Tampereen yliopisto

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REPRODUCING ORGAN INTERACTIONS IN BODY-ON-CHIPS

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ABSTRACT

Niklas Lostedt: Reproducing organ interactions in body-on-chips
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Since the development of drugs has taken strides, the traditional ways of screening drugs using laboratory animals or 2D *in vitro* models have not been sufficient because of their lack of physiological relevance compared to humans. To overcome this problem, organ-on-chip technology was created.

Organ-on-chips are small microfluidic devices that have been designed to mimic a specific organ or tissue in the human body. Organ-on-chips are capable of mimicking environmental factors such as temperature, air composition and mechanical forces. Because organ-on-chips only include one organ, relevant interactions between organs cannot be mimicked. Since organs communicate with each other using molecules that are carried via the bloodstream, the interactions between organs are hard to examine *in vitro*. This challenge has led to the development of body-on-chip technology, with which the interactions between organs can be studied in a small scale.

By culturing different cell types in different compartments and connecting these compartments to each other, it is possible to mimic organ interactions in a small scale. Body-on-chips can be defined as microfluidic devices on which two or more cell types are cultured and connected to each other to create a part of the human body. Understanding these interactions in a small scale gives an insight of how drugs would ultimately affect humans in a larger scale.

Different compartments in body-on-chips can be connected to each other in various different ways. It is possible to use external connections such as tubing, when creating a body-on-chip from several organ-on-chips. To recapitulate organ interactions on a single chip, organ compartments can be connected by porous membranes on either planar or vertical orientation, or by microfluidic channels. The simplest way of constructing a body-on-chip is to connect several organ-on-chips by tubing or by a different external connection. Even though external connections are the simplest way of connecting organs to each other, the most common way of building a body-on-chip is to use microfluidic channels.

In order to communicate, organs need to have fluidic flow between each other to send signals. In the human body, this would be done by secreting molecules in the blood stream. In body-on-chips, organs communicate via cell culture media. Because there is no such thing as a universal medium yet, body-on-chips are forced to use several different cell culture medias in different parts of the chip to enable communicating and providing the cells with nutrients.

The future for body-on-chips is filled with challenges, from which maybe the biggest challenge is to develop a universal cell culture medium as efficient as blood in the human body. The universal cell culture medium would have to both mimic blood as accurately as possible and support all used cell types. It can be assumed that in the future body-on-chips will develop to recapitulate the human body even more accurately.

Keywords: body-on-chip, organ-on-chip, organ interactions, drug screening

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TIIVISTELMÄ

Niklas Lostedt: Elinten välisten vuorovaikutusten jäljitteleminen body-on-chipeissä
Kandidaatin tutkielma
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Lääkekehityksen ottaessa yhä suurempia kehitysaskeleita, perinteiset lääketestausmenetelmät, kuten eläinkokeet ja *in vitro* kokeet, ovat fysiologisen ihmiseen vertautumattomuutensa vuoksi jääneet riittämättömiksi. Tämän ongelman ratkaisemiseksi on kehitetty teknologia, joka tunnetaan nimellä organ-on-chip.

Organ-on-chipit ovat pieniä mikrofluidistisia siruja eli laitteita, jotka on suunniteltu jäljittelemään jotakin tiettyä ihmisen kudosta tai elintä mahdollisimman tarkasti. Organ-on-chipit ovat kykeneviä jäljittelemään muun muassa oikeaa ympäristön lämpötilaa, ilmankoostumusta sekä kudokseen tai elimeen kohdistuvia mekaanisia voimia. Organ-on-chipit sisältävät kuitenkin vain yhden elimen, jolloin fysiologisesti tärkeitä elinten välisi vuorovaikutuksia ei voida jäljitellä. Koska elimet kommunikoivat toistensa kanssa verenkiertoon eritettyjen molekyylien välityksellä, elinten välisiä vuorovaikutuksia on haastavaa tutkia *in vitro*. Tämä haaste on johtanut body-on-chippien kehittämiseen, joiden avulla elinten välisiä vuorovaikutuksia on mahdollista tutkia.

Viljelemällä soluja sirun eri kammioissa ja kytkemällä nämä kammiot toisiinsa mikrofluidistisesti, elinten välisiä vuorovaikutuksia on mahdollista tutkia pienessä skaalassa. Body-on-chipit voidaan määritellä mikrofluidistisina laitteina, joissa kahta tai useampaa yhdistettyä soluviljelmää käyttäen voidaan luoda osa ihmisen elimistöä. Kun viljelmien välisi vuorovaikutuksia ymmärretään pienellä skaalalla, voidaan päätellä lääkeaineen vaikutus ihmiseen kehoon suuremmalla skaalalla.

Sirun eri kammioita voidaan kytkeä toisiinsa monilla eri tavoin. Kytkemisessä voidaan käyttää ulkoisia liitäntöjä, kuten putkia, kun halutaan luoda suurempi kokonaisuus monista eri siruista. Jos elinten välisiä vuorovaikutuksia halutaan jäljitellä yhdellä sirulla, voidaan elimet kytkeä toisiinsa käyttäen huokoisia kalvoja tai mikrofluidistisia kanavia. Yksinkertaisimmillaan body-on-chip voidaan luoda yhdistämällä useampi organ-on-chip toisiinsa käyttäen ulkoisia liitäntöjä. Vaikka ulkoiset liitännät ovatkin yksinkertaisimpia, yleisin tapa yhdistää elimiä toisiinsa on käyttää mikrofluidistisia kanavia.

Jotta elinten välinen vuorovaikutus on mahdollista, elimet tarvitsevat nestemäisen kierron. Ihmisen elimistössä elimet vuorovaikuttavat toistensa kanssa erittämällä molekyylejä verenkiertoon, kun taas body-on-chipeissä elimet vuorovaikuttavat kasvatusliuoksen avulla. Koska kasvatusliuokset on kehitetty spesifisti yhtä solutyyppiä kohden, body-on-chipeissä joudutaan käyttämään eri kasvatusliuoksia laitteiston eri osissa solujen kommunikaation ja ravinteiden saannin mahdollistamiseksi.

Body-on-chippien tulevaisuudessa riittää haasteita, joista kenties merkittävin on universaalin kasvatusliuoksen kehittäminen. Universaalin kasvatusliuoksen tulee jäljitellä mahdollisimman tarkasti ihmisen verta ja tukea samanaikaisesti kaikkia käytettäviä solutyyppejä. Onkin oletettavaa, että tulevaisuudessa body-on-chipit kehittyvät vastaamaan yhä tarkemmin ihmisen kehoa.

Avainsanat: body-on-chip, organ-on-chip, elinvuorovaikutukset, lääketestaus

Tämän julkaisun alkuperäisyys on tarkistettu Turnitin OriginalityCheck -ohjelmalla

PREFACE

The aim of this bachelor's thesis is to study how organ interactions are reproduced in body-on-chips in order to create a drug screening model as close to human as possible.

I would like to thank my instructor Mari Pekkanen-Mattila for presenting me a subject for my thesis and guiding me along the way.

Tampere, 26.4.2021

Niklas Lostedt

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1. INTRODUCTION

As science and the development of new drugs have taken strides, there has been a growing need for a reliable and safe drug screening platform capable of predicting the effects of drugs on the human body. Between the years 2006-2015, the average likelihood of a drug to make it through all the testing phases and be approved for clinical use was only 9.6% (Mullard, 2016). Based on these calculations it is easy to comprehend that the traditional ways of screening drugs are not decent enough to be called reliable and there is definitely room for improvement.

Traditionally drugs have been tested either on laboratory animals or 2D *in vitro* models, but neither of the mentioned are physiologically relevant in relation to humans and thus cannot provide accurate results (Lee, S. H. & Sung, 2018). The physiological events in a laboratory animal's organs are challenging to examine and thus thoroughly understanding the events based on animals alone is a challenge (Ramadan & Zourob, 2020).

The need of a drug testing platform has led to the development of organ-on-chips, on which different cells can be cultured in a human-like microenvironment. Organ-on-chips can be defined as microfluidic devices on which different cells can be cultured or co-cultured to mimic a specific organ or tissue. Organ-on-chips are capable of mimicking the microarchitecture of tissues as well as the extracellular environments. (Ramadan & Zourob, 2020) To achieve the most accurate environment possible, there are many parameters which can be affected. Organ-on-chips are capable of mimicking in example the right temperature, air composition and in some implementations even mechanical forces. (Kodzius et al., 2017)

Microfluidic chips are usually fabricated using polymethylsiloxane (PDMS) or glass to enable live-cell imaging (Ramadan & Zourob, 2020). Chips are often fabricated using a technique called soft lithography. In soft lithography, a liquid polymer such as PDMS is poured on an etched silicon substrate, which leads to formation of a rubber-like, optically clear material. By inverting the mold, it is possible to create so called microfluidic channels in which liquid, such as a cell culture medium, can flow. (Bhatia & Ingber, 2014)

In the human body, organs communicate with each other using different signals that are carried via the bloodstream. *In vitro*, it is challenging to examine these signals between different organs due to the complexity of the signaling. Body-on-chip technology allows us to examine these signals to better understand the interactions between organs. (Ramadan & Zourob, 2020) By culturing or co-culturing cells on a microfluidic device to create organs and connecting the cultures microfluidistically to each other thus creating a so-called body-on-chip or a multiorgan-on-chip, it is possible to understand how organs communicate with each other and how a drug ultimately affects

the whole body or a part of it. Organs can be connected to each other in various different ways depending on the design of the chip used. According to a study by Ramadan & Zourob (Ramadan & Zourob, 2020), the organs can be connected to each other by using membranes in different orientations. Another study (Rogal et al., 2017) states, that body-on-chips can be divided into static, semistatic and flexible systems based on how the tissues are connected to each other. Each of the different ways have their own limitations and advantages compared to the other designs.

There is some controversy concerning what is considered a body-on-chip and what is considered a multiorgan-on-chip. According to some, only a few organs on a chip is not enough for the chip to be called a body-on-chip and it should be called a multiorgan-on-chip instead. I will, however, define a body-on-chip in this thesis as a chip that contains at least two organs that are connected to each other. This can be done with either integrating multiple organs on a single chip or connecting several chips to each other.

In this thesis I will be studying the different ways of reproducing interactions between different organs in microfluidic chips. As said, in order to profoundly understand how drugs affect the human body, it is extremely important to have a drug screening system where organs interact with each other similarly to humans. First, I will be explaining the basic ways of connecting organs to each other in body-on-chips. After explaining each of the basic ways of connecting organs to each other in body-on-chips, I will be studying some example chips to see how body-on-chips are practically constructed. Finally, I will be compiling a table on all the different chips and systems and comparing the applications to each other while speculating the future of body-on-chips.

2. FLEXIBLE SYSTEMS

The simplest way of creating a system with multiple connected organs is to use an external connection, such as tubing or plug-and-play-style connectors. Systems, where single-organ chips are connected to each other with external connectors, can also be called flexible systems. (Rogal et al., 2017) The advantage of using an external connection is that it does not require micro-fabrication of several fluidic channels and it offers a simple option of connecting several different organs to each other creating a larger system. In addition, single organ-on-chips can be cultured separately with a cell specific media before constructing the system by connecting the chips to each other. (Ramadan & Zourob, 2020)

2.1 Tubing

Connecting single organ chips to each other with tubing is undoubtedly the easiest way to enable multiple organs or cell cultures to communicate with secreted factors. You can see an example of a system created using external tubing in Figure 1.

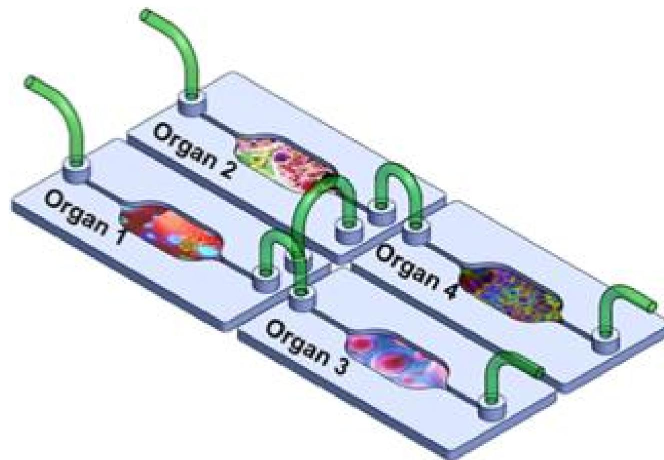

Figure 1. Organs connected externally via tubing (edited from Ramadan & Zourob, 2020).

The disadvantage of connecting organs to each other using tubing is that tubing does not support cell cultures that demand different culture medias (Ramadan & Zourob, 2020). This is because it is not possible to divide a flexible system into multiple subsystems. When the system cannot be divided into multiple subsystems, the whole system has to use the same culture media. In addition to not supporting different medias, tubing fails to mimic the natural flow between different organs. This is because unlike in the body, on a body-on-chip where organs are connected via tubing, the flow of media is constant throughout the system. (Ramadan & Zourob, 2020)

2.1.1 First pass metabolism using tissue slices

When drugs are consumed orally, they are metabolized significantly before reaching the systemic circulation. This metabolism of drugs is called the first-pass metabolism, and it happens mainly in the intestine and the liver. (Lee & Sung, 2018) The studying of first-pass metabolism is challenging *in vitro* since the transport and reactions of molecules happen simultaneously in various locations (Choe et al., 2017).

In an older study, van Midwoud et al. used precision cut liver and intestine slices from rats to study first pass metabolism (van Midwoud et al., 2010). The chip used in the study is constructed out of PDMS and contains six microchambers. Each of these microchambers contains either a liver slice or an intestine slice. An illustration of the chip's structure can be seen in Figure 2.

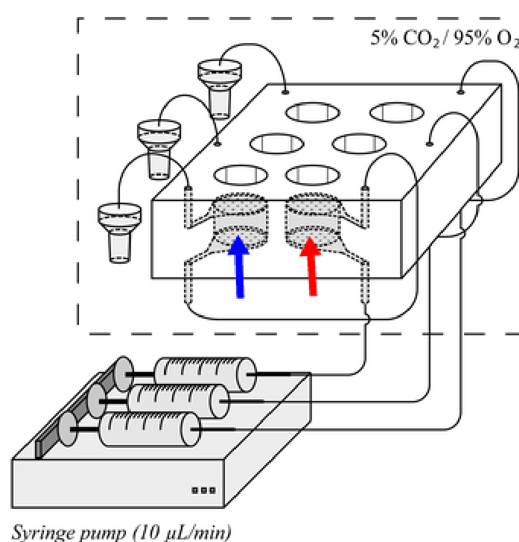

Figure 2. A microfluidistic chip mimicking first pass metabolism based on precision cut liver and intestine slices (edited from van Midwoud et al. 2010).

As it can be seen in Figure 2, the syringe pumps used to mediate medium flow are connected to the fluidic inlets of the microchambers that contain an intestine slice (red arrow). The flow rate of the chip is 10μl/min. The fluidic outlets of the microchambers containing intestine slices are connected to the inlets of the neighboring microchambers containing a liver slice (blue arrow) via external tubing. The data from the system is collected by connecting the fluidic outlets of the liver compartments to a collector setup. An illustration of one microchamber can be seen in Figure 3.

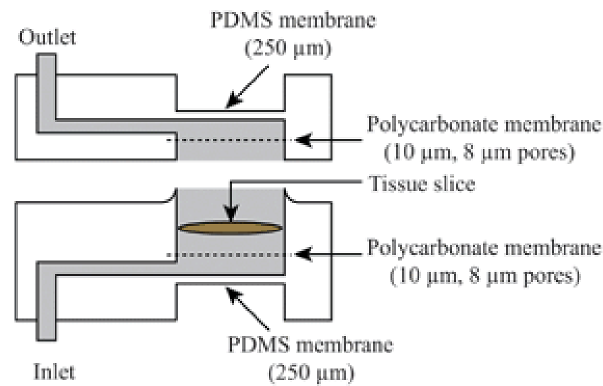

Figure 3. An illustration of the structure of one microchamber (edited from van Midwoud et al., 2010).

The system uses William's Medium E with Glutamax-I. The medium is supplemented with 25 mM d-glucose monohydrate, 50 µg mL−1 gentamicin and 2.5 µg mL−1 amphotericin B. As it can be seen from Figure 2, the system is kept in a humified box with 5% CO_2 and 95% O_2. Because PDMS is gas permeable, by using 250µm thick PDMS membranes in each of the microchambers (Figure 3), the gasses can penetrate the membrane and enter the medium, which enables the concentration regulation of the gasses in the medium. (van Midwoud et al., 2010)

The intestine slices were exposed to substrates, which led to formation of metabolites. By providing a continuous flow of medium through the system, the intestine metabolites travelled to the liver slices and ultimately affected the metabolism of the liver slices. First pass metabolism can hence be studied by studying the structure of individual organ slices and the metabolites travelling through the system. (van Midwoud et al., 2010)

Although the study using this system is over ten years old, the study demonstrates well how easily a body-on-chip can be created. This system also demonstrates the disadvantages of using external tubing, such as the similar flow conditions for all the connected organs. This can be a problem in cases where different cells require different flow conditions because of for example shear stress.

2.2 Other external connections

2.2.1 Lego®-like plug and play connector

Loskill et al. invented a Lego®-like plug and play connector called µOrgano with which single-organ cell cultures can be connected to form a system with multiple organs (Loskill et al., 2015). The components needed to connect single-organ cultures to each other can be seen in Figure 4.

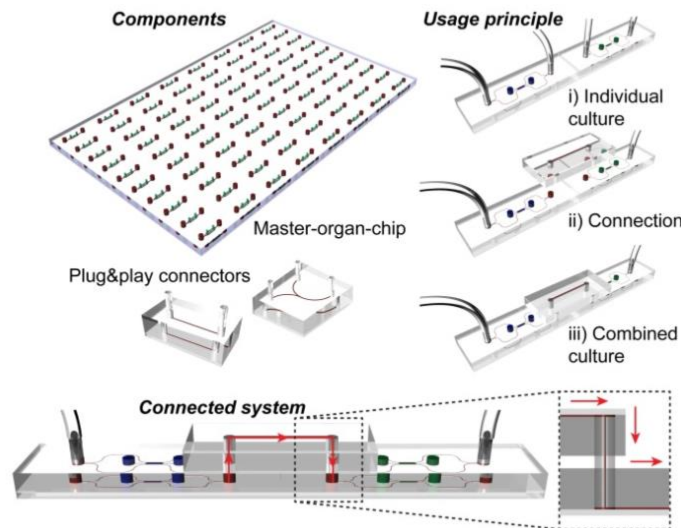

Figure 4. The basic components of μOrgano (edited from Loskill et al., 2015).

It is very simple to create a multi-organ system out of several single-organ cultures when using μOrgano. The main components that can be seen in Figure 4 are the master-organ-chip and the two different connectors. The master-organ-chip consists of several single-organ cultures, which have their own medium flow. When needed, the cultures can be connected to each other using either of the two connectors. As it can be seen in Figure 4, the connector allows medium flow from culture to another thus creating a system with two or more individual cultures connected to each other. (Loskill et al., 2015)

The study provides two different connectors. The first one is a linear connector which simply connect two cultures to each other so that medium is allowed to flow from one culture to another. The second one is a connector that can either divide one microfluidic channel into two separate microfluidic channels or join two channels into one. The choice of connector depends on the intended use. (Loskill et al., 2015) The structures of these two connectors can be seen in Figure 4.

Although the principle behind μOrgano is really simple, it seems to be a low-cost and effective way of creating a body-on-chip. The two different connectors allow μOrgano to be used in a wide array of ways thus making it possible to create a more complex system. The downside of using simple connectors like these is that it is not possible to use multiple mediums.

3. POROUS BARRIERS WITH VERTICAL ORIENTATION

In this configuration, porous materials are used to separate two vertically stacked chambers from each other (Figure 5). This allows the cells to be cultured or co-cultured close together. The cells in the upper compartment are cultured on top of the porous membrane and the cells in the lower compartment are cultured attached to the bottom of the membrane. This structure mimics the structure of many important parts of the human body such as blood vessels, skin or the lungs. (Ramadan & Zourob, 2020) In example blood vessels have a structure, where endothelial cells form a semi-permeable barrier (Pugsley & Tabrizchi, 2000). This structure could be reconstructed by culturing endothelial cells on a porous barrier with vertical orientation.

The pore sizes in this configuration can be adjusted to the desired size. Stacking two compartments on top of each other also allows the use of different culture medias and hence the use of different types of cells. In many studies, this configuration is used to study the absorption of substances from the lower compartment to the upper compartment. (Ramadan & Zourob, 2020)

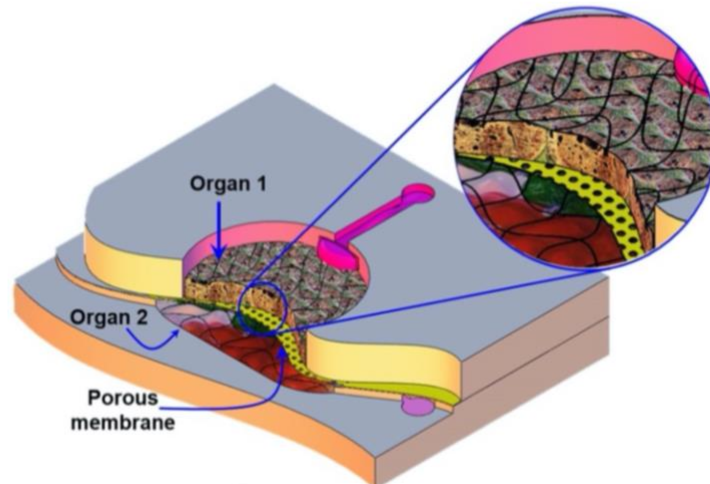

Figure 5. Organs connected via a porous membrane in a vertical orientation (edited from Ramadan & Zourob, 2020).

3.1 The human blood brain barrier

Park et al. aimed to mimic the function of the human BBB by constructing a two-channel body-on-chip out of PDMS (Park et al., 2019). An illustration of the chip's structure can be seen in Figure 6.

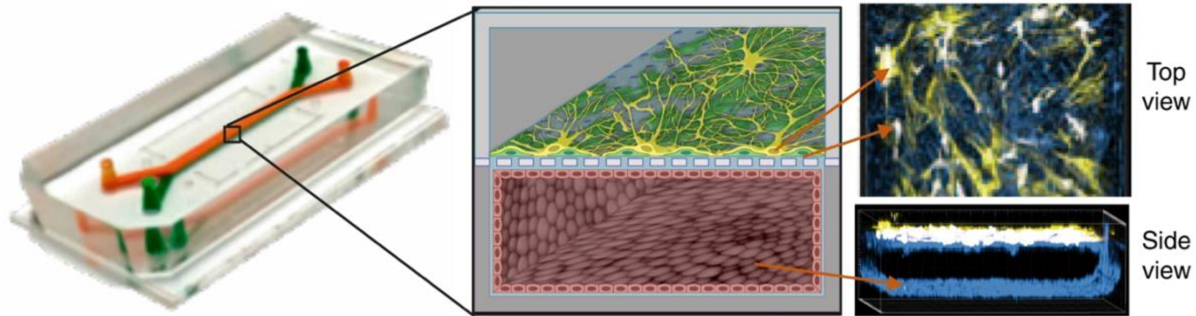

Figure 6. An illustration of a vertical human brain barrier chip (edited from Park et al., 2019).

As it can be seen from Figure 6, the body-on-chip constructed in the study consists of two vertically stacked compartments, which are separated by a porous membrane. The chip is constructed out of PDMS and the compartments are separated by a porous polyethylene terephthalate (PET) membrane. The membrane is coated by an ECM consisting of collagen type IV and fibronectin to allow the cells to have a natural environment to grow and attach to. (Park et al., 2019)

Astrocytes and pericytes are cocultured in the upper compartment of the chip on the apical side of the membrane to mimic the central neuronal system. The ratio of astrocytes to pericytes is 7:3. Human iPS-BMVECs (induced pluripotent stem cells from brain microvascular endothelial cells) are cultured in the lower compartment of the chip on the basal side of the membrane to mimic a blood vessel. (Park et al., 2019)

The chip uses two different mediums. The mixture of astrocytes and pericytes mimicking the central neuronal system use an astrocyte medium and the iPS-BMVECs use an endothelial medium with an additional 5% of retinoic acid. The chip is kept in continuous medium flow conditions, where the flow rate is 100µl/h. (Park et al., 2019)

The apparent permeability of the PET membrane is calculated to determine the function off the chip. This is done by injecting a known amount of dextran tracers to the vasculature channels, and the concentration of the tracers is calculated in the outlets of the vascular channels and the brain channels. (Park et al., 2019)

The results show that the iPS-BMVECs have covered all four walls of the lower compartment forming a hollow vascular lumen. The cells are able to form tight junctions and adherent junctions between each other. The astrocytes of the upper compartment are also able to come in contact with the cells on the basal side of the membrane through the pores. (Park et al., 2019) This shows that by using a porous membrane to connect two organs vertically to each other, it is possible to recapitulate organs interactions.

3.2 The human airway

Chronic respiratory diseases are one of the leading causes of death (Heron, 2019), so it is easy to comprehend how important it is to develop new platforms to study respiratory diseases. Benam et al. constructed a body-on-chip that aims to model lung diseases and drug responses in lungs (Benam et al., 2016). An illustration of the chip's structure can be seen in Figure 7.

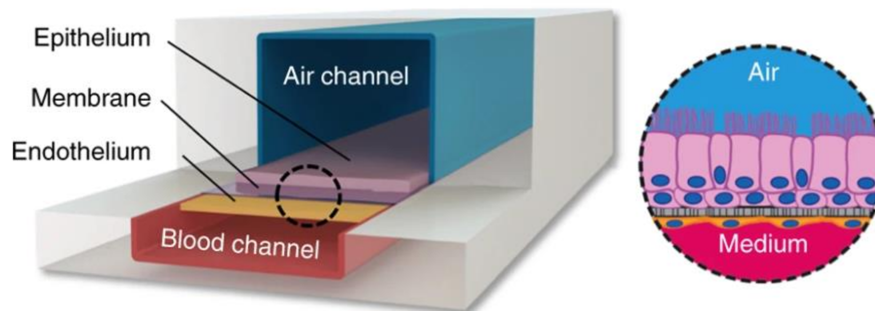

Figure 7. An illustration of the structure of a human lung chip (edited from Benam et al., 2016).

The chip is a two-channel body-on-chip that is constructed out of PDMS. The upper compartment is approximately 1mm of both height and width, and the lower channel 0.2mm high and 1mm wide. The two channels are separated by a porous polyester membrane, that is coated with type I collagen on both sides. (Benam et al., 2016)

The upper compartment is used to mimic the human lung. The lung is mimicked by a cell culture of human airway epithelial cells (hAECs), that have been differentiated to lung airway epithelial cells. The lower compartment is used to culture human lung microvascular endothelium cells to mimic a blood vessel. This creates a tissue interface on the porous polyester membrane. (Benam et al., 2016)

As it can be seen from Figure 7, the upper compartment of the chip has a constant flow of air. The lower compartment on the other hand has a constant flow of medium. The study uses Lonza EBM-2 endothelial cell basal growth medium supplemented with EGM-2MV SingleQuot Kit growth factors. The flow rate of the medium in the lower channel is 60µl/h. (Benam et al., 2016)

The results show that the epithelial cells create tight junctions and endothelial cells create adherent junctions. The chip has the same cellular structure that a healthy human lung would have, including a correct beating frequency and transport velocity. What is remarkable is that the chip successfully mimics a healthy human lung and is able to maintain these conditions for several weeks. (Benam et al., 2016)

4. POROUS BARRIERS WITH PLANAR ORIENTATION

In this configuration the cells are cultured physically in different compartments in a planar 2D structure. Even though the cells are cultured in different chambers, they are physically close together. This allows the organs to communicate with each other via secreted chemicals. (Ramadan & Zourob, 2020)

The chambers in this configuration can be separated with two types of different materials. The first option is to separate the chambers with a permeable thin solid barrier, which allows the organs to communicate with each other. (Ramadan & Zourob, 2020) You can see this option in Figure 8.

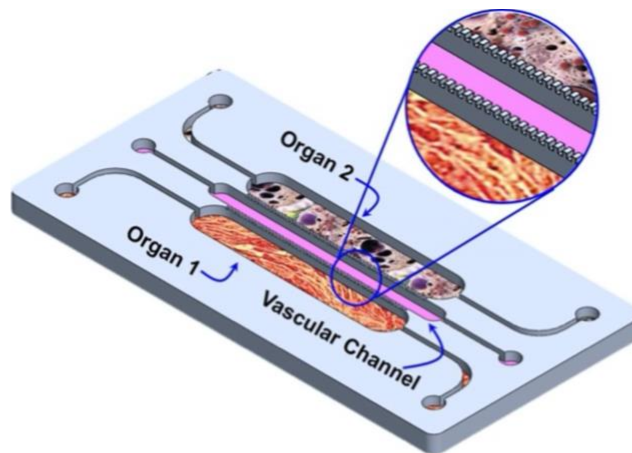

Figure 8. Organs connected in a planar orientation via a permeable thin solid barrier (edited from Ramadan & Zourob, 2020).

The second option is to separate the chambers with a porous material. This material can be filled with biomimetic materials such as a gel to enable the formation of vascular structures between the chambers. (Ramadan & Zourob, 2020) You can see this option in Figure 9.

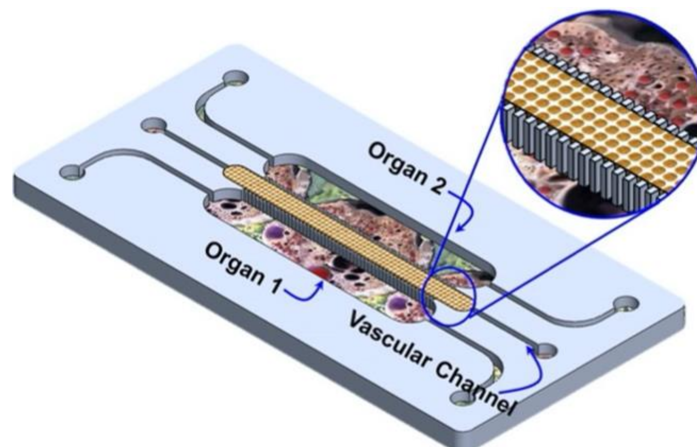

Figure 9. Organs connected in a planar orientation via a porous material (edited from Ramadan & Zourob, 2020).

As with vertical orientation, the pore sizes in the semi-porous material in this configuration can be adjusted to control what the barrier lets past. Unlike with external connectors, a system connecting organs with a semi-porous material can often use different culture medias in both chambers and hence it can be used to culture cells with different demands. (Ramadan & Zourob, 2020)

4.1 First pass metabolism using separate cell cultures

Choe et al. aimed to mimic first pass metabolism by culturing cells from the human intestine and the human liver in two different channels separated by a porous membrane (Choe et al., 2017). An illustration of the chip's structure can be seen in Figure 10.

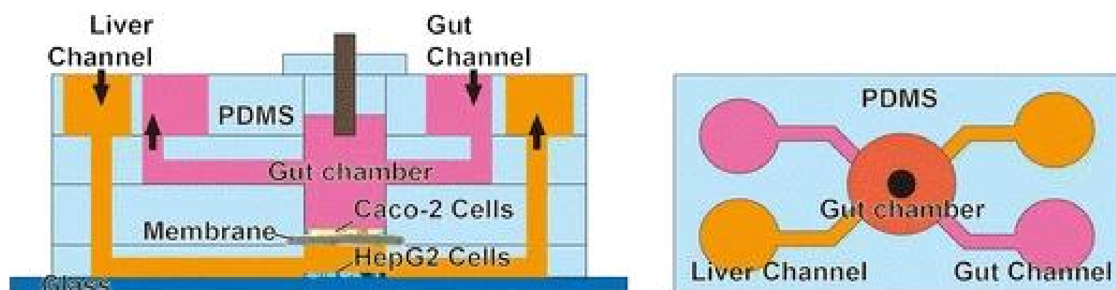

Figure 10. Top view (left) and side view of the microfluidic chip used to mimic first pass metabolism in a planar configuration (edited from Choe et al., 2017).

As it can be seen from Figure 10, the chip consists of two separate chambers that are connected by a membrane in a planar orientation. The gut chamber and the gut channel are illustrated with a pink color and the liver channel is illustrated with an orange color.

The study uses Caco-2 (human epithelial colorectal adenocarcinoma cell line) cells as gut cells and HepG2 (human hepatocellular carcinoma cell line) as liver cells. The gut cells are cultured in the gut chamber on a porous membrane under flow conditions. The liver cells are cultured on a glass surface with medium flow. (Choe et al., 2017)

Because the system does not use a pump, the medium flow is mediated by gravity flow. This makes it challenging to accurately control the medium flow rate. After many experiments, the best medium flow rate for the gut channel is determined to be 96µl/h. Because the gut channel is different in geometry compared to the liver channel, the medium flow rate in the liver channel is estimated to be approximately one third of the flow rate in the gut channel. (Choe et al., 2017) Because cells endure different amounts of shear stress, it may be needed to use different flow rates for different cell cultures.

Even though the cells are ultimately cultured in different channels, a porous membrane ensures a functioning interaction between the cells. When the cells are co-cultured together in the chip, high glucose DMEM (11995-065, Gibco) is used in both channels. (Choe et al., 2017)

4.2 Metastatic human breast cancer cell extravasation

It is known that most cancer deaths are a result of metastasis. Breast cancer has a relatively high metastatic potential meaning that breast cancer is likely to spread in the body. (Jin et al., 2020) Jeon et al. constructed a body-on-chip that enables the studying of metastatic human breast cancer cell extravasation within a bone mimicking microenvironment. The chip consists of two separate microfluidic channels that are separated by the bone mimicking microenvironment. (Jeon et al., 2015) An illustration of the chip's structure can be seen in Figure 11.

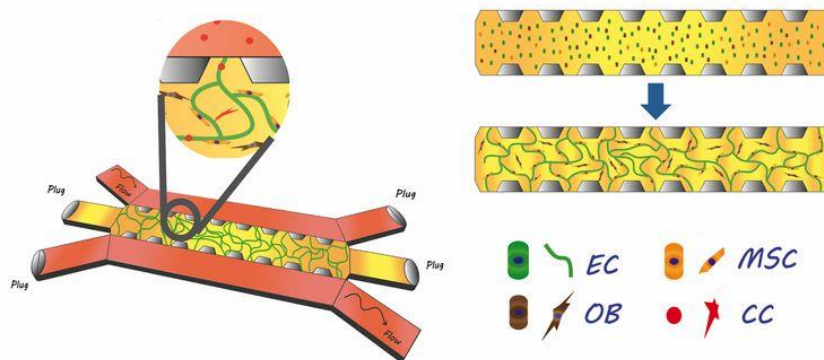

Figure 11. An illustration of a bone mimicking chip (edited from Jeon et al., 2015).

The chip aims to create a bone-mimicking microenvironment by seeding different cells into a fibrin gel. The microenvironment is created by embedding a triculture of primary human bone marrow-derived mesenchymal stem cells (hBM-MSCs), osteo-differentiated primary hBM-MSCs (which were differentiated using osteogenetic medium before seeding) and primary GFP-human umbilic vein endothelial cells (HUVECs) in a fibrin gel. This leads to the forming of an endothelial microvascular network, which is surrounded by a bone mimicking matrix constructed out of primary hBM-MSCs and primary osteo-derived hBM-MSCs. (Jeon et al., 2015) The vascular network can be seen on the right side of Figure 11.

As it can be seen in Figure 11, one end of each microfluidic channel is blocked by a plug. The outlet on the upper channel as well as the inlet of the lower channel are blocked by a plug. This forces the medium to flow through the bone mimicking section using the microvascular network. This way, when cancer cells are introduced through the medium, the cancer cells extravasate into the surrounding bone microenvironment. (Jeon et al., 2015)

The study does not specify what medium should be used when experimenting with this chip, other than it is a cell culture medium. In some of the experiments done with this chip, the medium flow rate is 120μl/h. (Jeon et al., 2015)

The chip is a good example of a case where medium is flowed through a material which contains a vascular channel (see Figure 9). The only flaw in this design is that the medium used needs to work with every used cell type, since this design cannot be used with several different mediums.

5. STATIC AND SEMISTATIC SYSTEMS

A static body-on-chip is a system, where the organs are connected to each other in a single device. The cells are seeded into different chambers at the same time and connected via one microfluidic channel. In static body-on-chips, the structure of the chip is permanent. This is also the most common way of building a body-on-chip. (Rogal et al., 2017)

Semistatic systems are based on Transwell® tissue inserts with integrated fluidic channels, pumps and censors. Unlike in a static system where cells are seeded simultaneously, in semistatic systems the tissues can be cultured before integration into the system. The tissues can also be replaced with other tissues to test different tissue combinations. (Rogal et al., 2017)

5.1 Static systems

5.1.1 Vitamin D_3 metabolism

In humans, vitamin D_3 is metabolized into 25-hydroxy vitamin D_3 in the liver, which is then further metabolized into 1,25-dihydroxy vitamin D_3 in the kidneys. Theobald et al. constructed a body-on-chip containing a liver chamber and a kidney chamber to study vitamin D_3 metabolism in humans. (Theobald et al., 2019) The structure of the chip used in the study can be seen in Figure 12.

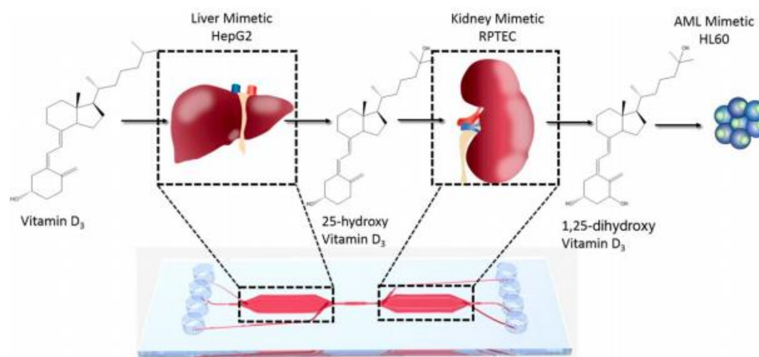

Figure 12. A liver-kidney chip used to study vitmain D metabolism in humans (Theobald et al., 2019).

HepG2 cells are cultured in their own chamber to mimic the liver and RPTEC cells are cultured on their own chamber to mimic a kidney. The liver chamber and the kidney chamber are connected to each other via a 150µm narrow fluidic channel. Hence, this chip would be considered to be a static system. (Theobald et al., 2019)

Vitamin D_3 is first introduced among growth medium via separate compartments to the liver which metabolizes the vitamin and transfers the metabolite molecules into the kidney. The kidney further

metabolizes the molecules which are then transferred to separate compartments which are located in the end of the chip. As it can be seen from Figure 12, there are also channels which pass either the liver or the kidney. This allows to study if the kidney can metabolize vitamin D_3 by itself or what metabolites the liver produces. (Theobald et al., 2019)

The system uses a low-pressure syringe pump to maintain a constant growth medium flow rate of 20µl/h. There is no re-circulation of media since the system is strictly linear. The study does not specify what the used medium is other than the medium can contain vitamin D_3. (Theobald et al., 2019) It can be assumed, that the medium is some kind of cell culture media that is accepted by both cell types in order for this system to work.

5.1.2 Pancreas-muscle microphysiological system

The human body depends on the correct volume of glucose in blood. This volume is controlled by for example the pancreas and the muscles. (Tirone & Brunicardi, 2001) Lee et al. constructed a chip with a pancreas compartment and a muscle compartment to study glucose metabolism (Lee, D. W. et al., 2019). The structure of the chip can be seen in Figure 13.

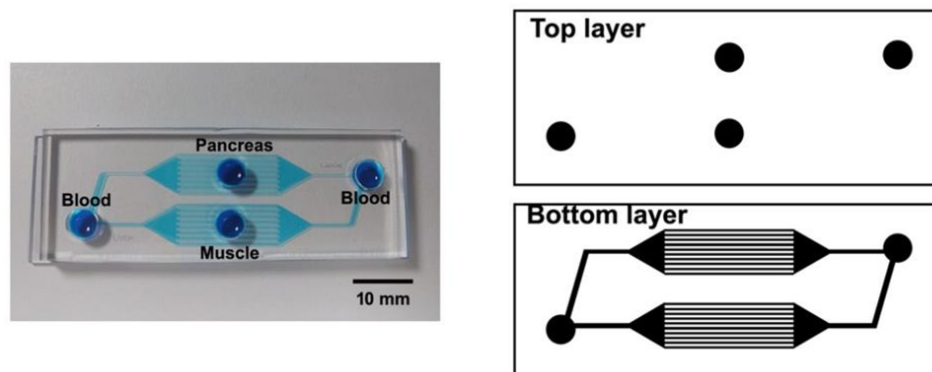

Figure 13. A body-on-chip used to study glucose metabolism (edited from Lee et al., 2019).

The chip consists of two layers, where the upper layer contains the organ compartments and medium reservoirs, and the lower layer contains the microfluidic channels that mimic blood flow. The top layer's organ compartments and reservoirs are connected to the bottom layer via microfluidic channels. The organs are cultured on top of porous membranes. (Lee et al., 2019)

To mimic the muscle, the chip uses C2C12 myoblasts. To mimic the pancreas, the chip uses INS-1 cells. (Lee et al., 2019) The cells are cultured in their own chambers as shown in Figure 13.

Both of the medium reservoirs contain 94.5µl of artificial blood with a total volume of 189µl. The medium flows freely from the medium reservoirs and no external pump is used. The flow rate of medium in the muscle is 167.6µl/h and in the pancreas the flow rate is 332.4µl/h. (Lee et al., 2019)

5.2 Semistatic systems

5.2.1 Four-organ chip

Maschmeyer et al. used Transwell® tissue inserts to construct a body-on-chip consisting of four different organs, the liver, the intestine, a kidney and skin. The chip is constructed out of PDMS and rests on top of a surface used to mount all the key components. (Maschmeyer, I. et al., 2015) The structure of the chip can be seen in Figure 14a.

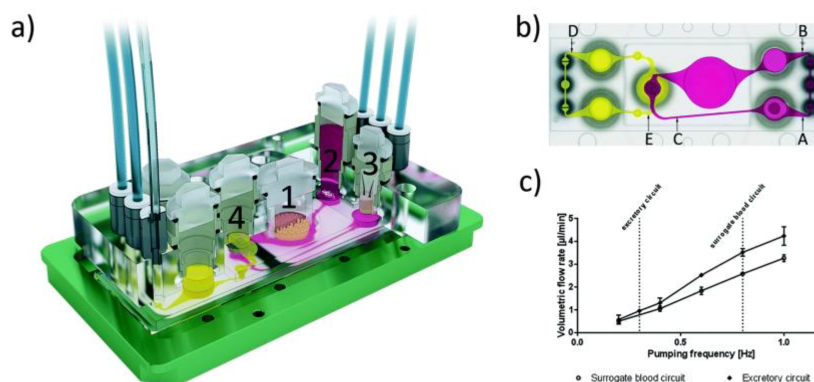

Figure 14. A four-organ chip using Transwell® inserts (edited from Maschmeyer et al., 2015).

The chip consists of two different fluid flow circuits which are operated by a peristaltic on-chip micropump. The yellow flow circuit in Figure 14b represents the excretory flow circuit and the pink circuit represents the surrogate blood flow circuit. The organs are numbered from one to four, where one is the intestine, two is the liver, three is skin and four is the kidney. The skin compartment is shielded from fluid flow by a Transwell® insert. As it can be seen, the two individual flow circuits overlap at the kidney compartment, where metabolites from the surrogate blood flow circuit are carried to the excretory circuit. The letters from A-E represent five different measurement spots used to collect data. (Maschmeyer et al., 2015)

The organs in this study are either 3D models of an organ or inserts that have been cultured in Transwell® inserts. The intestine is a reconstructed 3D model of the human small intestine constructed with epithelial cells, the liver is a 3D equivalent of the human liver constructed with HepaRG cells, the kidney is constructed with human tubule cells and the skin is a human biopsy which is loaded in a 96-well Transwell® insert. The tissues are removed every seven days for analysis. (Maschmeyer et al., 2015)

The study uses three different culture mediums. These are the small intestine culture medium, the liver tissue medium and the proximal tubule cell medium. The average flow rate can be seen in Figure 14c. For the excretory circuit, the average flow rate in approximately 60µl/h and for the surrogate blood circuit, the average flow rate is approximately 210µl/h. (Maschmeyer et al., 2015)

The chip used in the study demonstrates the advantages of semistatic systems quite clearly. The tissues can be cultured before inserting them to the chip and they can be removed for analysis or replaced with other tissues. Instead of one medium and one cell type, the chip can use three different mediums, that are circulated with on-chip pumps.

5.2.2 The human vasculature

Schimek et al. constructed a body-on-chip that aims to emulate the heart and the vessels of human vasculature (Schimek et al., 2013). The structure of this chip is similar to the four-organ chip. The structure of the chip can be seen in Figure 15a.

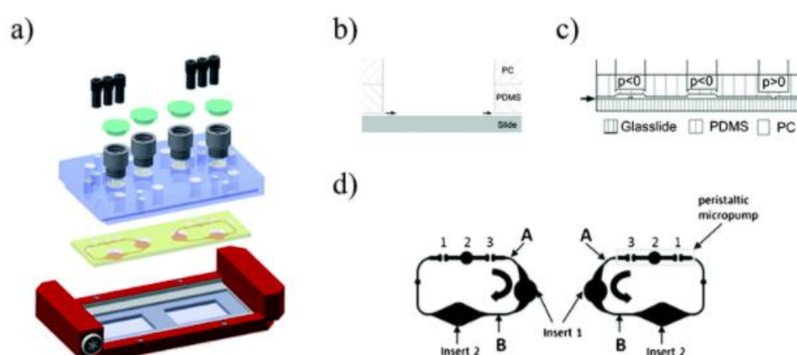

Figure 15. A body-on-chip emulating the human vasculature (Schimek et al., 2013).

The chip consists of two separate fluidic circuits which are operated by individual on-chip peristaltic pumps. Both circuits have two compartments for tissue inserts. The chip is placed on top of a heatable chip holder to maintain a constant temperature of 37°C. Each of the fluidic circuits contain two spots, where fluid flow and cell analysis can be carried out non-invasively. (Schimek et al., 2013) The locations of these spots can be seen in Figure 15d.

The fluidic circuits are covered with human dermal microvascular endothelial cells (HDMECs) to mimic vessels. The medium flow in these circuits can be operated so that the medium can flow in either direction. (Schimek et al., 2013) The structure of the pumps can be seen in Figure 15c.

The chip is designed to integrate tissue inserts into the system later on in the future. The placements of the inserts can be seen in Figure 15d and the structure of a single compartment can be seen in Figure 15b. The compartments are designed to contain tissue inserts with a mass up to 100mg. (Schimek et al., 2013)

Because the system is designed for future applications, there are no specifications regarding mediums or flow rates. The medium(s) can be chosen specifically for the intended inserts and the flow rate depends on the intended use.

The chip creates a suitable platform for future applications, where the interactions between two different organs may need to be studied. Because the chip can use Transwell® tissue inserts, the

inserts can be replaced to either study different organs or to use fresh inserts. The fluidic flow allows both the organs and the endothelial cells to have a natural environment to grow, which is naturally helpful when studying interactions. Furthermore, because the fluidic flow can be reversed, it is possible to study in interactions in an occasion where two organs interact in two directions, in example the intestine and the liver.

6. MULTIPLE DIFFERENT CONNECTIONS

6.1 The human neurovascular unit

The neurovascular unit (NVU) regulates the influx and efflux of in example drugs and nutrients between the systemic circulation and the central nervous system. The NVU consist of the brain endothelial cells and the cells with which they interact. (Banks, 2016)

The blood brain barrier (BBB) is a complex structure that controls the exchange of substances between the central neuronal system (CNS) and the systemic circulation. The complexity of the BBB causes challenges in developing drugs that can cross the barrier and affect the CNS. (Banks, 2016)

In a more recent study, Maoz et al. coupled three microfluidic chips to each other to mimic the human NVU. The system was constructed by connecting two BBB chips (BBB_{influx}, BBB_{efflux}) by tubing to each side of a brain chip. (Maoz et al., 2018) An illustration of the system's structure can be seen in Figure 16.

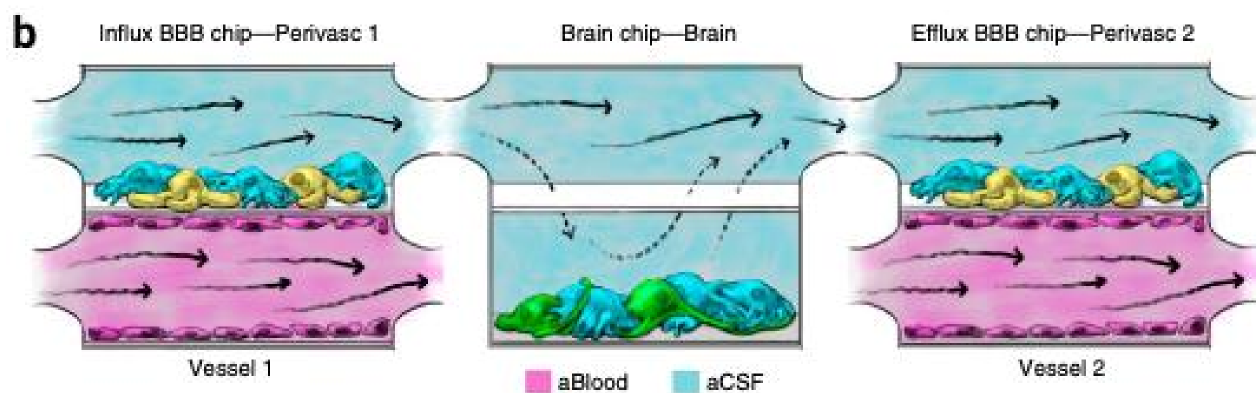

Figure 16. A microfluidic system mimicking the human neurovascular unit, side view (edited from Maoz et al., 2018).

The pink cells in Figure 16 represent the primary human brain microvascular endothelial cells (hBMVECs) which are cultured on the lower vascular compartments of the BBB chips. A mixture of astrocytes (blue) and pericytes (yellow) are cultured in the top compartments of the BBB chips. Human brain neuronal cells (green) are cultured alongside with astrocytes in the lower compartment of the brain chip. The compartments in each chip are separated from each other with porous membranes. The brain chip's membrane allows diffusion between the compartments. (Maoz et al., 2018)

The chips are connected to pressurized medium inlets and several peristaltic pumps that are used to flow the mediums through the system. There are several sample points, where data is collected

from the system. The chips are connected to each other physically by tubing, which allows for neuronal medium to flow through the whole system. There is also a porous membrane in the brain chip, which connects the upper fluidic flow to the lower part of the brain chip, which can be seen in Figure 16. (Maoz et al., 2018) An illustration of the whole system can be seen in Figure 17.

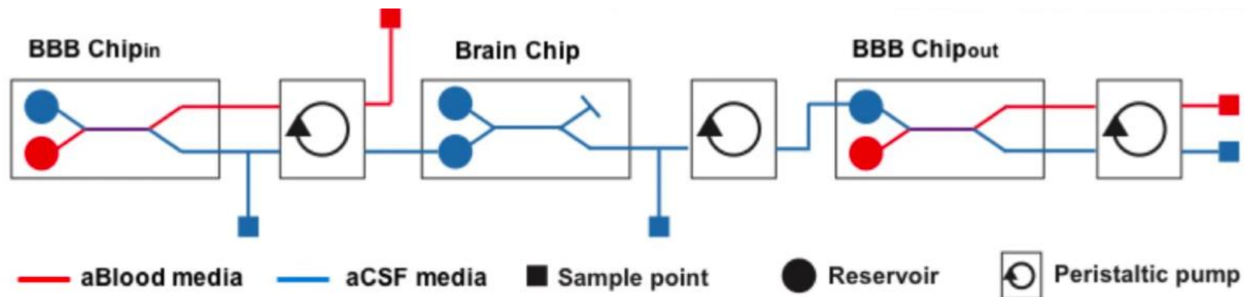

Figure 17. An illustration how chips are connected to each other in a system mimicking the human neurovascular unit (edited from Maoz et al., 2018).

The system uses two different mediums which are flown through the system at a rate of 60µl/h. As it can be seen in Figure 17, endothelial medium (artificial blood) is flown through the lower compartments of the two BBB chips individually. Neuronal medium (artificial cerebral spinal fluid, aCSF) is flown first through the upper compartment of the BBB_{influx} chip which is connected to the upper compartment of the brain chip. From the upper compartment of the brain chip, the aCSF is transferred to the upper compartment of the BBB_{efflux} chip. (Maoz et al., 2018)

7. CONCLUSIONS AND FUTURE DIRECTIONS

The goal of this thesis was to study how organ interactions can be produced in body-on-chips. In order to compare different ways of connecting organs to each other in body-on-chips, a table, which includes relevant information from the body-on-chips and systems studied in this thesis, was created (Table 1).

Table 1. The connections and chips studied in this thesis.

Connection	Organs	Chip	Cells/inserts used	Mediums used	Flow rate (µl/h)	Reference
External tubing	Intestine, liver	First pass metabolism with tubing	Intestine and liver tissue slices	William's Medium E with Glutamax-I	600	(van Midwoud et al., 2010)
Custom external connectors	Any chosen	Lego®-like connectors	Any chosen	Any chosen	Any	(Loskill et al., 2015)
Vertical membrane	Brain, blood vessel	The human BBB	iPS-BMVECs, astrocytes, pericytes	Astrocyte medium, endothelial medium	100	(Park et al., 2019)
Vertical membrane	Lung, blood vessel	The human airway	hAECs, endothelial cells	Lonza EBM-2 endothelial cell basal growth medium	60	(Benam et al., 2016)
Planar membrane	Intestine, liver	First pass metabolism with planar membrane	Caco-2, HepG-2	High glucose DMEM	96, ≈32	(Choe et al., 2017)
Planar membrane	Bone, blood vessels	Metastatic human breast cancer cell extravasation	hBM-MSCs, OD hBM-MSCs HUVECs	Unspecified cell culture medium	120	(Jeon et al., 2015)
Microfluidic channel	Liver, kidney	Vitamin D_3 metabolism	HepG2s, RPTECs	Cell culture medium with vitamin D_3	20	(Theobald et al., 2019)
Microfluidic channel	Pancreas, muscle	Pancreas-muscle microphysiological system	C2C12 myoblasts, INS-1	Artificial blood	167.6, 332.4	(Lee et al., 2019)
Microfluidic channel	Liver, intestine, kidney, skin	Four-organ chip	Tissue inserts, 3D reconstructions	Small intestine culture medium, liver tissue medium, proximal tubule cell medium	≈60, ≈210	(Maschmeyer et al., 2015)
Microfluidic channel	Blood vessels, any chosen organs	The human vasculature	HDMECs, any chosen inserts	Any chosen	Any	(Schimek et al., 2013)
Multiple connections	Blood brain barrier, brain	The human NVU	hBMVECs, pericytes, astrocytes	Endothelial medium, neuronal medium	60	(Maoz et al., 2018)

7.1 Used cell culture mediums and flow rates

The body-on-chips and systems studied in this thesis tend to use a specific cell culture medium, that suits the cultured cells. Since cell culture mediums are optimized for a particular cell type and are not universal to all cell types (Bhatia & Ingber, 2014), this often leads to a situation where the chip has to use more than one medium in order to successfully culture different cells in different compartments. Having several mediums forces the design of the chip to be more complex as far as microfluidic channels are concerned. As it can be seen from Table 1, most of the chips that use several cell types have to use more than one medium.

The lack of a universal medium that is supported by most of the cell types is certainly one of the biggest future challenges of body-on-chips, that would solve the problem of having to use several cell culture mediums. Blood can be thought to be a cell culture medium, which supplies the cells in our bodies with nutrients and removes unneeded metabolites. The artificial blood would have to include all the needed chemicals, nutrients and certain factors that individual organs need. In addition, the artificial blood would have to have a physiologically relevant composition. (Huh et al., 2012) At the moment, there is no artificial blood replacement that would be universal to all cell types in the way blood is.

In terms of cell culture medium flow rates, the flow rates differ from 20µl/h all the way to 600µl/h. The more recent studies seem to be using lower flow rates to presumably prevent tearing in the cell culture. The oldest study in this thesis (van Midwoud et al., 2010) uses the highest flow rate of all the body-on-chips. The flow rate is mediated by syringes. The chip uses tissue inserts from rats, so there is no significant tearing of cells and a higher flow rate can be used. A more recent study (Maschmeyer et al., 2015), that uses 3D cultured tissue inserts, uses flow rates of 60µl/h and 200µl/h. The flow rate is mediated by external pumps. The chip that uses tissue inserts from rats is the most uncomplicated of all the chips studied in this thesis, so it could be that there has been some development in body-on-chips where the flow rate is further optimized.

Different flow rates can be explained with shear stress. It is known that different cell types react differently to certain flow rates. If the flow rate of medium in the chip is too high, it can cause in example deformation of the cells or detachment of the growth surface. (Armistead et al., 2019) Because of this, the flow rates need to be optimized and thus some chips may use several different flow rates.

7.2 Used connections

The body-on-chips studied in this thesis use one of five ways to connect organs to each other. These five options are external tubing, custom external connectors, planar and vertical membranes and microfluidic channels.

As said, connecting organs with microfluidic channels is the most common way of constructing a body-on-chip. (Rogal et al., 2017) As it can be seen in Table 1, using microfluidic channels is the most common way of connecting organs in this thesis also. In total four different body-on-chips with four different purposes use microfluidic channels. Two of these chips use cells to mimic organs and two use some kinds of tissue inserts. The articles concerning these four chips have been written between the years 2013-2019.

External connections are the second most common way of connecting organs in this thesis, as it can be seen in Table 1. Three different chips use an external connection, from which two use tubing and one uses custom connectors. Since this is also the simplest way of connecting organ-on-chips to create a larger system, the oldest article in this thesis is about using external tubing to mimic first pass metabolism.

Vertical and planar membranes are the least common ways of constructing a body-on-chip in this thesis, although these are much more widely used in reality. All of the chips, where organs communicate via a porous membrane, use human cells to mimic organs. The articles are relatively new as they have been written between the years 2015-2019.

7.3 Combining multiple techniques

It cannot be denied that the ultimate goal in the field of body-on-chips would be to create a functional human on a chip. The chip would include multiple sampling points where data could be read from all the human subsystems. In order to achieve a chip that perfectly mimics the human body in all aspects, body-on-chips have to grow even larger and more complex.

Most likely when body-on-chips are developed even further, multiple techniques of connecting organs to each other will have to be used simultaneously in one body-on-chip system. Only one of the 11 body-on-chips studied in this thesis uses more than one technique when connecting organs to each other. In that system, three individual chips are connected to each other using tubing and one of the chips includes a porous membrane in vertical orientation which connects two different compartments to each other.

When body-on-chips are further developed, it can be assumed that the systems will grow even larger and more complex. It is challenging to construct a chip that will accurately mimic one subsystem of the human body, and in the future when it would be ideal to screen a drug in even larger subsystems, possibly the entire human body, smaller subsystems will have to be connected. In order to achieve this, different techniques will have to be used and the systems will grow even more complex.

When examining Table 1, it can be seen that when creating functional blood vessels, it would be beneficial to use a porous membrane in vertical orientation to ensure that the vessels function

properly. If the goal would be to create a body-on-chip with multiple individual chips that are connected via blood vessels, only one type of connection would be needed. Since the chips include different organs that are connected to each other inside one individual chip, even more types of connections are needed. It is thus easy to comprehend, that when systems grow more complex, multiple techniques will have to be used.

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