Materials Chemistry Horizons REVIEW

Application of Microfluidic Platforms in Cancer Therapy

Farnaz Dabbagh Moghaddam* & Francesca Romana Bertani*

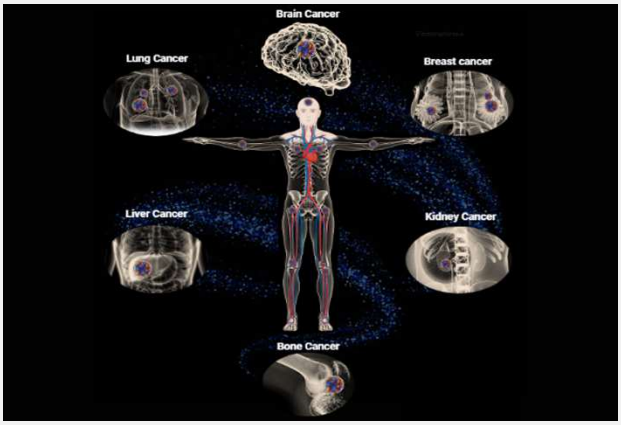
Institute for Photonics and Nanotechnologies, National Research Council, Via Fosso del Cavaliere, 100, 00133, Rome, Italy

Corresponding authors: farnaz.dabbaghmoghaddam@ifn.cnr.it (F. Dabbagh Moghaddam); francesca.bertani@ifn.cnr.it (F. Romana Bertani)

Mater. Chem. Horizons, 2022, 1(1), 69-88 10.22128/mch.2022.556.1007 OPEN ACCESS

ABSTRACT

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020. The implementation of new technological tools can improve prevention strategies, diagnostics, and treatment systems for this group of diseases. Microfluidic devices like Organs on a Chip are being considered a rising approach in biological cancer studies. They involve volumes down to less than microliters and usually do not require specialized machinery and materials to be produced. Therefore, they are potentially used in clinical settings without restriction. In addition, microfluidic platforms have a high potential for mimicking biological conditions. They are recognized as promising tools in cancer fields like single cell detection, fluid biopsy, drug screening modeling, angiogenesis, and metastasis. This review describes the fabrication methods and application of microfluidic platforms in cancer therapy.



Keywords: Microfluidic platforms, diagnosis, cancer, therapy

1.Introduction

Cancer, after cardiovascular diseases, is the second cause of death in the world [1]. The lack of efficient methods to detect cancer's early stages takes approximately 10 million lives annually [2]. Amongst the different types of cancer, about one-fifth belongs to breast cancer [3,4]. In various types of cancer, drug resistance and metastasis to other organs, including bone, liver, and lung, are the leading causes of death [5,6]. Today, despite the drawbacks, costs, and limited availability of some methods, technologies such as positron emission tomography, diagnostic magnetic resonance imaging, and computed tomography are used to plan cancer treatment after tumor biopsy [7]. Therefore, the need for a diagnostic method with higher speed and accuracy can be felt in studies related to cancer prevention, diagnosis, treatment, and prognosis. Microfluidic systems are characterized by an engineered fluid flow geometrically limited to small objects. In the past decades, this technology has been used for various biological and pharmaceutical antimicrobial applications, cell culture, design of drug release systems, DNA replication, synthesis of nanomaterials, and the development of monitoring systems [8]. Microfluidic devices have become important models in cancer-related studies in recent decades. They have been able to represent a new model compared to the *in vivo*, *ex vivo*, and *in vitro* experimental models traditionally used. Microfluidic instruments are being used as innovative platforms in cancer research, diagnosis, and monitoring. The current review highlights the fabrication methods of microfluidic platforms and their potential application in diagnosis and cancer therapy.

Received: June 07, 2022
Received in revised: June 24, 2022
Accepted: July 20, 2022

2. Materials for microfluidic chips fabrication

In the design of microfluidic systems, depending on the intended application, the architecture of the microchannel and microstructures must fit the requirements to provide the desired result. Therefore, also in consideration of this, choosing the best material for a specific objective and design is of the utmost importance. Considering the great variety of materials available today for the production of microfluidic devices, the selection of materials depends on intrinsic physicochemical properties, manufacturing process (casting, hot inlay, and injection molding to name a few), and environmental context (budget, equipment, etc.). The types of materials used to build microfluidic systems are introduced below.

2.1. Inorganic materials

Silicon has significant thermal conductivity, stable electroosmotic mobility, and high resistance to organic solvents: these properties have led to the use of this material as the first choice in the design of microfluidic systems [9]. Silicon is not transparent in the visible optical range, so bonding polymers or glass (transparent materials) is necessary for detecting fluorescence or fluid imaging [10]. Silicon has a high elastic modulus, so some examples of applications are in combination with droplet-based polymerase chain reactions or nanowires to detect unlabeled cardiac biomarkers [10]. After silicon, glass has been used to produce microfluidic chips [11]. In previous studies, glass or quartz capillaries were used for gas chromatography and capillary electrophoresis microchannels in the microfluidic field [11]. Glass is transparent and electrically insulating, compatible with biological samples, impermeable to gas, and amorphous [12]. This material is processed through wet etching or standard wet/dry photolithography [12]. In addition to being used in on-chip reactions, solvent extraction, droplet formation, and in situ fabrication, glass chips are widely used in capillary electrophoresis and allow to separate analytes in solutions of complex materials [13]. Restrictions on the use of glass are due to the difficulty and high cost of processing and production. Low temperature condensed ceramics are commonly used to design microfluidic devices too. They are based on aluminum oxide that initially takes the form of laminated sheets and, after assembly, is heated to a high temperature. This material is more economical than silicone and glass and takes less time to prepare [13].

2.2. Polymers

With the advancement of materials science, polymer-based chips replaced silicon/glass chips. These compounds are cheaper and more accessible and have become the most used materials in chip production [14]. Polymers are classified into three groups: elastomers, thermoplastics (more common in the fabrication of microfluidics), and thermosets [11]. Polydimethylsiloxane (PDMS) and thermoplastic elastomer (TPE) are the most commonly used elastomers, and due to having crosslinking following thermal treatment, they have a special elastic property. They can be stretched or compressed, and after the external force is removed, they return to their original form [11].

PDMS is a fast-building material that adheres strongly to PDMS, glass, and substrates and presents optical clarity, good elastomeric properties, and gas permeability [15]. PDMS has a low modulus of elasticity (300-500 kPa) and has been employed for manufacturing valves and pumps [10]. The tendency to adsorb and dissolve hydrophobic molecules that swell in contact with non-polar solvents is a consequence of the hydrophobicity of the PDMS surface [10]. Another feature of this material to be taken into account in the design of chips and assays, is the adsorption of some classes of biomacromolecules, such as proteins, on the surfaces exposed to the cell culture medium [16].

Thermostatic polyester is an insoluble material resistant to creep [11]. It is transparent and cheap, gas impermeable, unsuitable for long-term cell culture, and has significant mechanical and physical strength [11]. TPE is a hydrophobic and thermal material composed of the polymerization of polyester and styrene by ultraviolet or heat radiation [17]. Thermoplastics contain cross-linked polymers that can retain their shape as temperature decreases, but they are not suitable for long-term cell culture because they have a high hardness against the gas flow [10]. Thermoplastic polymers include (polycarbonate, polystyrene, polyethylene glycol diacrylate, polymethylmethacrylate, and Teflon (perfluorinated compounds (PFEP/ PFA/PFPE), polyurethane)) [10]. Polystyrene is a transparent, biocompatible, inert, rigid material, and its surface can be easily functionalized [10]. Polycarbonate is a durable and transparent compound that results from bisphenol A and phosgene polymerization and leads to carbonate groups [18]. Polymethylmethacrylate is an inexpensive polymer with the lowest hydrophobicity in common

plastics [10]. Polymethylmethacrylate is used in the design of microfluidic systems due to its low cost, significant mechanical properties, high optical transparency, ease of construction, and compatibility with electrophoresis [10]. Liquid Teflon is introduced in two perfluoroalkoxy and fluorinated ethylene propylene [10]. Polyurethane elastomers are used to design and construct microfluidic systems due to their mechanical strength, flexibility, and high abrasion resistance [16]. Polyurethanes show good biocompatibility properties, as they have also been used in medical applications, including those used to make artificial hearts, intra-aortic balloons, heart valves, hemodialysis membranes, or pacemaker leads [19].

2.3. Cellulosic materials

Another material that has received much attention in constructing microfluidic systems is a flexible, biocompatible, inexpensive, and cellulose-based filter paper [2]. Paper has a porous structure that provides flow, filtration, and separation in microfluidic systems [2] and is used as the substrate to be functionalized with biomolecules like aptamers in combination with optical or electrochemical detection to form biosensors with several demonstrated applications also in cancer detection [20].

2.4. Hydrogels

Hydrogels are important compounds because they are similar to the extracellular matrix and are mainly cell-related [13]. Hydrogels can be used to construct microchannels to transfer various solutions, cells, and materials because these compounds have a three-dimensional structure containing hydrophilic, porous, controllable polymer chains bioparticles [13]. **Table 1** showed the comparison of properties of different materials used in the construction of microfluidic platforms.

Table 1. Comparison of properties of different materials used in the construction of microfluidic systems [21].

Feature	Metal	Ceramic	Elastomer	Thermoplastic	Hydrogel	Paper
Low cost	Positive	Positive	Moderate	Positive	Positive	Positive
Ease of fabrication	Positive	Positive	Positive	Moderate	Moderate	Positive
Good mechanical properties	Positive	Negative	Positive	Positive	Moderate	Negative
Ease of sterilization	Not reported	Negative	Positive	Positive	Negative	Negative
Flexibility (Young's modulus) (GPa)	Negative (100–200)	Negative (65–250)	Positive (~0.0005)	Negative (1.4–4.1)	Positive (low)	Positive (0.0003–0.0025)
Oxygen permeability	Not reported	Positive (>1)	Positive (~500)	Variable (0.05–5)	Positive (>1)	Positive (>1)
Biocompatibility		Moderate	Positive	Positive	Positive	Positive
Chemical modification possibility	Not reported	Moderate	Moderate	Moderate	Positive	Moderate
Optical clarity	Negative	Negative	Slight autofluorescence	Positive	Positive	Negative
Smallest channel dimension	Not reported	>1 µm	<1 µm	<100 nm	>1 µm	>1 µm
Low absorption	Not reported	Positive	Positive	Positive	Moderate	Moderate
Rapid prototyping	Not reported	Negative	Negative	Positive	Moderate	Moderate
Tunable fluorescence	Negative	Negative	Positive	Negative	Moderate	Negative
Potential for cell ingrowth	Negative	Negative	Negative	Negative	Positive	Positive

3. Microfluidic fabrication techniques

Different methods are proposed to fabricate microfluidic devices. However, those methods have not been adapted to construct microfluidic devices for biological purposes. The most important items that should pay attention to for fabricating microfluidic devices are low-cost, easy operation, low waste, and high production. In this section, we will be discussed the well-suited fabrication methods, pros, and cons related to each method.

3.1. Chemical processes

Chemical processes include electrochemical discharge machining, wet etching, and dry [21]. The process of performing the electrochemical discharge machining technique using a spark generated (applying voltage between two electrodes) is electrochemically applied to the tool surface. This method is used with non-conducting materials such as ceramics and glass [21]. Wet technique due to fast etching speed and simultaneous processing of the examined items has been considered [21]. One of the limitations of this method is a significant safety and environmental risks that it entails [21]. In contrast, dry etching techniques have overcome some of the limitations of wet etching, but due to its very low speed, it is not preferred compared to wet etching [21].

3.2. Mechanical processes

Despite mechanical technique limitations (reduced accuracy and efficiency compared to lithographic methods), they are used to design and fabricate microfluidic systems based on silicon, glass, polymers, micro and nano-imprinting, and hot embossing [21]. Also, mechanical cutting techniques, abrasive jet machining, and ultrasonic machining are used to construct complex three-dimensional structures due to their low cost [21].

3.3. Laser-based processes

As a fast and simple fabrication technique, laser plotting/cutting has been used for rapid prototyping. This method creates a laser beam to engrave desired patterns onto the target substrate [21]. Using long and ultrashort pulses, small channel widths can be obtained. Different types of substrate materials can be utilized for this method, such as polymethylmethacrylate (PMMA), polycarbonate, polydimethylsiloxane (PDMS), etc. [21]. Laser fabrication methods are quick and expensive, so the application protocols to achieve a controllable cross-section lead to complex challenges [21].

3.4. Three-dimensional printing

One of the most powerful and popular methods of making microfluid systems is 3D printing, which has unique advantages (flexibility in design, reusability, high resolution, and easy manufacturing) compared to other techniques [22]. Polyjet printing, inkjet printing, molten sediment modeling, and stereolithography are among the various methods used for 3D printing and electrospinning [22].

3.5. Hybrid technologies

Due to the limitations of each method, Hybrid technologies are a new technique that was designed to overcome the challenges and limitations. One of the systems based on Hybrid technologies is a combined 3D system micromachine laser laminated printing is complex to design transparent microfluidic devices [21]. Another hybrid technology that integrates the advantages of xerography and thermal lamination in the printing process was a three-dimensional prototype, which enabled rapid production with high potential in construction [21].

4. Biological models: Tumor microenvironment

Solid microenvironmental tumors with heterogeneous and complex structures are very similar to organs and the tumoral microenvironment has recently been included in the description of the hallmark of cancer (besides self-sufficiency in signals growth, insensitivity to anti-growth signals, escape apoptosis, borderless proliferation potential, stable angiogenesis, tissue escape, and metastasis [23,24]), to testify to the importance of the environment in the growth and evolution of the tumoral population, actively supporting tumoral cell behavior [23].

The cellular component of tumor microenvironments includes different cell populations; besides cancer cells, fibroblasts, pericytes, adipocytes, infiltrating lymphocytes, and resident immune cells, exhibit their modulation function by inhibiting the activation of immune cells and inducing death or inhibition of immune cell proliferation [25].

The rapid production of ATP, synthesis of macromolecules, and regeneration are usually observed in cancer cells, on the other hand, they invade nearby tissues and use them to expand and develop [26]. The extracellular matrix

(ECM) is the main biochemical component of the cellular microenvironment and plays an important role in the migration, invasion, anchoring, and signaling of cancer cells [27].

Tumor microenvironments, hypoxia, and low pH nutrient deficiencies are critical metabolic features in phenotype determination (**Figure 1**). Various studies have shown that cancer cells, by changing metabolic pathways, give access to nutrients and oxygen so that they can have the energy and macromolecules needed to reproduce a cell, whose most important biochemical pathways are: fatty acid oxidation, lipid clearance, replacement of cell airways, and cell-mediated mechanisms that are performed under hypoxia and normoxia [28–30]. One of the factors contributing to cancer cells' survival in adverse and changing conditions is the reaction to lack of oxygen, which leads to limited oxidative phosphorylation or other aerobic reactions. This mode sets the redox equilibrium conditions that disrupt and affects cell signaling [31]. Numerous studies have shown that high levels of oxidative stress are seen in tumor microenvironments and that nutrients lead to cancerous cell proliferation [32]. Cancer can be considered in this perspective as a way to survive hypoxia, and glutamine metabolism as an alternative route for bioenergetic processes [33].

Collagen I, II, III, V, and IX form a firmer environment if compared to the non-tumoral microenvironment [27]. So, stiffening enhances cell growth, and the cell-cell junction integrity is compromised, impeding lumen formation [24]. Invasive irregular and non-polar colonies are formed without cell-to-cell binding protein, a significant cancer feature [24]. Another critical component in tumor microenvironments is the state of acidity. Cells cancers under the influence of hypoxia and anaerobic glycolysis show an increase in lactic acid production and decreased extracellular pH. Hence, the tumors with acidic microenvironments are more phenotypic; they move towards malignancy [34]. The acidic environment increased melanoma cells' growth, proliferation, and metastasis [35]. Because a fatty acid synthesis is so essential for the survival of cancer cells, acidic cancer cells' fat is re-synthesized, in the process of which Coenzyme A enzymes carboxylase and fatty acid synthase are crucial. Acidic and hypoxic environments express increased synthase fats in cancer cells, a dominant phenotype in various human cancers [36]. VEGF upregulation and oncogenes expression (Ras or Myc) can induce angiogenesis in the tumor [27]: VEGF expression leads to neovascularization in tumors, which creates a network of blood vessels inside cancer and complicates the tumor's microenvironment [27].

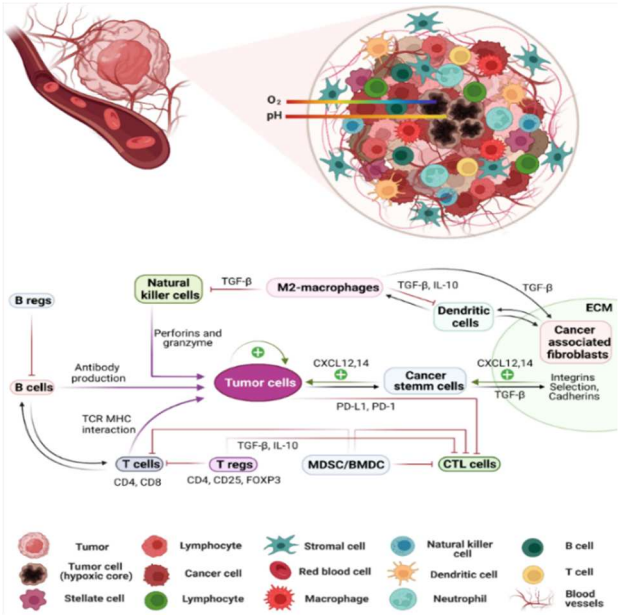


Figure 1. Components of the tumor microenvironment. The tumor microenvironment comprises a complex network system of heterogeneous, stromal, and various immune cells organized in an irregular vascular and collagen system. Poor perfusion and glycolytic tumor cells with dense packaging reduce oxygen levels, acidic pH, nutritional loads, anti-inflammatory cytokines, chemokines, and metabolic accumulation. The tumor microenvironment has important mechanisms and interactions. Green arrows: indicate the anti-tumor activity of the immune system; red arrows: show the inhibition of the anti-tumor activity of the immune system; ring in the middle: tumors themselves have strong positive effects on tumor growth.

5. Modeling and application of microfluidic platforms in cancers

One of the most important reasons that make cancer treatment difficult is the existence of complex and also dynamic microenvironments [37]. Although with the advancement of specialized sciences over the past few decades, cancer treatment approaches have been developed around surgery, radiation therapy, chemotherapy, hormone therapy, targeted therapies to inhibit angiogenesis or cell growth, immunotherapy, and the benefit of nanotechnology, the low survival rates of cancer patients and high variability of personal response to therapeutic approaches have become a matter of concern [38,39]. According to studies, about 90% of cancer deaths are due to metastasis at the primary cancer site, so early detection and metastasis prevention strategies can reduce mortality in patients [40]. The most common method of screening anti-cancer drugs as the first step of preclinical tests is based on two-dimensional culture models that represent poor tumor modeling since they don't account for the complexity of the clinical tumoral system.

On the other hand, more successful studies have been obtained by creating three-dimensional tumor models that are physiologically more relevant to the actual tumor condition. The microscopic tumor model has unique advantages that have been considered necessary in research studies compared to other models and can summarize the tumor microenvironment in a relatively common and simple way [41]. Microfluidic technologies have been developed with early advances and demonstrations focusing on on-chip system-targeted laboratory systems for bioassay and clinical diagnosis [41].

5.1. Non-microfluidic tumor models

There are three models for mimicking the tumor microenvironment that contains tumor tissue explants, animal-based and cell line-based tumor models [27]. In two-dimensional models, cell culture is performed as a single layer on flat surfaces of flasks and plates for culture as a single culture or simultaneously with different cell types [42]. In single culture, cells with the same phenotype are obtained, which don't represent effective tumoral heterogeneity. As explained in the previous sections, the tumor structure consists of cells with cell-cell communication and interaction, so this modeling is not practical because it consists of only one cell type [42].

Suspension culture, non-adherent surface methods, hanging drop methods, and scaffold-based culture are among the three-dimensional models [43]. The suspension culture leads to tumor formation by inhibiting the adhesion of the studied cells to the surfaces [44]. The non-stick surface method, like suspension culture, prevents cells from adhering to surfaces and promotes spherical formation directly through anti-fouling surface coatings [45]. The droplet method is another model that forces suspended cells to form regular, drooping spherical colonies [46]. In scaffold-based culture, tumor cell suspension creates three-dimensional structures by mixing with a hydrogel or solid scaffold, which can ultimately successfully mimic the extracellular matrix and provide successful information in microenvironment studies [47,48].

5.2. Microfluidic tumor models

The base of organs-on-chip models is for disease analysis and to mimic processes such as intra-tumor injection into scalable microfluidic formulations [49]. One of the limitations of *in vivo* studies of clinical models is the poor predictive power of the immunogenicity process and anti-cancer effects of therapeutic drugs [50]. On the other hand, the limitation of these studies at the level of animal studies and conventional cell culture models does not provide accurate predictions of human physiological and immunological responses to these drugs [50]. Mechanical studies in laboratory animals are complex, leading to the inability of researchers to closely monitor and control the microenvironment [50].

Microfluidic organs were designed and developed on chips to solve these limitations, which cultured primary human cells in microstructures [41]. This technique also can rewrite the chemical and mechanical microenvironment in human organs [41]. It can characterize the physiological features and structures of the human body compartments, such as blood capillaries, proximal renal tubules, liver, sinuses, coronary arteries, and other critical targets for models [51]. Cancer cells enter the bloodstream and spread throughout the body, leading to metastasis [52]. Hence, the unique architecture of blood vessel branching was simulated many years ago using microfluidic technologies, which received much attention in discussing tumor angiogenesis and cancer metastasis studies [51]. A chamber was designed for tumor cells containing a three-dimensional extracellular matrix imitation gel [51].

The system enabled real-time and high-resolution imaging of processes and close monitoring of endothelial barrier function [51]. Intravenous injection is one of the most widely used methods of delivering chemotherapy drugs but is usually associated with severe side effects. Different types of organs were designed and built on a chip according to the timeline provided: vascularized multi-tissue organ chips (2010), cancer cell intravasation chip (2012), angiogenesis, vasculogenesis and cancer cell extravasation chips (2013), breast cancer metastasis chip (2014), orthotopic primary breast cancer chip (2015) and orthotopic invasive and lung cancer chips [41]. Microfluidic systems have shown many benefits in studies based on modeling specific stages in cancer cascade (tumor growth and spread), angiogenesis, invasion, and progression and metastasis. Therefore, the following studies have been performed on various types of cancerous tissues and microfluidic systems.

5.2.1. Brain cancer/tumor on a chip

The brain is a complex organ consisting of blood vessels and neurons. This close interaction leads to the formation of controlled metabolic activity through the exchange of nutrients and waste products [53]. The brain structure consists of an inner vascular basement membrane secreted by endothelial cells and pericytes (containing extracellular matrix proteins such as collagen IV, laminin, nidogen, and perlecan) and the outer membrane of the outer parenchymal, which is made by astrocyte endfeet processes [54].

The blood-brain barrier is the structure that governs the central nervous system (CNS) and separates from the systemic circulatory (**Figure 2**). The CNS is created by microvascular endothelial cells that cover the brain's capillaries, pericardium, and the perivascular end of astrocytes [55]. The basement membrane protects the blood-brain barrier, pericardium, and astrocyte terminal processes that form a complex network around capillaries with unique functions [54]. One of the most critical roles of the blood-brain barrier is to maintain interstitial fluid homeostasis of the CNS, on which the normal function of neurons depends [55]. The factors involved in blood-brain barrier dysfunction can be applied to neurodegenerative disorders, stroke, infection, and brain tumors [55]. Brain metastases are the deadliest complication of cancer; the leading causes of brain tumors can be the abnormal growth of primary nerve cells and the migration of metastases from other parts of the body [56]. Some types of cancer can metastasize to the brain, so early detection can play an essential role to control [56]. Tracer compounds that enter the body cannot pass through specialized endothelial cell connections and enter the CNS [57].

Thus, no molecules pass through the blood-brain barrier because the endocannabinoid system of the blood-brain barrier abundantly expresses nutrient carriers and output transmitters and has a receptor-mediated level of transcytosis; these systems organize exchanges of the blood and CNS [57]. The interaction of tight junctions leads to high selectivity in the brain. It limits the entry of large particles (> 500 Da), which can significantly affect the brain's ability to absorb and succeed in drug delivery (< 10%) [58]. Treatment of CNS diseases has limitations for mimicking the blood-brain barrier properties since there is no effective blood-brain barrier *in vitro* models available [55].

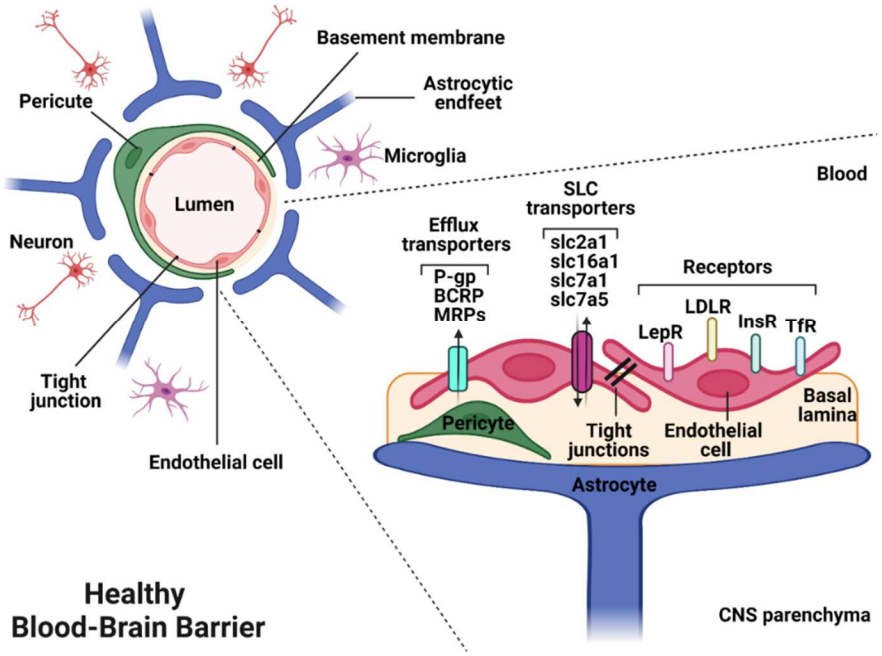


Figure 2. **The scheme of the healthy blood-brain barrier.** The blood-brain barrier structure consists of brain capillary endothelial cells joined by a basement membrane, tight junctions, pericytes, and astrocyte endfeet processes surrounding the capillaries. These structures, neurons, and microglia form the neurovascular unit. The schematic section (on the right) presents the various transporters and receptors expressed by brain endothelial cells. Active efflux transporters transport lipophilic molecules from the CNS towards blood: transporters such as P-glycoprotein, breast cancer resistance protein, and multidrug resistance proteins. The solute carriers transport nutrients such as glucose and amino acids into the brain and can be unidirectional or bidirectional, for instance, GLUT1/SLC2A1 (glucose), EAAT1/SLC1A3 (glutamate), SLC16A1 (lactate, pyruvate), SLC7A1 (cationic amino acids), and LAT1/SLC7A5 (neutral amino acids). Several receptors are present in the blood-brain barrier to meet the brain's metabolic demand: the transferrin receptor, insulin receptor, low-density lipoprotein receptor, and leptin receptor. P-gp: P-glycoprotein; BCRP: breast cancer resistance protein; MRPs: multidrug resistance proteins; SLC: solute carriers; TfR: transferrin receptor; InsR: insulin receptor, LDLR: low-density lipoprotein receptor; and LepR: leptin receptor.

The transwell cell culture system is the most used technique to create a blood-brain barrier [58]. For instance, transendothelial electrical resistance (TEER) was used to characterize the single endothelial cellular model and transwell culture systems to fabricate a blood-brain barrier model with various cells [58]. The findings showed that the presence of astrocytes leads to the thickening of the endothelial layer [58]. One of the limitations of transwell systems is the lack of dynamic flow; hence, they cannot induce shear stress to mimic what happens in the physiological environment of the brain [59]. These limitations were overcome by a dynamic microfluidic flow that included a trans volume membrane at the top and electrodes at the bottom [60]. The microfluidic co-culture system presented a different mechanism than previous techniques [59]. A microfluidic co-culture system showed a 1.5-fold increase in transendothelial electrical resistance [59]. The advantages of this system include pH adjustment, use of compounds with different molecular weights, tracking of molecules, and detailed analysis of permeability [59]. With the introduction of three-dimensional structures, a severe change in studies occurred. One of these structures is the neurovascular unit, with two vascular and cerebral chambers and a porous membrane separating the two chambers; it could mimic brain tissue much more successfully than other systems, allowing for a more detailed study of interactions variety of cells, drugs, and compounds with a blood-brain barrier [58].

Therefore, designing and inventing an advanced system can be helpful in the development process of treatment. The three-dimensional polydimethylsiloxane microfluidic system containing 16 independent functional units was designed to study the blood-brain barrier system (**Figure 3**) [55]. These functional units consist of four uniform areas of the blood-brain barrier and channels for injecting collagen or natural extracellular matrix astrocytes [55].

Animal lung, breast, and melanoma cancer models have a high potential and tendency to metastasize to the brain [61]. Brain metastasis results from tumor cells passing through the blood-brain barrier [61]. There are certain actions and reactions between cancer cells and astrocytes in the blood-brain barrier that directly affect the ability of cancer cells to pass through the brain [55].

Glioblastoma multiforme is one of the most malignant and dangerous brain tumors studied through various microfluidic systems [62]. For example, a 4-chamber microfluidic device was fabricated using PDMS soft lithography, which was used to culture C6 glioma cells in rats. The type and behavior of cells exposed to different colchicine concentrations were investigated. This study showed that morphological changes and cell death increase with increasing colchicine concentration or associated treatment time [63]. In another model, U-251-MG cells were treated with cytotoxic T lymphocytes, antiproliferative agent temozolomide, and hypoxia-activated anti-cancer drugs, hypoxia in association with collagen hydrogel bed [62].

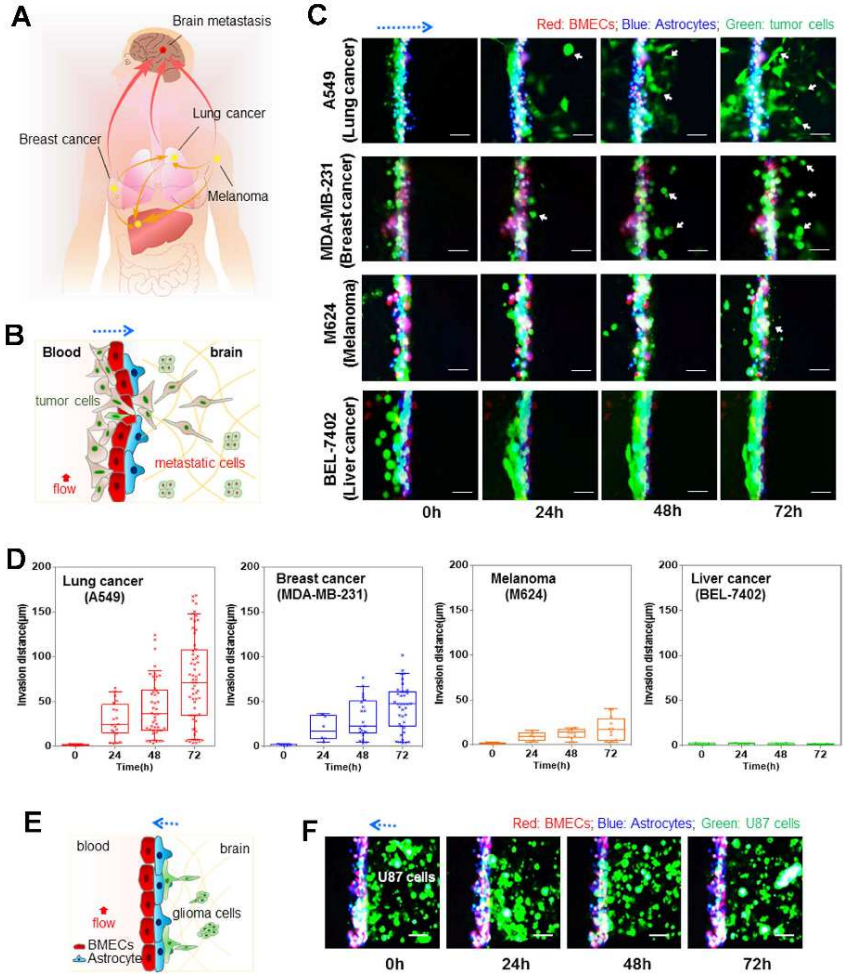


Figure 3. Brain metastatic via malignant cells. Brain cancer cells can metastasize to other organs such as lungs, breasts, and blood, then induce lung cancer, breast cancer, and melanoma (**A**). Exogenous cancer cells penetrate the brain by crossing the blood-brain barrier (**B**). Time-lapse images of extravasation of lung cancer cells (A549), breast cancer cells (MDA-MB-231), melanoma (M624), and liver cancer cells (BEL-7402) across the barrier on this blood-brain barrier system after 72 h; Red, BMECs; Blue, astrocytes; Green, cancer cells. Arrows metastatic cancer cells into the brain compartment by crossing the blood-brain barrier (**C**). Cell migration of different cancer cells crossing the blood-brain barrier shows invasion distance inside the box (**D**). Time-lapse migration of glioma U87 cells in the brain; after 72 h, these cells could not traverse the blood-brain barrier into the vascular compartment. Red, BMECs; Blue, astrocytes; Green, U87 cells (**E and F**). Reprinted with permission of Nature from reference [55].

The response of the Glioblastoma multiforme 8 (GBM8) human glioblastoma cell line to temozolomide was examined in the microfluidic system (multilayer soft lithography and PDMS) using tumor tissue instead of cancer cells. After culturing tissue sections on a porous polytetrafluoroethylene (PTFE) porous membrane, a simple diffusion model was used to deliver the drug [64]. Primary human brain endothelial cells of pericytes and astrocytes were cultured in a three-dimensional fibrin matrix. The results demonstrated that interstitial fluid flow is essential for angiogenesis and vascularization of brain endothelial cells [65].

The microfluidic cytometric imaging system has a high ability in quantitative analysis and single-cell proteome; it has clinical applications such as analyzing four proteins in the mTOR signaling pathway of human brain tumor samples and comparison with clinical immunohistochemistry [66]. Temozolomide (an alkylating agent with a high potential for DNA degradation) is the only choice of chemotherapy in the treatment of GBM [67]. Temozolomide not only does not increase the patient survival rate but also induces resistance of tumor cells [67]. The three-dimensional microfluidic system studied drug-induced programmed cell death, and the results showed that cells were significantly less sensitive to monolayer culture systems than to three-dimensional models [67]. However, the induction of apoptosis in the three-dimensional model is significantly lower than in monolayer cultures [67].

5.2.2. Lung cancer on a chip

Cell-cell interactions, cell-blood flow, and cell gas exchange are among the essential respiratory examinations for the most successful performance of drugs [68]. Lung cancer is a serious disease in today's communities with a high potential for metastasis to other organs [69].

According to its cellular origin, it is divided into two groups: Small cell lung cancer (SCLC) (15%) and non-small cell lung cancer (NSCLC) (85%). NSCLC is divided into squamous cell carcinoma, adenocarcinoma, and large cell carcinoma [69]. Cells that grow through the metastatic cascade must undergo various cellular processes, from the local and intravascular invasion of blood flow, survival in the bloodstream, and exit to colonization and growth in new organ environments [70]. These different stages are linked to various molecular programs that regulate cancer cells' migration, survival, and proliferation [70]. The molecular and cellular mechanisms that allow cancer cells to spread early through overcoming all barriers are not well understood due to the lack of metastatic specimens obtained from patients [70].

Microchips can successfully model metastasis and how it spreads outside the primary organ. One of these models is related to lung cancer and its metastasis to lung, liver, bone, and brain organs [41]. In this design, lung cancer cells growing on a lung cancer chip invade the vascular system channel where the blood circulation is shown with an arrow and successfully migrates to other chips. The role of the heart and the pumping of blood to other organs is mimicked by pumps embedded in the system [41] (**Figure 4**).

The microfluidic system, which included a multi-sensor platform, was designed to study the lung cancer specimen. At the same time, the operation of this device was based on the trans-epithelial electrical impedance of drug candidates [71]. After culturing the NCI-H1437 lung cancer cell line on a glass-based chip, the toxicity levels of different concentrations of doxorubicin and docetaxel were studied using a TEER impedance sensor [71].

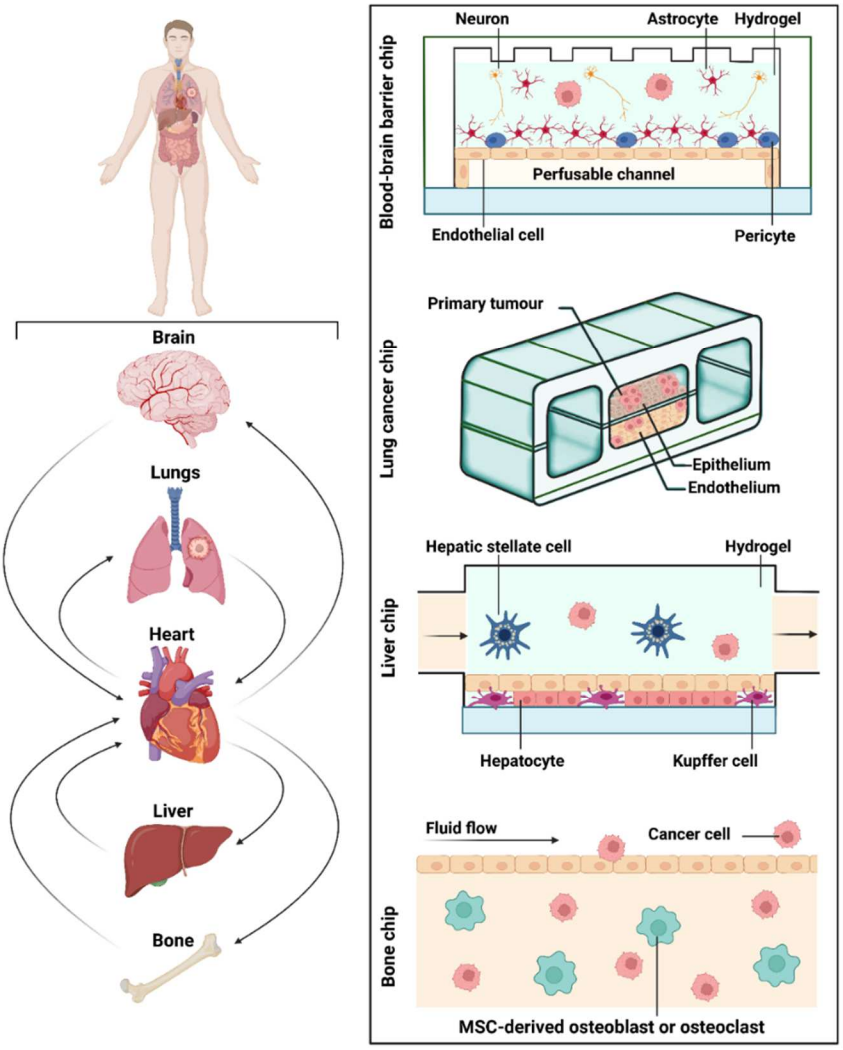


Figure 4. Monitoring lung cancer cells' migration to the liver, bone, heart, and brain. Metastatic spread of lung cancer cells and involvement of other organs using a fluorescent label that enters the circulatory system and is seen in remote tissues such as the liver, bone, or brain as the most susceptible and common target organs for lung metastasis.

5.2.3. Breast cancer on a chip

Another common and dangerous disease that has become a severe threat to women's health is breast cancer, and nearly 2.1 million breast cancer cases occur worldwide each year [72–74]. Therefore, specialized study of pathogenic pathways and drug metabolism systems and finding practical goals are essential in diagnosing and treating this disease [72]. Recently, two strategies have been developed rapidly, organoid culture techniques and organ on chip based on microfluidic technology. The first relies on the 3D culture of mammalian stem cells with the sequential addition of growth factors; in contrast, the organ on the chip can fine-tune the microenvironment based on the microfluidic device [72].

There are various *in vitro* breast cancer models on a microfluidic chip, such as the spheroid model (homotypic spheroid, heterotypic spheroid), lumen models (ductal breast model, breast-vasculature interaction model), designable patterned co-culture models, breast–other organ interaction model, and three-dimensional extracellular matrix in breast tumor-on-a-chip (matrix candidate, hypoxia model) [72]. In a spherical system, exactly like a solid tumor, cancer cells accumulate in the body while maintaining the ability and potential to multiply and invade [75]. In the homotypic spherical model, three-dimensional cultures are used to simulate tumor tissue in the body more accurately. For example, MCF-7 cells and fibroblasts prepared homotypic spheres in a microfluidic device [76].

Another study developed a microfluidic chip-based three-dimensional breast cancer model by co-culturing monodisperse breast tumor spheroids aggregated by T47D or MDA-MB-231 with monocytes in a three-dimensional collagen matrix to investigate macrophages and ECM effect on tumor cell migration [77]. Breast cancer T47D, together with HepG2 (hepatic cancer) and HCT116 (colon cancer) cells were used in a microfluidic system to form various spheres to study drug sensitivity and drug signaling pathways [78]. Heterotypic spheres can successfully mimic cancer tissue treatment by merging stromal cells with tumor cells (fibroblasts, endothelial cells, and fat cells) [78]. One of the dominant methods in studying cancerous tumors is the placement of immune cells, including monocytes, macrophages, and stellate cells, in a heterotypic sphere. For example, in several studies in this area, a spherical model including breast tumor cell lines (BT20, MCF-7, and MCF-10A) was designed to mimic tumor heterogeneity *in vitro* [79]. ECM-based hydrogels were used as scaffolding to facilitate epithelial fabrication in one structure. The microfluidic chip created a semi-channel U-shaped duct to mimic a tree-like duct system [80].

In breast cancer metastasis, breast tumor cells are separated from the primary carcinoma vessels and transported to distant organs to form secondary tumors. Therefore, arteries play an essential role in the metastasis process. The design of an artificial vessel embedded in an extracellular matrix to study the process of MDA-MB-231 breast cancer cells entering the vessel created a new approach to cancer-related studies [81]. National rod molds were used to produce three-dimensional lumen vessels and subsequently coated with human dermal microvascular endothelial cells. Invasion and migration of breast cancer cells were recorded by imaging over time in the matrix around the blood vessels [81].

Understanding critical physiological pathways and mechanisms in the progression of the disease, drug metabolites, and organ interactions are essential, so designing multi-organ systems on a common chip could be a new approach to cancer treatment research [82]. One of the studies in this regard was related to the design of a system in which lung, liver, and cancerous breast organs were placed on a microfluidic chip to study the pathways involved in the delivery of curcumin [82]. A549, HepG2 C3A, and MDA-MB-231 cancer cells created lung, liver, and breast cancer organs in three single microfluidic chambers, respectively [82]. Multi-organ microphysiological system showed that intravenous treatment significantly reduces breast cancer survival compared to inhalation therapy [82].

Because of the importance of ECM in maintaining cellular activities and functions (proliferation, migration, apoptosis, response to drugs), the imitation of a three-dimensional matrix in a microfluidic system is one of the most important considerations in organ engineering on a chip [72]. Porosity and permeability are two essential factors in modeling that hydrogels and electrospun fibers are the two supporting families in properties of natural hydrogels, including collagen [72]. Electrospun fibers with properties such as (adjustable diameter, mechanical stiffness, and embedding particles/compounds) play the role of the scaffold in three-dimensional cell culture; polycaprolactone and polylactic acid, due to biocompatibility and biodegradable nature, are two common materials in the made of electrospun fibers [72]. Different oxygen levels have been observed in solid tumors, and usually, the highest rate of hypoxia is in the central part of the tumor [83]. Hypoxia and its causes lead to tumor progression and resistance to treatment. Therefore, more specialized studies related to this index can be a big step in treating patients [83].

5.2.4. Liver cancer on a chip

The liver regulates the body's metabolism (protein synthesis/storage, carbohydrate metabolism, cholesterol, phospholipids, and bile salts). Parenchymal cells called hepatocytes and non-parenchymal cells include Kupffer cells, hepatic stellate cells, hepatic sinusoidal endothelial cells, and hepatic lymphocytes [84].

Knowing the role of the microenvironment in liver metastasis (from pre- to prometastatic niches) is too important. In the mouse model of metastatic pancreatic ductal adenocarcinoma study, the results presented that pancreatic ductal adenocarcinoma-derived exosomes taken up by hepatic Kupffer cells increase TGFβ production to raise fibronectin production by hepatic stellate cells and recruitment of bone marrow-derived macrophages. On the other hand, the macrophage migration inhibitory factor is necessary for premetastatic niche formation and metastasis. Also, in exosomes derived from patients with pancreatic adenocarcinoma of the pancreas, which later led to liver metastasis, migration inhibitory factors were higher than in patients whose tumors had not progressed. So, the identification and analysis of migration inhibitors to control pancreatic adenocarcinoma metastasis in the liver are effective (11). In exosome-fused Kupffer cells, the proinflammatory factors S100P and S100A8 were upregulated (**Figure 5A**).

Once blood cancer cells enter the liver, they are exposed to the body's first line of defense, which includes liver sinusoidal endothelial cells, Kupffer cells, and natural killer liver cells (18). The release of inflammatory mediators, such as IL1b, TNFα, and IL18, can also initiate a cascade that facilitates cells' rapid exit from the arteries into a less "toxic" microenvironment (**Figure 5B**).

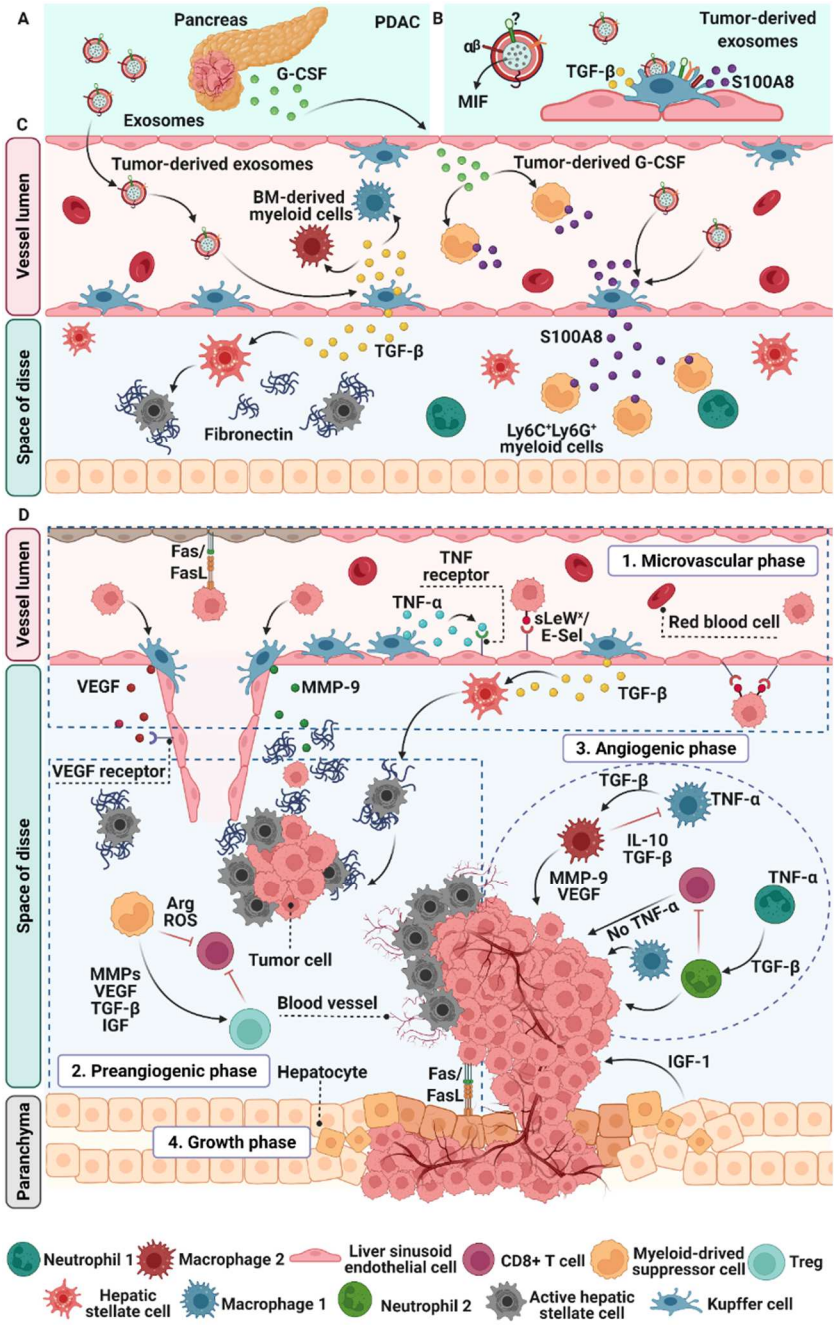


Figure 5. Schematic figure of premetastatic niche in the liver. Diagrammatic representation of a PDAC (**A**). Kupffer cell activated by PDAC-derived exosomes is shown (**B**). There are potential therapeutic strategies in the disease space: enhancing kupffer cell tumoricidal activities, inducing hematopoietic stem cells apoptosis, and inhibiting inflammatory (**C**). The multistep process of liver colonization by disseminated cancer cells. These four significant phases include the microvascular, proangiogenic, angiogenic, and growth phases. MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; Treg, regulatory T cell.

One of the salient features of the liver in research is the valuable information it provides regarding drug development and screening and cytotoxicity testing. Toxicity and side effects of drugs following the use of the drug itself, which is known as "direct toxicity" or metabolites produced (toxicity of side effects) in the liver or other organs [62].

A three-dimensional microfluidic system was used to study the human liver and investigate the role of extracellular vesicles caused by breast cancer in liver metastasis and cause the endothelial transmission to mesenchymal and destruction of vascular barriers. It also regulates β1 growth factor conversion in breast cancer-derived extracellular vesicles, fibronectin, an adhesive extracellular matrix protein, on hepatic sinus endothelial cells, which facilitates the adhesion of breast cancer cells to the liver microenvironment (**Figure 6**) [85]. To study the integration of three dimensions laboratory models with multi-organ technology on one chip, they developed one lung/liver. Development of a lung/liver-on-a-chip, connecting in a single circuit, normal human bronchial epithelial cells cultured at the air-liquid interface, and HepaRG™ liver spheroids. Aflatoxin B1 toxicity in normal human bronchial epithelial cells air-liquid interface tissues decreased when HepaRG™ spheroids were present in the same chip circuit, proving that the HepaRG™-mediated detoxification is protecting/decreasing from aflatoxin B1-mediated cytotoxicity [86].

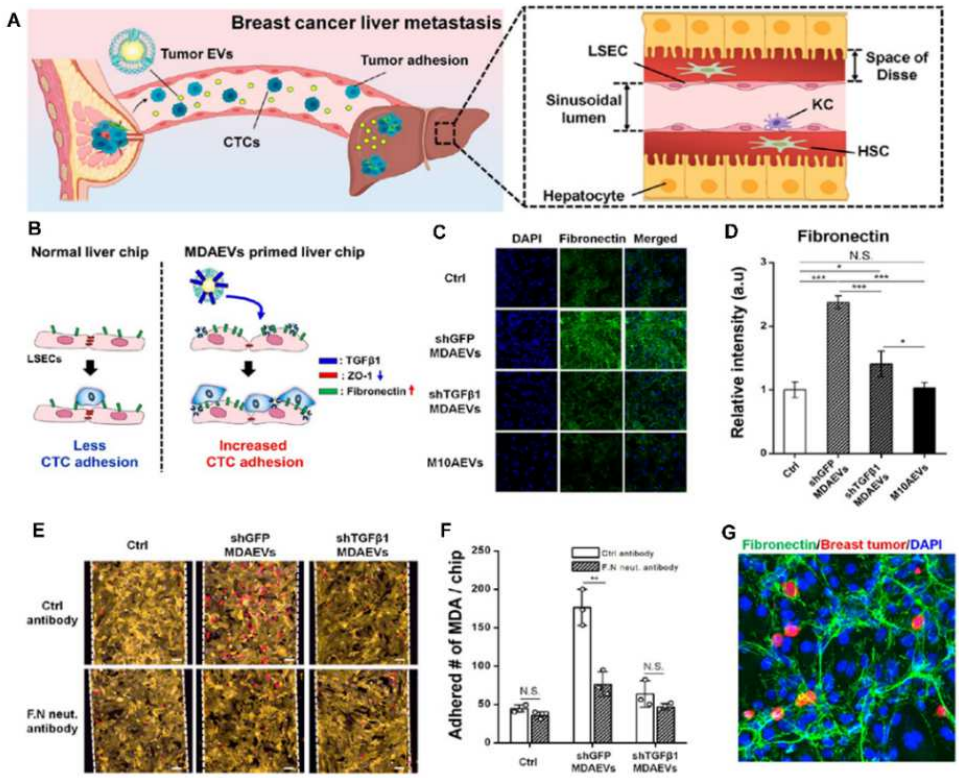


Figure 6. Microfluidic human liver chip recapitulates breast cancer liver metastasis. The breast cancer liver metastasis model after premetastatic niche formation by primary tumor-derived extracellular vesicles (KC: Kupffer cell, HSC: hepatic stellate cell) (**A**). The mechanism for primary breast cancer-derived extracellular vesicles-mediated premetastatic niche formation promoting breast cancer cell adhesion to the liver microenvironment. Representative images and quantification of fibronectin expression on LSECs in human liver chips after treatment with shGFP or shTGFβ1 MDAEVs and M10AEVs (**B and C**). Effect of LSECs fibronectin after treatment of MDAEVs with control antibody or fibronectin-neutralizing antibody on MDA cell adhesion to LSECs in the human liver chips (red: MDA; orange: LSECs (**D and E**). Representative image of breast cancer cells (red) co-localizing with fibronectin (green) on top of an LSECs layer in human liver chips (DAPI: blue) (**F**). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Reprinted with permission of ACS from reference [85].

5.2.5. Kidney cancer on a chip

The human urinary system comprises the kidneys, ureters, bladder, prostate, and urethra, which play an essential role in excreting waste products, regulating blood volume, and other critical activities. The kidney is of particular

importance in studies focusing on the effects of drugs and plays a role in eliminating foreign biotics and is highly effective in regulating blood pressure. Prostate and bladder cancers are more common than other cancer types of the urinary tract [62].

The primary function of the proximal tube is to reabsorb solutes and fluids, which have been similarly reconstructed in several tube systems on a chip with a membrane-based design [87]. The initial design consists of two chambers (a channel in the upper part to mimic urine production and a channel in the lower part that mimics the interstitial space) [88,89]. In this system, mouse distal tubular cells or Madin-Darby canine kidney (MDCK) cells were used [88].

A microfluidic chip was used to quantify the biomarker of bladder cancer and apolipoprotein A1. This three-dimensional system consists of two layers of air to control the mixer and valve; the inner layer was used for the fluid part, and a polydimethylsiloxane layer was used for microchip sealing micromixer and suction type micro-valve and magnetic beads. A four-layer, three-dimensional microchip was fabricated. The results were compared with the classical enzyme-linked immunosorbent assay method. The most crucial advantage of microchips is the faster detection of cancer cells than previous systems [90]. In another study, a three-dimensional microfluidic system was designed and built to simulate the microenvironment of bladder cancer. Bladder cancer cells (T24), stromal cells, fibroblasts, endothelial cells, and macrophages were used, and specialized channels were allocated to optimize access to four cell types. By examining the effect of various chemotherapeutic compounds on bladder cancer cells, it was proved that methotrexate, vincristine, doxorubicin, and cis-diamine platinum dichloride group are more sensitive candidates for chemotherapy [91].

5.2.6. Bone cancer on a chip

Bone is an organ that can be seriously targeted by cancer metastasis because it has favorable conditions for absorbing tumor cells. The unique mechanical properties of the extracellular matrix of bone, which is mainly composed of hydroxyapatite, affect several cellular responses in the tumor microenvironment, such as proliferation, migration, survival, and morphology as an angiogenic activity associated with bone metastasis [92]. Bone is a dynamic organ with inorganic structure, motor function, soft tissue structural support, hormonal regulation, and organizes the bone marrow compartment [93].

Currently, studies are focusing on bone cancer metastasis compared to other organ-on-chip systems. To evaluate the invasion of prostate cancer cells from the LNCaP and C4-2B cell lines with the mouse in combination with osteoblastic cells (MC3T3-E1s), these cell lines have been cultured in a microfluidic device equipped with the multi-photon imaging-based technique. Androgen-dependent behavior of LNCaP cells resulted in less aggressive than stromal cells, while androgen-independent C4-2Bs showed more aggressive behavior. Also, it was observed that when C4-2Bs were treated with conditioned media from a mixture of C4-2Bs and MC3T3-E1, the percentage of protruding C4-2Bs increased significantly. Osteoclasts stimulate cancer metastasis through the breakdown of the bone matrix (thereby releasing growth factors) and the direct secretion of osteoclast signals [93].

6. Conclusions and perspective

Although there is a massive amount of work going on in medical science developing new methods in diagnostic, therapy, and pharmacological protocols for cancer, there are still many challenges in these areas. One of the newest tools is represented by microfluidic systems, which have been considered for their high potential and have led to many advances in the cancer diagnosis and treatment debate over the past decade. Cancer is one of the leading causes of death worldwide. Microfluidic devices are promising in the diagnosis of cancer. Early cancer detection leads to a better chance of real-time treatment monitoring, with significant and minor side effects. Microfluidic tools are highly valuable for cancer research due to their high modularity, throughput, sensitivity, and low cost and material consumption, allowing for improved Spatio-temporal control. The most crucial point that is considered in the design and development of organ-on-chip models is the focus on the construction and implementation of a system that can satisfactorily mimic the physiological and pathophysiological conditions of the body. Only then can the exact behaviors of the types of cancers and the drugs used be evaluated. Microfluidic models can be perfused (e.g.,

continuous, cyclic, or alternating) differently from static models, including transformer wells, spheres, and organoids; they are more successful in studies that focus on neovascularization and invasion spread of cancer cells.

On the other hand, perfusion can increase the function and duration of cell survival. Including a perfused endothelium-lined vasculature also offers greater clinical relevance for studying drug delivery and modeling pharmacokinetics and pharmacodynamics, which are greatly influenced by drug transport into and across the vascular endothelium. They allow for the establishment of controlled chemical gradients, and air-liquid interfaces to replicate organ-relevant mechanical environments, fluid shear stresses, and hydrostatic pressures that are present *in vivo* tumor microenvironment. The ability to collect samples selectively from either the parenchymal, stromal, or vascular compartments for further molecular, cellular, genetic, or pharmacokinetic analysis are other advantages of this system. Organ on chips is more difficult to use than other 3D culture systems despite all these advantages. Usually, they have a lower throughput than can be obtained with highly multiplexed static culture chambers. Another challenge associated with these systems is technical robustness. The scale and complexity of microfluidic systems that experience controlled fluid flow must fully interact to achieve optimal performance and the scalability of the results, is still under discussion also, for long-term studies, the challenge of maintaining life and cell function and structural integrity of multiple textures and different types is different types of cells crucial for the reliability of results [41,51,94,95]: in these directions lot of scientific and technological effort is being put in place involving engineering, material science, biology, physics and medicine expertise.

Authors' contributions

All authors contributed to drafting and revising of the paper and agreed to be responsible for all the aspects of this work.

Acknowledgments

F.D.M. is supported by the PERSEO Project, funded in the framework of Gruppi di Ricerca Regional Call identified by protocol number A0375-2020-36656.

Declaration of competing interest

The authors declare no competing interest.

Funding

This paper received no external funding.

Data availability

Not applicable.

References

[1] M. Piñeros, L. Mery, I. Soerjomataram, F. Bray, E. Steliarova-Foucher, Scaling up the surveillance of childhood cancer: a global roadmap, JNCI J. Natl. Cancer Inst. 113 (2021) 9–15.
[2] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA. Cancer J. Clin. 68 (2018) 394–424.
[3] A. Bigham, V. Rahimkhoei, P. Abasian, M. Delfi, J. Naderi, M. Ghomi, F.D. Moghaddam, T. Waqar, Y.N. Ertas, S. Sharifi, Advances in tannic acid-incorporated biomaterials: Infection treatment, regenerative medicine, cancer therapy, and biosensing, Chem. Eng. J. (2021) 134146.
[4] M. Hassanpour, M.H. Shahavi, G. Heidari, A. Kumar, M. Nodehi, F.D. Moghaddam, M. Mohammadi, N. Nikfarjam, E. Sharifi, P. Makvandi, Ionic liquid-mediated synthesis of metal nanostructures: Potential application in cancer diagnosis and therapy, J. Ion. Liq. (2022) 100033.
[5] S.K. Singh, S. Singh, J.W. Lillard Jr, R. Singh, Drug delivery approaches for breast cancer, Int. J. Nanomedicine. 12 (2017) 6205–6218.
[6] P. Makvandi, M. Chen, R. Sartorius, A. Zarrabi, M. Ashrafizadeh, F.D. Moghaddam, J. Ma, V. Mattoli, F.R. Tay, Endocytosis of abiotic nanomaterials and nanobiovectors: Inhibition of membrane trafficking, Nano Today. 40 (2021) 101279.
[7] S. Akgönüllü, M. Bakhshpour, A.K. Pişkin, A. Denizli, Microfluidic Systems for Cancer Diagnosis and Applications,

Micromachines. 12 (2021) 1349.
[8] H.-Y. Cho, J.-H. Choi, J. Lim, S.-N. Lee, J.-W. Choi, Microfluidic Chip-Based Cancer Diagnosis and Prediction of Relapse by Detecting Circulating Tumor Cells and Circulating Cancer Stem Cells, Cancers (Basel). 13 (2021) 1385.
[9] S. Van den Driesche, F. Lucklum, F. Bunge, M.J. Vellekoop, 3D printing solutions for microfluidic chip-to-world connections, Micromachines. 9 (2018) 71.
[10] P.N. Nge, C.I. Rogers, A.T. Woolley, Advances in microfluidic materials, functions, integration, and applications, Chem. Rev. 113 (2013) 2550–2583.
[11] K. Ren, J. Zhou, H. Wu, Materials for microfluidic chip fabrication, Acc. Chem. Res. 46 (2013) 2396–2406.
[12] J. Hwang, Y.H. Cho, M.S. Park, B.H. Kim, Microchannel fabrication on glass materials for microfluidic devices, Int. J. Precis. Eng. Manuf. 20 (2019) 479–495.
[13] L. Mou, X. Jiang, Materials for microfluidic immunoassays: a review, Adv. Healthc. Mater. 6 (2017) 1601403.
[14] A. Alrifaiy, O.A. Lindahl, K. Ramser, Polymer-based microfluidic devices for pharmacy, biology and tissue engineering, Polymers (Basel). 4 (2012) 1349–1398.
[15] C.M.B. Ho, S.H. Ng, K.H.H. Li, Y.-J. Yoon, 3D printed microfluidics for biological applications, Lab Chip. 15 (2015) 3627–3637.
[16] E. Piccin, W.K.T. Coltro, J.A.F. da Silva, S.C. Neto, L.H. Mazo, E. Carrilho, Polyurethane from biosource as a new material for fabrication of microfluidic devices by rapid prototyping, J. Chromatogr. A. 1173 (2007) 151–158.
[17] E. Roy, M. Geissler, J.-C. Galas, T. Veres, Prototyping of microfluidic systems using a commercial thermoplastic elastomer, Microfluid. Nanofluidics. 11 (2011) 235–244.
[18] Y. Liu, D. Ganser, A. Schneider, R. Liu, P. Grodzinski, N. Kroutchinina, Microfabricated polycarbonate CE devices for DNA analysis, Anal. Chem. 73 (2001) 4196–4201.
[19] W.-I. Wu, K.N. Sask, J.L. Brash, P.R. Selvaganapathy, Polyurethane-based microfluidic devices for blood contacting applications, Lab Chip. 12 (2012) 960–970.
[20] Y. Wang, J. Luo, J. Liu, S. Sun, Y. Xiong, Y. Ma, S. Yan, Y. Yang, H. Yin, X. Cai, Label-free microfluidic paper-based electrochemical aptasensor for ultrasensitive and simultaneous multiplexed detection of cancer biomarkers, Biosens. Bioelectron. 136 (2019) 84–90.
[21] A.-G. Niculescu, C. Chircov, A.C. Bîrcă, A.M. Grumezescu, Fabrication and applications of microfluidic devices: A review, Int. J. Mol. Sci. 22 (2021) 2011.
[22] A.K. Au, N. Bhattacharjee, L.F. Horowitz, T.C. Chang, A. Folch, 3D-printed microfluidic automation, Lab Chip. 15 (2015) 1934–1941.
[23] D. Hanahan, Hallmarks of Cancer: New Dimensions, Cancer Discov. 12 (2022) 31–46.
[24] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell. 144 (2011) 646–674.
[25] J. Piñeiro Fernández, K.A. Luddy, C. Harmon, C. O'Farrelly, Hepatic tumor microenvironments and effects on NK cell phenotype and function, Int. J. Mol. Sci. 20 (2019) 4131.
[26] R.A. Cairns, I.S. Harris, T.W. Mak, Regulation of cancer cell metabolism, Nat. Rev. Cancer. 11 (2011) 85–95.
[27] M. Shang, R.H. Soon, C.T. Lim, B.L. Khoo, J. Han, Microfluidic modelling of the tumor microenvironment for anti-cancer drug development, Lab Chip. 19 (2019) 369–386.
[28] M.-D. Wang, H. Wu, S. Huang, H.-L. Zhang, C.-J. Qin, L.-H. Zhao, G.-B. Fu, X. Zhou, X.-M. Wang, L. Tang, HBx regulates fatty acid oxidation to promote hepatocellular carcinoma survival during metabolic stress, Oncotarget. 7 (2016) 6711.
[29] L.K. Boroughs, R.J. DeBerardinis, Metabolic pathways promoting cancer cell survival and growth, Nat. Cell Biol. 17 (2015) 351–359.
[30] T. Cheng, J. Sudderth, C. Yang, A.R. Mullen, E.S. Jin, J.M. Matés, R.J. DeBerardinis, Pyruvate carboxylase is required for glutamine-independent growth of tumor cells, Proc. Natl. Acad. Sci. 108 (2011) 8674–8679.
[31] J.E. Klaunig, Oxidative stress and cancer, Curr. Pharm. Des. 24 (2018) 4771–4778.
[32] M.-D. Wang, N.-Y. Wang, H.-L. Zhang, L.-Y. Sun, Q.-R. Xu, L. Liang, C. Li, D.-S. Huang, H. Zhu, T. Yang, Fatty acid transport protein-5 (FATP5) deficiency enhances hepatocellular carcinoma progression and metastasis by reprogramming cellular energy metabolism and regulating the AMPK-mTOR signaling pathway, Oncogenesis. 10 (2021) 1–11.
[33] J. Cao, H. Choi, D. Kranseler, A. Pantel, D. Mankoff, R. Zhou, Dynamic PET Imaging Marker of Glutaminase Inhibition in Triple Negative Breast Cancer, J. Nucl. Med. 60 (2019) 135.
[34] Y. Kato, S. Ozawa, C. Miyamoto, Y. Maehata, A. Suzuki, T. Maeda, Y. Baba, Acidic extracellular microenvironment and cancer, Cancer Cell Int. 13 (2013) 1–8.
[35] S. Sutoo, T. Maeda, A. Suzuki, Y. Kato, Adaptation to chronic acidic extracellular pH elicits a sustained increase in lung cancer cell invasion and metastasis, Clin. Exp. Metastasis. 37 (2020) 133–144.
[36] F. Röhrig, A. Schulze, The multifaceted roles of fatty acid synthesis in cancer, Nat. Rev. Cancer. 16 (2016) 732–749.
[37] F.R. Balkwill, M. Capasso, T. Hagemann, The tumor microenvironment at a glance, J. Cell Sci. 125 (2012) 5591–5596.
[38] F.D. Moghaddam, I. Akbarzadeh, E. Marzbankia, M. Farid, A.H. Reihani, M. Javidfar, P. Mortazavi, Delivery of melittin-loaded niosomes for breast cancer treatment: an in vitro and in vivo evaluation of anti-cancer effect, Cancer Nanotechnol. 12 (2021) 1–35.
[39] A. Moammeri, K. Abbaspour, A. Zafarian, E. Jamshidifar, H. Motasadizadeh, F. Dabbagh Moghaddam, Z. Salehi, P. Makvandi, R. Dinarvand, pH-Responsive, Adorned Nanoniosomes for Codelivery of Cisplatin and Epirubicin: Synergistic Treatment of Breast Cancer, ACS Appl. Bio Mater. 5 (2022) 675–690.
[40] X. Guan, Cancer metastases: challenges and opportunities, Acta Pharm. Sin. B. 5 (2015) 402–418.

[41] A. Sontheimer-Phelps, B.A. Hassell, D.E. Ingber, Modelling cancer in microfluidic human organs-on-chips, Nat. Rev. Cancer. 19 (2019) 65–81.
[42] J.P. Thiery, H. Acloque, R.Y.J. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell. 139 (2009) 871–890.
[43] T.G. Manning, J.S. O'Brien, D. Christidis, M. Perera, J. Coles-Black, J. Chuen, D.M. Bolton, N. Lawrentschuk, Three dimensional models in uro-oncology: a future built with additive fabrication, World J. Urol. 36 (2018) 557–563.
[44] B.-W. Huang, J.-Q. Gao, Application of 3D cultured multicellular spheroid tumor models in tumor-targeted drug delivery system research, J. Control. Release. 270 (2018) 246–259.
[45] E. Fennema, N. Rivron, J. Rouwkema, C. van Blitterswijk, J. De Boer, Spheroid culture as a tool for creating 3D complex tissues, Trends Biotechnol. 31 (2013) 108–115.
[46] M.J. Ware, K. Colbert, V. Keshishian, J. Ho, S.J. Corr, S.A. Curley, B. Godin, Generation of homogenous three-dimensional pancreatic cancer cell spheroids using an improved hanging drop technique, Tissue Eng. Part C Methods. 22 (2016) 312–321.
[47] S. Breslin, L. O'Driscoll, Three-dimensional cell culture: the missing link in drug discovery, Drug Discov. Today. 18 (2013) 240–249.
[48] G.Y. Lee, P.A. Kenny, E.H. Lee, M.J. Bissell, Three-dimensional culture models of normal and malignant breast epithelial cells, Nat. Methods. 4 (2007) 359–365.
[49] A.U.R. Aziz, C. Geng, M. Fu, X. Yu, K. Qin, B. Liu, The role of microfluidics for organ on chip simulations, Bioengineering. 4 (2017) 39.
[50] S. Gehmert, S. Gehmert, X. Bai, S. Klein, O. Ortmann, L. Prantl, Limitation of in vivo models investigating angiogenesis in breast cancer, Clin. Hemorheol. Microcirc. 49 (2011) 519–526.
[51] J.T. Borenstein, Microfluidic techniques for cancer therapies, Eur. Pharm. Rev. 22 (2017) 50–53.
[52] F.D. Moghaddam, S. Hamedi, M. Dezfulian, Anti-tumor effect of C-phycocyanin from Anabaena sp. ISC55 in inbred BALB/c mice injected with 4T1 breast cancer cell, Comp. Clin. Path. 25 (2016) 947–952.
[53] A.E. Urai, B. Doiron, A.M. Leifer, A.K. Churchland, Large-scale neural recordings call for new insights to link brain and behavior, Nat. Neurosci. (2022) 1–9.
[54] R. Aryal, A. Patabendige, Blood–brain barrier disruption in atrial fibrillation: a potential contributor to the increased risk of dementia and worsening of stroke outcomes?, Open Biol. 11 (2021) 200396.
[55] H. Xu, Z. Li, Y. Yu, S. Sizdahkhani, W.S. Ho, F. Yin, L. Wang, G. Zhu, M. Zhang, L. Jiang, A dynamic in vivo-like organotypic blood-brain barrier model to probe metastatic brain tumors, Sci. Rep. 6 (2016) 1–12.
[56] C.R. Oliver, M.A. Altemus, T.M. Westerhof, H. Cheriyan, X. Cheng, M. Dziubinski, Z. Wu, J. Yates, A. Morikawa, J. Heth, A platform for artificial intelligence based identification of the extravasation potential of cancer cells into the brain metastatic niche, Lab Chip. 19 (2019) 1162–1173.
[57] L. Kaplan, B.W. Chow, C. Gu, Neuronal regulation of the blood–brain barrier and neurovascular coupling, Nat. Rev. Neurosci. 21 (2020) 416–432.
[58] B. Miccoli, D. Braeken, Y.-C.E. Li, Brain-on-a-chip devices for drug screening and disease modeling applications, Curr. Pharm. Des. 24 (2018) 5419–5436.
[59] L.M. Griep, F. Wolbers, B. de Wagenaar, P.M. ter Braak, B.B. Weksler, I.A. Romero, P.O. Couraud, I. Vermes, A.D. van der Meer, A. van den Berg, BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function, Biomed. Microdevices. 15 (2013) 145–150.
[60] J.A. Brown, V. Pensabene, D.A. Markov, V. Allwardt, M.D. Neely, M. Shi, C.M. Britt, O.S. Hoilett, Q. Yang, B.M. Brewer, Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor, Biomicrofluidics. 9 (2015) 54124.
[61] J. Li, P. Cai, A. Shalviri, J.T. Henderson, C. He, W.D. Foltz, P. Prasad, P.M. Brodersen, Y. Chen, R. DaCosta, A multifunctional polymeric nanotheranostic system delivers doxorubicin and imaging agents across the blood–brain barrier targeting brain metastases of breast cancer, ACS Nano. 8 (2014) 9925–9940.
[62] N. Kashaninejad, M.R. Nikmaneshi, H. Moghadas, A. Kiyoumarsi Oskouei, M. Rismanian, M. Barisam, M.S. Saidi, B. Firoozabadi, Organ-tumor-on-a-chip for chemosensitivity assay: A critical review, Micromachines. 7 (2016) 130.
[63] W. Liu, P. Sun, L. Yang, J. Wang, L. Li, J. Wang, Assay of glioma cell responses to an anticancer drug in a cell-based microfluidic device, Microfluid. Nanofluidics. 9 (2010) 717–725.
[64] T.C. Chang, A.M. Mikheev, W. Huynh, R.J. Monnat, R.C. Rostomily, A. Folch, Parallel microfluidic chemosensitivity testing on individual slice cultures, Lab Chip. 14 (2014) 4540–4551.
[65] M.A. Winkelman, D.Y. Kim, S. Kakarla, A. Grath, N. Silvia, G. Dai, Interstitial flow enhances the formation, connectivity, and function of 3D brain microvascular networks generated within a microfluidic device, Lab Chip. 22 (2022) 170–192.
[66] J. Sun, M.D. Masterman-Smith, N.A. Graham, J. Jiao, J. Mottahedeh, D.R. Laks, M. Ohashi, J. DeJesus, K. Kamei, K.-B. Lee, A microfluidic platform for systems pathology: multiparameter single-cell signaling measurements of clinical brain tumor specimens, Cancer Res. 70 (2010) 6128–6138.
[67] E. Samiei, A. Seyfoori, B. Toyota, S. Ghavami, M. Akbari, Investigating programmed cell death and tumor invasion in a three-dimensional (3D) microfluidic model of glioblastoma, Int. J. Mol. Sci. 21 (2020) 3162.
[68] X. Liu, J. Fang, S. Huang, X. Wu, X. Xie, J. Wang, F. Liu, M. Zhang, Z. Peng, N. Hu, Tumor-on-a-chip: From bioinspired design to biomedical application, Microsystems Nanoeng. 7 (2021) 1–23.
[69] Â. Carvalho, G. Ferreira, D. Seixas, C. Guimarães-Teixeira, R. Henrique, F.J. Monteiro, C. Jerónimo, Emerging lab-on-a-chip approaches for liquid biopsy in lung cancer: status in CTCs and ctDNA research and clinical validation, Cancers (Basel). 13

(2021) 2101.
[70] J. Ko, M.M. Winslow, J. Sage, Mechanisms of small cell lung cancer metastasis, EMBO Mol. Med. 13 (2021) e13122.
[71] M.A.U. Khalid, Y.S. Kim, M. Ali, B.G. Lee, Y.-J. Cho, K.H. Choi, A lung cancer-on-chip platform with integrated biosensors for physiological monitoring and toxicity assessment, Biochem. Eng. J. 155 (2020) 107469.
[72] K. Song, X. Zu, Z. Du, Z. Hu, J. Wang, J. Li, Diversity Models and Applications of 3D Breast Tumor-on-a-Chip, Micromachines. 12 (2021) 814.
[73] A.D. Dey, A. Bigham, Y. Esmaeili, M. Ashrafizadeh, F.D. Moghaddam, S.C. Tan, S. Yousefiasl, S. Sharma, A. Maleki, N. Rabiee, Dendrimers as nanoscale vectors: Unlocking the bars of cancer therapy, in: Semin. Cancer Biol., Elsevier, 2022.
[74] F.D. Moghaddam, P. Mortazavi, S. Hamedi, M. Nabiuni, N.H. Roodbari, Apoptotic effects of melittin on 4T1 breast cancer cell line is associated with up regulation of Mfn1 and Drp1 mRNA expression, Anti-Cancer Agents Med. Chem. (Formerly Curr. Med. Chem. Agents). 20 (2020) 790–799.
[75] M. Huerta-Reyes, A. Aguilar-Rojas, Three-dimensional models to study breast cancer, Int. J. Oncol. (2021).
[76] L. Zhao, M. Shi, Y. Liu, X. Zheng, J. Xiu, Y. Liu, L. Tian, H. Wang, M. Zhang, X. Zhang, Systematic analysis of different cell spheroids with a microfluidic device using scanning electrochemical microscopy and gene expression profiling, Anal. Chem. 91 (2019) 4307–4311.
[77] T. Yuan, D. Gao, S. Li, Y. Jiang, Co-culture of tumor spheroids and monocytes in a collagen matrix-embedded microfluidic device to study the migration of breast cancer cells, Chinese Chem. Lett. 30 (2019) 331–336.
[78] Y. Chen, D. Gao, H. Liu, S. Lin, Y. Jiang, Drug cytotoxicity and signaling pathway analysis with three-dimensional tumor spheroids in a microwell-based microfluidic chip for drug screening, Anal. Chim. Acta. 898 (2015) 85–92.
[79] S. Chandrasekaran, Y. Geng, L.A. DeLouise, M.R. King, Effect of homotypic and heterotypic interaction in 3D on the E-selectin mediated adhesive properties of breast cancer cell lines, Biomaterials. 33 (2012) 9037–9048.
[80] M.M.G. Grafton, L. Wang, P.-A. Vidi, J. Leary, S.A. Lelièvre, Breast on-a-chip: mimicry of the channeling system of the breast for development of theranostics, Integr. Biol. 3 (2011) 451–459.
[81] A.D. Wong, P.C. Searson, Live-cell imaging of invasion and intravasation in an artificial microvessel platform, Cancer Res. 74 (2014) 4937–4945.
[82] P.G. Miller, C. Chen, Y.I. Wang, E. Gao, M.L. Shuler, Multiorgan microfluidic platform with breathable lung chamber for inhalation or intravenous drug screening and development, Biotechnol. Bioeng. 117 (2020) 486–497.
[83] S.M. Grist, S.S. Nasseri, L. Laplatine, J.C. Schmok, D. Yao, J. Hua, L. Chrostowski, K.C. Cheung, Long-term monitoring in a microfluidic system to study tumour spheroid response to chronic and cycling hypoxia, Sci. Rep. 9 (2019) 1–13.
[84] W. Seo, W.-I. Jeong, Hepatic non-parenchymal cells: Master regulators of alcoholic liver disease?, World J. Gastroenterol. 22 (2016) 1348.
[85] J. Kim, C. Lee, I. Kim, J. Ro, J. Kim, Y. Min, J. Park, V. Sunkara, Y.-S. Park, I. Michael, Three-dimensional human liver-chip emulating premetastatic niche formation by breast cancer-derived extracellular vesicles, ACS Nano. 14 (2020) 14971–14988.
[86] D. Bovard, A. Sandoz, K. Luettich, S. Frentzel, A. Iskandar, D. Marescotti, K. Trivedi, E. Guedj, Q. Dutertre, M.C. Peitsch, A lung/liver-on-a-chip platform for acute and chronic toxicity studies, Lab Chip. 18 (2018) 3814–3829.
[87] J. Bahnemann, A. Enders, S. Winkler, Microfluidic systems and organ (human) on a chip, in: Basic Concepts 3D Cell Cult., Springer, 2021: pp. 175–200.
[88] K.-J. Jang, K.-Y. Suh, A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells, Lab Chip. 10 (2010) 36–42.
[89] K.-J. Jang, A.P. Mehr, G.A. Hamilton, L.A. McPartlin, S. Chung, K.-Y. Suh, D.E. Ingber, Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment, Integr. Biol. 5 (2013) 1119–1129.
[90] Y.-H. Lin, Y.-J. Chen, C.-S. Lai, Y.-T. Chen, C.-L. Chen, J.-S. Yu, Y.-S. Chang, A negative-pressure-driven microfluidic chip for the rapid detection of a bladder cancer biomarker in urine using bead-based enzyme-linked immunosorbent assay, Biomicrofluidics. 7 (2013) 24103.
[91] P. Liu, Y. Cao, S. Zhang, Y. Zhao, X. Liu, H. Shi, K. Hu, G. Zhu, B. Ma, H. Niu, A bladder cancer microenvironment simulation system based on a microfluidic co-culture model, Oncotarget. 6 (2015) 37695.
[92] J. Ahn, J. Lim, N. Jusoh, J. Lee, T.-E. Park, Y. Kim, J. Kim, N.L. Jeon, 3D microfluidic bone tumor microenvironment comprised of hydroxyapatite/fibrin composite, Front. Bioeng. Biotechnol. (2019) 168.
[93] A. Mansoorifar, R. Gordon, R.C. Bergan, L.E. Bertassoni, Bone-on-a-Chip: Microfluidic Technologies and Microphysiologic Models of Bone Tissue, Adv. Funct. Mater. 31 (2021) 2006796.
[94] C. Zhao, Z. Ge, C. Yang, Microfluidic techniques for analytes concentration, Micromachines. 8 (2017) 28.
[95] L. Vaccari, G. Birarda, G. Grenci, S. Pacor, L. Businaro, Synchrotron radiation infrared microspectroscopy of single living cells in microfluidic devices: advantages, disadvantages and future perspectives, in: J. Phys. Conf. Ser., IOP Publishing, 2012: p. 12007.

Authors Biography

Farnaz Dabbagh Moghaddam received her Ph.D. in Developmental and Cellular Biology from Azad University, Science and Research Branch, Tehran, Iran, in 2021. Currently, she is a Postdoctoral Research Fellow at the CNR-IFN Institute for Photonics and Nanotechnologies National Research Council, Rome, Italy. Her current research interests include the design of materials for biomedical applications.

Francesca Romana Bertani received her Ph.D. in Human Pathology from Sapienza University of Rome in 2008. She's currently permanent researcher at the Instituto for Photonics and Nanotechnologies of the Italian National Research Council. Her research interests include the development of tools, approaches and instruments (combining optics, microfluidics, coculture models) to study biological processes and pathophysiology at cell scale.