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REVIEW

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# Microfluidic endothelium-on-a-chip development, from *in vivo* to *in vitro* experimental models

ADRIANA-ELENA BULBOACĂ[1)], PAUL MIHAI BOARESCU[1)], CARMEN STANCA MELINCOVICI[2)], CARMEN MIHAELA MIHU[2)]

[1)]*Department of Functional Biosciences, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[2)]*Department of Morphological Sciences, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania*

## Abstract

In the last years, animal testing in medical research has been a controversial topic because of various reasons, such as ethical considerations and species differences. Therefore, more attention has been given to develop new technologies that can replace animal experiments and create *in vitro* models. Organ-on-a-chip (OOC) technology is a new and advanced technology based on microfluidic devices that can mimic the structure and function of entire organs and tissues as *in vitro* models. OOC models are miniature tissues and organs that assign characteristics for three-dimensional (3D) cell culture representation that resemble the original organs, together with their specific microenvironment microfluidic systems and specific biophysical processes, in order to mimic the normal physiological conditions and functionalities of the organs. Existing OOC models, such as liver, pancreas, heart, skin, brain, kidney, vessels, have been developed and designed for a specific function study. This review focuses on the main knowledge concerning OOC research and especially vascular endothelium-on-a-chip (EOC) model, developed in order to offer specific tools for studying vascular functions in physiological and pathological conditions. The field of OOC devices is still at the beginning, but in the future, this technology may have important roles in developing novel therapeutic approaches, offering new therapeutic molecules and providing the first step towards personalized medicine.

***Keywords***: organ-on-a-chip, endothelial cells, microfluidic systems, cell culture.

## Introduction

Organ-on-a-chip (OOC) technology is a new developed technology designed to mimic the organs' structure and create conditions for functional connections between cells by signaling molecules pathways. Therefore, OOC models assign features for three-dimensional (3D) representation of cellular complexity of the organs together with their natural microenvironments' microfluidic systems and specific physical conditions, in order to recapitulate the as close as possible natural biology of the structures [1]. This *in vitro* advanced technology is able to replace animal experiments that are very important for preclinical screening of new therapeutic molecules, but which have various issues, such as time-consuming process, species differences and ethical considerations. Because cell culture systems are not exactly reproducing the entire organ function and they are also not in functional interaction with other types of cells, a system that permit multiple cell culture in a specific microenvironment and that are able to communicate through signaling molecules developed as a new advanced technology for experimental studies. This technology is able to offer new therapeutic molecules more rapid and that are highly reproducible in clinical studies. By cultivating patient's cells-on-a-chip platform is also providing the first step to a personalized medicine [2, 3]. Furthermore, the OOC systems are further developed for creating a multiorgan platform able to mimic the interactions between multiple organs [4]. Once created, multiorgan-on-a-chip (MOC) models can represent an ideal pharmacokinetic and pharmacodynamic tool for assessing the efficacy of pharmaceutical compounds on multiple organs [5]. A large number of MOC models were proposed but, because of the dynamic nature of human environment, the integration process of many organs into a chip remains a significant challenge in the future.

Despite the advantages of OOC compared with two-dimensional (2D) cultures and animal models, the major challenge to assign various OOC models consists in maintaining the cells viability as long as possible, for an adequate support of pathophysiological mechanism studies, but also to assess morphological and biochemical consequences associated with various disorders [6, 7]. This advanced technology raised from the necessity to develop experimental models close related to natural conditions that can mimic multiple diseases and for a more rapid implementation of therapeutic strategies that can be transferred to clinical trials in a future personalized medicine. There are already serious concerns about the time to develop new therapeutic molecules. To meet these requests is necessary to create a model designed for a specific experiment that is testing a precise function of the organ by biomarkers. The parameters that serve as biomarkers have to have a precise assessing tool and have to offer the possibility to implementation in clinical studies. OOC already developed models correspond to this goal leading to a new technology in medical research field represented by laboratory-on-a-chip [8]. OOC models also permit the cell's "cross-talking" by signalizing molecules, such as cytokines or chemokines, synthesized by various

types of cells after they activation. The activation process can consist in triggering molecules for oxidative stress, inflammation, or apoptotic process. Existing OOC models, such as liver, pancreas, heart, skin, brain, kidney, are designed for a specific function study. After a better understanding of their functions, these models can serve to test various drugs in order to improve their delirious function resulting from a specific disorder [9]. Moreover, there is a growing interest in MOC models for drug testing due to the opportunity to study the systemic effects of new therapeutic molecules [10]. In this way, OOC can offer a viable solution that can contribute to a more realistic experimental model for a patient-specific disease and for a proper treatment. In this order, the cell culture on the chip can be represented by patient's cells sample that are set on a chip in specific-organ microenvironment and the model can be used to test the efficiency of various drugs for each patient.

## Endothelium-on-a-chip (EOC) as a main model for specific functions study

One of the most important OOC is represented by vessels-on-a-chip. In order to create a model that can reproduce the specific functions of endothelial structures, efforts were made to mimic the structure fabrication related to specific functions. Even there is yet not possible to have all the functions and implied structures on the same chip, several characteristics on endothelial cells in accord with their functions were successful used for research in order to replace animals in biomedical experiments [11, 12]. Vessel's wall permeability changes can constitute one of the most important components of pathophysiological mechanisms involved in diseases from all pathology fields. On the other hand, endothelial cells constitute the most important structure involved in atherosclerosis, onset of thrombotic process or in cancer metastasis. Structure changes of vessels wall contribute essentially to platelet activation and adhesion to the vessel wall glycoproteins, and further will contribute to the coagulation cascade initiation due to specific surface receptors exposure. These phenomena occur consequently to exposure of extracellular membrane proteins, such as collagen and laminin, and release of von Willebrand factor (vWF) [13, 14]. The entire thrombotic process is also requiring platelet participation by expression of their adhesion to extracellular membrane proteins *via* glycoprotein Ib (GPIb) and glycoprotein VI (GPVI) [15]. Platelet activation and release of adenosine diphosphate (ADP) and thromboxane A2 (TxA2) together with platelet aggregation *via* glycoprotein IIb/glycoprotein IIIa (GPIIb/GPIIIa) homodimers are the ultimate processes designated to the first step of hemostasis [16]. Platelet activation is a consequence of endothelial structural changes [16].

Creating a model for vessels-on-a-chip is frequently addressed only to endothelium cells that can maintain their viability for days, in order to study the effects of endothelial damage on platelet activation. They can also serve to study the permeabilization for various molecules that are released in circulation and can further influence the specific tissues' structures or functions. The main functional systems that were considered to be important for endothelium involvement in specific disorders, in order to be reproduced on a chip, are presented in Table 1 [17–35].

**Table 1 –** ***Pathological processes associated with endothelial structural damage and dysfunction***

| Function | Associated structures |
|---|---|
| Endothelial structure as a barrier | ▪ Vasodilatation molecules: NO [17] is synthesized continuously in endothelial cells from L-arginine by the constitutive eNOS enzyme [18]; in physiological quantities, exerts a protective role by maintaining vasomotricity vessels equilibrium; synthesized in excess, it is a component of nitro-oxidative stress with harmful effects [19].<br>▪ Soluble antiplatelet molecules (aggregation and adhesion inhibitors).<br>▪ PGI2 [20]; shear stress upregulates COX-2 activity and increase the production of PGI2; PECAM-1 regulates vascular inflammatory responses [21].<br>▪ Anti-adhesive molecules in vessel wall structure – glycocalyx [17]; glycocalyx destruction is mediated by ROS, MMPs and other enzymes, such as heparanase and sialidases; glycocalyx degradation has as a result increasing of vessel wall permeability, leukocyte–endothelium interactions, thrombosis onset and vascular inflammation [22].<br>▪ Anticoagulant molecules – thrombomodulin (anti-coagulation effect by binding to thrombin and inactivating factors Va and VIIIa by increasing the generation of activated protein C) [23].<br>▪ Endothelial EP4 receptor (for PGE2) on endothelial cells has an antithrombotic role [24]. |
| Increased shear stress | ▪ Increased shear stress induces the nuclear accumulation of class I, class II HDACs and DNA methyltransferases in endothelial cells, promoting oxidative stress and inflammation with endothelial cells dysfunction as a consequence [25].<br>▪ Activation of endothelial cells is associated with vWF factor releasing [26]; platelet-derived factor V released from activated platelets is an important contributor to arterial thrombosis, together with increased cGMP levels and overexpression of P-selectin on the platelet surface [27]. |
| Platelet activation | ▪ Endothelial cells lesion is leading to collagen exposure; in damaged vessels, vWF factor binds to subendothelial collagen fibers and to platelet GPIb–IX–V receptor complex, thus mediating platelets adhesion to the injury site [28].<br>▪ Upon adhesion, the primary platelet activators (collagen, thrombin, ADP, TxA2 and epinephrine) will induce multiple platelet responses (platelet morphology changes from discoid shape to other forms, activation of $\alpha$IIb$\beta$3 integrin promoting platelet aggregation and synthesis of TxA2 [29]; in the following phase, an irreversible platelet activation will result by: secretion of platelet dense and alpha-granules, exposure of membrane PS on the platelet surface activating the coagulation cascade, as well as formation of dense fibrin networks and platelet-derived microparticles [29].<br>▪ Activated platelet release vWf and factor V [30]. |
| Oxidative stress and inflammation | ▪ ROS molecules induce hyperadhesive vWF assembly [31].<br>▪ Vascular injury induces recruitment of pro-inflammatory cytokines (TNF$\alpha$), which increases expression of surface adhesion molecules (***e.g.***, ICAM-1, VCAM-1) on endothelial cells and release of vWF [32]. |

| Function | Associated structures |
|---|---|
| Tumor metastasis, angiogenesis | ▪ The metastatic process is initiated by destruction of extracellular matrix due to MMPs, released by tumor cells. These proteolytic enzymes are responsible for degradation of basement membrane components (collagen IV, laminin) [33, 34].<br>▪ Tumor angiogenesis is promoted by PDGF and VEGF [35]. |

ADP: Adenosine diphosphate; cGMP: Cyclic guanosine monophosphate; COX-2: Cyclooxygenase-2; DNA: Deoxyribonucleic acid; eNOS: Endothelial nitric oxide synthase; GPIb–IX–IV: Glycoprotein Ib–IX–V; HDACs: Histone deacetylases; ICAM-1: Intercellular adhesion molecule-1; MMPs: Matrix metalloproteinases; NO: Nitric oxide; PDGF: Platelet-derived growth factor; PECAM-1: Platelet endothelial cell adhesion molecule-1; PGI2: Prostaglandin I2; PS: Phosphatidylserine; ROS: Reactive oxygen species; TNF*α*: Tumor necrosis factor alpha; TxA2: Thromboxane A2; VCAM-1: Vascular cell adhesion molecule-1; VEGF: Vascular endothelial growth factor; vWF: von Willebrand factor.

## Microfluidic vessels models

In order to provide tools for the study of vascular phenomena, various *in vitro* microfluidic vessels models have been developed. They are complex in architecture and physiology, designed to offer solutions for artificial organ vascularization. The fabrication methods had to consider mimicking not only the histology of the vessels, but also to recreate an environment close to physiological conditions, corresponding to the organs or tissues where they were designated. Other models are designed to study the mechanisms associated with pathological conditions in order to understand the vessels contribution in onset or progression of various diseases. Using the advances in microfluidics, biomaterials and stem cells technology, the perfusable microvessels models can offer platforms that can imitate the tumor microenvironment, occluded microvessels or perfused capillary networks [36, 37]. These microfluidic platforms can constitute an important advanced step in experimental studies, targeting not only the understanding of the vessel's physiology and their contribution in various disorders, but also implementation of new therapeutic strategies. Due to a very complex architecture of human vessels, that have a particular structure and environments in each organ or tissue, there is not yet possible to reproduce the exact vessels' models. Still, according with specific goals, different models are designed to study the effect of shear stress on vessels' wall [38], the effects of exposure of the subendothelial structures to bloodstream or to shear stress with platelet activation and adhesion (thrombosis onset) [39], as well as the transendothelial transport phenomena [40].

One of the most used models for transendothelial transport mechanisms assessment consists in analysis of a fluorescent tracer that can across the tight junctions of endothelial cells [41]. The above properties of the vessels have a common important function that refers to the role of the barrier exerted by vessels wall. Thus, barrier integrity constitutes one of the most important features to study, in almost each microfluidic vessel model. Targeting this goal, efforts were made in order to develop instruments that are able to quantify the permeability of vessels wall. The existing technique offers the possibility for quantitative assessment of the permeability of vessels wall by measuring transepithelial/transendothelial electrical resistance (TEER), as well as paracellular diffusion of radiolabeled permeability markers (*e.g.*, Mannitol) [40]. For qualitative assessment of membrane structure, different methods can be used: freeze-fracture electron microscopy of transmembrane fibrils or immunohistochemical analysis of tight junction-related protein expression [40]. As for the study of trans-endothelial transport mechanisms, the permeability of vessels' wall can be study by measuring the ability of a well-known size molecule that serve as a fluorescent tracer. By assessment the tracer passage in surrounding medium, the leakage can be estimated and is expressed in terms of permeability (P) (cm/s) [42]. Typical molecules used in these models are fluorescent-labeled Dextran or fluorescent small molecule dye that can further be identified into the interest region, and florescence intensity measured according to the time interval [43]. There are conditions where the permeability offers valuable information about the intensity of the pathological process, *e.g.*, an inflammation after an acute toxic aggression on endothelium, where the permeability is expected to be increased compared with normal endothelium function. The increased permeability can be transitive or permanent, and this characteristic can be also studied by assessment of vascular permeability in different time intervals. The same phenomenon is expected to be present when vessels' architecture is anarchic, *e.g.*, in novel vessels formation due to ischemic condition or due to neoplastic processes. The leakage can express the intensity of ischemia [vascular endothelial growth factor (VEGF) synthesis is related to ischemia magnitude] or may depend on tumor characteristics [36]. One of the most important transport functions studied on microvessel models is the penetrability of endothelium barrier by drugs or by drug delivery systems, such as nanocarriers [44]. Drugs' penetrability through microvessels on *in vitro* models are most studied mechanisms for tumors treatments or for cancer metastasis prevention [44, 45].

According to the literature data, the main studies focusing on structural and functional properties of vessels and their modeling in order to study various phenomena are presented in Table 2 [36, 41, 42, 46–67].

**Table 2 –** ***Vessels-on-a-chip models: structural and functional properties***

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|---|---|
| **Endothelial structures models** | After the ECs are seeded into the channels, they are incubated for a period of time (between 1–3 days) in order to get confluences. The formation of the monolayer of ECs with confluences can be faster if the microenvironment of the cells is provided with fibronectin, prior the introduction of ECs. The ECs monolayers are growing in a cylindrical channel within collagen gels and their diameters can increase to 75–150 μm after maturation. In this model, the density of the cells is approximately $10^7$ cells/mL and offers a five days viable endothelial barrier [42]. Adherence between ECs can be tested by identification of specific junction protein, such is VE–cadherin. There are specific models that reproduce human ECs barrier that can support repeated measurements for permeability coefficient and where focal gaps can be detected [41]. Most used human ECs lines are HUVECs and HDMECs [36]. |

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| **Vascular flow, pressure, and stability** | Mimicking the vascular shear stress represents one of the greatest challenges for scientists, due to the complex system of forces that can be a modeling factor for endothelial structures. Unidirectional shear stress with a constant pressure produces endothelial lesion that are reproducing the real human conditions. Improving the stability and longevity of the ECs has to be based on pulsatile flow conditions. Shear stress and transmural pressure are the dominant mechanical signals that impose the rhythm of cells proliferation [46]. Pulsatile flow creates conditions for reorganization of the cytoskeleton and junctions modeling [41]. The intensity of pulsatile flow can be adapted also to various pathologies in order to mimic various disorders, such as atherosclerosis or stenosis conditions. Vascular cell adhesion molecules geometries correlate with shear stress on the vessels wall [47]. The variation of the pressure can be obtained by computer-controlled valve system that can modulate the recirculating fluid system physical properties [41]. More complex approaching is needed to create endothelial models that can recapitulate the human BBB. Using a commercially available microfluidic platform, Brown *et al.* developed a human microfluidic BBB model, using co-culture of primary human astrocytes and hCMEC/D3, resembling the human brain capillaries [48]. The model allows to study the junction between ECs under a physiological shear stress, acting for more than 24 hours, by assessing the phenotypical tight junction biomarkers [48]. |
| **Barrier function** | The ECs barrier function is tested by permeability coefficient that represents the quantity of molecules with a certain size that are allowed to pass through endothelial junctions [36].<br>The barrier function is tested with fluorescent substances, such as Dextran. *In vivo* vessels permeability coefficients have normal range between $10^{-8}$ to $10^{-6}$ cm/s for 70 kDa Dextran [36].<br>The permeability of endothelial barrier is expected to be normal in physiological conditions and increased in pathological conditions (inflammation, apoptosis or necrosis), that lead to an activated endothelium with increased cells metabolic activity [49]. There are also significant differences between the permeability of the vessels with normal architecture and new vessels with anarchic architecture where the increased leakage is one of the most important features. The neovessels anarchic architecture in tumors contributes to the tumor cells spreading and metastasis [50]. Tumor's vessels have an irregular diameter, abnormal branching, without typical characteristics of arterioles, capillaries or venules, being unable to be classified. Their basal membrane is incomplete and has abnormal pericytes coat resulting large-caliber with thin walls [51]. The expression of various receptors, integrins or growth factors is also abnormal [52].<br>Cationic liposomes are able to avid binding to pathological endothelium (associated with inflammatory process or tumors). This characteristic facilitates therapies with liposomes as carriers for various drugs [53].<br>Building OOC BBB is useful for quantification of drug targeting brain tissue study and is more complex due to its particular structure. The tight junction between ECs and the presence of polarized efflux pumps on luminal surface has to be modeled [54]. OOC BBB needs to be integrated in a functional network, known as neurovascular unit and contains neurons, pericytes, astrocytes, microglial cells, and ECM [55]. The BBB permeability is increasing in all the pathological situations of the central nervous system associated with cerebral edema. Disruption of BBB is associated with various neurological diseases, such as epilepsy, central nervous system tumors, ischemic stroke, multiple sclerosis or Alzheimer's disease [55]. |
| **Cell motility and morphological parameters assessment** | The morphology of the ECs is an important parameter to evaluate cell viability. In normal conditions, ECs are elongated, with the long diameter in the direction of shear stress. Any transition from the normal morphology to a pathological shape can indicate a dysfunction of the ECs. This can be from a mild dysfunction, such as the transition from cobblestone shape to a spindle-like morphology under shear stress, to severe changes that could indicate apoptosis or necrosis [56, 57]. ECs in brain vessels resist to shear stress and do not elongate under these mechanical forces. This is an important characteristic that was demonstrated on BBB-on-a-chip model, in a specific microfluidic environment. This may indicate that ECs in BBB are programmed to resist to elongation under the shear stress due to their special and unique phenotype [38]. Tight junctions are forming in static conditions that will decrease the motility of the cells that prevent up regulation of proteins synthesis. Increasing the structural protein synthesis rate is associated with cell's structural and functional changes [58]. If the shear stress conditions are increased due to hypertension or other systemic vascular diseases, will occur barrier function impairment, which could be reversed by therapeutic interventions [59].<br>The effect of share stress on dhBMECs was evaluated using live cells imaging, observing the cell morphology, cells speed, proliferation and apoptosis rate. Confluent monolayer is maintained in microfluidic conditions, on OOC device, and proteins and gene expression serve as biomarkers for various functions assessments (immunofluorescence method) [58]. Tight junctions can be evaluated by identification of several highly express adhesion proteins, such as VE-cadherin, ZO-1, occludin and claudin-5 [60].<br>Cells motility increase under the expression of mitogenic growth factors, such as FGF, EGF, VEGF, and can be considered as a parameter of cells activity. Cells speed can be evaluated by imaging of the cells and tracking the position of individual cell [38]. Moreover, ECs-on-a-chip model can be arranged as microfluidic network of channels containing proper constituents for red blood cells adding. In this way, a more complex platform results and this platform can also offer conditions for an *in vitro* model of *in vivo* circulation with communication between different cell types [61].<br>OOC models containing ECs that mimic vessels wall can be used as microfluidic platforms for studying the effect of drugs on morphological and structural changing in pathological conditions. There is also the possibility to integrate different drugs concentration on these platforms, in order to assess the dose-dependent effects. These microfluidics systems have all the advantages of lab-on-a-chip technology that allowed cell operations, such as seeding, culture, stimulation and staining into a chip [62]. In order to study liver drug toxicity and metabolism or the efficacy of anticancer molecules, microfluidics-based *in vitro* systems were realized [63–65]. The proliferation rate assessment can be used as a parameter to evaluate the efficiency of anticancer drugs [66]. |
| **Capillary perfusion** | To study the function of capillary network, the capillary perfusion can be evaluated. Capillary perfusion can be defined as the ratio of fluorescence intensity of the substance in vascularized tissues compared with unvascularized tissues [36]. A variety of pathological conditions can be assessed by this method, such as increased capillary perfusion due to hyperemia associated with inflammation or decreased capillary perfusion in ischemic conditions [36]. The fluorescence method is using fluorescence-labeled Dextran as a molecule that has the property to remain in the vessel lumen, if there is no increased permeability, or to increase the fluorescence, if is accumulating in an ischemic capillary network [42]. |
| **ECs activation** | Using live-cell microscopy, it is possible to analyze the cells by tracking their positions in a time-defined interval. Image analysis can provide also a quantitative assessment of mitosis rate, apoptosis process or motility of the cells [36]. |

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|---|---|
| **Neoangiogenesis** | Engineered tissue technique aims to create platforms that are able to mimic original structures in human body. Starting with ECs culture, microfluidic technology can create an environment-on-a-chip that can recapitulate the vessels structure. By integration in an ECM and by adding fibroblasts, this platform can create proper conditions for branching and anastomosis. Finally, a capillary network will result that can be designed to study the physiological and pathological conditions details. The angiogenesis resulting from this technique is a valuable tool for the study of various triggers, such as shear stress or cytokine influence [67]. |

BBB: Blood–brain barrier; dhBMECs: Derived human brain microvascular endothelial cells; ECM: Extracellular matrix; ECs: Endothelial cells; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; hCMEC/D3: Human cerebral microvascular endothelial cell line; HDMECs: Human dermal microvascular endothelial cells; HUVECs: Human umbilical vein endothelial cells; OOC: Organ-on-a-chip; VE: Vascular endothelial; VEGF: Vascular endothelial growth factor; ZO-1: *Zonula occludens*-1.

## Future perspectives of EOC models in pathology

Two of the most important pathological conditions, ischemia and uncontrolled cells proliferation during tumor formation, are the main targets for EOC platforms development. Therefore, these models are the most advanced models and the technology implemented is the most complex one.

### The role of EOC models designed for tumor growth and metastasis studies

Tumor hallmarks include several characteristics represented mainly by continuous producing of growth molecules by tumor cells, evading molecules that function as suppressors, decreased apoptotic signals, facilitating replicative immortality, angiogenesis and enabling metastasis [68, 69]. Tumors are also characterized by genetic instability and associated inflammation. They are also recruiting normal cells that contribute to the tumor microenvironment. Every type of tumor can recruit a specific repertoire of normal cell that will contribute to the specific metabolic feature of the tumor microenvironment [68]. To survive and growth, tumor provide neovessel formation. Neovessels serves for tumor nutrition but they are also one of the most important pathways for metastasis. Therefore, one of the main objectives in antitumor therapeutic strategies is to suppress neovessel proliferation and function. Metastatic tumor cells are spreading to the other organs or tissues through two steps: intravasation and extravasation [68]. The process of intravasation needs macrophages recruitment (tumor-associated macrophages that facilitate transendothelial migration of cancer cells) [70]. This process is very complex and incomplete understood, taking place at the interface between tumor and neovessels and having as participants numerous types of cells and signaling mechanisms. The goal of *in vitro* vascular models is to create a specific microenvironment that is able to mimic tumor environment and to offer a tool for studying details of metastatic process cascade [71, 72]. Is already known that neovessels have an abnormal structure (less smooth muscle cells and pericytes), and consequently, an increased permeability, that facilitate tumor metastasis [73]. There is a linear relationship between the neovessels network density and the metastatic process, as well as between leakages size and tumor invasion [73].

Tumor metastasis process is also correlated with the microvessel diameter, beginning in microvessels larger than 30 μm [73]. The tumor cells can be added to an extracellular matrix (ECM) component in order to observe the interface between ECM and new formed tumor microvessels [71]. Detachments of tumor cells, their transendothelial migration and invasion process can be observed [74]. The spreading of the tumor into surrounding tissues involves few steps, represented by detachment of the tumor cells from primary tumor, degradation of ECM and migration process [75, 76]. Tumor microenvironment plays a crucial role for metastasis rate [70]. Using real-time imaging was reported that vascular permeability is transient, depending on tumor microenvironment, macrophages cooperation, and signaling molecules synthesis, which finally will influence tumor cell dissemination [70]. The metastasis is also time depending on neovessels formation, angiogenesis being a fast process designed to tumor cells growth, proliferation and surviving [68]. The observation of interaction between endothelial cells and tumor cells is made by live cells microscopy fluorescent-labeled proteins being used to identify various pathological aspects, such as increased permeability or intensity of various signaling pathways [77]. OOC models comprise the structures required for a specific function. A special attention is paid to recreate the specific tumor microenvironment and to maintain a viable cells network, in order to offer a valuable tool for tumoral processes that controls metastasis cascade. Understanding the underlying mechanisms of tumor metastasis is an important step for therapeutic strategies applied to those patients who already present a metastatic tumor.

### Ischemia and EOC models

Occluded microvessels models are important *in vitro* platform for vascular diseases, such as atherosclerosis, myocardial infarction or ischemic stroke. The complexity of such a model resides from the complexity of the targeting organs structures. Creating an entire OOC model could bring these platforms closer to the real pathogenetic processes associated with vascular diseases. Therefore, the efforts are made toward heart-on-a-chip or brain-on-a-chip models fabrication that can mimic the structure and biological process inside the organ [78, 79]. The most difficult task is to create a proper microfluidic environment for a correct cell signaling pathways [80–82]. The endothelial cells play an important role in these models due to their crucial contribution to the barriers that compose the interface between the blood and a specific tissue. One of the most complex structures that assign the barrier function is the blood–brain barrier (BBB). The endothelial cells morphology, function, specific protein expressions and the microenvironment has to recapitulate the brain real physiological conditions of flow dynamics. After the building of the proper model, the microvessels can be obstructed manually or specific condition for thrombosis onset can be reproduced [55]. Perfusable tissue-engineered microvessel models can serve for studying the morphology and function of endothelial

cells studying under the turbulent flow associated with atherosclerosis [47]. There is already possible to create *in vivo* models that are able to reproduce the specific 3D geometry of aneurysms, stenosis, bifurcations, in order to study the hemodynamic complex consequences of different pathological shear stress patterns [47]. It is believed that thrombotic mechanism onset is conditioned by hemodynamic particularities, endothelial cells morphology changes and platelet activation [83]. Mannino *et al.* created a vasculature model by seeding a monolayer of endothelial cells on a support that respect a circular distribution geometry, able to decrease the platelet aggregability; by introducing a disturbing flow factor, the platelet adhesivity and aggregability can be increased [47]. This model can serve as an *in vitro* support to study the processes associated with platelet activation and thrombosis onset. *In vitro* models can also create the possibility for studying endothelial injury under occluded and non-occluded microvessels [84]. Already existing models can also reproduce flow conditions in order to study platelets–endothelial cells interactions, that is a dynamic process yet incompletely understood [85, 86]. The potential of microfluidic device technologies in this field bring hopes for a better understanding of pathophysiological mechanism and for a more efficient therapeutic strategies implementation.

## Conclusions

The existing EOC models were developed in order to assign specific tools for vascular functions in physiological and pathological conditions, such as cells viability, transport properties, mitosis rate, proliferation, angiogenesis, apoptosis, permeability and metastasis. No models can perfect mimic the human conditions, but they offer the possibility to assess endothelial functions by replacing animal experiments with *in vitro* studies. The challenge is to fabricate EOC models as close as possible to *in vivo* conditions. EOC technology can be also used as a tool for therapeutic molecules efficiency researches. By incorporating the patient's cell lines in these models of chip platform, there are already creating conditions for the first steps in a precise personalized medicine.

### Conflict of interests

The authors declare that they have no conflict of interests.

### References


[1] Kimura H, Sakai Y, Fujii T. Organ/body-on-a-chip based on microfluidic technology for drug discovery. Drug Metab Pharmacokinet, 2018, 33(1):43–48. https://doi.org/10.1016/j.dmpk.2017.11.003 PMID: 29175062

[2] Caballero D, Kaushik S, Correlo VM, Oliveira JM, Reis RL, Kundu SC. Organ-on-chip models of cancer metastasis for future personalized medicine: from chip to the patient. Biomaterials, 2017, 149:98–115. https://doi.org/10.1016/j.biomaterials.2017.10.005 PMID: 29024838

[3] Arrigoni C, Gilardi M, Bersini S, Candrian C, Moretti M. Bioprinting and organ-on-chip applications towards personalized medicine for bone diseases. Stem Cell Rev Rep, 2017, 13(3):407–417. https://doi.org/10.1007/s12015-017-9741-5 PMID: 28589446

[4] Lee SH, Sung JH. Organ-on-a-chip technology for reproducing multiorgan physiology. Adv Healthc Mater, 2018, 7(2):1700419. https://doi.org/10.1002/adhm.201700419 PMID: 28945001

[5] Zhao Y, Kankala RK, Wang SB, Chen AZ. Multi-organs-on-chips: towards long-term biomedical investigations. Molecules, 2019, 24(4):675. https://doi.org/10.3390/molecules24040675 PMID: 30769788 PMCID: PMC6412790

[6] Skardal A, Shupe T, Atala A. Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. Drug Discov Today, 2016, 21(9):1399–1411. https://doi.org/10.1016/j.drudis.2016.07.003 PMID: 27422270

[7] Gröger M, Dinger J, Kiehntopf M, Peters FT, Rauen U, Mosig AS. Preservation of cell structure, metabolism and biotransformation activity of liver-on-chip organ models by hypothermic storage. Adv Healthc Mater, 2018, 7(2):1700616. https://doi.org/10.1002/adhm.201700616 PMID: 28960916

[8] Gorjikhah F, Davaran S, Salehi R, Bakhtiari M, Hasanzadeh A, Panahi Y, Emamverdy M, Akbarzadeh A. Improving "lab-on-a-chip" techniques using biomedical nanotechnology: a review. Artif Cells Nanomed Biotechnol, 2016, 44(7):1609–1614. https://doi.org/10.3109/21691401.2015.1129619 PMID: 26758969

[9] Shanti A, Teo J, Stefanini C. *In vitro* immune organs-on-chip for drug development: a review. Pharmaceutics, 2018, 10(4):278. https://doi.org/10.3390/pharmaceutics10040278 PMID: 30558264 PMCID: PMC6320867

[10] Phan DTT, Wang X, Craver BM, Sobrino A, Zhao D, Chen JC, Lee LYN, George SC, Lee AP, Hughes CCW. A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. Lab Chip, 2017, 17(3):511–520. https://doi.org/10.1039/c6lc01422d PMID: 28092382 PMCID: PMC6995340

[11] Herrmann K, Pistollato F, Stephens ML. Beyond the 3Rs: expanding the use of human-relevant replacement methods in biomedical research. ALTEX, 2019, 36(3):343–352. https://doi.org/10.14573/altex.1907031 PMID: 31329258

[12] Mathur T, Singh KA, Pandian NKR, Tsai SH, Hein TW, Gaharwar AK, Flanagan JM, Jain A. Organ-on-chips made of blood: endothelial progenitor cells from blood reconstitute vascular thromboinflammation in vessel-chips. Lab Chip, 2019, 19(15):2500–2511. https://doi.org/10.1039/c9lc00469f PMID: 31246211 PMCID: PMC6650325

[13] Bergmeier W, Hynes RO. Extracellular matrix proteins in hemostasis and thrombosis. Cold Spring Harb Perspect Biol, 2012, 4(2):a005132. https://doi.org/10.1101/cshperspect.a005132 PMID: 21937733 PMCID: PMC3281566

[14] White-Adams TC, Berny MA, Patel IA, Tucker EI, Gailani D, Gruber A, McCarty OJT. Laminin promotes coagulation and thrombus formation in a factor XII-dependent manner. J Thromb Haemost, 2010, 8(6):1295–1301. https://doi.org/10.1111/j.1538-7836.2010.03850.x PMID: 20796202 PMCID: PMC4367539

[15] Watson SP, Auger JM, McCarty OJT, Pearce AC. GPVI and integrin $\alpha IIb\beta 3$ signaling in platelets. J Thromb Haemost, 2005, 3(8):1752–1762. https://doi.org/10.1111/j.1538-7836.2005.01429.x PMID: 16102042

[16] Woulfe D, Yang J, Brass L. ADP and platelets: the end of the beginning. J Clin Invest, 2001, 107(12):1503–1505. https://doi.org/10.1172/JCI13361 PMID: 11413156 PMCID: PMC200202

[17] Mitra R, O'Neil GL, Harding IC, Cheng MJ, Mensah SA, Ebong EE. Glycocalyx in atherosclerosis-relevant endothelium function and as a therapeutic target. Curr Atheroscler Rep, 2017, 19(12):63. https://doi.org/10.1007/s11883-017-0691-9 PMID: 29127504 PMCID: PMC5681608

[18] Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol, 2012, 10(1):4–18. https://doi.org/10.2174/157016112798829760 PMID: 22112350

[19] Klemenska E, Beresewicz A. Bioactivation of organic nitrates and the mechanism of nitrate tolerance. Cardiol J, 2009, 16(1):11–19. PMID: 19130411

[20] Reddoch KM, Montgomery RK, Rodriguez AC, Meledeo MA, Pidcoke HF, Ramasubramanian AK, Cap AP. Endothelium-derived inhibitors efficiently attenuate the aggregation and adhesion responses of refrigerated platelets. Shock, 2016, 45(2):220–227. https://doi.org/10.1097/SHK.0000000000000493 PMID: 26555740

[21] Russell-Puleri S, Dela Paz NG, Adams D, Chattopadhyay M, Cancel L, Ebong E, Orr AW, Frangos JA, Tarbell JM. Fluid shear stress induces upregulation of COX-2 and $PGI_2$ release in endothelial cells *via* a pathway involving PECAM-1, PI3K, FAK, and p38. Am J Physiol Heart Circ Physiol, 2017, 312(3):H485–H500. https://doi.org/10.1152/ajpheart.00035.2016 PMID: 28011582 PMCID: PMC5402016

[22] Sieve I, Münster-Kühnel AK, Hilfiker-Kleiner D. Regulation and function of endothelial glycocalyx layer in vascular diseases. Vascul Pharmacol, 2018, 100:26–33. https://doi.org/10.1016/j.vph.2017.09.002 PMID: 28919014

[23] Wang M, Hao H, Leeper NJ, Zhu L; Early Career Committee. Thrombotic regulation from the endothelial cell perspectives. Arterioscler Thromb Vasc Biol, 2018, 38(6):e90–e95. https://doi.org/10.1161/ATVBAHA.118.310367 PMID: 29793992 PMCID: PMC6218174

[24] Hao H, Hu S, Wan Q, Xu C, Chen H, Zhu L, Xu Z, Meng J, Breyer RM, Li N, Liu DP, FitzGerald GA, Wang M. Protective role of mPGES-1 (microsomal prostaglandin E synthase-1)-derived $PGE_2$ (prostaglandin $E_2$) and the endothelial $EP_4$ (prostaglandin E receptor) in vascular responses to injury. Arterioscler Thromb Vasc Biol, 2018, 38(5):1115–1124. https://doi.org/10.1161/ATVBAHA.118.310713 PMID: 29599139 PMCID: PMC5920701

[25] Lee DY, Chiu JJ. Atherosclerosis and flow: roles of epigenetic modulation in vascular endothelium. J Biomed Sci, 2019, 26(1):56. https://doi.org/10.1186/s12929-019-0551-8 PMID: 31387590 PMCID: PMC6685237

[26] Marin T, Gongol B, Chen Z, Woo B, Subramaniam S, Chien S, Shyy JYJ. Mechanosensitive microRNAs – role in endothelial responses to shear stress and redox state. Free Radic Biol Med, 2013, 64:61–68. https://doi.org/10.1016/j.freeradbiomed.2013.05.034 PMID: 23727269 PMCID: PMC3762952

[27] Ren M, Li R, Chen N, Pang N, Li Y, Deng X, Wang L, Luo M, Liu Y, Hao H, Liu Y, Sun H, Wu J. Platelet-derived factor V is a critical mediator of arterial thrombosis. J Am Heart Assoc, 2017, 6(7):e006345. https://doi.org/10.1161/JAHA.117.006345 PMID: 28673898 PMCID: PMC5586322

[28] Ruggeri ZM, Mendolicchio GL. Adhesion mechanisms in platelet function. Circ Res, 2007, 100(12):1673–1685. https://doi.org/10.1161/01.RES.0000267878.97021.ab PMID: 17585075

[29] Tomaiuolo M, Brass LF, Stalker TJ. Regulation of platelet activation and coagulation and its role in vascular injury and arterial thrombosis. Interv Cardiol Clin, 2017, 6(1):1–12. https://doi.org/10.1016/j.iccl.2016.08.001 PMID: 27886814 PMCID: PMC5154246

[30] Westein E, van der Meer AD, Kuijpers MJE, Frimat JP, van den Berg A, Heemskerk JWM. Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner. Proc Natl Acad Sci U S A, 2013, 110(4):1357–1362. https://doi.org/10.1073/pnas.1209905110 PMID: 23288905 PMCID: PMC3557050

[31] Chung DW, Chen J, Ling M, Fu X, Blevins T, Parsons S, Le J, Harris J, Martin TR, Konkle BA, Zheng Y, López JA. High-density lipoprotein modulates thrombosis by preventing von Willebrand factor self-association and subsequent platelet adhesion. Blood, 2016, 127(5):637–645. https://doi.org/10.1182/blood-2014-09-599530 PMID: 26552698 PMCID: PMC4742551

[32] Jain A, van der Meer AD, Papa AL, Barrile R, Lai A, Schlechter BL, Otieno MA, Louden CS, Hamilton GA, Michelson AD, Frelinger AL 3rd, Ingber DE. Assessment of whole blood thrombosis in a microfluidic device lined by fixed human endothelium. Biomed Microdevices, 2016, 18(4):73. https://doi.org/10.1007/s10544-016-0095-6 PMID: 27464497 PMCID: PMC4963439

[33] Hotary K, Li XY, Allen E, Stevens SL, Weiss SJ. A cancer cell metalloprotease triad regulates the basement membrane transmigration program. Genes Dev, 2006, 20(19):2673–2686. https://doi.org/10.1101/gad.1451806 PMID: 16983145 PMCID: PMC1578694

[34] Wirtz D, Konstantopoulos K, Searson PC. The physics of cancer: the role of physical interactions and mechanical forces in metastasis. Nat Rev Cancer, 2011, 11(7):512–522. https://doi.org/10.1038/nrc3080 PMID: 21701513 PMCID: PMC3262453

[35] Nash GF, Turner LF, Scully MF, Kakkar AK. Platelets and cancer. Lancet Oncol, 2002, 3(7):425–430. https://doi.org/10.1016/s1470-2045(02)00789-1 PMID: 12142172

[36] Bogorad MI, DeStefano J, Wong AD, Searson PC. Tissue-engineered 3D microvessel and capillary network models for the study of vascular phenomena. Microcirculation, 2017, 24(5):e12360. https://doi.org/10.1111/micc.12360 PMID: 28164421 PMCID: PMC6726128

[37] Bogorad MI, DeStefano J, Karlsson J, Wong AD, Gerecht S, Searson PC. Review: *in vitro* microvessel models. Lab Chip, 2015, 15(22):4242–4255. https://doi.org/10.1039/c5lc00832h PMID: 26364747

[38] Reinitz A, DeStefano J, Ye M, Wong AD, Searson PC. Human brain microvascular endothelial cells resist elongation due to shear stress. Microvasc Res, 2015, 99:8–18. https://doi.org/10.1016/j.mvr.2015.02.008 PMID: 25725258 PMCID: PMC4426013

[39] Becker BF, Jacob M, Leipert S, Salmon AHJ, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. Br J Clin Pharmacol, 2015, 80(3):389–402. https://doi.org/10.1111/bcp.12629 PMID: 25778676 PMCID: PMC4574825

[40] Douville NJ, Tung YC, Li R, Wang JD, El-Sayed MEH, Takayama S. Fabrication of two-layered channel system with embedded electrodes to measure resistance across epithelial and endothelial barriers. Anal Chem, 2010, 82(6):2505–2511. https://doi.org/10.1021/ac9029345 PMID: 20178370 PMCID: PMC2839931

[41] Price GM, Tien J. Methods for forming human microvascular tubes *in vitro* and measuring their macromolecular permeability. Methods Mol Med, 2011, 671:281–293. https://doi.org/10.1007/978-1-59745-551-0_17 PMID: 20967637

[42] Chrobak KM, Potter DR, Tien J. Formation of perfused, functional microvascular tubes *in vitro*. Microvasc Res, 2006, 71(3):185–196. https://doi.org/10.1016/j.mvr.2006.02.005 PMID: 16600313

[43] Wong AD, Ye M, Levy AF, Rothstein JD, Bergles DE, Searson PC. The blood–brain barrier: an engineering perspective. Front Neuroeng, 2013, 6:7. https://doi.org/10.3389/fneng.2013.00007 PMID: 24009582 PMCID: PMC3757302

[44] Moya M, Tran D, George SC. An integrated *in vitro* model of perfused tumor and cardiac tissue. Stem Cell Res Ther, 2013, 4(Suppl 1):S15. https://doi.org/10.1186/scrt376 PMID: 24565445 PMCID: PMC4029276

[45] Song JW, Cavnar SP, Walker AC, Luker KE, Gupta M, Tung YC, Luker GD, Takayama S. Microfluidic endothelium for studying the intravascular adhesion of metastatic breast cancer cells. PloS One, 2009, 4(6):e5756. https://doi.org/10.1371/journal.pone.0005756 PMID: 19484126 PMCID: PMC2684591

[46] Price GM, Wong KHK, Truslow JG, Leung AD, Acharya C, Tien J. Effect of mechanical factors on the function of engineered human blood microvessels in microfluidic collagen gels. Biomaterials, 2010, 31(24):6182–6189. https://doi.org/10.1016/j.biomaterials.2010.04.041 PMID: 20537705 PMCID: PMC2884145

[47] Mannino RG, Myers DR, Ahn B, Wang Y, Rollins M, Gole H, Lin AS, Guldberg RE, Giddens DP, Timmins LH, Lam WA. "Do-it-yourself *in vitro* vasculature that recapitulates *in vivo* geometries for investigating endothelial–blood cell interactions". Sci Rep, 2015, 5:12401. https://doi.org/10.1038/srep12401 PMID: 26202603 PMCID: PMC4894411

[48] Brown TD, Nowak M, Bayles AV, Prabhakarpandian B, Karande P, Lahann J, Helgeson ME, Mitragotri S. A microfluidic model of human brain (μHuB) for assessment of blood brain barrier. Bioeng Transl Med, 2019, 4(2):e10126. https://doi.org/10.1002/btm2.10126 PMID: 31249876 PMCID: PMC6584314

[49] Yetkin-Arik B, Vogels IMC, Neyazi N, van Duinen V, Houtkooper RH, van Noorden CJF, Klaassen I, Schlingemann RO. Endothelial tip cells *in vitro* are less glycolytic and have a more flexible response to metabolic stress than non-tip cells. Sci Rep, 2019, 9(1):10414. https://doi.org/10.1038/s41598-019-46503-2 PMID: 31320669 PMCID: PMC6639367

[50] Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM. Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol, 2000, 156(4):1363–1380. https://doi.org/10.1016/S0002-9440(10)65006-7 PMID: 10751361 PMCID: PMC1876882

[51] Papadimitrou JM, Woods AE. Structural and functional characteristics of the microcirculation in neoplasms. J Pathol, 1975, 116(2):65–72. https://doi.org/10.1002/path.1711160202 PMID: 168332

[52] Dvorak HF, Detmar M, Claffey KP, Nagy JA, van de Water L, Senger DR. Vascular permeability factor/vascular endothelial

growth factor: an important mediator of angiogenesis in malignancy and inflammation. Int Arch Allergy Immunol, 1995, 107(1–3):233–235. https://doi.org/10.1159/000236988 PMID: 7542074

[53] Thurston G, McLean JW, Rizen M, Baluk P, Haskell A, Murphy TJ, Hanahan D, McDonald DM. Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice. J Clin Invest, 1998, 101(7):1401–1413. https://doi.org/10.1172/JCI965 PMID: 9525983 PMCID: PMC 508718

[54] Löscher W, Potschka H. Blood–brain barrier active efflux transporters: ATP-binding cassette gene family. NeuroRx, 2005, 2(1):86–98. https://doi.org/10.1602/neurorx.2.1.86 PMID: 15717060 PMCID: PMC539326

[55] Jiang L, Li S, Zheng J, Li Y, Huang H. Recent progress in microfluidic models of the blood–brain barrier. Micromachines (Basel), 2019, 10(6):375. https://doi.org/10.3390/mi10060375 PMID: 31195652 PMCID: PMC6630552

[56] Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ Res, 2007, 100(2):174–190. https://doi.org/10.1161/01.RES.0000255690.03436.ae PMID: 17272819

[57] Davies PF. Flow-mediated endothelial mechanotransduction. Physiol Rev, 1995, 75(3):519–560. https://doi.org/10.1152/physrev.1995.75.3.519 PMID: 7624393 PMCID: PMC3053532

[58] DeStefano JG, Xu ZS, Williams AJ, Yimam N, Searson PC. Effect of shear stress on iPSC-derived human brain microvascular endothelial cells (dhBMECs). Fluids Barriers CNS, 2017, 14(1):20. https://doi.org/10.1186/s12987-017-0068-z PMID: 28774343 PMCID: PMC5543552

[59] Garcia-Polite F, Martorell J, Del Rey-Puech P, Melgar-Lesmes P, O'Brien CC, Roquer J, Ois A, Principe A, Edelman ER, Balcells M. Pulsatility and high shear stress deteriorate barrier phenotype in brain microvascular endothelium. J Cereb Blood Flow Metab, 2017, 37(7):2614–2625. https://doi.org/10.1177/0271678X16672482 PMID: 27702879 PMCID: PMC5531355

[60] Katt ME, Xu ZS, Gerecht S, Searson PC. Human brain microvascular endothelial cells derived from the BC1 iPS cell line exhibit a blood–brain barrier phenotype. PLoS One, 2016, 11(4):e0152105. https://doi.org/10.1371/journal.pone.0152105 PMID: 27070801 PMCID: PMC4829259

[61] Genes LI, Tolan N, Hulvey MK, Martin RS, Spence DM. Addressing a vascular endothelium array with blood components using underlying microfluidic channels. Lab Chip, 2007, 7(10):1256–1259. https://doi.org/10.1039/b712619k PMID: 17896007

[62] Liu D, Wang L, Zhong R, Li B, Ye N, Liu X, Lin B. Parallel microfluidic networks for studying cellular response to chemical modulation. J Biotechnol, 2007, 131(3):286–292. https://doi.org/10.1016/j.jbiotec.2007.06.014 PMID: 17706314

[63] van Midwoud PM, Verpoorte E, Groothuis GMM. Microfluidic devices for *in vitro* studies on liver drug metabolism and toxicity. Integr Biol (Camb), 2011, 3(5):509–521. https://doi.org/10.1039/c0ib00119h PMID: 21331391

[64] Popovtzer R, Neufeld T, Popovtzer A, Rivkin I, Margalit R, Engel D, Nudelman A, Rephaeli A, Rishpon J, Shacham-Diamand Y. Electrochemical lab on a chip for high-throughput analysis of anticancer drugs efficiency. Nanomedicine, 2008, 4(2):121–126. https://doi.org/10.1016/j.nano.2008.03.002 PMID: 18482873

[65] Naoghare PK, Kwon HT, Song JM. An automated method for *in vitro* anticancer drug efficacy monitoring based on cell viability measurement using a portable photodiode array chip. Lab Chip, 2007, 7(9):1202–1205. https://doi.org/10.1039/b708341f PMID: 17713621

[66] Torisawa YS, Shiku H, Yasukawa T, Nishizawa M, Matsue T. Multi-channel 3-D cell culture device integrated on a silicon chip for anticancer drug sensitivity test. Biomaterials, 2005, 26(14):2165–2172. https://doi.org/10.1016/j.biomaterials.2004.05.028 PMID: 15576192

[67] DiVito KA, Daniele MA, Roberts SA, Ligler FS, Adams AA. Microfabricated blood vessels undergo neoangiogenesis. Biomaterials, 2017, 138:142–152. https://doi.org/10.1016/j.biomaterials.2017.05.012 PMID: 28570946

[68] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell, 2011, 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013 PMID: 21376230

[69] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol, 2013, 14(10):1014–1022. https://doi.org/10.1038/ni.2703 PMID: 24048123 PMCID: PMC4118725

[70] Harney AS, Arwert EN, Entenberg D, Wang Y, Guo P, Qian BZ, Oktay MH, Pollard JW, Jones JG, Condeelis JS. Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by TIE2hi macrophage-derived VEGFA. Cancer Discov, 2015, 5(9):932–943. https://doi.org/10.1158/2159-8290.CD-15-0012 PMID: 26269515 PMCID: PMC4560669

[71] Wong AD, Searson PC. Live-cell imaging of invasion and intravasation in an artificial microvessel platform. Cancer Res, 2014, 74(17):4937–4945. https://doi.org/10.1158/0008-5472.CAN-14-1042 PMID: 24970480 PMCID: PMC4155070

[72] Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. *In vitro* tumor models: advantages, disadvantages, variables, and selecting the right platform. Front Bioeng Biotechnol, 2016, 4:12. https://doi.org/10.3389/fbioe.2016.00012 PMID: 26904541 PMCID: PMC4751256

[73] Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res, 1974, 34(5):997–1004. https://doi.org/ PMID: 4841969

[74] Burden-Gulley SM, Qutaish MQ, Sullivant KE, Lu H, Wang J, Craig SEL, Basilion JP, Wilson DL, Brady-Kalnay SM. Novel cryo-imaging of the glioma tumor microenvironment reveals migration and dispersal pathways in vivid three-dimensional detail. Cancer Res, 2011, 71(17):5932–5940. https://doi.org/10.1158/0008-5472.CAN-11-1553 PMID: 21862632 PMCID: PMC3173511

[75] Nakada M, Nakada S, Demuth T, Tran NL, Hoelzinger DB, Berens ME. Molecular targets of glioma invasion. Cell Mol Life Sci, 2007, 64(4):458–478. https://doi.org/10.1007/s00018-007-6342-5 PMID: 17260089

[76] Teodorczyk M, Martin-Villalba A. Sensing invasion: cell surface receptors driving spreading of glioblastoma. J Cell Physiol, 2010, 222(1):1–10. https://doi.org/10.1002/jcp.21901 PMID: 19688773

[77] Hoffman RM. The multiple uses of fluorescent proteins to visualize cancer *in vivo*. Nat Rev Cancer, 2005, 5(10):796–806. https://doi.org/10.1038/nrc1717 PMID: 16195751

[78] Kitsara M, Kontziampasis D, Agbulut O, Chen Y. Heart on a chip: micro-nanofabrication and microfluidics steering the future of cardiac tissue engineering. Microelectron Eng, 2019, 203–204:44–62. https://doi.org/10.1016/j.mee.2018.11.001

[79] Frimat JP, Luttge R. The need for physiological micro-nanofluidic systems of the brain. Front Bioeng Biotechnol, 2019, 7:100. https://doi.org/10.3389/fbioe.2019.00100 PMID: 31134196 PMCID: PMC6514106

[80] Yang K, Park HJ, Han S, Lee J, Ko E, Kim J, Lee JS, Yu JH, Song KY, Cheong E, Cho SR, Chung S, Cho SW. Recapitulation of *in vivo*-like paracrine signals of human mesenchymal stem cells for functional neuronal differentiation of human neural stem cells in a 3D microfluidic system. Biomaterials, 2015, 63:177–188. https://doi.org/10.1016/j.biomaterials.2015.06.011 PMID: 26113074

[81] Murphy AR, Laslett A, O'Brien CM, Cameron NR. Scaffolds for 3D *in vitro* culture of neural lineage cells. Acta Biomater, 2017, 54:1–20. https://doi.org/10.1016/j.actbio.2017.02.046 PMID: 28259835

[82] Brimmo A, Goyette PA, Alnemari R, Gervais T, Qasaimeh MA. 3D printed microfluidic probes. Sci Rep, 2018, 8(1):10995. https://doi.org/10.1038/s41598-018-29304-x PMID: 30030464 PMCID: PMC6054653

[83] Ku DN, Flannery CJ. Development of a flow-through system to create occluding thrombus. Biorheology, 2007, 44(4):273–284. PMID: 18094451

[84] Zilberman-Rudenko J, Itakura A, Wiesenekker CP, Vetter R, Maas C, Gailani D, Tucker EI, Gruber A, Gerdes C, McCarty OJT. Coagulation factor XI promotes distal platelet activation and single platelet consumption in the bloodstream under shear flow. Arterioscler Thromb Vasc Biol, 2016, 36(3):510–517. https://doi.org/10.1161/ATVBAHA.115.307034 PMID: 26769048 PMCID: PMC4767639

[85] Prieto JH, Sampoli Benitez BA, Melacini G, Johnson DA, Wood MJ, Komives EA. Dynamics of the fragment of thrombomodulin containing the fourth and fifth epidermal growth factor-like domains correlate with function. Biochemistry, 2005, 44(4):1225–1233. https://doi.org/10.1021/bi0478852 PMID: 15667216

[86] Vera RH, O'Callaghan P, Fatsis-Kavalopoulos N, Kreuger J. Modular microfluidic systems cast from 3D-printed molds for imaging leukocyte adherence to differentially treated endothelial cultures. Sci Rep, 2019, 9(1):11321. https://doi.org/10.1038/s41598-019-47475-z PMID: 31383888 PMCID: PMC6683170

***Corresponding author***
Carmen Stanca Melincovici, Lecturer, MD, PhD, Discipline of Histology, Department of Morphological Sciences, Iuliu Haţieganu University of Medicine and Pharmacy, 4–6 Louis Pasteur Street, 400349 Cluj-Napoca, Romania; Phone +40740–219 735, e-mail: cmelincovici@yahoo.com

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