

MICROFLUIDIC DEVICES AS A TOOL FOR DRUG DELIVERY AND DIAGNOSIS: A REVIEW

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ABSTRACT

Microfluidic devices are a good example of the collaboration of chemical, biological, and engineering sciences. Microfluidic devices emerge as an in fluent technology which provides an alternative to conventional laboratory methods. These devices are employed for the precise handling and transport precise quantities of drugs without toxicity. This system is emerging as a promising platform for designing advanced drug delivery systems and analysis of biological phenomena on miniature devices for easy diagnosis. Microfluidics enables the fabrication of drug carriers with controlled geometry and specific target sites. Microfluidic devices are also used for the diagnosis of cancer circulating tumor cells. In the current review, different microfluidic drug delivery systems and diagnostic devices have described.

Keywords: Microfluidic drug delivery system, Circulating tumor cells, Cancer diagnosis, Lab on chip

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INTRODUCTION

Microfluidic devices are an excellent tool for drug delivery and evaluation of drug effectiveness at the cellular level. Microfluidics devices consist of a micro-electro-mechanical system. In recent years the microfluidic devices are emerging as an important tool for cancer detection. These devices are used to analyze the fluids which are present in the micrometer-sized channels. There are many types of microfluidic devices that have been developed [1]. Cancer is also known as a malignant neoplasm, which includes a broad group of diseases having unregulated cell growth. In this disease, there is uncontrollable division and growth, which causes the formation of malignant tumors. This malignant tumor can invade multiple organs and spread to the whole body. The leading cause of death throughout the world is due to cancer [2]. At present many treatment options are available for cancer, which includes surgery, chemotherapy, radiation therapy, and palliative care. All the above treatments depend upon the type, location, and grade of cancer [3]. These treatments had disadvantages as these are very expensive and had various side effects. Circulating tumor cells (CTCs) are rare cancer circulating in the blood vessels in cancer metastasis. The detection of CTCs provides a rapid and less painful diagnosis method as compared to tissue biopsy. This method is a very good alternative to the previous invasive biopsy. It is also termed as liquid biopsy as only blood is withdrawn for cancer detection [4]. Rapid research is going on to develop microfluidic devices for drug delivery and detection of CTCs. The most widely accepted devices are chromatography, quantum dots, surface plasmon resonance, amperometric based sensing, and nuclear magnetic resonance [5]. In this review, types of microfluidic devices, drug delivery, and screening have been described. The review is based on data obtained from Pubmed (<http://www.ncbi.nlm.gov/pubmed>) and Google scholar with preference given to the data obtained during the last 10 y. The research items were varied. The main research terms were microfluidic devices, microfluidic drug delivery system, microscale channels, microneedles for drug delivery, circulating tumor cells, diagnosis of CTCs by the microfluidic system, lab-on-chip and various combinations of these terms.

Microfluidic drug delivery system

Drug delivery using microfluidic devices on unhealthy tissues is done with high precision and a localized manner. Drug delivery is done by small particles in the form of bio capsules, microparticles, and nanoparticles. Another drug delivery system used is the microneedles. Microfluidic devices have many advantages over the conventional delivery system, such as precise target delivery,

controlled release, precise dosage, and multiple dosages. These devices are used for the direct drug delivery and fabrication of drug carriers. The side effects of another system that are overcome in this system are toxicity, inflammation, and wastage. Microfluidics drug systems are used for peptides, proteins, and DNA based drugs to the target sites. This method also protects drugs from enzymatic degradation and the immune response of the host body [6].

Fabrication of drug carriers

The drugs for proper action should reach the target organ without any damage. It should be protected from stomach acid, enzymes, and immune cells. The drug carriers of different polymer materials are used as drug carriers [7]. Now day's microfluidic devices are used for the fabrication of drug carriers by mixing different particles. The drug carrier should be biodegradable, biocompatible, and nontoxic. The polymers used as drug carriers are made more suitable by modifying using microfluidic devices [8]. This system helps to fabricate uniform particles and thus provides better bioavailability and controlled release of drugs. A microfluidic device fabricates to produce nanoparticles from PLGA-PEG and PLGA. The Microfluidic system also enhances the ratio of drug load into the particle and encapsulation of multiple drugs into one particle by mixing particles of different nature and phases [9].

Microfluidic devices can fabricate drug carriers from nano to micro size and also maintain the uniform size. The self-assembled microfluidic fabrication is done by simple micromixer, hydrodynamic flow focusing and staggered herringbone micromixer (fig. 1-3). In hydrodynamic flow, a core of carrier solution, which consists of a surfactant mixer, is focused on the microchannel by surrounding streams of the miscible buffer. The microfluidic devices can also control the uniformity of the drugs carrier by geometry, flow rates, and diffusion coefficient of miscible streams. Micromixers are of two types active and passive depends upon the force applied. In an active mixer, the external source is used to mix two fluids of different phases. The forces like acoustic and ultrasonic waves, magnetic particles are used to exert force for mixing the particles. In the passive type, mixing depends upon the hydrodynamic flow of fluids no external force is applied as shown in fig. 1 [10]. Staggered herringbone mixer depends upon the chaotic advection. It consists of repeated patterns of grooves on the bottom surface of the microchannel. The grooves internally contain two channels of different lengths (one longer and another shorter) which are connected at an angle of 45 ° as shown in fig. 2. Helical motion is created to move the fluid inside the channel by the grooves. This method provides high efficiency of mixing drug carriers [11].

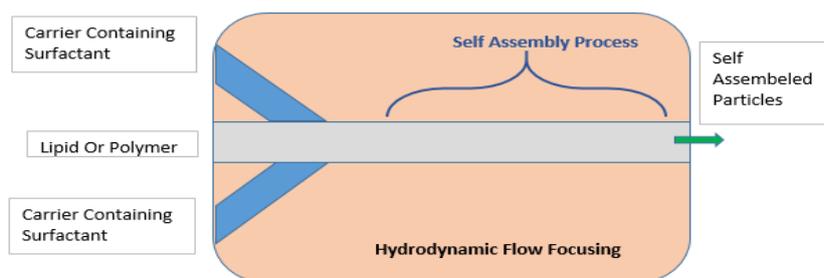


Fig. 1: Hydroderma flow micromixer for the fabrication of microfluidic drug carriers [12]

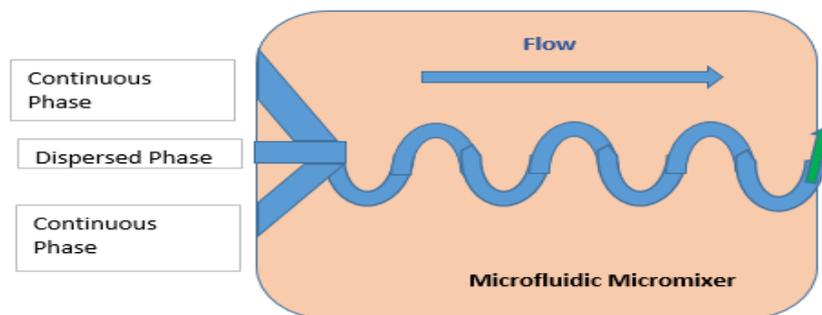


Fig. 2: Simple microfluidic mixer for the fabrication of microfluidic drug carriers [12]

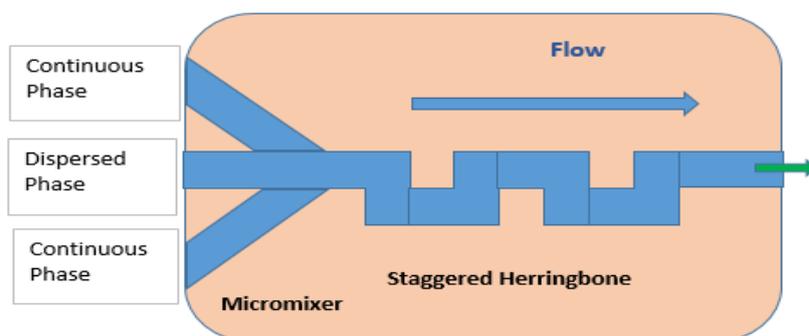


Fig. 3: Staggered herringbone mixer for the fabrication of microfluidic drug carriers [12]

Droplet-based carriers

It is the most widely used method for the fabrication of carriers with high reproduction and homogenous drug-loaded particles. This method is also used for the production of microcapsules, microbubbles, and microgels [13]. This method produced a particle of larger size (microparticles), which are more stable and deliver a high amount of drugs for a longer time. T junction, co-flowing, and flow-focusing are corner geometries used in the fabrication of drugs carriers. T junction two immiscible fluids are mixed by an intersection at a point where two channels converge to form a "T" shape. In co-flow, the flow of the fluids is in the same orientation. Two microchannels are present, which are immersed in each other. The inner channel contains the continuous phase and the outer channels have a dispersed phase. Inflow focus both phases are in contrast directions and mixed at the intersect present at the end of the inner channel as shown in fig. 4 [14].

Direct delivery of drugs

Microfluidic devices are also used for the direct delivery of drugs to the target tissues. The transdermal delivery is the most important method for direct delivery into the skin. The most widely used methods for transdermal administration of the drugs are hypodermic needles, topical creams, and transdermal patches. The skin has a barrier layer stratum corneum layer, which prevents the

entry of drugs into the body and needles are a very painful mode of drug delivery. A new form of a delivery system called the microneedles helps to enhance the delivery of the drug by penetrating this skin barrier and overcoming the various problems associated with the conventional method of delivery. The primary principle involves disruption of the skin layer, thus creating micron size pathways that lead the drug directly to the epidermis or upper dermis region from where the drug can directly go into the systemic circulation without facing the barrier. Microneedles puncture the skin, excluding the nerve-rich area and get penetrates the epidermis of the skin. The drugs transport drugs directly into the organs across the skin barriers [15]. Microneedles are sharp and less painful, which puncture the skin with a small dimension. After puncturing the skin, needles are removed and drugs are inserted into the skin in the form of microgels and microlotions. In some microneedles, the drugs are coated on the needles and the drugs are dissolved into the skin directly. Microneedles are also made up of biodegradable material and after the insertion, the microneedles get dissolved inside the body, and drugs are released [16].

Camptothecin, which is an anticancer drug, has encapsulated with polymer polyvinyl alcohol and poly lactic-co-glycolic for better delivery in cancer patients. Doxorubicin is another anticancer drug that uses microfluidic devices as a carrier for hepatic cancer treatment [14].

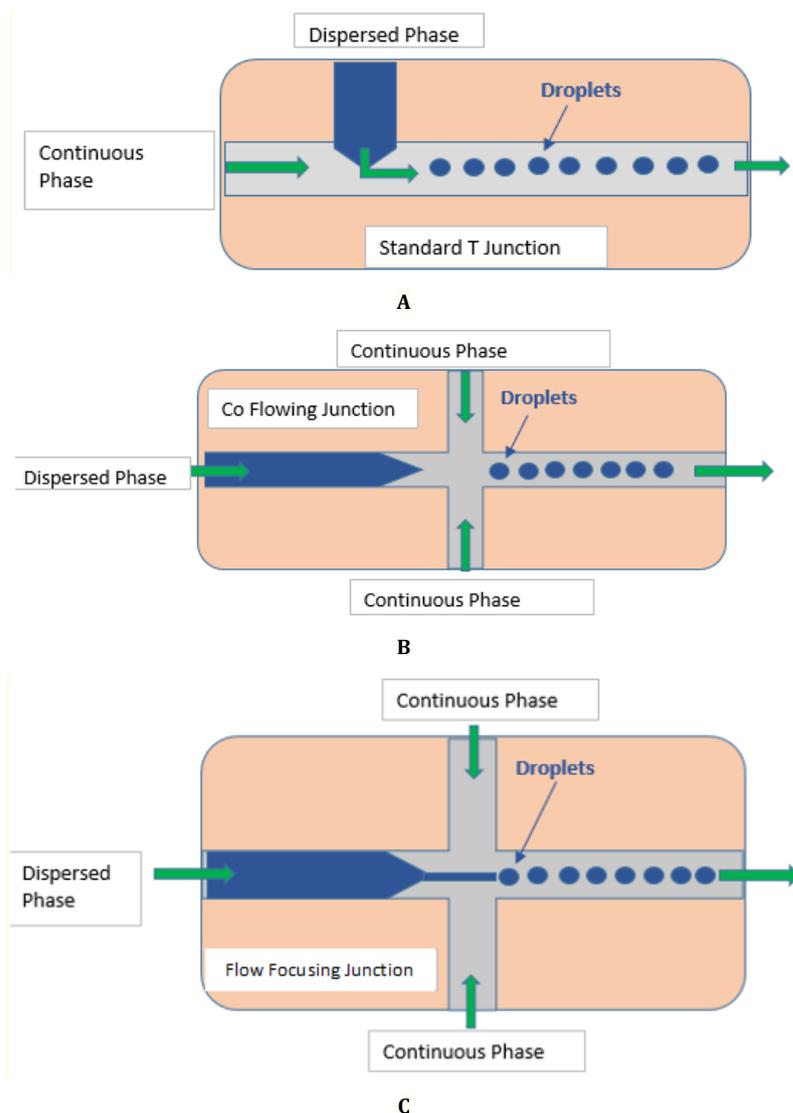


Fig. 4: Droplets microfluidic platforms (A) T-junction; (B) co-flowing junction and (C) flow-focusing junction [12]

Diagnosis of cancer by microfluidic devices

Labeled microfluidic devices

Immunomagnetic affinity-based capture: This method is based on the affinity of the target cells. A specific antibody is functionalized to the microchannel walls and the microstructure. This method involves two strategies positive enrichment in this the sample containing CTCs are pumped through the microchannel and the cells with the same affinity to the functionalized antibody get captured and the rest of the sample move freely through the microchannel. In the negative enrichment, the CTCs cells flow freely through the sample and the sample gets captured by the functionalized surface [17].

A new generation of microfluidic devices are developed by combining different methods such as cell immunoaffinity to the magnetophoresis in which a force is exerted by a nonuniform magnetic field over a superparamagnetic particle. This force helps to pull and push the particles according to the magnetic susceptibility of particles. If the particle has higher susceptibility than the suspending medium, particle is pulled towards the zone of high gradient and vice versa [18]. CellSearch device is the only clinically approved testing system by FDA for CTCs detection. In this, the magnetic field is used to capture CTCs labeled with ferrofluid nanoparticles. Then CTCs are stained with pan-cytokeratin C biomarker specific for CD45 CTCs. This device is widely used for breast, prostate, and colorectal cancer. Isoflux is another device that

worked on the same technique as above. In this, the sample is passed slowly through the device. This method has high sensitivity in the detection of CTCs from hepatocellular carcinoma cancer [19, 20].

Dielectrophoresis based capture

In this method, the electrokinetic force is used to capture the CTCs. The device has a nonuniform electric field generated by an electrode array. This nonuniform electric field induces a polarizing effect on the neutral cells. The force exerted is of two types: positive in which the particle is attracted to the electric field when the particle is more polarized than the medium and negative when the particle is less polarized and gets repelled from the electric field [21]. The method depends upon the dielectric potential and gradient of the electric field square. This method is further categorized into 3 types. eDEP in which electrode array is used to generate an electric field square gradient. iDEP (Insulator DEP) in which an insulator is added into the (electrode DEP) to creates an electric gradient and cDEP (contactless DEP) in which liquid electrode is used to generate the electric gradient and separated by an insulator from the channel [20]. This method is low cost, more specific, and small sample volume. DEP microfluidic devices are modified by integrated with the chip to improve the performance of the device. The chips are made up of polydimethylsiloxane (PDMS). The lap on a chip device is used to detect MCF-7 human breast cancer from HCT-116 breast cancer [22].

Labeled free microfluidic devices

Size based filtration: This method is based on the size of cells and filters are used for the separation of cells. CTCs and blood cells are separated based on morphology. The sizes of CTCs are in between 17-55 μm , which is larger than the normal blood cells. VyCap micro sieves are an example of a microfluidic device based on the filtration system. This device has a silicon nitride membrane with a pore size of 5 μm . The sample is passed through the filter and cells get separated based on size and morphology [23].

Density-based filtration: In this method, separation is done based on a density gradient. The sample is centrifuged and blood forms different layers: Upper plasma layer, middle buffy coat layer, and at bottom RBCs layer based on cell density. The CTCs are identified by immune-fluorescence staining [24].

Chromatography: The sample having the target particle (mobile phase) passes through the stationary phase and the particle are separated by different speeds. The system of this device is very complex. GM-MS has been reported to identify biomarkers of various kinds of cancers. HPLC and LC-MS are other useful tools reported to detect body fluid and cancer [25].

Surface plasmon resonance

It is an optical bioassay, high sensitivity, and real-time monitoring method. It is a label-free method which depends upon the changes in the refractive index close to the surface. The method depends upon the resonant oscillation of free electrons on the surface of the metal which presents near a dielectric medium. When a molecule binds to the metal surface the dielectric properties of the interface change and detected by the photodetector [18]. Various studies have shown the detection of cancer cells by this system such as VEGF breast cancer (100 pg/ml), AFP liver cancer (500 pg/ml), f-PSA prostate cancer (100 fg/ml) [26, 27, 28]. This method is improved by installing a CCD camera for image capturing [29]. Also, the system is combined with the classical sandwich enzyme assay. In this, the system antigen is a sandwich between a primary antibody and a labeled antibody [30]. This system is also coated with

nanoparticles (Au and silver) which amplify the signal and are rapidly detected by the detector [31].

Other microfluidic systems

A system was prepared for the detection of A549 cancer cells by integrating the surface of a microfluidic device with a piezoelectric sensor. The system works by suspending superparamagnetic microbeads into a nickel pillar array and the detection was done by the analyses of the accumulated mass of cells resonant character at the piezoelectric sensor. This is a low cost and high efficient system [32].

A biomicrofluidic device was designed to detect a breast cancer cell line (MCF-7). The system is fabricated by lithography based microfabrication technique. In this device, a weir-type filter is present which capture the fluorescent-labeled cells. The detection is seven times stronger than the conventional glass slide method [33].

GILUPI cell collector is an antibody-coated device, which captures the target cells *in vivo*. The device is inserted through a standard venous cannula into the cancer patient's vein. The blood is volume-limited in the traditional immunoaffinity devices overpower as it can detect rare CTCs in 1.5-3 liters of blood in 30 min. This device consists of stainless steel wire (16 mm) with a rounded tip (2 mm). The tip has a 3D antiEpCAM antibody functioned layer which captures the rare CTCs flowing in the blood of cancer patients [34].

A label-free novel microfluidic device was designed for the improvement of the diagnostic technique. In this, the cell size and deformability are used as a biomarker for diagnosis. A high throughput optical detection system was integrated with the lateral equilibrium position of cell deformability [35]. Exosomes and ctDNA are also used as a biomarker in cancer detection. CTCs are very rare in the early stage of cancer so very difficult to detect. Exosomes have reported for ready detection of the early stage of pancreatic, melanoma, and glioblastoma cancer. ctDNA is the fragments of DNA circulating the blood system by dying cancer cells. The ctDNA can be detected by using a technique like PCR, RT-PCR, and next-generation sequencing [36]. Recently new microfluidic devices have developed for the better detection of rare CTCs as shown in table 1.

Table 1: Microfluidic devices for the detection of rare circulating tumor cells

S. No.	Microfluidic devices	Material	Detection	References
1	Chitosan microcarriers	PDMS	Breast cancer cells	[37]
2	Rapa delivery	PDMS	RAPA drug delivery	[38]
3	Labyrinth microfluidic devices	PDMS channel bonded to glass slides	Hepatocellular carcinoma	[39]
4	A novel integrated microfluidic device	PDMS	Colorectal cancer	[40]
5	optically-induced-dielectrophoresis (ODEP)-based microfluidic system	-	Leukocytes	[41]
6	3D scaffold gelatin-microchip	PDMS scaffold skeleton	MCF-7 cells	[42]
7	Hyaluronic acid-functionalized electrospun PLGA nanofibers embedded in a microfluidic chip	PLGA	CD44+carcinoma	[43]
8	DNA-templated magnetic nanoparticle-quantum dot (QD)-aptamer copolymers (MQAPs)	PDMS	Rare CTCs	[44]

Drug toxicity

Microfluidic devices are used to create a well-controlled microenvironment. This microenvironment provides more advantages than conventional methods to check drug toxicity. The advantages of the microfluidic system over other methods are more optimum conditions as *in vivo* system. The current drug methods are *in vitro* tests in labs and animal models to check the activity and toxicity screening of drugs [45]. The screening of drug toxicity is a key feature in the development of the new drug. The preclinical trials using animal models are very expensive and low accuracy than the actual *in vivo* activity of drugs. Only 10 % of drugs get to pass through the Phase I clinical trials and approved for marketing [46]. Microfluidic devices are emerging as an *in vitro* system for the screening of drug toxicity prediction in the *in vivo* environment. The various microfluidic devices are developed which mimic the microenvironment of the target organs and check the drug toxicity in organ-level conditions [47].

Liver-on-chip

The liver is an important organ for the screening of drug toxicity. All the metabolism of compounds occurs in the liver and the toxicity analysis in microfluidic devices having the same effect as *in vivo* liver system provides the actual activity of that drug. The metabolism can also impact the drugs and the drug carrier's liver on-chip is an important tool for drug toxicity [48]. The 3D microfluidic devices resemble more closely the *in vivo* situation inside the organ system. The controlled flow of fluids can be blood flow. *In vitro* testing using 3D models of microfluidic have hepatocytes cells and with metabolic enzymes and hepatic transporters [49].

The pH, composition of media, and oxygen requirement can be easily controlled by the fluid flow into the microfluidic devices. The devices also reproduce the metabolic process equal to the *in vivo* conditions. The system also generates concentration gradients, pumps, valves, and other organs [50]. The microdevices are made up of plastic, glass,

elastomers materials and are easily detectable. In liver-based microfluidic devices various *in vitro* models are used such as cell-free, subcellular fractions cell lines, primary hepatocytes cells, and tissues. The uses of different cell lines are used in the study to analyze the molecular mechanisms of drug toxicity. The drug toxicity screening can be more accurately predicted by the use of different cell lines in one microfluidic device. Most of the drugs are metabolized in the liver cells and after that, it can produce toxic metabolites having an impact on other organs [51]. In 2004 Shular and coworker designed a microfluidic system for the screening of drug toxicity on multiple organs. In this microsystem made up of silicon and have three different cell lines (lung, adipocytes, and liver cell). Naphthalene can be analyzed by using this microfluidic system as this drug can cause drug toxicity on the lung cells. 3D- μ FCCS is another 3D microfluidic device using kidney, adipocyte, and liver cells separately integrated into PDMS material [52]. Lung on-chip is a similar organ on a chip microfluidic devices are used to check the toxicity of drugs on the lung epithelium cells [53]. The kidney on-chip system had designed to study drug toxicity on the renal cells. In this system, renal and nephron cells are integrated into a PDMS chip with a continuous flow of inner tubular fluid [54]. Heart on-chip system analyses the cardiac tissue contraction under the exposure of different chemical or drugs [55]. Brain on-chip is used to detect the effect of drugs on endothelial cells and brain interactions. Human-on-chip is used to screening the toxicity and metabolism of drugs at an organism level. In this system interconnected chambers having different organ cells are connected with a microfluidic circulatory system [56].

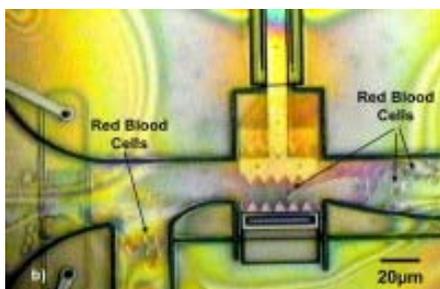


Fig. 5: bioMEMS microfluidic device actuated with 'microteeth' [57]

BioMEMS is such devices developed by Sandia National laboratories, USA for the safe delivery of proteins or pharmaceuticals product to

specific target cells. This device contains silicon microteeth that trap single red blood cell and pump it through 20 μ m channel as shown in fig. 5 [58].

Advantages and drawback of microfluidic devices

The microfluidic devices are based on the highly targeted delivery of specific drugs at a specific time. There is no sufficient toxicological data for the newly synthesized drugs like HIV and anticancer cytotoxic drugs doxorubicin, rubitecan, gemcitabine, and daunerubicin. These drugs were also observed to have an apoptosis effect on the adjacent normal cells. The concentrations of drugs are also a very critical point in drug delivery. The drugs have a minimum threshold functional concentration and the slight amount above threshold concentration could cause irreversible damage to functional organs [59]. Novel drug delivery systems are used to control the release of drugs to a specific site and also target to disease site [60].

The resemblance between the active sites of drug receptors and other proteins can also cause various side effects. The microfluidic devices can deliver the drugs to specific target sites in a minimum functional concentration rather than the traditional nonspecific drug delivery systems. The nonlinear drug delivery is also problems in the case of growth hormones delivery to the specific target tissue as it should be very precise for the proliferation of the tissue and the linear delivery could be achieved by the microfluidic devices so that no damage to the acute organs. The microfluidic drug delivery system ensures the bioavailability of drugs at specific target receptors with the desired dose of release [61]. Laminar flow is characteristic feature of microfluidic system and it depends upon Reynolds number (Re). Re is a dimensionless number represent the viscous flow of fluids. At low Re the fluid moves smoothly and shows a laminar flow. At high Re the mixing is not linear but shows turbulent flow as shown in fig. 6. In micro-scale system laminar flow is observed and in macro-scale turbulent flow is dominant [62].

The roughness of the surface is a very important parameter in the micro-scale systems as the surface area to volume ratio increases so the flow of fluid depends upon the surface phenomenon. Roughness affects fluid flow and creates a pressure drop. To overcome this problem nano finished microchannel with elevated temperature and less drops in the pressure are used [64]. The friction factor is another parameter that has been observed over a small range of relative roughness under low Re. In the blood flow through the microchannels, surface roughness has an impact on the blood viscosity and blood flow [65].

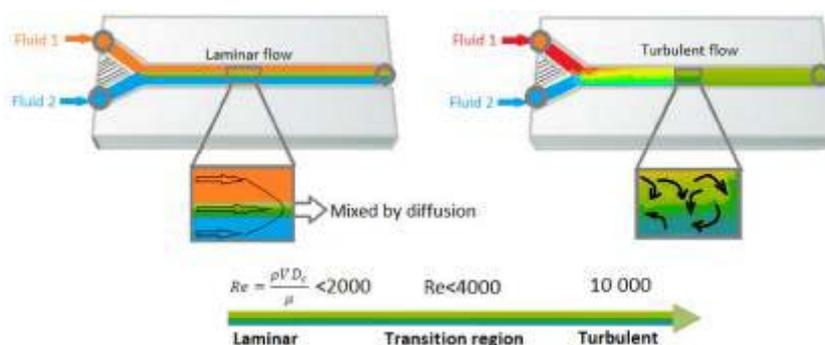


Fig. 6: Laminar and turbulent flow in microfluidic system [63]

The chemical reaction between the liquid and material is a major problem. This causes fouling in which the sample and reagents get stick to the surface of the microchannel device. The fouling in the channel can cause clogging and encrustment of the system [66]. To avoid the fouling cleaning process should be done. For example in blood flow there are some macromolecules like NaCl which can bind to the surface or the uncharged proteins upon unfolding can become charged and can react with the materials; the fouling can also affect

the pressure as the clog of molecule creates hindrance in the fluid flow and also the molecule which gets clogged will be missing from the samples and it could cause inaccuracy in the results [67].

The fouling can cause more problems in the diagnosis with time as more samples will be passed from the microchannel. To overcome the problem of fouling in glass-based microchannel coating with bovine serum albumin can be done but this solution has many

drawbacks as the small molecules can be lost due to adsorption to the coated surface [68]. Self-assembled monolayer coating and PDMS materials have been used to overcome this problem. But these materials are also not the 100% solution to the fouling of surface and any kind of research is going on to solve this problem [69]. In small scales, the regulation of capillary forces is very difficult and it affects the flow rate of the liquid in a microchannel. For the

regulation of capillary flow valves, electrochemical and static forces are employed in microchannel devices. In glass-based microfluidic devices, the weak capillary force is observed so the fluid moves very slowly from the glass microchannels. Nanorods are employed to improve the capillary force [70]. Different materials like glass, silicon and polymer are used for fabrication of microfluidic devices as described in table 2.

Table 2: Different materials for the fabrication of microfluidic devices

S. No.	Microfluidic chip material	Application	Disadvantages	References
1	Silicon	High resistance towards organic solvents, highly stable, interneers, high resistance for oxidation	High cost, not easy to handle, less flexible, opaque in color	[71]
2	Glass	Transparent, high thermal conductivity	High cost, hardness	[72]
3	PDMS	Easy to fabricate, stretchable, Transparent, Suitable for valve and pumps fabrication, inertness, less cost of fabrication	Absorption of hydrophobic molecules	[73]
4	Thermostat polyester	Insoluble, inexpensive, don't absorb biomolecules	Stiff, high cost	[74]
5	Thermoplastics	Highly crosslinked polymer, high production at low cost	Rigidity	[75]
6	Paper microfluidic	Flexible, cheap, easy degradation	Complex fabrication	[76]
7	Hydrogels	Highly porous, suitable for cellular biology studies, Biodegradable, low toxicity	Limited hydrophobic drug delivery, low mechanical strength	[77]

CONCLUSION

Microfluidic technologies are an important role in the field of a cancer diagnosis as well as the progress of medicine. Microfluidic systems have emerged as a tool for direct and localized drug delivery to target sites. The advantage of these systems is the delivery of an exact and small quantity of drug doses, reducing the need for using high concentrations of drugs with significant side effects. The diagnosis of CTCs is a better alternative to the conventional biopsy for cancer detection. Microfluidic devices are a potential tool that enables earlier cancer molecular diagnosis. This method is for better-targeted cancer therapy and improved follow-up care. Novel microfluidic devices with improved design should be employed for more precise drug delivery and diagnosis.

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AUTHORS CONTRIBUTIONS

All the author has contributed equally.

CONFLICTS OF INTERESTS

Declared none

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