft and photo-lithographic fabricated devices have a potential to control the diffusion and flow of liquids in channels. The fabricated microfluidic devices of unique geometry facilitate excellent control over extracellular microenvironment and soluble factor interactions. The current devices do not only replace the *in-vitro* approaches but also provide a new understanding of biomolecules separation and flow in the network of microchannels. The appropriate physiological responses of cells in biological studies or drug screening analysis require engineering of active cells outside the body to enhance their maximum potential for best cellular therapy. This review work mainly focuses on the current methodologies involved in the fabrication process and limitations of cell array technology. The information is given on considerable advantages of microfluidic devices. Towards the end, potential applications covering microfluidic-based *in-vitro* drug analysis and analytical-based separation/detachment are discussed with potential future viewpoints.

Keywords: Microfluidics; Fabrication; Cell array technology; Applications; *In-vitro* drug analysis

1. Introduction

Microfluidic technology is acknowledged as regulating fluids at the micrometer level. Multidisciplinary research prospects to meet the bulk of biomedical tasks is a major research endeavor at present. Various technologies and methodologies have been introduced with the advancements in the field of microelectronics which enable researchers to design and fabricate different device structures for drug screening and biomolecules separation [1]. Traditionally, photo-lithography and etching techniques were used in semiconductor industry for patterning and production of microstructures on glass or silicon. This approach is fraught with high cost, specialized equipment requirements, and prohibited rapid prototyping. A major development has been to develop a replica molding carried after the advent of soft lithography [2]. Glass and Silicon were considered as expensive materials because they were feasible only for a single device manufacturing [3-6]. Today, various types of materials such as ceramics, plastics, glass, metal, paper, elastomers, and gels are used for device fabrication process due to their unique characteristics and advantages in bio-applications [7]. Other factors including heating, cooling, detection and controls are also important to the couple during a microfluidic device fabrication. The introduction of cheap and transparent polydimethylsiloxane (PDMS) and master mold usage for several microfluidic devices fabrication has replaced the conventional tools in biomedical research.

PDMS, a well-known elastomeric polymer allows the fabrication of precise microchannels network. Its inherent characteristics such as biocompatibility, flexibility, and gas permeability have revolutionized the current *in-vitro* research [6, 8]. The choice of material and technology is reliant on the design of microchannels and the application of the device. The development of exceptional capabilities in fabrication processes stemmed from some discoveries especially in life sciences studies such as human genome sequencing [9-11], biochemical screens [12, 13] and droplets generation for molecules and cells separation [14, 15]. Micro-technology enhances the efficacy of various biological and chemical applications by reducing the cost of reagents as well as experiment time. For example, although microscopic analyses have some advantages for population variance calculations online observation, comparison and high-throughput drastically increase the importance of miniaturized devices [11]. It is difficult to establish a macroscopic setup for single cell analysis during drug screening. In the present study, a synergistic discussion of microfluidic technology is presented with insights into various fabrication approaches, advantages, and limitations. In this work, the current methodologies involved in the fabrication process including soft lithography, casting, and micro-contact printing are reviewed. The information is also given on considerable merits and demerits of cell array technology. Towards the end, potential applications covering microfluidic-based *in-vitro* drug analysis and analytical-based separation/detachment are discussed with potential future viewpoints.

1.1 PDMS soft lithography, Casting and Micro-contact printing

The noteworthy binary strategies explained here; PDMS casting and micro-contact printing are often identified as PDMS soft lithography to fabricate microfluidic frameworks devoted to cell biology research as well as for typo-graphing molecular micro-patterns on cell culture substrates. Figure 1 illustrates a classic process flow for developing a microfluidic device. The most frequent method used for PDMS microfluidic devices fabrication is molding method [3, 16, 17]. Photolithography method for mold creation needs a spin coater as well as committed UV lamp. Monolayer microfluidic device fabrication process carried out by using conventional soft lithographic techniques. The association of low Young's modulus of PDMS allows the micro-valves assimilation during the multilayer devices fabrication [18, 19]. The softness and low surface energy render it difficult to accomplish metal deposition on PDMS surface. The suitability of glass substrate helps to develop clean room procedures for silicon-based microelectronic engineering, and it is possible before plasma bonding to coat metallic as well as dielectric layers clearly on the glass substrate during PDMS fabrication practice [20].

[Figure 1 to be inserted here]

1.2 Non-specific assimilation

The hydrophobic properties of PDMS permit smooth dispersion in nonpolar natural solvent variables. The immersion of hydrophobic particles into PDMS, change the cell behavior would lead in exogenous chemicals that take to cytotoxicity. Many analysts have declared lessening non-specific retention as well as dispersion into PDMS (e.g., using inert

fabrication material, surface modification, and extraction). Culbertson's team administered PDMS by utilization of sol-gel and tetraethyl ortho-silicate integration for silica filling inside polymer matrix efficiently reduced the absorption of the hydrophobic material. After treatment, the oxygen infusion, device transparency, and biocompatibility were also evaluated [21]. Millet and co-workers [22] discarded platinum impetus, un-cross-linked oligomers, and soluble factors constantly by using extractions of local PDMS with different solvent components. Figure 2 shows PDMS-based microfluidic devices for embryo culture [23].

[Figure 2 to be inserted here]

1.3 Evaporation

Evaporation in cell culturing process can become harmful in microfluidic structures if a small volume of liquid medium evaporates. This is attributed to the sudden enhancement in osmolality, which is very effective on the cell physiology (e.g., Growth, Signaling, Ion balancing and Metabolism) [24]. Various approaches have been conceived to solve this major issue. Takayama's group put a solution to resolve this major problem by developing a hybrid membrane of PDMS-ptylene that could be useful to stop evaporation [25]. The utilization of thin hybrid membrane for non-moistened situation supported the cultivation of different pre-implanted sensitive embryo and endothelial of mouse and humans respectively. Several biological types of research require a low level of oxygen (1–2%) in microfluidic devices (e.g. tissue engineering and cancer). It was reported that minimal oxygen pressure was needed in microfluidic devices for soft materials due to gas diffusion. The development of hypoxic conditions and lower evaporation in the device permitted cell culture effortlessly [26].

1.4 Bubble

It is considered critical that the assimilation of microfluidic arrays required biocompatibility, unproductiveness along with inhibition of bubbles formation and accumulation. These issues can be overcome with different viable approaches. Though bubbles formations during the connection or eradicating of tubes to the devices usually occur [24]. It is important to avoid bubbles formation and accumulation, particularly during fluid circulation. Researchers have used different approaches for bubbles eradication such as ultrasonic waves, surface modification or mechanical traps. Sung and Shuler [27] formulated devices consisted of two layers, upper layer bigger in size for bubbles trapping and bottom layer smaller in size containing channels for flow. These fabricated traps enhance medium circulation in the device and mimic the blood circulation. Mehta et al. [28] fabricated two different types of traps in the device for bubbles trapping with advantages of simple, stable as well as no intervention with fluid flow. Bubbles catching was executed by the addition of a little tube-shaped chamber at the association of inlet and microchannel. The connection of the bypass channel through trapping channel facilitates bubbles removal from channels.

1.5 Flow rate impact on cell culture

The external pumping for fluid flow inside the microchannels could be a source of bulk applications and functions. This process commonly comprises a syringe pump use for controlling of liquid flow and pneumatic PDMS valves to arouse liquid flow inside the microfluidic channel. During experimental analysis, flow rate variation could be the requirement for better investigation, (setting range from zero to required volume). Zhou and co-workers [29] fabricated microfluidic devices with hard and soft materials to check the response of fluid flow in microchannel. They investigated response time and found flow rate variation is rapid in the hard material rather than soft material.

2. Advantage of cell array technology

In difference to bio-detecting advancements transferred on molecular recognition, the utilization of entire cells as detecting bodies permit the analysis of tested sample rather than its components identity. Incorporation of cellular expression can ascertain cellular reactions, metabolic reaction, cell viability and genotoxicity computing either unconventional or wild biochemical impacts of the objective analyte(s) [30]. Also, living cells study provide an opportunity to check the produced biochemical effects in real-time and non-destructive manners with less reagent consumption, no requirement for preliminary as well as systematic steps including hybridization or staining. The multiple impacts of multiplexed sensors are enhanced in microarray studies if compared to the standard values nose [31] and tongue [32] electronically. A miniaturized array allows real-time monitoring of analyzed samples, better than current microtiter plate-based investigating methodologies. The miniaturized array ultimately prompts the improvement of a versatile portable device for both research center as well as outdoor use, for procedures like mutagenicity and toxic substances assessment. The fast progressing field of chemical and biological science undoubtedly utilized such apparatuses for unraveling complex biochemical processes.

3. Limitations/Drawbacks of cell array technology

There are limitations during PDMS utilization in biological studies at the cellular level. The specific characteristics of PDMS of absorbing biomolecules of hydrophobic nature and the adsorption of drugs usually happened outside the media distinguished PDMS from other soft materials [33]. The extraction and removal of organic compounds trace from solution carried out by using PDMS due to its absorption characteristics. Analysts have observed many problems in cellular studies due to adsorption of proteins molecules on the surface of PDMS. Cell signaling experiments and drug dose reaction and response, the PDMS involvement can strongly distort the result. Various PDMS surface coverage has been created particularly to diminish the adsorption issue. Furthermore, the permeability to water vapor of PDMS permitting to dissipation within the device is another paramount issue. Medium osmolality alterations or entire desiccating over time are allowed after this development. Employing hydration channel networking, controlled environment of hygrometry or culture having renewal system are commonly adopted methods to reduce this issue. Exquisitely to balance device hygrometry, before employing PDMS device should be accustomed to several hours [34]. They have been considered to cope with the new cell microarray technology and all microarray plugs, ~150 μm in length and ~100 cells of harbor [35]. The

results of a phenotypic study of gene overexpression are understandable by using cell microarrays having *cDNA* transfection. In emerging techniques, cell microarrays could be versatile which ease the gene underexpression study quick. The currently identified phenomenon: RNA interference (RNAi) of dsRNA-induced in animals as well as plants the sequence-specific posttranscriptional gene silencing [36, 37]. To expand microfluidic application in plant cell manipulation, Wang et al. [38] presented a conceptual attempt at protoplast fusion in the microfluidic device (Figure 3). Various drawbacks are yet to be solved for the pragmatic biosensors application. Currently, analytical techniques include chromatography specifically gas and liquid chromatography and mass spectrometry [39].

[Figure 3 to be inserted here]

4. Potential Applications

4.1 Microfluidic approach for *in-vitro* drug analysis

The manageable assembling of microfluidic array permit its utilization at the cellular level in biology. Within the local cellular microenvironments, the range of micro-channels imitates well [40, 41]. Cell culture having microfabrication devices have latterly been explained on silica, glass and PDMS substrates besides with hepatocytes, lung, and insect cells, with none yet for the purpose of executing a unified test system [42-44]. Figure 4 Configuration of the integrated microfluidic system. (A) Schematic representation of the functional circuit used for cell-microenvironmental interactions. The responsibilities of different valves are illustrated by their colors: red for regular valves (for isolation) and yellow valves (for communication of the adjacent chambers). (B) Optical image of the actual device. The various channels were loaded with food dyes to help visualize the different components of the microfluidic chip; the colors correspond to those in (A), with green indicating the fluidic channels. (C) Composition of the microfluidic device (four layers sequentially from top to bottom, including the fluidic layer, control layer, thin PDMS layer, and glass slide) [38].

[Figure 4 to be inserted here]

Primarily, Hung *et al.* constructed an integrated microfluidic system with an application gradient developer to enable cellular monitoring for the long-term duration [45]. The system comprises of an application developer as well as 10×10 arrays of microchambers. That allows execution of 100 experiments in collateral as well as a distinguished environment in all chambers. Utilizing cells of HEK having microchannels fabricated with two alternative plastics, polystyrene (PS) as well as Cyclo-Olefin Polymer (COP), and silicone elastomer, cytotoxicity is investigated [46]. PS and COP could be much suitable for PDMS devices when utilized in the selection of hydrophobic drugs that is explained in their analysis. The initial lab-on-a-chip platform was compatible to implement entire stages necessary for mammalian cell cultivation, firstly cell seeding, secondly progression, thirdly detachment and lastly reseeding for through mammalian cell culture on the fresh surface investigated by [47].

The traditional protocols of mammalian cell culture commonly specify generated carbon dioxide and also comparative humidity. These dimensions for the system of micro fluids are

retained by positioning the complete system of micro fluids within an environmental chamber and also having permeability for gas materials: PDMS. Forry and Locascio [48] constructed a device that enabled on-chip to check the partial pressure of carbon dioxide by moving pre-equilibrated aqueous media along with control channels beside the device. Long-lasting mammalian cells culture within the microfluidic systems allowed without involving an incubator of cell culture or carbon dioxide independent media. Such introducing experimental projects are outstanding models which highlight the ability of microfluidic techniques for upgrading HTS through successfully controlled fluid handling as well as unescorted by the need for complex robotics. Hamon and Hong [49] had explained a new technique for analyzing values of cell-based IC by utilizing a microfluidic device comprising a unit of generation a gradient as well as micro-chambers for cell harboring. The cell-based analyses could be exhibited by utilizing 100-150 cells and nanoliter scale inhibitors on the current device. The three (H_2O_2 , Triton X-100, and CdCl_2) cytotoxic molecules IC_{50} values from the analogs dose response of every inhibitor on fibroblast NIH/3T3 cells successfully identified in on-chip investigations. The given device has the capability for small molecules screening on different types of cells as well as examining other cell behavior factors. Cell-culture platforms for drug testing, in microfluidic-relayed, vital tasks are efficiency, quantification as well as immobilization of cell specimens in a specific place for the cause of investigated drug exposure and calculations. Immobilization of cells profiles dynamically lies into the following categories: (1) cells adhesion on the surface of the substrate, (2) cells entrapment in porous polymeric materials, and finally, (3) cells apprehending behind membrane materials. Cells immobilization on the surface of the substrate for two-dimensional cell cultivation easily marked by suspended cells processed for increasing cell stickiness. Active cells adhesiveness and proliferation remarkably identified on the surface of the substrate based on several factors, includes chemistry [50], wettability [51], surface charge [52], microstructure [53] as well as the roughness of surface [54]. It is notable to efficiently administer surface factors like as to unify the cellular behavior on a surface. That is essential for two-dimensional cell culture-based drug testing. The evolution of efficient as well as user-friendly identification protocols is anticipated that they would increase the broad utilization of cell-culture based on microfluidic systems in drug testing in the coming era [41].

4.2 Microfluidics approach for analytical studies

Owing to some considerable benefits e.g. less reagent as well as strong utilization, limited reaction period, chances for *in-situ* use, less price, dynamic design, microfluidic designs intended for cellular studies have gained massive consideration. In addition to tissue engineering, microfluidic-based practices have been initiated to execute a crucial function in explorations within cellular biology, neurology, and medicine [55]. Assay reminiscent of cell-based is measured as capable for the functioning categorization along with detection of drugs, pathogens, toxicants, also odorants. A comprehensive re-evaluation on microfluidics concerning cellomics has been in print using Berg and Andersson that discussed the micro-devices used for cell sampling, trapping, and sorting, cell therapy, along with chemical analysis of cell [56]. Lee and colleagues [57] incorporated microfluidic devices for handling

of the cell, cytometry, di-electrophoretic cellular manipulation sorting, and common analysis of cells. Manz and co-workers have explained developmental history as well as miniaturization theory, microfluidic systems fabrications, and also microfluidic standard applications comprising preparation of the sample, sample injection, manipulation of the sample, reaction, separation, and detection [58-60]. Biological applications based on microfluidic techniques include cell culture, PCR, separation of DNA, sequencing of DNA, together with medical diagnostics were also verified. Currently, PDMS-based micro-devices exploited and evaluated in flow cytometry, cells seeding, in addition to diverse flow-based cell testing systems [61-63]. After many decades of progression, microfluidic devices have considered themselves as strong apparatus designed for cell-based calculations. Numerous cell directing techniques may be exploited keen on microfluidic devices used for cell-based calculations like magnetical, optics, mechanical, and also electrical utilizing procedure. The magnetical procedure is a vivid, versatile, as well as non-invasive cell utilization process. Due to huge experience and also well-configured procedures for a vast range of samples, the magnetically manipulated procedure can be freely unified along with microfluidics for cell-based assay. It is thought that the magnetic administration procedure will become more precise because of recent magnetic bead materials manufacturing as well as refinements method for alloying different ligands to magnetic beads.

Microscale dispensation techniques expedite the deliverance of new medicines to the souk abridged dispensation expenditure along with enhancing patient benefit. The study comprises the revised of entity bioprocess operations on the microliter scale exploiting any micro-well or microfluidic designs. Automation can improve investigational output; will aid the corresponding testing of challenging biocatalyst along with development opportunities [64]. Conversely, molecular drugs, numerous of the core doctrine incorporated in micro-well along with microfluidic trialing have corresponding applications in biological as well as regenerative drug process. Primarily, the capacity to accurately define automated environmental settings with normalizing operations have the colossal perspective to abet the stem cells differentiation plan [1, 65]. During the microfluidic device diffusional integration of laminar flow incessantly generates a constant chemical slope to substantiate this perception. Researchers investigated and customized chemotactic responses of *P. aeruginosa* PAO1 by using peptone and trichloroethylene (TCE) as an attractant and repellent with mutant PC4. The micro-device evidently explains that *P. aeruginosa* was tempted and repelled from peptone and TCE respectively. The microfluidic system does not merely smooth the progress of the chemotaxis information, also gives a handy perceptive of the motility method of *P. aeruginosa*. Nevertheless, it is a constructive device for the swift supervision in the investigational approach of bacterial chemotaxis [66].

The miniaturization, parallelization, as well as integration enabled by microfluidic systems, is somewhat similar to miniaturization as well as the integration of electronic components in microprocessors, which has transformed society. It has been regretted that microfluidics is a solution looking for a “killer” application [67]. The capability to use microfluidics for “digital biology” in which many assays are exhibited at the single molecule, single cell, or single-organism level is likely to be more important [68]. Major restriction in single cell analysis is solitary time-point calculations, except the probability to

analyze behavioral alterations of single cell/progenies. Incorporation of a spatiotemporal introverted cell lab-on-a-chip structure with controlled cultivation device allows entity cell formation underneath précised expansion surroundings together with semi-automated cell reclamation and advanced kinetic organization of the distinct cell progenies [69].

Various principle evidence of experiments has proven that such technique can be implemented to mechanisms of biology, promising to many broad bottlenecks which restrict progression in recent biology [70-73]. Controversially, such kinds of improvements are predominantly precise along with resolute by enterpriser's inquisition in commercializing microfluidics. Even though, the exploring factions continue to scrutinize the diverse aspects of microfluidics. Above all, universities have the indispensable infrastructure for constructing various, if not each, of the devices elaborated in this. In support of such universities which don't have the outline or skills, microfluidic creators are at hand at official websites of Caltech and Stanford Universities. Such creators will invent norm planned molds in addition to micro-devices based on PDMS at a less significant price to the consumer.

In the succession of the microfluidic system, incorporation of detection methods will be the vital key. Newly investigated detectors are quite capable, each constructed over optics devices, NEMS/MEMS or else CMOS reliant chemical sensors [74-76]. Thriving adaptation of novel techniques besides with microfluidics will construct more standardized plus flexible devices, furthermore, can corollary in larger recognition sensitivities than these recently practicable. Biological science has protracted ever since eight strip PCR tubes also ninety-six well plates, narrative apprehension has previously been given to numerous microfluidic-based assays into the device plus operation of natural systems, and the region of microfluidics will progress auxiliary to influence natural science [77]. The propinquity of one dimension in an array from 1 to 100 nm along with the Debye length, the dimension of biomolecules for example DNA or proteins, or constant slip length, supplementary to the tremendous power of the geometry provides distinctive characteristics to nano-fluidic devices [78].

5. Concluding remarks and future perspective

Recently, a range of microfluidic devices has been made available with diverse potentialities for biomolecule separation purposes. The operational bacterial array has also been used to present many obstacles that must be overcome before the industrial science mellow. An integrated lab-on-a-chip demonstrative framework, in light of the innovations summarized in this review, is promising. Various capacities e.g. fabrication process, and also precise bi-identification can also be achieved by joining with other propelled advancements. The arrival of the whole-cell biosensors and the entire cell array, both, setting a stage for the next step evolution. In the future, we expect to see additional examples of methods of detection, sample preparation, separations, and fluid manipulation incorporated onto microchips, with further emphasis on the production of self-contained and truly integrated lab-on-a-chip systems.

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All authors contributed equally by analyzing and reviewing the literature data. Final version is revised, read and approved by all authors.

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Figure 1. Schematic display of single layer microfluidics device generation.

Figure 2. PDMS-based microfluidic devices for embryo culture. (A) The WOW system, (B) A microfunnel (C) A microfluidic IVC channel. In (B) and (C), the left and right figures show reservoir or microfluidic channels of the system and embryo and fluid motions, respectively (Reproduced from Ref. 23, an open access work, published under CC BY-NC-SA 3.0 license).

Figure 3. Configuration and function of the microfluidic device. A. Schematic representation of the device with five culture chambers arranged in a pentagonal array and applied for the parallel culture of protoplasts. A center sample input was designed for the introduction of various liquids, including protoplast suspension, rinsing solution, culture medium, and dye. The square in the red dotted line corresponds to B. Double micro-column lines in each chamber were designed to promote trapping of protoplasts while the seeding process (B). The gap between micro-columns was 20 μm . C. Plan (up) and elevation (down) of the microfluidic device. Hydrostatic pressure was used to realize material transportation using a small volume difference. D-I. Chemical fusion of the tobacco mesophyll protoplasts was performed in the microfluidic device using polyethylene glycol (PEG). The time when the two protoplasts contacted is considered as the start of fusion (i.e., 0 s) (D), followed by 10 s (E), 30 s (F), 50 s (G), 80 s (H), and 190 s (I) (Reproduced from Ref. 38, an open access work, published under CC BY-NC-SA 3.0 license).

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