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Applications of Microfluidics in Biomedical and Pharmaceutical Fields -An Overview

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Abstract: ***The precise manipulation of fluids at the microscale level within minuscule channels measuring tens to hundreds of micrometres is the subject of the multifaceted field of microfluidics. This technology has transformed the pharmaceutical industry by enabling miniaturized, high-throughput, and economical solutions for drug discovery, formulation, and delivery. The creation of sophisticated drug delivery systems like nanoparticles and liposomes has become far simpler due to this method that can precisely control fluid dynamics, which also enables faster reaction kinetics and better drug encapsulation. Beyond drug formulation, microfluidic platforms also enable disease modeling, toxicity assessment, and pharmacokinetic/pharmacodynamic analysis, providing a quick and efficient alternative to conventional techniques. In addition to this, devices like microfluidic chips also combine several analysis processes into a single device with less reagent consumption and with enhanced research encouragement. Furthermore, microfluidics plays an important role in personalized medicine and point-of-care diagnostics, offering rapid, more accurate testing for a customized treatment strategy. The increased use of microfluidics in pharmaceutical research is promising to facilitate faster drug discovery, enhance individualized medicine, and improve point-of-care diagnostic testing. This paper discusses the definition, importance, and uses of microfluidics in the pharmaceutical field based on its implications for the future of drug discovery and healthcare.***

Keywords: Microfluidics, Drug Discovery, Pharmaceutical Research, Point-of-Care Diagnostics, Personalized Medicine.

Abbreviations:
OOC: Organ-on-a-Chip
STCM: Surface-Tension-Confined Microfluidic
POC: Point-of-Care
PM: Placental Malaria
CSA: Chondroitin Sulfate A
5-FU: 5-Fluorouracil
mCCA: Microfluidic Cell Culture Assay
SERS: Surface-Enhanced Raman Scattering
6MP: 6-Mercaptopurine
LoC: Lab-on-a-Chip
IVD: In-Vitro Diagnostic

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I. INTRODUCTION

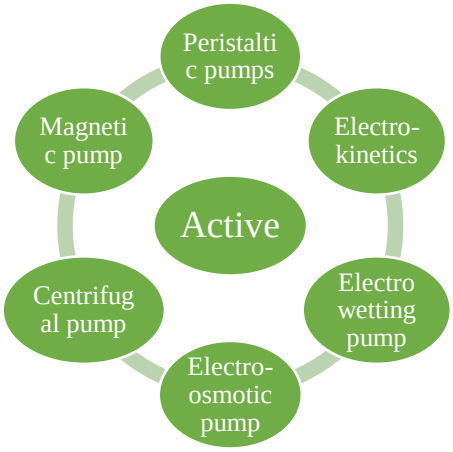
As Whitesides stated, microfluidics is "the science and technology of systems that process or manipulate small amounts of fluids, using channels with dimensions of tens to hundreds of micrometers" [1]. In simple terms, microfluidics is a constantly changing technology that deals with the maneuvering of fluids limited to minute channels. Microfluidics manipulate the fluid flow inside a microchannel system. Droplet-based microfluidics generate and manipulate monodisperse drops ranging from femtoliters to nanoliters in volume within an immiscible phase. This method benefits from its microscale nature and allows the integration of sample preparation, analysis, and detection with high throughput and precise control [2]. The ability to compartmentalize cells within picoliter droplets in microfluidic devices has opened up a wide range of strategies to extract information at the genomic, transcriptomic, proteomic, or metabolomic levels from large numbers of individual cells [3].

Before the advent of Microfluidics technology, scientists used a variety of liquid-handling methods for assays that involved petri dishes, culture bottles, and microtitre plates (also called microplates) [4]. In the 1980s, microfluidics technology gained popularity and helped several disciplines, including the biomedical and pharmaceutical fields. By utilising fewer samples and reagents, facilitating quick detection, and simulating actual biological conditions, it aids in the development of reasonably priced devices in biomedical research [5].

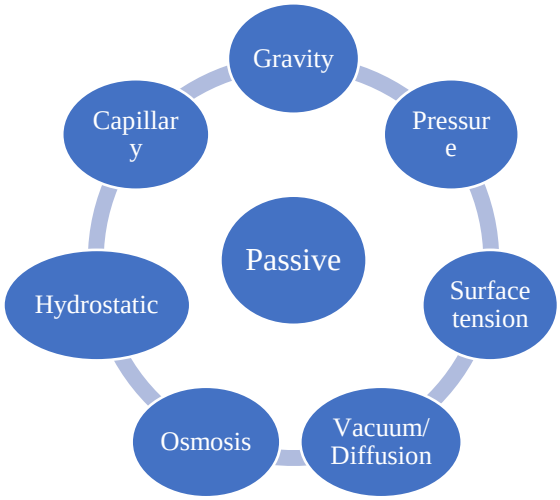
This interdisciplinary field combines principles from various domains, such as engineering, physics, chemistry, biology, and nanotechnology, to create devices, often called lab-on-a-chip systems. These devices have many applications, particularly in medical diagnostics, chemical analysis, and biological research.

II. TECHNIQUES OF MICROFLUIDIC OPERATION

These techniques determine the manner of fluid flow on the microfluidic devices. They are bifurcated as active and passive methods as depicted in Fig 1 and 2. An external source or pump is used to analyse, move, or transport biological samples in the active method, and passive methods do not employ an external source for the same [6].



[Fig.1: Active Microfluidic Operation]



[Fig.2: Passive Microfluidic Operation]

III. PRINCIPLES USED IN MICROFLUIDICS

A. Laminar Flow

In many microfluidic workflows, processing particles, cells, and droplets for coating, labeling, analysis, and reactions is essential but multi-step procedures are frequently laborious and slow. The laminar flow in microchannels can be used to speed up and simplify these processes. This can be accomplished by controlling the movement of items within the streams using different forces or by modifying the flow streams surrounding target objects for localised perfusion [7].

B. Surface Tension

Surface tension is crucial for droplet generation, transport, mixing, and manipulation in microfluidic systems because it outweighs gravitational and inertial forces. Surface-tension-confined microfluidic (STCM) device is an emerging class of microfluidic systems that use surface energy to control fluid movement [8].

C. Diffusion

It is a process driven by the presence of a concentration gradient within a fluid, where molecules naturally move from regions of higher concentration to areas of lower concentration. This movement occurs to achieve equilibrium and is influenced by the type and size of the molecules involved [9]. This process helps in the movement of fluids passively across the channels on a microfluidic device.

IV. APPLICATIONS OF MICROFLUIDICS IN BIOMEDICAL AND PHARMACEUTICAL FIELDS

Microfluidics, a field that combines precise analysis methods and miniature devices, holds great promise for biomedical applications such as medication administration, DNA amplification, cell culture, point-of-care (POC) diagnostics, and more [10]. The applications of LOC devices, which are on the rise, are:

A. Drug Discovery and Development

Microfluidic technology finds its major application in the pharmaceutical industry as a tool for drug discovery. Traditional methods of drug discovery are often tedious, labor-intensive, and expensive. Microfluidic chips can conduct thousands of biological or chemical reactions simultaneously, allowing pharmaceutical companies to rapidly screen large libraries of compounds for potential drugs. To back this claim Lambert et al. developed a modular microfluidic platform for crystallization research, providing a versatile and adaptable instrument that doesn't require surfactants. Irbesartan, Rimonabant, and Aripiprazole were the stable and metastable drug forms whose solubility in different solvents was tested by researchers. Additionally, they investigated the nucleation patterns of sulfathiazole in acetonitrile and water, finding that cooling speeds affect both polymorphism and nucleation. This microfluidic platform enabled them to conduct solubility studies, nucleation statistics, and polymorph screening. In conclusion, they discovered three unknown forms of Sulfathiazole whose XRD patterns and Raman spectra did not match any referenced form. They inferred from these findings that their microfluidic platform was a potential game changer for polymorph screening that could be used in the pharmaceutical industry to discover new forms of active pharmaceutical ingredients (API) [11].

Tissue/Organ-on-a-chip (OOC) technology augments the drug development process by providing alternatives to pre-clinical (animal) testing of the API. Animal models are not the best representatives of the human physiologic environment due to this reason; in vitro tests lack physiological relevance. These constraints are overcome by microfluidic organ-on-a-chip models, which replicate physiological flow conditions and three-dimensional cell development. By connecting artificial organs on a single chip, these models enable researchers to assess drug pharmacokinetic profiles, improving the precision and predictiveness of drug testing, hence minimizing the risks of failure in later stages of clinical trials [12].

B. Disease Modelling

Since direct observation of biological molecules' interference is limited, human diseases are governed by complex mechanisms that are inherently challenging to comprehend. Therefore, disease modeling techniques are very important for comprehending the pathophysiology of diseases and creating cutting-edge treatment plans [10].

There is growing interest in creating microfluidic organs or tissues-on-a-chip for two main reasons: first, direct human experimentation is not permitted, and second,

animal models do not accurately represent human physiology. Additionally, these gadgets might save costs and accelerate the drug discovery and testing process [13].

To understand disease modelling better, we can consider a study conducted by Mosavati et al. where a team of researchers developed a placenta-on-a-chip model to mimic the nutrition exchange between the mother and foetus when placental malaria (PM) is present, the model simulates the placental barrier by cultivating human umbilical vein endothelial cells (foetal side) and trophoblast cells (maternal side) on opposing sides of an extracellular matrix gel in a microfluidic device. This model helped in evaluating the permeability of the placental barrier for glucose to compare the effects of the presence of CSA (chondroitin sulfate A) - adherent malaria-infected and uninfected erythrocytes. The results from this study would be beneficial for comprehending placental malaria pathology and developing appropriate treatments for the same [14]. Similarly, Miny et al. drafted a review, where they presented the efficacy of microfluidic devices and models for the in vitro study of neurodegenerative disease pathophysiology and pharmacology. They found that these devices enabled researchers to investigate the propagation of peptides or molecules along neurons and synapses, along with some neuroinflammatory aspects involved in these diseases. They concluded that OOCs like Brain-on-a-chip could be used for CNS and PNS modelling to examine molecular interactions and to perform pharmacological studies along with drug screening [15].

Disease-on-chip models attract the curiosity of many due to their potential to mimic the disease microenvironment, regulatory factors, and physiological circumstances surrounding organs. The effects of the environment, cell patterning, cell-to-cell communication, and other factors can be controlled for emulating the organ and relevant diseases.

C. Pharmacokinetics and Pharmacodynamics

Determining the target drug's pharmacokinetic, pharmacodynamic, toxicokinetic, and toxicodynamic properties is essential for the drug development protocol [16]. Understanding pharmacokinetics (how a drug affects the body) and pharmacodynamics (how a drug impacts the body) requires a thorough grasp of lab-on-a-chip technology.

Sung et al. worked on a microfluidic system for drug testing that was created using a pharmacokinetics-pharmacodynamics (PK–PD) model. To explain the PK–PD behaviour of 5-Fluorouracil (5-FU), a mathematical model was created and matched to experimental data from a microfluidic cell culture assay (mCCA). Through the integration of mCCA and PK-PD modelling, this platform provides a new in vitro/in silico method for more precise and effective drug testing [17].

Fei et al. introduced a programmable microfluidic device for the pharmacokinetic investigation of numerous medications in diverse cell types. The study used intracellular surface-enhanced Raman scattering (SERS) spectra to track the pharmacokinetics of methimazole (MMI) and 6-mercaptopurine (6MP) in various cells. According to the findings, both medications entered cells in 4 minutes and were eliminated 36 hours later. The distribution of drugs within cells was further monitored by SERS mapping. This study shed light on the synergistic effects of drugs. The study concluded with the fact that microfluidic system is a useful tool for drug design and research because it allows for high-precision, real-time monitoring of drug behaviour [18].

D. Personalized Medicine

Personalized medicine tailors treatment to the individual characteristics of each patient, and Lab-on-a-chip (LoC) technology is a key enabler of this approach. Lab-on-a-chip devices can analyse a patient's specific genetic makeup, biomarkers, and other individual factors in real-time. This can lead to more precise drug prescriptions, dosage adjustments, and treatment plans based on an individual's unique biological profile.

For instance, exosomes are small extracellular vesicles (30–150 nm) carrying lipids, proteins, mRNAs, miRNAs, and DNA, which play a significant role in cell-to-cell communication and have great promise for diagnostic and therapeutic purposes. Their ability to remain stable in bodily fluids makes them useful as biomarkers, making liquid biopsies possible. However, problems including limited yield, lengthy processing periods, expensive prices, and a lack of standardisation make it difficult to isolate and analyse pure exosomes. Starting from exosome isolation to exosome detection and analysis, microfluidics has now made it possible to tackle these issues efficiently. This goes on to prove that personalised liquid biopsies can also be done using this technique [19]. The domain of personalised or precision medicine has made great progress in oncological medicine thus, propelling cancer therapy to new heights [20].

E. Cancer Diagnosis

Cancer is still one of the primary causes of death all over the world. In this case, by the time symptoms start to show, it might be too late for a patient. Early detection and ongoing monitoring can significantly impact a patient's chances of survival [21]. The optimal treatment for each patient is defined by personalized cancer therapy which incorporates information from a variety of diagnostic tests and the patient's medical history [22].

Keeping this in mind, Wie et al. created the Auto-ICell system, a 3D-printed microfluidic chip combined with algorithms that analyse real-time images. They incorporated droplet microfluidics technique which offers a distinctive approach for single-cell and molecular analysis, allowing high-throughput screening with utmost precision. Uniform droplets with sizes ranging from 70 µm to 240 µm, produced at a high throughput of 1,500 droplets per minute, enclose breast cancer cells. Blister formation in cell and cell circularity are detected and measured in the fluorescent field. They concluded their research by saying that the Auto-ICell system enables swift, economical device fabrication and automatic monitoring of single-cell morphology and apoptosis. It improves the capabilities of droplet-based single-cell analysis while lowering costs and increasing efficiency, which makes it a useful tool for clinical and biological research [23].

Mollica et al. also worked on a microfluidic chip that intended to duplicate important stages of the cancer metastasis cascade in

a controlled environment. With a combination of matrigel and breast cancer cells, the chip's two interconnected channels simulate malignant tissue and a vascular compartment lined with endothelial cells, respectively. Through pro-inflammatory stimulation that modifies vascular permeability, the model successfully mimics the intravasation, vascular adhesion, extravasation, and invasion of cancer cells. This platform offers a useful resource for researching the processes of metastasis and evaluating possible therapeutic approaches in a regulated in vitro setting [24].

Recently, Lipreri et al. presented a polydimethylsiloxane (PDMS)–agarose microfluidic system, used for creating tumour spheroids originating from patients and assessing individual treatment responses. Reliable drug screening is made possible by the device's effective production of 20 uniform spheroids (about 300 µm in diameter) in 24 hours. The study evaluated the effects of doxorubicin on osteosarcoma and chondrosarcoma spheroids using a proprietary imaging index and found that the maximum dosage (10 µM) reduced cancer cell viability by around 75%. Within 48 hours, osteosarcoma spheroids showed increased sensitivity. They finished by concluding that owing to this platform, personalised cancer therapy has a potential in vitro model, despite significant implementation issues [25].

F. Point-of-Care Diagnostics

Quick and accurate diagnosis of samples of various body fluids is made possible by LoC (microfluidic) devices. (13) The PoC tests need to be simple enough to be performed by untrained individuals at the patient site. These tests must produce rapid, accurate, sensitive, and specific results at a low cost, preferably in a short time of a few seconds to a few hours. This will enable non-trained personnel to input a sample of extracted body fluid (such as blood, urine, saliva, sweat, etc.) into the machine and receive informative results with minimal user involvement. Fully integrated lab-on-a-chip (LoC) technologies, encompassing all necessary analysis stages in a single device, have the potential to greatly improve point-of-care medical diagnostics [26].

Sista et al. demonstrated a Digital Microfluidic (DMF) platform based on electrowetting that combines testing and sample processing on a single chip. They solidified its utility firstly, by illustrating an immunoassay for cardiac troponin I based on magnetic beads that use whole blood and yield results in less than eight minutes. Secondly, A droplet was moved between two heat zones to complete a 40-cycle real-time PCR in under 12 minutes. Thirdly, sample preparation involves the use of magnetic beads to detect human genomic DNA and bacterial and fungal illnesses (methicillin-resistant *Staphylococcus aureus* and *Candida albicans*). The electronic control of fluid movement makes this DMF platform incredibly portable. They concluded by saying that a single chip may be redesigned for multiple diagnostic tests due to its modular and scalable design, which makes multifunctional testing affordable and available at the point of care [27].

Similarly, Li et al. also foresaw the advantages of microfluidic technology, and together they presented a handheld microfluidic liquid handling device that is completely automated and controlled by a smartphone. Its Important characteristics include the usage of a small pneumatic system and elastomeric on-chip valves, which can execute a sandwich immunoassay using beads without the need for human assistance. It is light and has small dimensions. It operates for 8.7 hours using less power. Seeing these benefits, they came to the conclusion that complex liquid handling for a range of biochemical and cell-based tests, including PCR, flow cytometry, and nucleic acid sequencing, can be automated using this portable system. Its incorporation with biosensors may advance the development of portable in-vitro diagnostic (IVD) instruments [28].

These diagnostics are particularly valuable in developing countries or areas with limited healthcare infrastructure. For instance, microfluidic devices have been used for the rapid detection of infectious diseases such as HIV, tuberculosis, and malaria. The data collected can guide pharmaceutical interventions and help track the effectiveness of treatments [29].

G. Drug Delivery Systems

Microfluidic chips can be designed to control the release of drugs at specific rates, locations, or in response to specific physiological triggers. This is particularly beneficial for drugs that require precise dosing or for therapies where the drug must be delivered to a particular part of the body. Such microfluidic delivery systems offer benefits such as accurate dosage, focused delivery, continuous and regulated drug release, the potential for multiple dosing, and minimal side effects [13].

Core-shell drug carrier particles are made especially for regulated drug release at specific sites, reducing adverse effects and enhancing therapeutic effectiveness. The creation of core-shell drug carriers using microfluidic chips is more economical than conventional approaches and allows for the production of particles with consistent sizes and shapes at both the nanometer and micrometer scales. This goes onto prove that microfluidic systems are a possible substitute for traditional technologies in drug delivery applications due to their significant advantages, which include better efficiency, repeatability, and integration capabilities [30].

Another novel drug delivery system known as Nanoparticles, are beneficial in cancer treatment as they act as functional carriers that make it possible to administer both hydrophilic and hydrophobic medications. The development of drug delivery systems based on nanoparticles has been completely transformed by the advent of microfluidic platforms, which provide an accurate and effective production method. To improve therapeutic efficacy in both in-vitro and in-vivo applications, microfluidics enables the fabrication of nanoparticles with customisable physicochemical properties such as size, size distribution, and morphology [31]. Along the same lines, Sartipzadeh et al. tested Chitosan (CS) based nanoparticles, which were made using droplet microfluidics.

The test results revealed that adjusting various parameters could produce CS droplets of different sizes and geometries, customized for applications like drug delivery, tissue engineering & cell encapsulation, biosensing & bioimaging [32]. These evidence further solidify the usefulness of microfluidics in improving

various drug delivery systems.

H. Toxicity Testing

One of the major challenges in pharmaceutical research is ensuring that new drugs are safe for human use. Traditional toxicity testing methods, often involving animal testing, are not only expensive and time-consuming but also raise ethical concerns. These microfluidic chips provide a more ethical and efficient alternative for toxicity testing. Analysing drug toxicity is imperative for the safe advancement of new medications which typically rely on animal and cell-based models, but conducting in vivo tests has drawbacks such as ethical concerns, high expenses, and the inability to perform quantitative analysis or high throughput screening [33].

Cellular or animal models are usually required for toxicity or drug safety testing [16]. Microfluidic chips can be engineered to imitate the human body machinery, often referred to as "organ-on-a-chip" systems [34]. These models can replicate the physiological conditions of the liver, heart, lungs, or kidneys, allowing researchers to observe how a drug affects these tissues in real-time [35]. This provides a more accurate prediction of a drug's toxicity and reduces reliance on animal testing. For example, Toh et al. created a 3D HepaTox Chip, a microfluidic device intended for in vitro drug toxicity assessment, with the purpose of predicting liver toxicity [36]. The microchannels present on this device preserve the metabolic and synthetic processes of hepatocytes by forming a three-dimensional microenvironment. This chip then allowed for drug testing at several dosages at once, generating concentration gradients for investigation of dose-dependent responses and a strong relationship between in-vivo LD_{50} values and chip IC_{50} values. Another study by Tirella et al. presented a microfluidic gradient maker (GM) for in-vitro toxicology analysis of local anesthetics bupivacaine and lidocaine on cell cultures [37]. The design of this device was made using the COMSOL Multiphysics technique and the microchannels were Fabricated using soft lithography with PDMS [38].

V. CONCLUSION

With the advancement of technology, it is a given that the medical field must improve with time. Microfluidic systems can be used for drug screenings in the preclinical phase and are more reliable and have better control than traditional in vitro assays, to accurately imitate the various organ systems in humans. Hence, this technology is modifying the pharmaceutical industry by offering faster, more accurate, and cost-effective solutions for drug discovery, development, and diagnostics. From high-throughput screening and pharmacokinetic simulations to personalized medicine and advanced drug delivery systems, Microfluidics can drastically augment the efficiency of pharmaceutical processes. As this technology continues to evolve, it is likely to play an even greater role in advancing personalized therapies and reducing the costs of bringing new drugs to market, ultimately benefiting patients worldwide.

DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

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- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Authors Contributions:** The authorship of this article is contributed equally to all participating individuals.

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