https://doi.org/10.33263/BioMed11.002008

ISSN: 2668-6007 https://biomedicalengineering.international

Biomedical Engineering International

Volume 1, Issue 1, Pages 0002-0008
2019

Review

Received: 15.09.2019
Accepted: 25.09.2019
Published: 30.09.2019

Microfluidics – Organ-on-chip

Iulia Ioana Lungu [1,2], Alexandru Mihai Grumezescu [1,*]

[1] Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, Romania
[2] National Institute of Laser, Plasma and Radiation Physics (NILPRP), Bucharest-Magurele, Romania
* Correspondence: alexandru.grumezescu@upb.ro Scopus ID: 36503987100

Abstract: This review is an introduction into the world of organ-on-chip models. By briefly explaining the concept of microfluidics and 'lab-on-chip', the main focus is on organs-on-chip and body-on-a-chip. The usual method to test the toxicity of a drug is through animal testing. However, the results do not always correlate to humans. In order to avoid animal testing, but also attain useful results, human-derived cell cultures using microfluidics have gained attention. Among all the different types of organ-on-chip devices, this review focuses on three distinct organs: heart, skin and liver. The main requirements for each organ-on-chip, as well as recent researches are presented. There have been considerable advancements with organ-on-chip models; however, even these have their limitations. Due to the fact that the system mimics a single organ, the systemic effect of drugs cannot be fully tested. Therefore, body-on-a-chip systems have been developed; which basically are a composed of a single chip that has several chambers, each chamber accounting for a distinct organ. Multi-organ-on-chip systems have been investigated, and even commercialized, the field still being under extensive research.

Keywords: microfluidic devices, organ-on-a-chip, body-on-a-chip.

1. Introduction

Microfluidics is a scientific term referring to the control of fluids inside channels of micro-dimensions. Commonly, small amount of fluids are manipulated in channels that have dimensions of up to hundreds micrometers. Regarding microfluidics, the term "Lab-on-chip" has been introduced as a mean to mimic laboratory experiments in miniaturized conditions. This concept gained a lot of attention from researchers, which soon moved up to applications such as organs-on-chip. The idea of organs-on-chip developed rather fast in areas such as medicine and biology due to the clear advantages, such as reduced costs, small dimensions that makes them easily transportable, reduced experimental times and ability to perform *in vitro* experiments with a better control over the parameters. These outstanding progresses in microfluidics started from physics researches, where small volumes, from microliters to femtoliters, needed to be handled. Micro-scale observation of bulk flow is an appropriate example; whereas turbulent flow can only be observed at macro-scale, laminar flow is predominant at micro-scale [1].

Pharmacokinetics are usually predicted through animal testing. The toxicity information gained through these animal tests is used to determine the efficiency of drugs on the human body. However, since 2013, the European Union (EU) has strictly forbidden animal testing in the cosmetic industry. As an alternative, the focus has been shifted to using human-derived cells for *in*

Cite This Article: Lungu, I.I.; Grumezescu, A.M. Microfluidics – Organ-on-chip. Biomed Eng Int **2019**, 1, 0002-0008. https://doi.org/10.33263/BioMed11.002008

vitro testing. Still, these alternatives have their drawbacks, mainly due to the incapacity to assess the communication between organs [2]. As a modern method, cell cultures using microfluidics provide several advantages over conventional techniques in regard to the manipulation, as well as analyzing cultures at a micro-scale. As technology continues to move forward, portable diagnostic techniques are becoming a real possibility. The purpose of organs-on-chip is to realistically mimic the behavior and the biological activity of tissues and organs. However, in order to achieve that specific parameters need to be included onto these platforms, such as osmotic pressure, flow rate, pH, the existence of nutrients as well as toxins, and many more [1].

Figure 1 describes the basic principal that is used for the general process of designing organ-on-a-chip models. Firstly, the microfluidic chip is designed taking into consideration all the required features for the desired organ (i.e. chambers and/or channels). Secondly, considering the organ that one wants to mimic, the required cells are cultured inside the chip in an appropriate medium so that they can grow, differentiate and acquire the desired functionality. Lastly, the data is collected through different tests, either physical or chemical [1].

Regardless of all the advantages that organ-on-chip devices have, there are still challenges that need to be further investigated and overcome, from both a biological and a design point of view. For example, in order to attain a useful *in vitro* model, it has to not only monitor, but also register all the physiological reaction determined by particular biological stimuli. Moreover, a considerable issue is generated by the sources of cells used in these devices. Due to the fact that immortal cells are generally derived from cancer cells, they lose their initial organ functional activity. Therefore primary human derived cells could be ideal candidates to determine the kinetics of drug delivery, however there are considerable limitation due to their availability and cost [2].

This review intends to shed some light on the meaning of organ-on-chip models, the recent advancements on the most common types, as well as highlighting the human-on-chip idea.

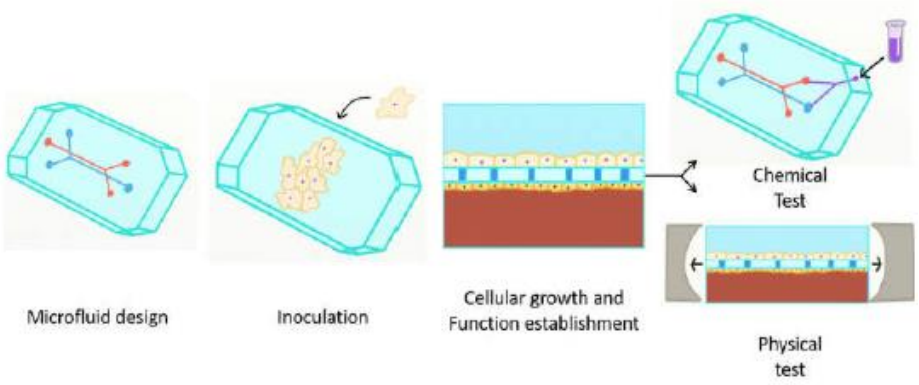

Figure 1. Fundamental principal for designing organ-on-a-chip devices [1]. Reprinted from an open access source.

2. Heart-on-chip

The use of microfluidic devices in order to better understand the behavior of the heart provides several advantages when engineering disease and drug screening models. These models have the capacity to stimulate the tissue at a micro-meter level and reduce the difference between *in vitro* and *in vivo* models. As a benefit over classic static cell cultures, the microfluidic environment can provide regulations over the conditions of the culture, both from a physical, as well as a chemical point of view. As mentioned earlier, the results of drug testing on animal models cannot be fully correlated to the effects of the same drugs, on humans [3].

Cardiovascular toxicity is the leading cause of failure of phase I drug testing. . As mentioned earlier, the results of drug testing on animal models cannot be fully correlated to the effects of the same drugs, on humans. Therefore, this led to the advancement of engineering human cardiac organ-on-chip models. The main consideration is the mechanism through which the heart blood, which is

a contraction process generated as a response to the electrical signal received from the cardiovascular system. Therefore, as a minimal requirement for the functionality of the heart muscle, a responsive and contractile bundle of cardiac muscle fibers are needed. With this in mind, several design considerations have been taken into account when discussing heart-on-chip models, including the use of human induced pluripotent stem cells derived from cardiomyocytes, the addition of fibroblasts and endothelial cells for support, electrochemical stimulation, as well as real-time records of the contraction of the cardiac muscle [4-6].

To asses cardiac contractility, Ahn et al., developed a mussel-inspired 3D chip. In order to mimic the extracellular matrix environment of the myocardium, polydopamine and polycaprolactone nanofibers were used to test the effects of titanium oxide and silver nanoparticles. Moreover, the effect of these nanoparticles on the contractions of the cardiac tissue was monitored non-invasively through integrated sensors [7].

Experiments are being conducted on microfluidic devices based on cardiomyocytes, derived from human induced pluripotent stem cells, clusters arranged in clusters in order to evaluate drug compounds. These cardiac spheroid-on-a-chip devices were coupled with confocal high content imaging and used to determine the correlation between outgrowing cell numbers and drug concentrations. Therefore making them potential candidates for the monitoring of drug effects on long-term cultures [8].

3. Skin-on-chip

Skin is the largest organ in the human body and therefore it is vital when it comes to assessing the absorption of drugs, as well as their cutaneous action. Skin-on-a-chip devices are excellent models to test the bioavailability of pharmaceutical active components that cross the endothelium into the bloodstream. Therefore, it is crucial when designing a skin-on-a-chip model to replicate the multilayers from which the skin is composed [4].

Since skin is the principal organ that protects the body from external factors, a main requirement when designing skin-on-a-chip models is to test them against all different stress factors that cause various effects. Moreover, these devices are an alternative to classical *in vitro* and *in vivo* models, therefore reducing the use of animal tests [1]. Moreover, the incorporation of several *in situ* biosensors into the skin-on-a-chip devices provides several benefits. It is a non-invasive way to acquire real-time reports on the function of the skin tissue, as well as feedback on the action of the drugs [9].

Mori et al., designed a skin-on-a-chip model based on perfusable vascular channels embedded with endothelial cells, which were fixed into a culture device linked to external pumps and tubes. Results showed a traditional dermal/epidermal structure, confirmed its barrier function and the supply of required nutrients from the vascular channels to the device. Moreover, it was confirmed that the device can be used to study vascular absorption due to the data obtained from the molecule permeation tests into the vascular channels through the epidermal layer [10].

A multi-chamber microfluidic skin-on-a-chip device designed for penetration studies was investigated. Penetration testing was attained on three different lipophilic chemicals and the results indicated that the device can be suitable for testing absorption and permeation of chemical compounds through skin [11].

4. Liver-on-chip

Liver is a major component of the human body due to its metabolic activity; making it the main organ that is associated with drug metabolism. Even though it is a regenerative tissue, it can go through severe injuries from chronic diseases and viral infections. Drug metabolism is achieved through the main cells found in the hepatic lobule: hepatocytes. One of the main drawbacks of classical *in vitro* screening is that the hepatocytes go through a decrease in functionality over a certain period of time [1,2,4].

Some of the main requirements for the proper functionality of a liver-on-a-chip device include the use of human originated hepatocytes, replicating the micro-structure of the liver, appropriate oxygenation, as well as the addition of flow of medium. Microarchitecture is especially important due to the fact that it may have a significant effect on the functionality and,

therefore, the toxicity predictions. For liver-on-a-chip devices, the chip part is usually designed from optically clear polymers consisting of micro-sized channels (50-500 μm). In order to attain the required functionality, these channels incorporate either hepatocytes monocultures, or co-cultures of hepatocytes and hepatic non-parenchymal cells or additional stromal cells [12].

A liver-on-a-chip device engineered for the purpose of *in vitro* drug screening was studies by Delalat et al. The hepatotoxicity was tested on primary hepatocytes with three different drugs, acetaminophen, chlorpromazine, and tacrine. The architecture of the chip provided an excellent environment for the cells and the results showed that this liver-on-a-chip device can be used in drug screening as a substitute to animal models [13].

5. Body-on-chip

Pharmaceutical drug development is a generally extensive, as well as expensive, process. In order for the Food and Drug Administration (FDA) to approve a new pharmaceutical, there are several evaluations that need to take place. Initially, the drugs need to provide beneficial results on cell cultures, *in vitro* and on animal models, *in vivo*. If the results are good enough, they move to clinical trials, which rely on testing the drug, in a controlled manner, onto human subjects. However, even though most drugs provide favorable results in *in vitro* and *in vivo* tests, they usually fail in clinical trials. Recently, many researchers have focused on organs-on-a-chip in order to evaluate the toxicity of drugs in a controlled environment, mimicking the human one. Even though there has been great improvement with organ-on-chip devices, they also have their drawbacks. The main disadvantage is the fact that the systemic effect of the pharmaceuticals cannot be tested, due to the fact that there is only one replicated organ. This led to a progress toward the development of different organs models within the same chip, namely body-on-chip. These types of devices contain several cell types which are cultured in independent chambers inside a single chip, and every chamber is a model of a distinct organ. Depending on the natural interactions between the organs, the chambers are linked through channels. Therefore, the effect of a drug can be tested on several organs at the same time [14].

One of the first body-on-chip models was developed in 2004 at Cornell University and contained three distinct chambers "lung-liver-other" on a silicon chip. The device was tested in combination with a physiologically based pharmacokinetic model [15,16].

Imura et al., developed and tested a microfluidic chip accounting for an intestine and liver system. The appropriate cells were cultures for the intestine and the liver, as well as carcinoma cells in order to test the activity of anticancer agents. The results showed that the device could test the intestinal absorption, metabolic activity of the liver, and the bioactivity related to the desired cells [17].

In 2009, a 3D microfluidic chip was designed and compartmentalized in order to replicate three different organs: liver, lung, and kidney, and the adipose tissue. Moreover, growth factors were added to the medium to optimize the functionality of the cells. The results indicated that the multi-organ-on-chip model could be used for drug screening, either as an aid, or even to replace animal models altogether [18].

More recently, Oleaga et al., engineered a pumpless four-organ model attributed to heart, muscle, neuronal, and liver system. The devices` toxicity was tested against five known drugs. The results were in agreement with the available toxicity results from both animal and human testing [19].

Table 1 presents some of the (multi) organ-on-chip devices that have reached the market.

Table 1. Organ-on-chip technologies advance to market [16].

Company	Systems	Reference
Hesperos, Inc.	Multi-organ devices with incorporated biological sensors	[19,20]
TissUse	Multi-organ-on-a-chip devices with open configuration chips	[21,22]

Company	Systems	Reference
CN Bio Innovations	Single and multi-organ microphysiological devices	[23,24]
Charles Stark Draper Laboratory	Multiple-organ system that mimics the female reproductive system (EVATAR)	[25]
Emulate, Inc.	Lung, liver, intestine, kidney, and brain – on-chip	[26-28]
Alveolix	Lung-on-a-chip system	[29]
Nortis	Kidney, and liver –on-chip models	[30,31]
Quorum Technology	Arthery-on-a-chip	[32]
AxoSim	Nerve-on-a-Chip®	[33]
Synvivo	Microchip engineering – from actual images of tissue microvasculatures	[34,35]
AIM Biotech	Microvasculature-on-a-chip models	[36,37]

6. Conclusions

Even though there are various examples of multi-organ-on-chip devices published, and the research in this area has improved tremendously, there is still no full body-on-chip that can be used for systemic drug testing. Ideally, the drug would be introduced in the chip through either the skin or gut equivalent mimicking the subcutaneous and, respectively, oral administration. The drug would be monitored and evaluated on the interaction with every component of each chamber (organ). Therefore, the toxicity of the drug could be assessed systemically in similar environment as the human body, especially if the used cells are of human origin. This can also be of use in the understanding the diseases pathology and action mechanism. There has been tremendous progress up to day and research is still ongoing; and as technology advances, so will the progress in the field.

Funding

This research received no external funding

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors declare no acknowledgments.

References

1. Sosa-Hernández, J.E.; Villalba-Rodríguez, A.M.; Romero-Castillo, K.D.; Aguilar-Aguila-Isaías, M.A.; García-Reyes, I.E.; Hernández-Antonio, A.; Ahmed, I.; Sharma, A.; Parra-Saldívar, R.; Iqbal, H.M.N. Organs-on-a-chip module: A review from the development and applications perspective. *Micromachines* **2018**, *9*, 536. https://doi.org/10.3390/mi9100536
2. Kimura, H.; Sakai, Y.; Fujii, T. Organ/body-on-a-chip based on microfluidic technology for drug discovery. *Drug Metabolism and Pharmacokinetics* **2018**, *33*, 43-48. https://doi.org/10.1016/j.dmpk.2017.11.003
3. Kitsara, M.; Kontziampasis, D.; Agbulut, O.; Chen, Y. Heart on a chip: Micro-nanofabrication and microfluidics steering the future of cardiac tissue engineering. *Microelectronic Engineering* **2019**, *203-204*, 44-62. https://doi.org/10.1016/j.mee.2018.11.001
4. Ronaldson-Bouchard, K.; Vunjak-Novakovic, G. Organs-on-a-chip: A fast track for engineered human tissues in drug development. *Cell Stem Cell* **2018**, *22*, 310-324. https://doi.org/10.1016/j.stem.2018.02.011

5. Giacomelli, E.; Bellin, M.; Sala, L.; van Meer, B.J.; Tertoolen, L.G.; Orlova, V.V.; Mummery, C.L. Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Development (Cambridge, England)* **2017**, *144*, 1008-1017. https://doi.org/10.1242/dev.143438
6. Zhang, N.; Stauffer, F.; Simona, B.R.; Zhang, F.; Zhang, Z.-M.; Huang, N.-P.; Vörös, J. Multifunctional 3d electrode platform for real-time in situ monitoring and stimulation of cardiac tissues. *Biosensors and Bioelectronics* **2018**, *112*, 149-155. https://doi.org/10.1016/j.bios.2018.04.037
7. Ahn, S.; Ardona, H.A.M.; Lind, J.U.; Eweje, F.; Kim, S.L.; Gonzalez, G.M.; Liu, Q.; Zimmerman, J.F.; Pyrgiotakis, G.; Zhang, Z., *et al.* Mussel-inspired 3d fiber scaffolds for heart-on-a-chip toxicity studies of engineered nanomaterials. *Analytical and bioanalytical chemistry* **2018**, *410*, 6141-6154.
8. Christoffersson, J.; Meier, F.; Kempf, H.; Schwanke, K.; Coffee, M.; Beilmann, M.; Zweigerdt, R.; Mandenius, C.F. A cardiac cell outgrowth assay for evaluating drug compounds using a cardiac spheroid-on-a-chip device. *Bioengineering (Basel, Switzerland)* **2018**, *5*. https://doi.org/10.3390/bioengineering5020036
9. Zhang, Q.; Sito, L.; Mao, M.; He, J.; Zhang, Y.S.; Zhao, X. Current advances in skin-on-a-chip models for drug testing. *Microphysiological Systems* **2018**, *2*. https://doi.org/10.3390/mi9100536
10. Mori, N.; Morimoto, Y.; Takeuchi, S. Skin integrated with perfusable vascular channels on a chip. *Biomaterials* **2017**, *116*, 48-56. https://doi.org/10.1016/j.biomaterials.2016.11.031
11. Alberti, M.; Dancik, Y.; Sriram, G.; Wu, B.; Teo, Y.L.; Feng, Z.; Bigliardi-Qi, M.; Wu, R.G.; Wang, Z.P.; Bigliardi, P.L. Multi-chamber microfluidic platform for high-precision skin permeation testing. *Lab on a Chip* **2017**, *17*, 1625-1634. https://doi.org/10.1039/c6lc01574c
12. Starokozhko, V.; Groothuis, G.M.M. Judging the value of 'liver-on-a-chip' devices for prediction of toxicity. *Expert Opinion on Drug Metabolism & Toxicology* **2017**, *13*, 125-128. https://doi.org/10.1080/17425255.2017.1246537
13. Delalat, B.; Cozzi, C.; Rasi Ghaemi, S.; Polito, G.; Kriel, F.H.; Michl, T.D.; Harding, F.J.; Priest, C.; Barillaro, G.; Voelcker, N.H. Microengineered bioartificial liver chip for drug toxicity screening. *Advanced Functional Materials* **2018**, *28*, 1801825. https://doi.org/10.1002/adfm.201801825
14. Shanti, A.; Teo, J.; Stefanini, C. In vitro immune organs-on-chip for drug development: A review. *Pharmaceutics* **2018**, *10*, 278. https://doi.org/10.3390/pharmaceutics10040278
15. Mross, K.; Niemann, B.; Massing, U.; Drevs, J.; Unger, C.; Bhamra, R.; Swenson, C.E. Pharmacokinetics of liposomal doxorubicin (tlc-d99; myocet) in patients with solid tumors: An open-label, single-dose study. *Cancer chemotherapy and pharmacology* **2004**, *54*, 514-524. https://doi.org/10.1007/s00280-004-0825-y
16. Zhang, B.; Radisic, M. Organ-on-a-chip devices advance to market. *Lab Chip* **2017**, *17*, 2395-2420. https://doi.org/10.1039/C6LC01554A
17. Imura, Y.; Sato, K.; Yoshimura, E. Micro total bioassay system for ingested substances: Assessment of intestinal absorption, hepatic metabolism, and bioactivity. *Analytical chemistry* **2010**, *82*, 9983-9988. https://doi.org/10.1021/ac100806x
18. Zhang, C.; Zhao, Z.; Abdul Rahim, N.A.; van Noort, D.; Yu, H. Towards a human-on-chip: Culturing multiple cell types on a chip with compartmentalized microenvironments. *Lab Chip* **2009**, *9*, 3185-3192. doi https://doi.org/10.1039/B915147H
19. Oleaga, C.; Bernabini, C.; Smith, A.S.; Srinivasan, B.; Jackson, M.; McLamb, W.; Platt, V.; Bridges, R.; Cai, Y.; Santhanam, N., *et al.* Multi-organ toxicity demonstration in a functional human in vitro system composed of four organs. *Scientific reports* **2016**, *6*, 20030. https://doi.org/10.1038/srep20030
20. Chen, H.J.; Miller, P.; Shuler, M.L. A pumpless body-on-a-chip model using a primary culture of human intestinal cells and a 3d culture of liver cells. *Lab Chip* **2018**, *18*, 2036-2046. https://doi.org/10.1039/C8LC00111A
21. Dehne, E.M.; Hasenberg, T.; Marx, U. The ascendance of microphysiological systems to solve the drug testing dilemma. *Future science OA* **2017**, *3*, FSO185. https://doi.org/10.4155/fsoa-2017-0002
22. Rebelo, S.P.; Dehne, E.M.; Brito, C.; Horland, R.; Alves, P.M.; Marx, U. Validation of bioreactor and human-on-a-chip devices for chemical safety assessment. *Advances in experimental medicine and biology* **2016**, *856*, 299-316. https://doi.org/10.1007/978-3-319-33826-2_12
23. Tsamandouras, N.; Chen, W.L.K.; Edington, C.D.; Stokes, C.L.; Griffith, L.G.; Cirit, M. Integrated gut and liver microphysiological systems for quantitative in vitro pharmacokinetic studies. *The AAPS Journal* **2017**, *19*, 1499-1512. https://doi.org/10.1208/s12248-017-0122-4
24. Edington, C.D.; Chen, W.L.K.; Geishecker, E.; Kassis, T.; Soenksen, L.R.; Bhushan, B.M.; Freake, D.; Kirschner, J.; Maass, C.; Tsamandouras, N., *et al.* Interconnected microphysiological systems for quantitative biology and pharmacology studies. *Scientific reports* **2018**, *8*, 4530. https://doi.org/10.1038/s41598-018-22749-0
25. Xiao, S.; Coppeta, J.R.; Rogers, H.B.; Isenberg, B.C.; Zhu, J.; Olalekan, S.A.; McKinnon, K.E.; Dokic, D.; Rashedi, A.S.; Haisenleder, D.J., *et al.* A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. *Nature Communications* **2017**, *8*, 14584. https://doi.org/10.1038/ncomms14584
26. Jain, A.; Barrile, R.; van der Meer, A.D.; Mammoto, A.; Mammoto, T.; De Ceunynck, K.; Aisiku, O.; Otieno, M.A.; Louden, C.S.; Hamilton, G.A., *et al.* Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. *Clinical pharmacology and therapeutics* **2018**, *103*, 332-340. https://doi.org/10.1002/cpt.742

27. Sances, S.; Ho, R.; Vatine, G.; West, D.; Laperle, A.; Meyer, A.; Godoy, M.; Kay, P.S.; Mandefro, B.; Hatata, S., *et al.* Human ipsc-derived endothelial cells and microengineered organ-chip enhance neuronal development. *Stem cell reports* **2018**, *10*, 1222-1236. https://doi.org/10.1016/j.stemcr.2018.02.012
28. Workman, M.J.; Gleeson, J.P.; Troisi, E.J.; Estrada, H.Q.; Kerns, S.J.; Hinojosa, C.D.; Hamilton, G.A.; Targan, S.R.; Svendsen, C.N.; Barrett, R.J. Enhanced utilization of induced pluripotent stem cell-derived human intestinal organoids using microengineered chips. *Cellular and molecular gastroenterology and hepatology* **2018**, *5*, 669-677 e662. https://doi.org/10.1016/j.jcmgh.2017.12.008
29. Stucki, A.O.; Stucki, J.D.; Hall, S.R.; Felder, M.; Mermoud, Y.; Schmid, R.A.; Geiser, T.; Guenat, O.T. A lung-on-a-chip array with an integrated bio-inspired respiration mechanism. *Lab Chip* **2015**, *15*, 1302-1310. https://doi.org/10.1039/C4LC01252F
30. Weber, E.J.; Chapron, A.; Chapron, B.D.; Voellinger, J.L.; Lidberg, K.A.; Yeung, C.K.; Wang, Z.; Yamaura, Y.; Hailey, D.W.; Neumann, T., *et al.* Development of a microphysiological model of human kidney proximal tubule function. *Kidney International* **2016**, *90*, 627-637. https://doi.org/10.1016/j.kint.2016.06.011
31. Vernetti, L.A.; Senutovitch, N.; Boltz, R.; DeBiasio, R.; Shun, T.Y.; Gough, A.; Taylor, D.L. A human liver microphysiology platform for investigating physiology, drug safety, and disease models. *Exp Biol Med (Maywood)* **2016**, *241*, 101-114. https://doi.org/10.1177/1535370215592121
32. Yasotharan, S.; Pinto, S.; Sled, J.G.; Bolz, S.S.; Gunther, A. Artery-on-a-chip platform for automated, multimodal assessment of cerebral blood vessel structure and function. *Lab Chip* **2015**, *15*, 2660-2669. https://doi.org/10.1039/C5LC00021A
33. Huval, R.M.; Miller, O.H.; Curley, J.L.; Fan, Y.; Hall, B.J.; Moore, M.J. Microengineered peripheral nerve-on-a-chip for preclinical physiological testing. *Lab on a Chip* **2015**, *15*, 2221-2232. https://doi.org/10.1039/C4LC01513D
34. Brown, T.D.; Nowak, M.; Bayles, A.V.; Prabhakarpandian, B.; Karande, P.; Lahann, J.; Helgeson, M.E.; Mitragotri, S. A microfluidic model of human brain (muhub) for assessment of blood brain barrier. *Bioengineering & translational medicine* **2019**, *4*, e10126. https://doi.org/10.1002/btm2.10126
35. Pradhan, S.; Smith, A.M.; Garson, C.J.; Hassani, I.; Seeto, W.J.; Pant, K.; Arnold, R.D.; Prabhakarpandian, B.; Lipke, E.A. A microvascularized tumor-mimetic platform for assessing anti-cancer drug efficacy. *Scientific reports* **2018**, *8*, 3171. https://doi.org/10.1038/s41598-018-21075-9
36. Xiao, Y.; Kim, D.; Dura, B.; Zhang, K.; Yan, R.; Li, H.; Han, E.; Ip, J.; Zou, P.; Liu, J., *et al.* Ex vivo dynamics of human glioblastoma cells in a microvasculature-on-a-chip system correlates with tumor heterogeneity and subtypes. *Advanced Science* **2019**, *6*, 1801531. https://doi.org/10.1002/advs.201801531
37. Aref, A.R.; Campisi, M.; Ivanova, E.; Portell, A.; Larios, D.; Piel, B.P.; Mathur, N.; Zhou, C.; Coakley, R.V.; Bartels, A., *et al.* 3d microfluidic ex vivo culture of organotypic tumor spheroids to model immune checkpoint blockade. *Lab on a Chip* **2018**, *18*, 3129-3143. https://doi.org/10.1039/C8LC00322J