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Microfluidics In Oncology: Revolutionizing Cancer Detection And Therapy

¹Tisha Pramanick*, ²Nandine Dash, ³Nisha Patel, ⁴Surabhi Singh, ⁵Haney Solanki

¹Student at Smt. B.N.B Swaminarayan Pharmacy College, ²Student at Smt. B.N.B Swaminarayan Pharmacy College, ³Student at Smt. B.N.B Swaminarayan Pharmacy College, ⁴Student at Smt. B.N.B Swaminarayan Pharmacy College, ⁵Student at Smt. B.N.B Swaminarayan Pharmacy College

¹ B.Pharm

¹Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi, India

Abstract:- Deaths from cancer continue to be a major worldwide problem, often as a result of insufficient diagnosis and therapeutic approaches. As a cutting-edge development, microfluidic technology is essential to automation and miniaturization because it can effectively handle tiny material and sample sizes, from microliters to femtoliters. These tools help create nanoparticles for targeted medication administration in addition to making it easier to identify biomarkers linked to cancer in biological fluids. Numerous cancer treatment modalities, like as gene delivery, radiation therapy, and chemotherapy, may be investigated and improved using microfluidics. Microfluidics has many benefits in cancer, such as high throughput, remarkable sensitivity, low material consumption, and cost-effectiveness. The purpose of this study is to draw attention to the potential features, advantages, and therapeutic uses of microfluidic devices in the detection and management of cancer.

Keywords:- Cancer, diagnosis, drug delivery, gene delivery, microfluidics, radiotherapy.

1. Introduction

Unchecked cell proliferation inside tissues leads to malignant growth. Six essential traits are acquired as cancer progresses: resistance to anti-proliferative signals, autonomous growth, endless replication, prolonged vascularization, evasion of programmed cell death, and the capacity to penetrate tissues and spread(1). Globally, a significant number of cancer-related fatalities and new cases have been documented in recent years. Despite their widespread usage, the current conventional treatments chemotherapy, surgery, and radiation therapy frequently cause side effects include nausea, discomfort, diarrhea, muscular atrophy, skin irritation, mouth inflammation, vascular damage, nerve impairment, and toxicity to healthy tissues(2). Furthermore, tissue sample and biomarker measurement both of which are often intrusive procedures that result in tissue damage, restricted accessibility, and severe patient discomfort are the mainstays of conventional cancer diagnosis. Researchers are thus still looking into other and more efficient methods of diagnosis and treatment(3). Microfluidic technology is one of the cutting-edge innovations advancing cancer detection and therapy. This creative system has advanced quickly since it was created to handle chemicals on tiny platforms. Microfluidics was first presented as a theory that regulated the regulated passage of tiny reagent quantities through sub-millimeter-sized chambers. It eventually served as the basis for lab-on-a-chip technology, which transformed biomedical research(4). Compared to conventional approaches, microfluidic devices provide better cell culture techniques and allow for the reproduction of extracellular conditions that are almost in vivo for drug research involving cells. Their use in

cell isolation minimizes needless patient sampling by drastically lowering the amount of solutions and samples needed. Additionally, microfluidics offers cutting-edge methods for early cancer diagnosis and detection with excellent sensitivity and accuracy(5). This study is to investigate the function of microfluidic systems and their applications in cancer research and therapy, given the increased interest in using cutting-edge technology for the diagnosis and treatment of life-threatening illnesses(6).

2. Microfluidic systems

The exact control of the flow of tiny reagent quantities inside microchannels ranging from 1 millimeter to 1000 micrometers was made possible by the introduction of microfluidic technology thirty years ago as a biological instrument(7). Because liquid flow is absolutely laminar in these circumstances, target molecule concentrations may be precisely controlled. Initially, silicon and glass were the main materials used in microfluidic devices. Polymer-based materials including cyclic olefin copolymer (COC), polydimethylsiloxane (PDMS), and polymethyl methacrylate (PMMA) have been in use for decades and have gained popularity over time. The methods used to fabricate microfluidic devices have mostly been taken from electronic manufacturing procedures, which are still some of the most used methods(8).

A small layer of light-sensitive polymer is applied to a substrate in photolithography, one of the fundamental manufacturing techniques, and channel patterns are then created by exposing the substrate to UV light using either a shadow mask or direct contact patterning. In certain situations, particular resist types are exposed using electron beams to produce very high-resolution details. Similar to negative lithography, which is then utilized to remove certain regions selectively, the resist may dissolve in the right solvent depending on the kind of exposure, revealing the substrate below(9). A flat surface with distinct topographical patterns is then left behind when the prepared resist substrate is completely removed and used as an etch mask. Enclosed channels without physical dead volume are created by joining a second smooth substrate and adding input and output ports.

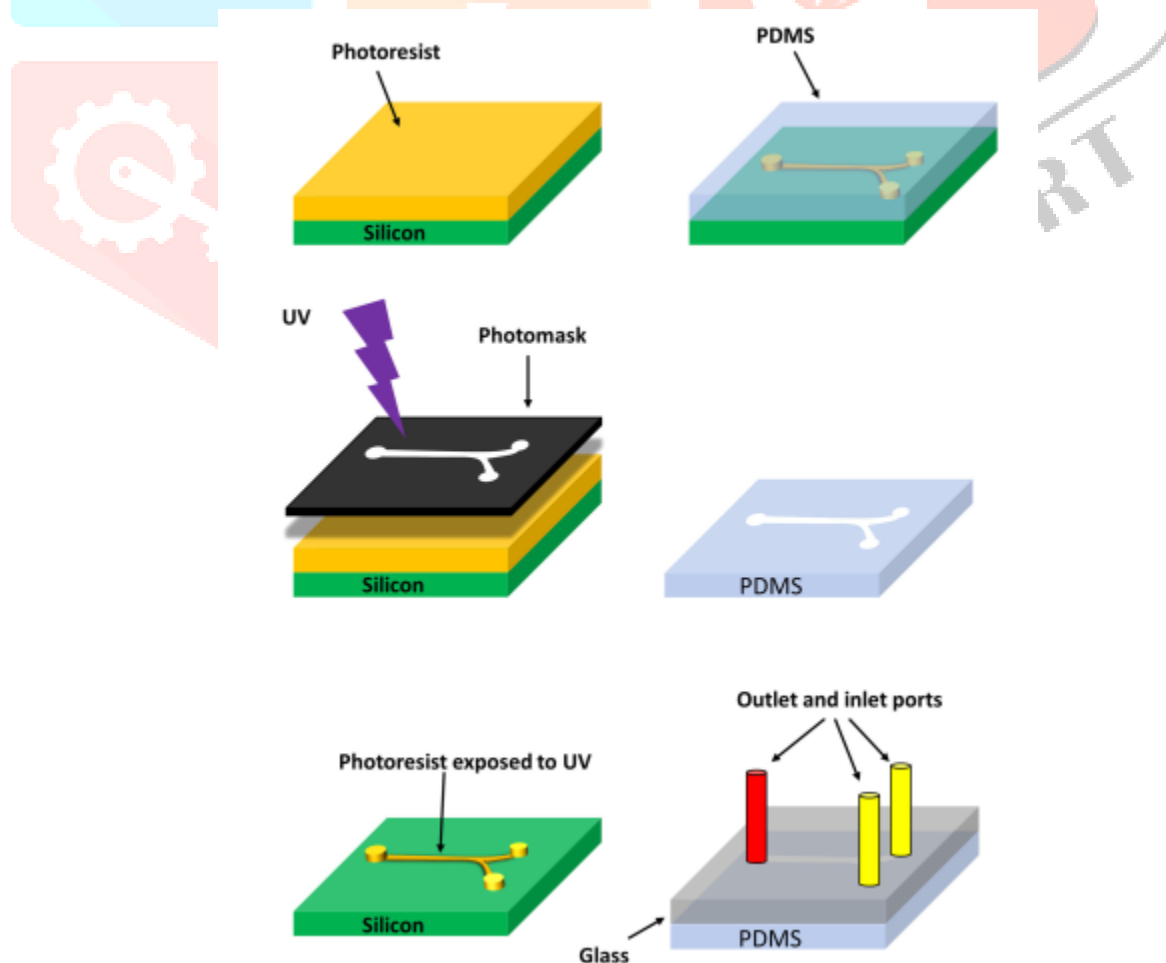


Figure 1 Schematic image of PDMS-based microfluidic device fabrication.

In order to optimize microfluidic systems, material selection is essential. Researchers are concentrating on different biocompatible metals and shape-memory alloys. In order to create organ-on-a-chip devices, microfluidics has recently been combined with biometrics, cell biology, and microengineering(10). By adding scaffolds and necessary stimuli to facilitate the development of functioning tissues and organ structures, these systems mimic biological microenvironments. When paired with microengineering techniques, microfluidic platforms enable the precise control of liquid flow and pressure at the microscopic level, facilitating the creation of three-dimensional scaffolds and controlled microenvironments with distinct physicochemical and biochemical characteristics.

These cutting-edge methods are now often used to produce physiologically accurate organ models, which are essential resources for pharmaceutical applications, disease modeling, and biological research(11). Metastasis has a major impact on death rates in cancer research, which emphasizes the need of using in vitro models that mimic real settings to study metastatic processes. For a variety of applications, such as drug screening, cellular response analysis, biomaterial research, and therapy development, microfluidic on-chip technology provides an extremely effective method. A variety of materials and production procedures, each suited to a particular scientific application, are essential to the success of microfluidic devices. Notably, the domains of biology and medicine have begun to pay more attention to biomedical microfluidic devices that are intended for high-throughput applications(12).

2.1. Biomedical microfluidic systems with high throughput (BMSWHT)

Because silicon and glass are biocompatible and work well with electronic components in microfluidic designs, they are the main materials utilized in the creation of biomedical microfluidic systems with high-throughput (BMSWHT)(13). Another popular method for making channels on microfluidic chips is photolithography. Polydimethylsiloxane (PDMS), one of the most widely used polymer substrates in microfluidics, is very biocompatible and appropriate for both in vitro and in vivo investigations(14). High transparency, permeability, and stability are among of its primary characteristics that make PDMS a great material for creating BMSWHT that is employed in a variety of applications, including drug screening, organ-on-a-chip models, and biomedical research. In order to generate high-throughput models in BMSWHT, PDMS is usually carved into several channels or shaped into certain templates, which makes it helpful for both pharmacological research and cell separation(15).

But it's important to take into account that PDMS has a number of limitations. These include the dispersion of unbonded particles from the PDMS substrate, limited absorbency for hydrophobic substances, non-specific molecule adsorption, inconsistency with different fluids, and noticeably high prices(16). Alternative cost-effective polymers, such as polymethyl methacrylate (PMMA), cyclic olefin copolymer (COC), thermoset polyester (TPE), polycarbonate (PC), polystyrene (PS), and perfluoropolyether (PFPE), have been developed for BMSWHT in order to overcome these limitations. These polymers have a lot of potential in the microfluidic industry.

Because of its adaptability, COC is often employed in X-ray crystallography and may be utilized in a variety of microfluidic settings(17). Cells from organs including the liver and kidney are cultured and studied using PFPE-based biochips. PC is a useful material for temperature-sensitive applications because it efficiently transfers heat through glass when used as a substrate for microfluidic devices. In one study, scientists created a microfluidic method for identifying DNA point mutations by combining PC and PMMA and using UV-based conventional techniques. Finally, TPE is a potential material for a variety of biomedical research applications because of its great compatibility with glass surfaces and simplicity of synthesis, which make it an advanced material in microfluidic fabrication(18).

2.1.1. Organ and cell-based drug screening by BMSWHT

Because they accurately mimic the physiological and anatomical features of target cells and human bodily activities on a laboratory scale, biomedical microfluidic systems with high-throughput (BMSWHT), which were created after cell-based or organ models, provide an efficient platform for drug screening(19). When compared to conventional animal research, this method drastically cuts down on time and expense. These platforms, which

closely resemble real in vivo settings, enable cell growth inside a three-dimensional extracellular matrix (ECM), such as hydrogels, scaffolds, and droplets. They provide a sophisticated approach to medication assessment by tracking insulin responses and metabolism at the organ level(20).

High-contrast imaging, large-scale throughput investigations, and improved drug research efficiency are all made possible by BMSWHT's ability to combine numerous systems with automated procedures and miniature structures(21). They are perfect for multi-drug screening applications since they may be used to evaluate drug effectiveness and cytotoxicity at several dosages at the same time. These benefits result from the ECM used in BMSWHT's exceptional permeability and structural flexibility(22).

To aid in drug testing and monitor in vivo performance, a breast-on-a-chip model was recently created that incorporates elements such tumor spheroids, extracellular matrix, and vascular systems(23). This model showed that carbon-dot nanoparticle-based medication delivery may successfully cross endothelial barriers in three hours without endangering the target cells. The adaptability and precision medicine potential of the controlled drug release system have been shown by its effective application to both non-TNBC cells, such as T47D, and triple-negative breast cancer (TNBC) cells, such as BT549(24).

2.1.2. Cancer diagnosis

2.1.2.1. Cancer-related biomarkers.

Malignancy-related biomarkers, including proteins, circulating tumor cells (CTCs), mRNA expression patterns, and free tumor nucleic acids, are important for determining biological activity and the pharmacological response to treatment plans in cancer patients(25). When these biomarkers are accurately identified, crucial clinical data is provided, allowing doctors to evaluate the course of the illness and decide on treatment strategies. Microfluidic systems are a useful tool for identifying cancer biomarkers because of their sophisticated bioanalysis capabilities(26).

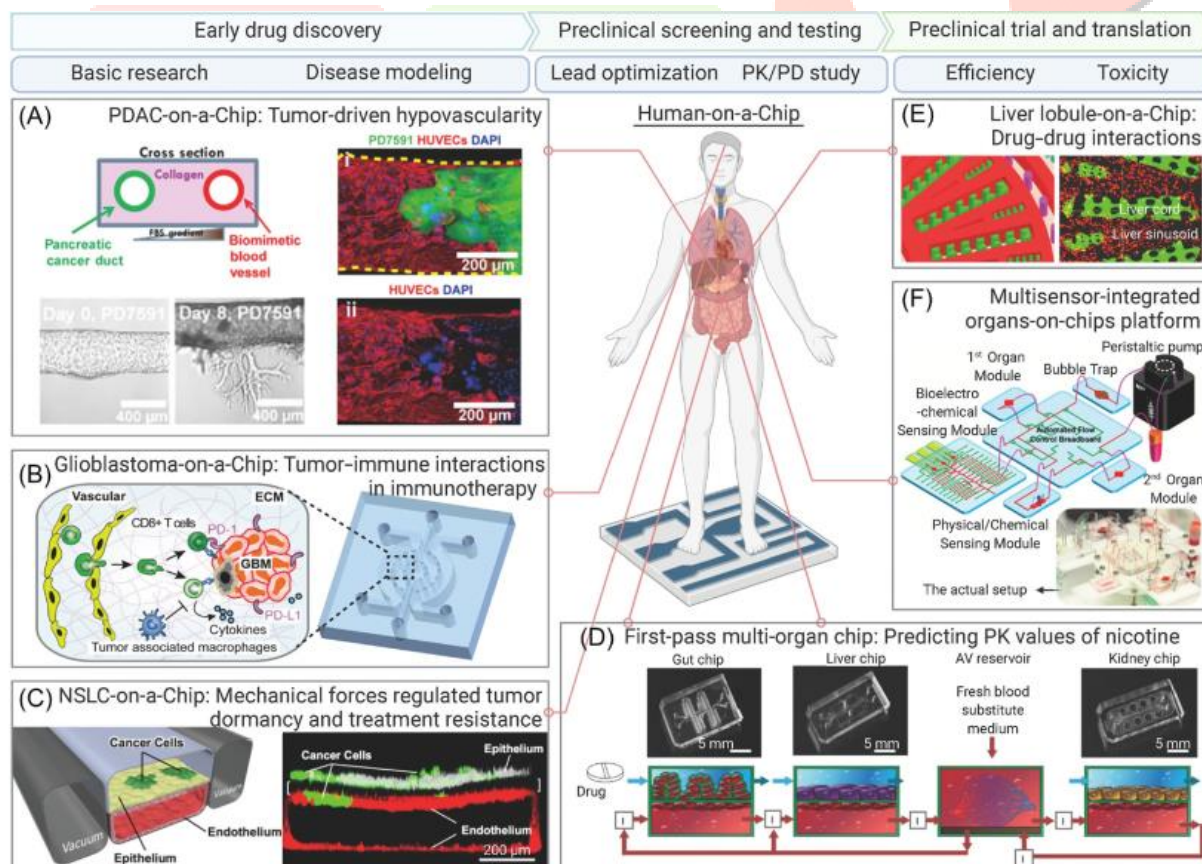


Figure 2 Organ-on-a-chip and advantages. (A) schematic illustration of pancreatic ductal adenocarcinoma (PDAC)-on-a-Chip alongside the vascular biometric system (confocal images). Studies on the PD7591cell line

(green part) was indicated, what makes to contribute to hypovascularity is the Activin-ALK7 pathway. (B) interactions of the immune system with a glioblastoma brain tumor on-a-chip is indicating that immunosuppression restraining implemented by tumor-associated macrophages increase the effectiveness of anti-PD1 immunotherapy. (C) A microchip designed to investigate non-small cell lung cancer (NSCLC) shows that physiological forces during breathing lead to drug resistance. (D) A first-pass multi-organ device was used to determine the pharmacokinetic (PK) parameters of nicotine. (E) A liver lobule-on-a-chip was employed to study the side effects of drugs and interactions on the liver. (F) A multi-organ platform.

For example, a specific biochip was created to concurrently identify many genes linked to prostate cancer, such as PCA3, SChLAP1, TMPRSS2-ERG gene fusion, and KLK2, in genetic testing for prostate cancer utilizing patient-derived bodily fluids. Liquid biopsies based on serum and urine were used to make this detection(27). Functional components of the biochip device include labeling with peroxidase-mimicking nanozymes (nanomaterials with enzyme-like properties), performing modified solid-phase isothermal amplification through nanofluidic regulation, electrically lysing target cells within a minute to release specific genes, and using electrochemical detection techniques. When these cutting-edge technologies are combined, the full diagnostic procedure may be finished in 30 minutes and the sensitivity level is high enough to identify as little as 50 copies of the target biomarker(28).

2.1.2.2. Circulating tumor cells (CTC) detection.

Cancer cells may move through the circulation as single cells or in clusters after separating from their original or metastasized tumor sites(29). In circulating tumor cell (CTC) research, the major goal of improved technology is to efficiently detect and separate single and clustered CTCs in enough amounts while preserving high purity from blood samples larger than 5 mL. In order to minimize cellular damage, this procedure must also be carried out under low stress(30).

The majority of CTCs display epithelial surface indicators, such as epithelial cell adhesion molecules (EpCAM), which are not present in blood cells, according to studies on these cells(31). The "CTC-chip," the first microfluidic device created to separate CTCs from blood samples, using microposts coated with anti-EpCAM antibodies. By altering the nano- or micro-structures inside microchannels, it is possible to increase the contacts between CTCs and antibody-coated surfaces, hence enhancing the CTC capture rate(32). Magnetic nanoparticles linked with EpCAM antibodies may also be used to mark CTCs. These can then be separated using a micromagnetic shifter or sorted in a microfluidic device(33).

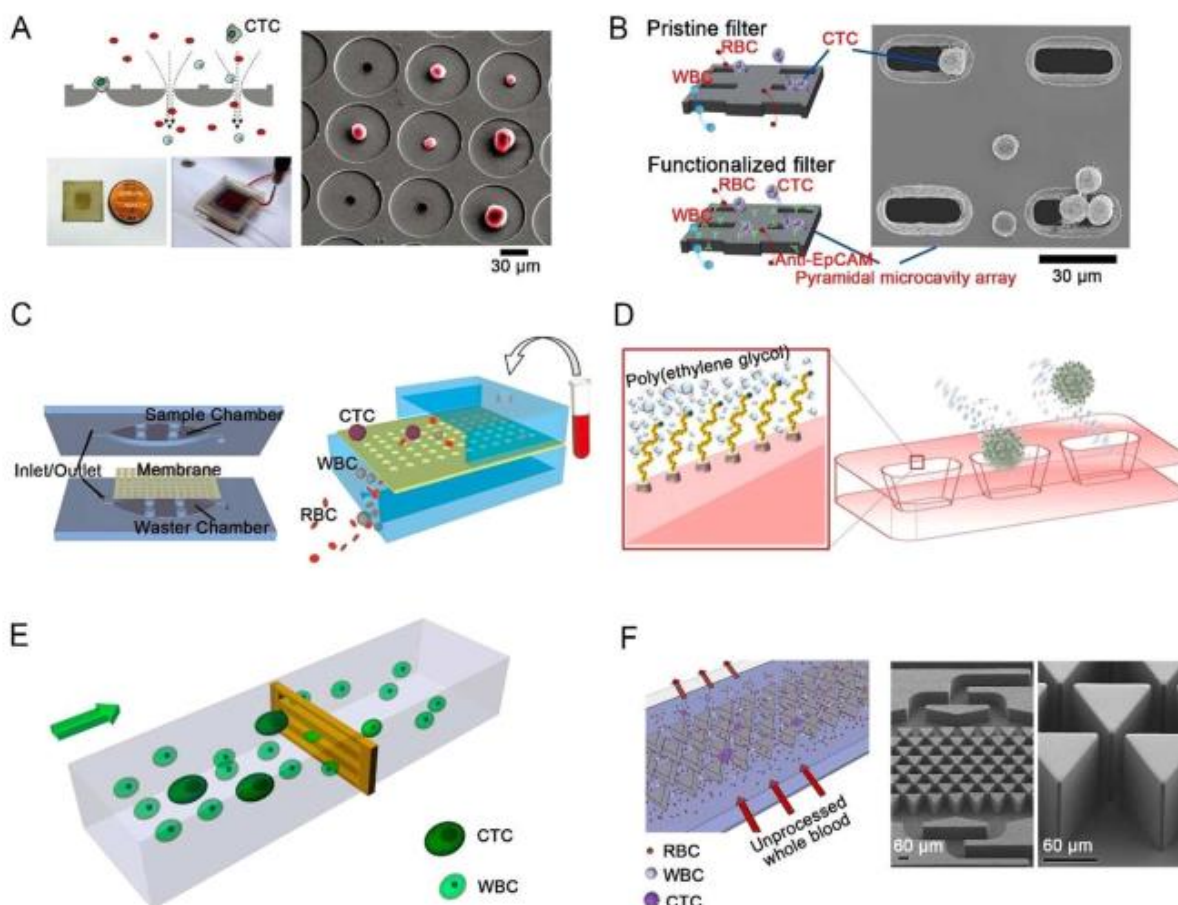


Figure 3 CTCs isolation methods. (A) The size-based separation of CTCs and scanning electron microscope (SEM) picture of trapped MCF-7 cells. (B) Schematic picture of CTCs enrichment. (C) CTCs sifting with PDMS membrane. (D) CTCs isolation by applying PEG-modified tapered-slit SU8 filter. (E) CTCs separation by filtering method. (F) CTCs clusters trapped by the chip.

Human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor (EGFR) are two additional surface molecules that may be used for CTC capture; however, the expression of these cancer markers differs greatly depending on the kind of tumor(34). When using just immunoaffinity-based microfluidic separation methods, a significant percentage of CTCs are often overlooked because to this variation in tumor marker expression(35). An other strategy is to use panels of aptamers rather than particular antibodies; however, as they are first created using cancer cell lines, they may not be able to identify CTCs with enough accuracy. The physical properties of CTCs, including as density, size, deformability, compressibility, and electrical impedance, may also be used to separate them in order to overcome the drawbacks of affinity-based isolation techniques(36).

2.1.2.3. Tumor exosomes isolation.

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2.1.2.4. Cell-free DNA tracking.

Cells going through apoptosis actively release cell-free DNA (cfDNA) and other apoptotic byproducts. Mutant cfDNA, often referred to as circulating tumor DNA (ctDNA), is released by cancer cells and is utilized as a predicting tool for certain cancers. It is regarded as a very promising diagnostic marker(45).

The reference range for cfDNA in whole blood in healthy people is normally 1–10 ng/mL, however these levels may rise dramatically in cancer patients(46). Blood cfDNA levels often overlap with those of healthy people, despite being connected to tumor growth and development. Therefore, for effective identification, it is essential to evaluate the ratio of mutant to wild-type genes (ctDNA to cfDNA) rather than only detecting cfDNA levels(47). One new development in this area is the detection of altered cfDNA in blood samples using electrochemical sensors, specifically nano-fabricated microelectrodes.

2.1.2.5. Measurement of proteins in cancer.

Unusual levels of measurable biomarkers, including cytokines, hormones, and peptide growth factors, are released by cancer cells and may be used as diagnostic indicators in cancer patients. Consequently, it is crucial to identify panels of these proteins for detection in tiny blood samples(48).

One of the most cutting-edge and successful innovations in this area is microfluidic devices, which have successfully entered the market(49). These devices make it possible to test many proteins at once and collect plasma from a small droplet of whole blood. Before microfluidic immunoassay systems can be completely incorporated into clinical settings for cancer biomarker detection, a number of experimental issues need to be resolved(50).

Potential issues with protein measurement using microfluidic techniques include protein aggregation, instability, and extended measurement times(51). However, improvements in microfluidic methods have been made to get beyond these restrictions, increasing the precision and effectiveness of protein detection(52).

3. Microfluidics technology and cancer treatment

3.1. Drug delivery

Microfluidic technology may be used to distribute regulated, tiny doses of drugs in a precise and targeted manner. When given systemically, these properties make it easier to administer drugs with cytotoxic effects or short half-lives(53). Additionally, by facilitating the creation of microneedles or needle-free injection systems, microtechnology may help conventional delivery techniques like invasive and uncomfortable injections.

The creation of efficient drug carriers is a crucial component of drug delivery research. These carriers need to reduce unwanted side effects, improve bioavailability, and control medication release(54). Additionally, they ought to enhance the absorption of medications that are relatively unstable and poorly soluble. In order to penetrate the intestinal mucosal barrier and reach the circulation, oral delivery vehicles also need to be tiny

enough, resistant to the stomach's acidic environment, and coated with the right indicators(55). A key component of drug carriers' effectiveness is their capacity to regulate and modify drug release, which is closely related to their size, shape, homogeneity, and formulation. Nanoparticles less than 10 nm, for instance, are quickly removed by the kidneys, while larger particles might be phagocytosed by immune system cells(56).

Drug carriers have significantly improved thanks to microfluidic technologies(57). By producing tailored nanoparticles with consistent sizes, shapes, and surface structures, these systems can effectively manage tiny sample quantities and improve controlled release and drug delivery(58). Microvortices, chaotic flow techniques, droplet-based procedures, and flow-focusing approaches are a few popular ways to create appropriate nanocarriers utilizing microfluidic devices.

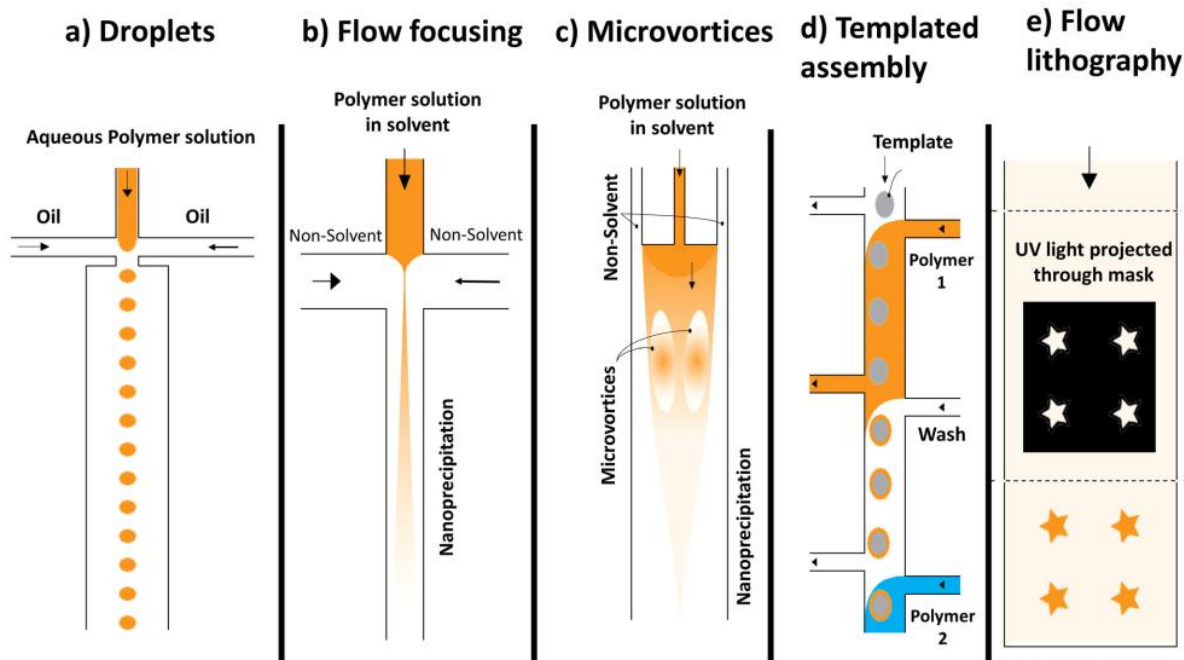


Figure 4 Nanoparticle fabrication methods by microfluidics.

It is possible to specially construct microfluidic systems that operate at Reynolds numbers higher than 100 in order to create nanoparticles for medication delivery via controlled microvortices(59). Researchers have used two-dimensional microfluidic systems, whose microvortices are crucial in producing organized forms, to dynamically manipulate three-dimensional chemical profiles by making minor modifications to intake pressures(60). In comparison to conventional stirring-based nanoparticle synthesis, lipid-polymeric nanoparticles have been produced at the intersection of three inlets using this microvortex model, showing a noticeably greater efficiency in this process(61). According to studies, changing the Reynolds number has an impact on the size of nanoparticles; as the number rises from 30 to 150, the diameter of the particle decreases from 93 nm to 55 nm.

Laminar flow in conventional microchannels is another microfluidic technique that avoids instability in typical circumstances. Within channels, specialist equipment like herringbone mixers are employed to increase mixing efficiency(62). By using geometric templates to create transverse flow patterns that compress and stretch liquid volumes inside a microchannel cross-section, a new approach known as chaotic advection has been created to increase mixing yield. The herringbone mixer's simplicity in production and ease of use have drawn a lot of attention(63). In order to create turbulence within a continuous flow, this system uses an organized arrangement of "herringbone grooves" on one or more channel surfaces. By distributing the reagent uniformly over the microchannel cross-section, this design optimizes residence duration and drastically lowers Taylor dispersion(64). These mixers have been used to create lipid-based nanoparticles in experiments. Subsequent research has shown that the size of the nanoparticles is directly influenced by the density of the lipids stirred by herringbone mixers, with lower lipid densities resulting in lipid particles that are about 30 nm in size(65). These mixers have also been used to produce a variety of drug-based nanoparticles, including lipid-based siRNA carriers and doxorubicin-loaded lipid nanoparticles.

Another high-throughput method for carrier synthesis that provides exact control over the creation of drug-loaded nanoparticles is droplet-based microfluidics. Under laminar flow circumstances, this technique manipulates discrete liquid volumes in immiscible phases, such as liquid-gas or water-oil systems(64). With carefully managed interfaces and regulated flow rates, microfluidic devices' capacity to produce distinct droplets has been extensively used for the creation of nanoparticles. For microparticle platforms, a wide variety of organic and inorganic materials have been investigated. PDMS-based systems or microfluidic capillary devices are often utilized to create modifiable monodisperse emulsions(66). Drug release rates are greatly influenced by the size of drug carriers; since smaller PLGA particles have a greater surface-area-to-volume ratio than bigger ones, they carry pharmaceuticals more quickly. Researchers have used a droplet-based microfluidic technique to create Janus nanoparticles in a single step. By combining many intake channels, this system creates a regulated precipitation flow that produces Janus nanoparticles with unique characteristics(67). These nanoparticles have the ability to encapsulate hydrophilic medications like doxorubicin on one side and hydrophobic medications like paclitaxel on the other. Different physical and chemical characteristics may be quickly assembled and integrated into Janus nanoparticles for dual-drug delivery thanks to this microfluidic architecture.

Another popular microfluidic method for precise mixing and regulated chemical reactions is hydrodynamic focussing. When solutions with different flow velocities enter a conduit side by side, this phenomena happens. Three-inlet microfluidic devices, in which lateral fluids encircle the main sample stream, are a popular method. Precision reagent addition during reactions is ensured by microfluidics' capacity to provide quick and even liquid mixing(68). This method has been used to create drug-loaded polymeric nanoparticles with reduced polydispersity, appropriate size, and enhanced drug-loading efficiency, such as PLGA-PEG particles. PEG incorporation in the nanoparticles is improved while preserving the appropriate particle size via the self-assembly process, which is modulated by nanoprecipitation through regulated mixing. Furthermore, a single-layer microfluidic device with successive inlets for vertical and horizontal flow focusing has been incorporated into a three-dimensional hydrodynamic flow focusing system(69). Even with high molecular weight polymer precursors, continuous nanoparticle manufacturing is made possible by this 3D flow pattern, which successfully inhibits polymer adherence to microchannel walls, a typical problem in 2D systems. These nanoparticles have shown improved tumor cell targeting effectiveness when pre-mixed with different medications(70).

Subsequent research has contrasted dialysis-based and microfluidic approaches for creating drug-encapsulated nanoparticles for the treatment of cancer. The results indicate that micelles made using microfluidic techniques had better drug-loading efficiency than those made using dialysis, with smaller nanoparticle sizes and lower polydispersity indices(71). Another creative method included creating chitosan-based nanoparticles filled with bioactive substances using a lithography-based microfluidic system on a silicon wafer(72). This technique created semi-spherical nanoparticles with regulated polydispersity by using a modified ionic-gelation process within microfluidic devices. Furthermore, when compared to traditional ionic-gelation techniques, these microfluidic-fabricated nanoparticles showed higher cytotoxicity, greater apoptosis-inducing effects, and improved drug-loading capability on cancer cell types(73).

3.2. Radiation therapy of cancer

Depending on the kind of radiation employed, radiotherapy may be used for anything from diagnosing disorders to treating and managing them(74). It is an essential tool in the medical sector. For example, because of their capacity to target malignancies by targeted radiation absorption, several radioactive isotopes that generate alpha or beta radiation are often used in therapeutic operations(75). Although the circulatory system usually carries these isotopes to their destinations, their distribution is often restricted to a small number of organs. Encapsulating or loading them into specific carriers, such as dendrimers, liposomes, polymeric micelles, or nanoparticles, increases therapeutic efficacy while reducing harm to nearby healthy tissues(76). Furthermore, radiation impacts vital biological processes and a number of interior cell structures, including the nucleus. Advanced systems that can precisely replicate biological processes and guarantee maximum operation during radiation investigations are necessary to comprehend these repercussions(77).

By offering a way to preserve biopsies or replicate organ function using cutting-edge technologies like organ-on-chip devices, microfluidics provides a solution. These developments provide scientists the ability to precisely examine biological reactions to radiation. By making biomarker analysis easier, microfluidics contributes to radiobiology in addition to basic and biological research(78). In medical research, particularly radiation studies, biological markers are very valuable because they may be used to measure the radiation resistance of cancer cells, quantify the amount of collateral damage to healthy tissues, and establish the right radiation dose for a successful course of therapy.

In one investigation, apoptotic cell responses in radiation-treated head and neck cancer cells were identified using microfluidic technology. Key markers of apoptosis, such as cytochrome C, lactate dehydrogenase (LDH), and caspase-3-cleaved cytokeratin-18, were examined by the researchers. Prior to applying the microfluidic device to radiation-exposed human cancer cells, the system was first calibrated using rat liver samples. As shown by low LDH levels, the method successfully maintained liver and cancer cell samples without radiation for over two weeks. Cancer cell samples were cultured in the system overnight and exposed to different radiation doses for five days in order to evaluate radiation-induced apoptosis(79). Three days after radiation, immunostaining verified a considerable rise in the apoptotic index when compared to untreated samples, even if biochemical indicators such as cytochrome C and LDH did not demonstrate significant cytotoxicity. It's interesting to note that untreated tissues in the microfluidic system showed apoptotic reactions that were comparable to those in conventional cell cultures. The study's results may have been enhanced, however, if it had directly compared irradiation samples in traditional and microfluidic environments(80). The microfluidic device successfully preserved cell viability by creating a 3D environment that was close to physiological conditions. It also used tissue-based indicators to show how radiation exposure and apoptosis are related.

In a different experiment, the combined effects of chemotherapy and radiation on soft tissue sarcoma spheroids were examined using a microfluidic system. Five linked chambers with tiny cubic compartments for spheroid formation were part of the system, which was built using a specific polymer. In order to guarantee exposure to regulated treatment conditions, this two-layer arrangement included culture chambers at the base and a linear channel on top. Different radiation dosages may be administered to each chamber while it was being treated with a particular chemotherapeutic drug(81). Two distinct sarcoma cell lines were used to create spheres, all of which were kept the same size for uniformity. Doxorubicin treatment was administered at various concentrations together with radiation doses of varied intensities. Precise monitoring of treatment responses was made possible by the organized grid arrangement of the culture chambers. After treatment, spheroids were separated into individual cells for examination using flow cytometry and colony-forming tests. Results showed that radiation treatment induced alternate cell death pathways, whereas doxorubicin exposure caused apoptosis in sarcoma cells. This work demonstrated how microfluidic devices may be used to mimic intricate therapeutic interactions and improve focused treatment plans(82).

3.3. Gene delivery

Since nucleic acid structures have been discovered and molecular pathways in a variety of illnesses have been understood, replacing damaged genes with functioning ones has become a sophisticated therapeutic approach known as "gene therapy." This method uses expression constructs to increase the production of vital proteins in cells(83). Furthermore, it has been shown that suppressing genes that are damaged or targeted may significantly improve the treatment of a variety of illnesses. Gene delivery has been the subject of much investigation in recent years as a potentially effective cancer therapy strategy(84). According to some experts, the development of cancer may be influenced by a variety of genetic changes, including mutations in important genes like P53, the deactivation of tumor suppressors, or the suppression of apoptosis-related pathways. Therefore, replacing a mutant gene with a normal one or silencing a detrimental gene may provide a revolutionary approach to cancer treatment.

Key traits for successful gene therapy have been established by subject-matter experts. It should start with a gene expression system based on plasmids. Second, certain therapeutic proteins must be produced by the inserted gene. In order to shield the plasmid from enzymatic breakdown and enable its transportation to the targeted site of

action, an effective delivery mechanism must be in place(85). Various vectors, including viral carriers as well as non-viral alternatives such as cationic liposomes and polymers, are exploited for gene transfer. Conventional transfection techniques do have several drawbacks, however, such as complicated processes, poor specificity, decreased accuracy, low cell survival, and restricted efficacy. Microfluidic technology advancements have offered creative ways to improve gene therapy and delivery using a variety of methods.

By driving plasmids toward the anode with an electric field and then promoting gene transfer, electroporation provides a way to introduce genes into cells(86). Microfluidic electroporation improves cell survival rates by using lower voltage levels than traditional electroporation procedures. Metallic electrodes on a specialized layer combined with microfluidic tubes that concentrate cells in a specific area make up a microfluidic device intended for electrotransfection. Different materials, electrode layouts, and microfluidic channels have all been investigated in order to improve transfection efficiency. Low direct current voltage has been shown to dramatically improve gene transfer efficiency while maintaining cell viability.

Hydrodynamic focusing is another technique to maximize gene delivery efficacy; it was first created for flow cytometry to increase cell counting precision. Additionally, this method has been modified for use in microfluidic applications. Hydrodynamic focusing has been used into electroporation-based microfluidic devices by researchers to enable gene transfer with low direct current voltage. Furthermore, optical gene delivery is a cutting-edge method for gene transfer in microfluidic devices(87). Light is an electromagnetic wave that can be concentrated and amplified to create laser beams. The energy levels of these beams may be changed to cause regulated stress on cells. Through this regulated exposure, genetic material may be introduced into live cells—a process called "optical transfection."

A variety of laser sources, like as pulsed and continuous-wave lasers, are used in optical transfection. Through targeted heating, continuous-wave and nanosecond pulsed lasers produce temporary holes in cell membranes(88). Additionally, these lasers produce microbubbles and thermoelastic stress, which help break down cellular membranes and deliver biofactors without the need for micropipette penetration. In one study, scientists created a microfluidic apparatus that uses hydrodynamic focusing to accurately match laser beams with individual cells(89). This microfluidic technology showed promise for continuous, high-throughput genetic material delivery by efficiently transferring fluorescent dye into human embryonic kidney cells.

4. Conclusion

Significant advancements in microfluidic methods and microfluidics-based evaluations have been made in recent years, underscoring the importance of this new strategy. medication screening, cancer diagnosis, medication administration, and treatment approaches including chemotherapy, radiation, and gene therapy are just a few of the areas of cancer research where these systems have been widely used. Microfluidic technology makes it possible to create drug carriers in well regulated environments and makes it easier to assess encapsulated medications for first screening, guaranteeing their effectiveness against cancer cells. Moreover, organ-on-a-chip, a technique that mimics human organ functions using microfluidics, enables researchers to evaluate the safety of novel therapeutic medications prior to their clinical implementation. It is anticipated that the use of microfluidics in drug delivery research will be crucial to the advancement of preclinical studies, saving money and time. These days, researchers and patients may diagnose cancer quickly and affordably thanks to microfluidic technology. A key benefit of this technology is its ability to detect specific diagnostic markers (like cancer biomarkers, circulating tumor cells, cell-free DNA, exosomes, and proteins) with high precision using a small sample size, making it a better option than conventional diagnostic techniques.

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