TECHNISCHE UNIVERSITÄT WIEN

DISSERTATION

Rapid prototyping, manufacturing and application of organ-on-a-chip and cell based lab-on-a-chip systems

ausgeführt zum Zwecke der Erlangung des akademischen Grades einer/eines Doktorin/Doktors der technischen Wissenschaften/der Naturwissenschaften/ der Sozial- und Wirtschaftswissenschaften unter der Leitung von

(Betreuung)

Univ. Prof. Dipl.-Ing. Peter Ertl
Name

E 164
Institutsnummer

Institute of Chemical Technologies and Analytics
Institutsbezeichnung

eingereicht an der Technischen Universität Wien

Faculty of Technical Chemistry
Fakultät

von

(VerfasserInn)

Sebastian Rudi Adam Kratz
Name

01528338
Matrikelnummer

Wien, am 15.09.2020 **eigenhändige Unterschrift**

Rapid prototyping, manufacturing and application of organ-on-a-chip and cell-based lab-on-a-chip systems

Dissertation

Author: Sebastian Rudi Adam Kratz

Main advisor: Univ. Prof. Dipl.-Ing. Dr. Peter Ertl

Co-advisor: Dr.nat.techn., MSc Mario Rothbauer

September 2020, Vienna Austira

Vienna University of Technology
Faculty of Technical Chemistry
Institute for Chemical Technologies and Analytics
Institute for Applied Synthetic Chemistry

Cell Chip Group

"*Everything not saved will be lost.*" – Nintendo "Quit Screen" message

Acknowledgments

I would like to thank you from the bottom of my heart:

Peter Ertl
Mario Rothbauer
Barbara Bachmann
Christoph Eilenberger
Gregor Höll
Doris Roth
Sarah Spitz
Rudolf Zinell

The cellchip group

Sarah Oberländer

Abstract

Nowadays there are basically two pre-clinical ways to gain a better understanding how physiological parameters and drugs can influence the human organism: well-established 2D cell culturing and complex animal models. Both approaches suffer either from a lack of complexity to recapitulate human physiology or come with species-specific differences and are ethically questionable. To overcome these limitations, organ-on-a-chip and cell-based lab-on-a-chip devices have been developed to create more complex human 3D cell models. Such micro physiological systems have shown to provide dynamic, reliable and reproducible measurement conditions, which are crucial in gaining a deeper knowledge into human pathological and physiological processes. Additionally, in recent years several optical and electrical sensing strategies have been employed in organ-on-a-chip devices to detect dynamic cellular responses with high spatial and temporal resolution. Despite these scientific advancements of *in vitro* cell-based assays and organ-on-a-chip devices, their translation into industrial products is still in its infancy. One major challenge in the development cycle of microfluidic devices is the time needed to go from initial idea to functional prototypes that can be readily translated into industrial manufacturing processes. To overcome this research to application gap, new rapid prototyping techniques are needed to ensure efficient device development including the integration of biosensors and the construction of multi-material composed devices. This thesis therefore reviews the current state of biosensing integration in micro physiological organs-on-a-chip and body-on-a-chip systems and investigates new tools and strategies for the rapid prototyping and manufacturing of organ-on-a-chip and cell-based lab-on-a-chip devices. A new rapid prototyping method is rated in regard to effort and accuracy in microfabrication and biocompatibility as well as the bonding ability to other materials and assembly of multi-featured devices including sensor integration. As a practical application, the developed rapid prototyping and manufacturing methods are employed to create a functional human chip-based organ model.

Kurzfassung

Heutzutage gibt es grundsätzlich zwei vorklinische Möglichkeiten, um besser zu verstehen, wie physiologische Parameter und Medikamente den menschlichen Organismus beeinflussen können: 2D-Zellkultivierung und komplexe Tiermodelle. 2D Zellkulturen schaffen es jedoch nur im geringen Ausmaß, die Komplexität der menschlichen Physiologie widerzuspiegeln und Tiermodelle können nie den Unterschied zwischen Spezies überwinden und zählen generell zu einer Praxis, die ethisch zu hinterfragen ist. Organ-on-a-Chip- und Lab-on-a-Chip-Systeme wurden entwickelt, um komplexere 3D-Zellmodelle zu erstellen, die dynamischen Umgebungsbedingungen durch Mikrofluidik ausgesetzt sind. Diese Modelle helfen durch die Integration von Biosensorik, tiefgreifende Kenntnisse über das Zellverhalten und menschliche Physiologie zu erlangen.

In den letzten Jahren wurden verschiedene Möglichkeiten etabliert, um eine physiologischere Micro-Umgebung zu modellieren sowie das Einbetten von nicht-invasiven Sensoren, um Einflüsse auf die Zellen in Kultur besser detektieren zu können. Eine große Herausforderung bei der Entwicklung von Mikrofluidiksystemen und Organ-on-a-chip Systemen sind die vielen Iterationsschritte, die vom ersten Entwurf bis zum fertigen und voll funktionsfähigen Arbeitsgerät mit integrierter Biosensorik unternommen werden müssen. Daher besteht ein Bedarf an Rapid Prototyping und Fertigung, die die Entwicklung neuer Organ-on-a-Chip- und zellbasierter Lab-on-a-Chip-Systeme ermöglichen.

In dieser Arbeit wird zunächst der aktuelle Stand der Integration von Biosensoren in mikrophysiologische Organ-on-a-Chip- und Body-on-a-Chip-Systeme untersucht und neue Werkzeuge und Strategien für das Rapid Prototyping sowie die Herstellung von Organ-on-a-Chip und zellbasierten Lab-on-a-Chip-Geräte untersucht. Die neuen Methoden werden hinsichtlich des Aufwands, der Genauigkeit bei der Mikrofabrikation und Biokompatibilität sowie der Fähigkeit der Verklebung mit anderen Materialien bewertet, um funktionelle Geräte aufzubauen und Sensorintegration zu ermöglichen. Diese Arbeit wird das gewonnene Wissen nutzen, um die entwickelten Rapid Prototyping- und Herstellungsmethoden zur Etablierung Organ-on-a-Chip- und zellbasierter Lab-on-a-Chip-Systeme zu nutzen.

Content

Introduction 1

1. Problem definition & State-of-the-art 1

2. Aim 11

3. Methods 12

4. Brief summary of the publications 15

5. Conclusion – Scientific value of the Dissertation 22

Results – Publications 25

I. Latest Trends in Biosensing for Microphysiological Organs-on-a-Chip and Body-on-a-Chip Systems 26

II. Characterization of four functional biocompatible pressure-sensitive adhesives for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems 27

III. A compression transmission device for the evaluation of bonding strength of biocompatible microfluidic and biochip materials and systems 28

IV. Optimized plasma-assisted bi-layer photoresist fabrication protocol for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes 29

V. A lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells 30

VI. Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing 31

References 32

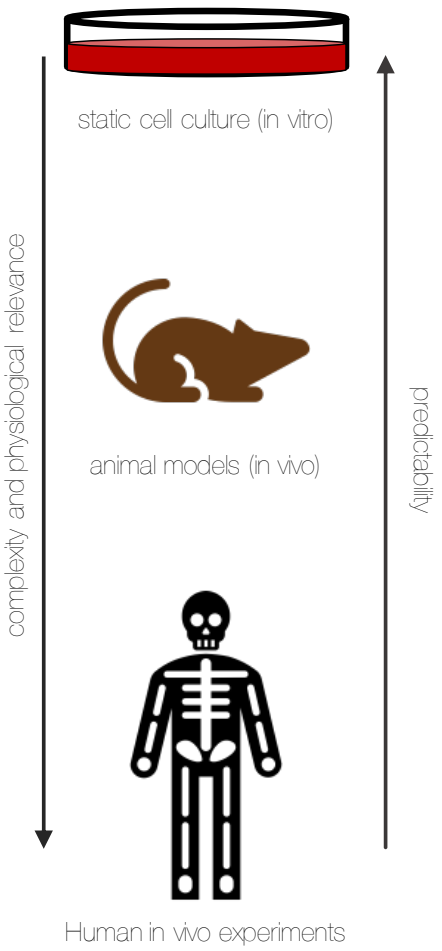
Introduction

1. Problem definition & State-of-the-art

The need of new physiological relevant human organ models

Human physiology is the understanding of the human body and its organ systems. Knowledge of human physiology is essential to understand behavior and characteristics of healthy tissues and organs as well as to interpret dysfunction, pathogenesis and their regeneration. Consequently, human physiology together with drug development and toxicology provides fundamental knowledge and progress for medicine and healthcare [1].

Nowadays there are three different, well established methods to gain this essential knowledge: i) the direct investigation by human *in vivo* (Latin for "within the living") experiments; ii) the indirect investigation by *in vivo* experiments in model organisms as animals; iii) *in vitro* (Latin for "within the glass") cell culture experiments [2] (Schematic 1). The straight approach of human *in vivo* experiments delivers very reliable results but due to risk and ethical concerns experiments can only be conducted carefully in special scenarios and a safe setup [3]. *In vivo* experiments with animal models can overcome those limitations, besides the question if animal testing is ethically justifiable, there is the drawback of always modeling a non-human physiology [4, 5]. On the other hand, *in vitro* cell culture experiments, where cells are grown and maintained in a controlled environment, can recreate human origin but are not capable of recreating physiology in terms of



Schematic 1 physiological models

a dynamic micro environment, microarchitecture and tissue to tissue communication [6].

In drug development, before a compound can be used in a clinical trial (human *in vivo*), first the pharmacodynamics and pharmacokinetics of compounds are assessed in preclinical *in vitro* 2D cell culture studies. As a next step the compounds are validated in animal models and after successful trials tested on humans [7]. Due to differences in species, animal models often aren't capable of reproducing human experiments, which leads to a high rate of failure of clinical trials for new drugs [8]. All these limitations, failures and linked costs prove the need for new physiological relevant human organ models.

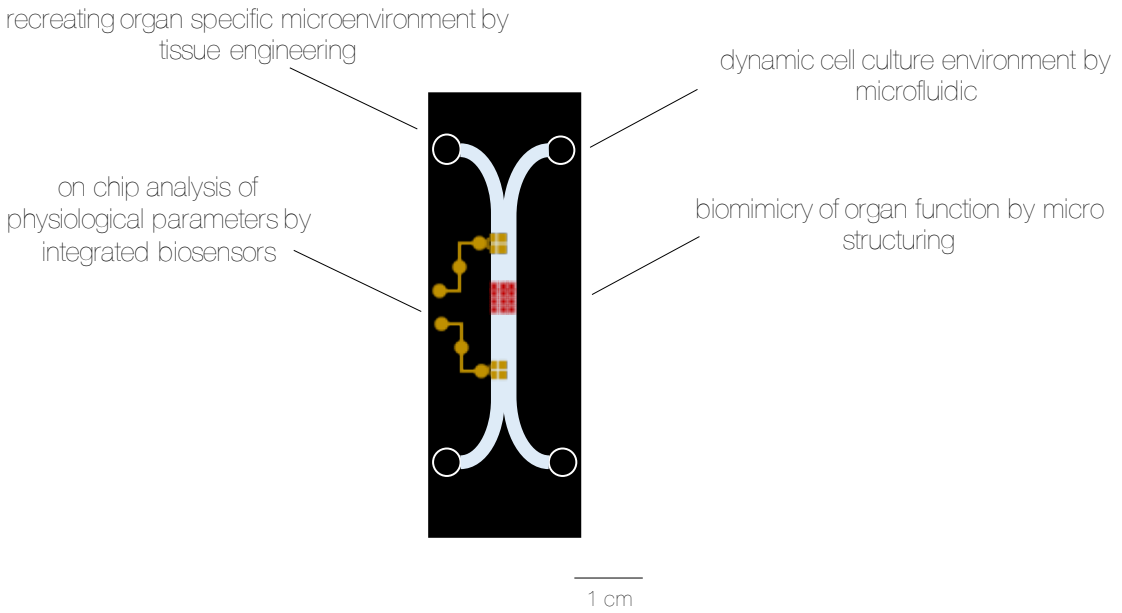
To tackle those drawbacks steady effort is invested to increase the physiological capability of static cell culture [9]. For example, to recapitulate tissue interfaces, cells can be cultured on top of permeable membranes within a two-compartment cell chamber. This model is used to investigate chemotaxis across tissue barriers [10]. Aside from that the shift from 2D culture towards 3D cultures, where either cells are cultured in spheroidal accumulations, called organoids or through the 3D self-assembly of cells within *in vivo* like hydrogels, enables a recreation of a more physiological model [11]. Due to the still static culture procedure of those technologies, there is quiet a lack of the impact of a dynamic cell microenvironment to mimic the natural tissue environment.

An alternative *in vitro* technology capable of overcoming some of the limitations of existing tissue models is based on microfluidic systems and created by microfabrication and microchip, which have recently been used to establish the next generation of *in vitro* cell-based systems with *in vivo* characteristics [12]. Microfluidics technology implement handling and manipulations of small sub-milliliter amounts of fluids down to a few pico liters in micro structured networks of microchannels. Various controlled biochemical reactions at very small volumes are conducted [13]. A major benefit of microdevices is their flexibility regarding microchannel network and chamber geometries as well as their ability to readily integrate assay functions such as pumps, heaters, mixers, micro-actuators and biosensors to create cell-based lab-on-a-chip systems [14]. Through the integration of a variety of cell types and artificial microtissues in this physical microenvironment as well as digital read outs, on-chip assays for molecular and cellular analyses ranging from *in vitro* diagnostic, personalized medicine and infectious disease monitoring can be performed [15]. Microfluidic and lab-on-a-chip technology provide the necessary tools and techniques for recreating micro scaled physiologically relevant, *in vivo*-like cell microenvironments as well as tissue-like architectures for *in vitro* cell cultures. Microfluidics further offer local and time depending control over cell

growth and can stimulate cells through microstructured surfaces with engineered biochemical properties. Mechanical stimuli of the extracellular matrix as well as precisely regulating the transport of fluids and soluble factors can be realized through microfluidic channels [16]. The trend towards *in vivo*-like and complex *in vitro* tissue culture platforms also for cell-based lab-on-chip systems through the integration of biosensing has enhanced on-chip analysis and the understanding of physiological behavior [17]. By reestablishing functions and the physiology of human organs and tissues on cell-based lab-on-a-chip systems with the advanced techniques of tissue engineering and stem cell biology, new physiological relevant human organ models are created - termed organs-on-a-chip [18].

Organ-on-a-chip and cell-based lab-on-a-chip systems

Organ-on-a-chip systems fuses micromachining, microfluidic technologies as well as biomimicry to reproduce crucial aspects of the physiology of human organs by enabling cell to cell interactions and rebuilding representative microarchitecture and extracellular microenvironments. These microenvironments can cause mechanical stimuli such as shear stress and mechanical stretching [19]. Those novel platforms gain insight into organ-level structures and functions and help to understand dysfunction, pathogenesis and the regeneration of human organs. The organ-on-a-chip technology enables a spectrum of possibilities for new human *in vitro* models. These models help to understand fundamental principles in the formation of organs and the progress of diseases, which furthermore helps to enhance target discovery as well as toxicity screening for drug development and hopefully replacing the need for animal experiments [20].



Schematic 2 a new physiological relevant model - organ-on-a-chip

To develop new physiological relevant organ-on-a-chip systems, one must first define a few crucial parameters of the organ specific microenvironment such as geometrical, mechanical and biochemical characteristics [21]. Geometrical properties as well as mechanical stimuli have to be considered for the functional chip design and the manufacturing of the microdevice [22] (Schematic 2). Representative examples are simple single channels to represent the vascular system [23]. Furthermore, barrier models where a two channel system is separated by a permeable membrane made of polymers or hydrogels to represent an organ or intra tissue interface like the placenta barrier [24] or the blood-brain-barrier [25]. Mechanobiological models as flexible structures to enable contraction of muscle [26, 27] and heart tissue [28]. To recapitulate mechanical stimuli such as the stretching of human pulmonary alveoli as well as connective tissue [29] or compression of bone tissue [30], actuated flexible systems are used. Also these systems can recreate an air-liquid interface for lung cells [19] or stimulate cells through shear force from laminar, pulsatile and interstitial flow [31].

Through the cultivation of different tissues in this microenvironment, micro physiological models can be established. The biological and therefore physiological relevance of the organ-on-a-chip system strongly depends on the cell source used to create it [32]. Cells sources are either immortalized cell lines, embryonic and induced pluripotent stem cells or primary cells [33]. Mostly cell lines are used to initially validate new organ-on-a-chip systems. While being able to recapitulate key aspects of human physiology [34], serial passaging of

cells and genetic changes of immortalized cell lines change the genotype and phenotype [35]. Stem-cell-derived progenitor or primary cells of patients can be used to gain higher clinical relevance and enhance accurate disease modelling [36]. Primary, mature cells directly represent the building blocks to model the human tissue, but because of collecting these in adequate amounts from their native environment and the tendency of those cells to often dedifferentiate or change their functionality in culture, the use for organ-on-a-chip systems is limited [37]. Embryonic and induced pluripotent stem cells, gained by reprogramming of somatic cells, are highly proliferative and can be differentiated into plenty specific cell types *in vitro* due to their pluripotent character and is therefore a promising approach. Furthermore, organ-on-a-chip technology offers new ways of stem cell differentiation through dynamic microenvironment as mechanical stimuli [38].

Patient specific introduced pluripotent stem cells in combination with organ-on-a-chip technology lead to personalized models that help to understand particular drug responses and advanced drug dosing for individual patients [39]. Therefore, drug response studies with a broad spectrum of genetic backgrounds can be conducted. Additionally the comparison of drug responses of gen mutated induced pluripotent stem cells from patients with gen modified healthy stem cells resulting in relevant personalized tissue models is possible [40]. Through the combination of multiple cell models within one device, multi-organs-on-a-chip are created to recapitulate organ to organ and tissue to tissue interactions and mimic aspects of the human metabolism [41]. In particular, drug absorption (gut), biotransformation of a prodrug into an effective metabolite (liver), long-term therapeutic effects and the potential of harmful side effects (kidney) can be studied with the help of multi-organ-on-a-chip systems representing important organ axis like the gut-liver-kidney axis [42].

Because of the reliability compared to static two-dimensional cell cultures and the relative low costs in contrast to animal testing organ-on-a-chips have the potential to establish a new standard for drug development and personalized medicine [43]. Also, the possibility of high-throughput, parallelization and multiplexing due to the small scale of the devices increase the economical aspect of organs-on-a-chip as a tool for drug and toxicity screening [44].

To study cell behavior and the impact of biochemical compounds as well as electrical and physical stimuli, organ-on-a-chip experiments are still predominated by microscopical and of-chip analysis. Due to the major establishment of image acquisition based on microscopy for histology and cell-culture staining and procedures like enzyme-linked immunosorbent

assays and polymerase chain reaction in biological research, these techniques are the primary analyzing methods for organ-on-a-chips [45]. The emerging field of biosensing, where an analytical device can detect chemical substances through the combination of biological components with a physicochemical detector and deliver digital read outs [46], has sparked the field of organ-on-a-chip technologies [47]. Essential parameters in cell culture like oxygen and glucose concentration as well as pH-value can be quantified through on-chip integration of optical biosensors. Oxygen can be sensed by fluorescence measurements of ruthenium dye, which shows oxygen sensitive quenching effects[48]. Optical refractive-index sensors are integrated to detect the activity of immobilized glucose oxidase [49]. On the basis of the absorption of light of phenol red the pH-value can be measured [50]. To sense physical deformation and study mechanobiology, either optical deformation of micro pillars can be detected to quantify the contraction of muscle cells [51] or the deflection of heart tissue is measured by the change of optical or electrical properties of deformed cantilevers [52]. Based on electrochemical activity, specific compounds can be measured label-free and time-resolved by electrode integration within the organ-on-a-chip device.

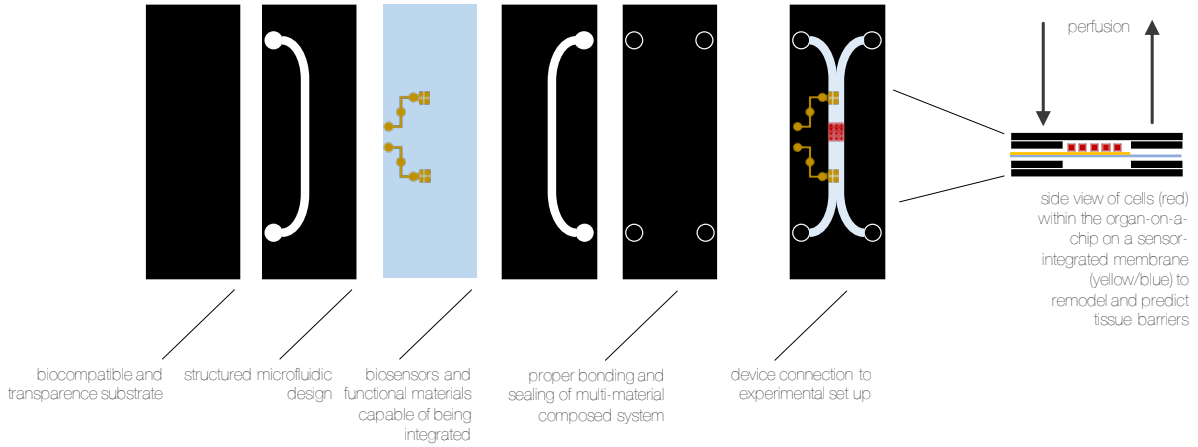
Aa an example to electrochemical measure, the presence of hepatic and cardiac biomarkers is immobilizing the needed recognition molecules with the help of magnetic microbeads [53], or the impedance measurement of the conversion by creatine kinase through microelectrodes functionalized by aptamers [54]. Other than electrochemical assays, integrated electrodes can be used to detect alteration of electrical parameters within the microenvironment. Electrodes are also capable of measuring deflection of the respiratory movements of cell culture membranes through changes in the impedance [55] or the contraction of muscle cells [56]. Micro electrode arrays are able to detect on-chip electrical activity of pancreatic islets [57, 58] as well as motoneuron and cardiomyocyte activity due to the electrical signaling of those cells [59]. Through electrode placing in the direct cell environment, electric cell substrate impedance sensing can be performed to assess confluency, identify cytotoxicity, cell proliferation or wound healing properties [60]. Barrier integrity of epithelial and endothelial cell layers is gauged by transepithelial or endothelial electrical resistance. The interlinkage of the cells through tight junctions and therefore the barrier tightness is directly correlated to the overall electrical resistance across the cell layer. In several organ-on-a-chip devices, trans epithelial electrical resistance is used to prove

barrier tightness for remodeling barrier function of skin [61], lung and gut [62] as well as the blood-brain barrier.

Although a trend towards sensor integrated platforms is steadily growing for organ-on-a-chip technologies, the biosensing functions are still in their infancy compared to the vast spectrum of already established bio- and microsensors, capable to being embedded into organ-on-a-chips. The increasing interdisciplinarity of organ-on-a-chip systems also increases the complexity and resources to operate those. Implementation, installation and placing of micro- and biosensors requires a more complex and elaborate infrastructure in addition to the facilities needed for chip manufacturing [63]. Therefore, new methods for prototyping and manufacturing of biosensor integrated organ-on-a-chip and cell-based lab-on-a-chip systems are needed.

Limitations of manufacturing organ-on-a-chip and cell-based lab-on-a-chip systems

The development of new devices and therefore the integration of biosensors often requires several design iterations and overall changes of possible experimental set ups until key aspects such as microfluidic, sensing, read-out and biology work properly.



Schematic 3 consideration for manufacturing multi-material composed organ-on-a-chip and cell-based lab-on-a-chip systems

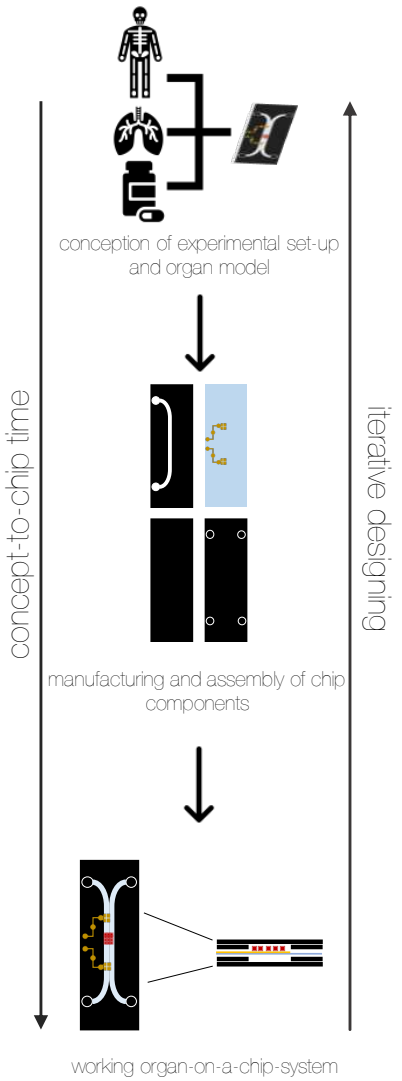
Recent manufacturing methods of biocompatible microfluidic devices are still relying on several individual steps of the procedure until a working prototype is finalized (Schematic 3). The first microfluidic devices were realized through the production methods developed for printed circuit boards. First liquid photoresist is applied and then geometrically structured

with UV-curing through photomasks. The residual uncured photoresist is removed and the exposed surface of the circuit board can be etched to bare the conduction elements [64]. Based on this procedure molds with a resolution of several µm can be created and used for soft lithography, where elastomers are micro structured through the curing process within these molds. This so called replica molding process is conducted by the predominant use of polydimethylsiloxane (PDMS), a biocompatible silicon-based organic polymer [65]. To bond individual layers of micro structured PDMS as well as PDMS with glass plasma activation of surfaces is used to covalent bond different layers of microdevices together [66]. Due to its capability of being micro structured, being easily bonded to glass and other PDMS parts and its characteristics as biocompatibility, gas permeability and optical transparency, microdevices made from PDMS are established as the standard in organ-on-a-chip and cell-based lab-on-a-chip systems [67]. Nevertheless, PDMS with a hydrophobic character tends to absorb small molecules, which is a major drawback of conducting drug studies on PDMS-based organ-on-a-chip devices [68]. Furthermore, PDMS needs an extensive procedure of mold production over several hours of curing to manual plasma bonding which hinder the industrial upscaling of the manufacturing procedure. Resistance to compound absorption and industrial scalable production is a necessary key aspect for the introduction of organ-a-chip technology as a new standard in drug validation within the pharmaceutical industry [69]. Therefore, constant effort being invested to overcome these limitations by either enhancing PDMS characteristics [70] or establishing new polymer based devices. An obvious candidate is polystyrene (PS), which is used for the manufacturing of petri dished and microtiter plates and thereby the material of choice in cell culture [71]. Also, other optical transparent and biocompatible polymers as polymethylmethacrylate (PMMA), polylactic acid (PLA), cyclic olefin polymer (COP), cyclic olefin copolymer (COC) and polyethylene terephthalate (PET) as well as polycarbonate (PC) are investigated as a possibility to overcome the limitations mentioned before [72]. Single designs or layers can be milled individually [73]. For a manufacturing of polymer-based microfluidic systems, which is more industrial relevant, injection molding and hot embossing are considered as the established standard. The major drawback of these manufacturing methods is the requirement of immense infrastructure, investment and time for every redesign because of necessity to reset the equipment as well as the costs for molds and tooling. A new promising approach to combine individual chip designs with fully automated production is the emerging field of 3d-printing and bioprinting [74]. Recently, effort is invested to overcome the still remaining

limitations such as printing resolution, printing speed, overall dimensioning, mechanical properties, optical transparency and a level of sufficient biocompatibility [75].

Rapid Prototyping of multi-material and sensor integrated systems

To develop new organ-on-a-chip and cell-based lab-on-a-chip systems with the aspects described before, iterative design improvements are necessary to successively build up complex devices (Schematic 4). For that reason, rapid prototyping, which reduces concept-to-chip time and cost by high level of flexibility in design, fast manufacturing and a simple assembly of biochip is needed. Because of the requirement of new mold and tools for every new design the methods and material mentioned before need a costly multi-step manufacturing process and are thereby not appropriate for one-step rapid prototyping.



Schematic 4 from model idea to working experimental setup

One possibility is to microstructure precured PDMS foil with xurography using a cutting plotter. Channel and compartment designs with a resolution down to several hundred micrometers can be plotted within minutes and bounded via plasma activation to glass or other micro structured PDMS layers. This two-step procedure results in a short concept-to-chip time, where it only takes several hours from the digital design to the final assembled device [76]. A major disadvantage is the that PDMS only provides sufficient bonding to glass and itself, which limits the assembly of durable multi-material composed devices [77]. In case of mimicking tissue barriers, where two different compartments are separated by distinguished cell layers, the widespread design is the integration of porous polymer membranes. Those membranes are layered between top and bottom microchannels to separate apical and basal culture compartments with independently addressable fluid streams to remodel physiological barrier [78]. To bond PET and other polymers to PDMS, intense effort was invested to develop a lot of

different extensive protocols by the use of silanization and solvent bonding [79-83]. The realization of multi-composed chips is an important aspect to enlarge the applications of organ-on-a-chip through the integration of functional materials and units. Thiol–ene-epoxy, a functional polymer for biochips with permanent and adjustable oxygen absorbing characteristics, can be used to create hypoxia cell microenvironments [84]. Functional units such as microvalves or micromechanical actuators for physical cell stimulation, which are composed of elastic structures, have to be permanently combined with rigid micro channel systems [85]. Newly developed organic-inorganic hybrid materials combine the advantages of both organic and inorganic properties such as rigidity, porosity, optical transparency, biocompatibility, cell adhesion, self-organization with mechanical strength and toughness [86]. These new materials often require a long multi-step chip manufacturing procedure to overcome issues like suffering from shrinkage and the establishment of new bonding protocols resulting in a long concept-to-chip time [87]. Due to its inorganic and bioinert nature, high optical transparency character as well as allowing cell adhesion, glass is established as the standard in microscopical imaging and is therefore the organ-on-a-chip device substrate of choice. Because of its electrical insulating properties, electrical sensor integration is often performed by sputtering thin micro electrodes on the top of glass slides to be placed in the direct cell microenvironment for applications like detecting electrical signaling of cells or impedance sensing and trans epithelial electrical resistance [88]. These thin-film electrodes are sensitive to the bonding processes resulting in a long multi-step chip manufacturing procedure leading also to a long concept-to-chip time.

All these limitations and drawbacks show that new methods are needed to realize novel organ-on-a-chip and cell-based lab-on-a-chip systems, which can be rapidly prototyped and are capable of building up multi-composed microdevices to enhance biosensor integration.

The development of new physiological relevant human organ models - organ-on-a-chip and cell-based lab-on-a-chip systems - will help to learn the behavior and characteristics of healthy tissues and organs as well as to interpret dysfunction, pathogenesis and their regeneration.

2. Aim

Organ-on-a-chip and cell-based lab-on-a-chip technology has demonstrated remarkable possibilities to dynamically remodel human physiology and gain insight into tissue behavior as well as pharmacodynamics and pharmacokinetics.

However, today most organ-on-a-chip and cell-based lab-on-a-chip systems still fail to take advantage of the potential offered by multi-disciplinary approaches linking microsystem manufacturing and biosensing. Due to the scientific nature of this technology, developing new systems often need several redesigns to overcome material limitations until a proof-of-concept is realized. Developing new systems is often hindered by long concept-to-chip time of several hours up to days and tricky biosensor integration, resulting in laborious iteration steps for each redesign. The goal of the work presented in this thesis is to develop tools and strategies, that can enable rapid prototyping and manufacturing of multi-material and sensor integrated devices for maintenance and monitoring of new *in vitro* micro physiological models.

First recent progress in biosensing for micro physiological organs-on-a-chip and multi-organs-on-a-chip systems need to be reviewed to evaluate the current status of sensor integrated systems. Then a new method needs to be developed for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems with the goal to match requirements like optical transparency, oxygen permeability, vapor permeability, bonding strength (tensile and shear force), resolution in micro structuring, bonding height and biocompatibility. Furthermore, the goal is to allow rapid prototyping within one hour, one-step manufacturing as well as easy realization of sensor integration and multi-material composition. The capability of manufacturing multi-material composed systems must be evaluated in regard to capacity of bonding for biocompatible microfluidic and biochip materials and systems. To demonstrate the applicability of the new rapid prototyping and manufacturing method, new organ-on-a-chip and cell-based lab-on-a-chip systems realization based on these methods need to be presented.

3. Methods

In this thesis, first of all the recent progress of biosensor integration in organ-on-a-chip and cell-based lab-on-a chip systems is reviewed to summarize the current problems, challenges, approaches and solutions. Afterwards a new rapid prototyping method is developed to enhance concept-to-chip time for cell-based lab-on-a-chip and organs- on-a-chip systems. Based on the newly developed rapid prototyping method the manufacturability in regard to bonding strength of biochip and microfluidic biocompatible materials is evaluated. To demonstrate the different application possibilities of the newly developed rapid prototyping and manufacturing methods in biosensor integrated organ-on-a-chip and cell-based lab-on-a chip systems, two new systems are realized.
The first system, a lab-on-a-chip for nanoparticle risk assessment on the placental barrier is established by embedding porous membrane-based impedance biosensor arrays within the system as well as the second system, a synovium-on-a-chip for monitoring three-dimensional tissue-level remodeling during inflammatory arthritis with non-invasive light scattering biosensing.

To review the recent state-of-the-art of possibilities and approaches of the integration of biosensor in organ-on-a-chip and multi-organs-on-a-chip systems (Publication I - Review: Latest Trends in Biosensing for Microphysiological Organs-on-a-Chip and Body-on-a-Chip Systems) literature is selected with following criteria: i) being up-to-date (published < 3 years ago), ii) relevant organ- and micro physiological models (each system representing a key function of an organ or tissue; excluding literature with simple cell culture observed with sensors), iii) without imaging through microscope or fluorescence techniques, iv) only on-chip data acquisition and v) biosensors are integrated into the organ- or body-on-a-chip device. Furthermore, the term “biosensor” also includes cell-based microsensors where living cells serve as biorecognition elements. To develop a reliable method for rapid prototyping of organ-on-a-chip and cell-based lab-on-a-chip systems in less than 1h (Publication II - Article: Characterization of four functional biocompatible pressure-sensitive adhesives for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems), pressure sensitive biocompatible adhesives are micro structured and incorporated between conventional glass slides. The microstructured biocompatible adhesives within the devices

are characterized in regard to the key aspects of cell-based lab-on-a-chip and organ-on-a-chip systems: optical properties, oxygen permeability, vapor permeability, bonding strength (tensile and shear force), resolution in micro structuring, bonding height and biocompatibility. As a proof of principle, a cell-based lab-on-a-chip micro-structured adhesive-bonded device is realized to study the uptake of non-toxic fluorescently nanoparticles on human endothelial cells depending on time and the explosion to shear force.

To evaluate a key aspect of manufacturability, the capability of sealing and bonding of combinations of well-established materials and bonding protocols in cell-based lab-on-a-chip and organ-on-a-chip systems, tensile and shear strength of bonding is quantified (Publication III - Article: A compression transmission device for the evaluation of bonding strength of biocompatible microfluidic and biochip materials and systems). First of all, mechanical stress simulations are carried out to analyze the stress distribution by the design geometry of the compression transmission device as well as the influence of geometry and size of the bonding sample. The outcomes of the simulations for shear and tensile forces are compared to values from the experimental setup. Tensile or shear stress is applied on the bonding sample by mounting the device into a conventional press enhanced with a precision tension and compression load cell. The measured peak force is taken to identify the maximum load each sample can handle. The combination of common organic and inorganic materials in biochips such as glass, PDMS, PET, PC, COP, COC, PMMA and Ostemer 322 crystal clear are bonded and sealed with well stablished methods. Those methods cover adhesives, plasma and solvent bonding as and also adhering through additional amino-silane monolayers. Functional biochip polymers as thiol-ene-epoxy, which inhibit intermediate adhesive layers are investigated, too. All bonding samples are tested in regards to tensile and shear force as well as bonding strength directly following the bonding procedure and after the impact of cell culture conditions for 7 days (37° C and 100% humidity) to evaluate the material combined with the bonding procedure for the manufacturing of cell-based lab-on-a-chip and organ- on-a-chip systems.

The developed and evaluated methods for rapid prototyping and manufacturing is used to establish a lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on the placental barrier. First an optimized bi-layer lift of method for fabricating thin-film electrodes on commercially available porous polymeric membranes is developed by using a combination of LOR3A and AZ521E photoresists for micro-structuring down to 2.5 µm (Publication IV -Article: Optimized plasma-

assisted bi-layer photoresist fabrication protocol for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes). The established manufacturing methods for pressure sensitive adhesives are used to realize the integration of the membrane-based thin-film electrodes for the on-chip setup. The on-chip set up is used to quantify the barrier resistance of BeWo trophoblast epithelial cells and compare to a standard Transwell assay measured with an EVOM2 volt-ohm meter. Then the on-chip setup is transferred to a cell-based lab-on-a-chip device to study the impact of nanoparticles on the placental barrier (Publication V -Article: A lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells). Here the integration of interdigitated impedance biosensors located on top of a free-standing porous membrane that separates maternal and fetal circulation systems in the placenta-on-a-chip is manufactured through the rapid prototyping methods of biocompatible pressure sensitive adhesives. The device is characterized using fluorescent dextran permeability assays and tetrapolar trans-epithelial resistance measurements. Nano-risk assessment is carried out with the placental Bewo cell line and the application of the three most frequently encountered nanoparticles in daily life, which are titanium dioxide (TiO2), silicon dioxide (SiO2), and zinc oxide (ZnO) and validated against viability and ROS detection assays. Furthermore, a synovium-on-a-chip is established to remodel inflammatory arthritis (Publication VI - Article: Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing). Primary cells, fibroblast-like synoviocytes derived from rheumatoid arthritis patients depleted from immune cells for at least five passages (e.g. T-cells, B-cells and macrophage-like synoviocytes) are cultured within the microfluidic organ-on-a-chip device. With a dominant role in inflammatory rheumatoid arthritis, artificial biochemical stimulus tumor necrosis factor alpha is added to triggering and enhanced proliferation and cytokine production for a pathogen model. The on-chip synovial organoids are also analyzed through histological analysis, immunostaining, viability and metabolic activity assays through enzyme-linked immunosorbent assay to quantify inflammatory stimulation, extracellular matrix structures and cell proliferation.

For the detailed description of Material & Methods please refer to the corresponding publications in the section Results – Publications.

4. Brief summary of the publications

I. Latest Trends in Biosensing for Microphysiological Organs-on-a-Chip and Body-on-a-Chip Systems

Sebastian Rudi Adam Kratz, Gregor Höll, Patrick Schuller, Peter Ertl and Mario Rothbauer

Biosensors 9, 110 (2019); doi:10.3390/bios9030110

Organ-on-a-chips and Body-on-a-chip systems are microfluidic devices that recreate dynamic micro physiological tissue microenvironment. The recreations of representative functional units of tissue and organs (as 3D tissues e.g. organoids and spheroids or tissue barriers) aims to reduce animal testing by generating human organ models. Drug development can be facilitated and conducted by personalized medicine where tissue is created from patient-derived cells and patient-derived induced pluripotent stem cells. To maintain and understand cell and tissue behavior as well as to quantify the health status, different sensing and read-out strategies are needed. Through the integration of biosensors continuous and high content data of dynamic tissue response can be accessed. The review comprises the latest trends over the last 3 years for optical, physical and electrochemical biosensors in micro physiological organ-on-a-chip and body-on-a-chip systems.

Author Contribution: S.R.A. Kratz wrote the manuscript together with G. Höll, P. Schuller, M. Rothbauer and P. Ertl. S.R.A. Kratz finalized the visualization together with G. Höll, and M. Rothbauer. M. Rothbauer did the overall conceptualization.

II. Characterization of four functional biocompatible pressure-sensitive adhesives for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems

Sebastian Rudi Adam Kratz, Christoph Eilenberger, Patrick Schuller, Barbara Bachmann, Sarah Spitz, Peter Ertl & Mario Rothbauer

Scientific reports 9.1 (2019): 1-12; https://doi.org/10.1038/s41598-019-45633-x

Nowadays the standard procedure to build cell-based lab-on-a-chip and organ-on-a-chip systems is the use of polydimethylsiloxane (PDMS) for soft-lithography. While performed for broad applications in the academic setting the procedure is time and material consuming due to the curing time of PDMS and the need for new molds for every design iteration. Especially in the academic setting fast design iterations and rapid prototyping with a good level of adaptability are necessary. To shorten the concept-to-chip-time the process to structure pressure sensitive adhesives enables a high degree of freedom in design, rapid fabrication as well as fast and simple biochip assembly. Due to the toxicity of most adhesives for living biological systems, biocompatibility is one of the key factors to establish pressure sensitive adhesives for cell-based lab-on-a-chip and organ-on-a-chip systems. In this study four biocompatible pressure sensitive adhesives for rapid prototyping (less than 1 hour) are assessed in regard to micro structuring precision, physical and optical properties and biocompatibility. Besides the similar biocompatibility of all four pressure sensitive adhesives, big differences are observed in cutting behavior, bonding strength to polymers and glass as well as gas permeability. In addition, a cell-based lab-on-a-chip system was assembled with micro structured adhesives and used to investigate the time- and shear dependent uptake of non-toxic fluorescently labelled nanoparticles of human endothelial cells. The results demonstrate that, from plain to multilayered set ups, microdevices can be designed and assembled within 1h. The devices are capable of withstanding the conditions in cell culture for weeks and can be used to recreate phycological microfluidic cell-based lab-on-a-chip and organ-on-a-chip systems.

Author Contribution: S.R.A. Kratz is equally contributed to this work with C. Eilenberger and M. Rothbauer. S.R.A. Kratz conceived all experiments and performed the experiments together with C. Eilenberger and M. Rothbauer. S.R.A. Kratz analyzed the data with C. Eilenberge and M. Rothbauer. S.R.A. Kratz prepared the figures with C. Eilenberger, M. Rothbauer, B. Bachmann and S. Spitz. S.R.A. Kratz wrote the manuscript with C. Eilenberger, M. Rothbauer and P. Ertl.

III. A compression transmission device for the evaluation of bonding strength of biocompatible microfluidic and biochip materials and systems

Sebastian Rudi Adam Kratz, Barbara Bachmann, Sarah Spitz, Gregor Höll, Christoph Eilenberger, Hannah Goeritz, Peter Ertl & Mario Rothbauer

Scientific reports 10.1 (2020): 1-13; https://doi.org/10.1038/s41598-020-58373-0

Bonding and sealing of biocompatible microfluidic biochips is still an issue due to the combinations of a broad spectrum of different inorganic and organic polymers, which are needed to establish a sufficient multi-layered biochip. Often the experimental setup and sensor integration determine the material combination, consequently proper sealing and bonding methods must be chosen to ensure functionality and stability. Quantification of bonding strength of biochips is either carried out as a burst test or with expensive mechanical multi-test stations. Due to the dimensions of biochips, proper mounting of multi-layered samples is complicated and can lead to false results. Here a simple 3D printed compression transmission device for evaluating bonding strength is presented. The device translates compressive force into either shear or tensile force. The right sample shape and size were evaluated with experiments and mechanical stress simulations. The device is capable of quantifying the force to failure in regard to shear and tensile force for the most common bonding methods combined with the corresponding, most common used materials for biochips. All tested materials are biocompatible and established in cell-based biochip devices with established bonding methods such as solvent, adhesive and plasma adhering, and also bonding by using of amino-silane monolayers.

Author contributions: S.R.A. Kratz conceived the experiments together with M. Rothbauer and H. Goeritz. S.R.A. Kratz performed the experiments with B. Bachmann and M. Rothbauer. S.R.A. Kratz analyzed the data with G. Höll. S.R.A. Kratz wrote the manuscript together with M. Rothbauer and P. Ertl.

IV. Optimized plasma-assisted bi-layer photoresist fabrication protocol for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes

Patrick Schuller, Mario Rothbauer, Christoph Eilenberger, Sebastian Rudi Adam Kratz, Gregor Höll, Philipp Taus, Markus Schinnerl, Jakob Genser, Peter Ertl, Heinz Wanzenboeck

MethodsX 6 (2019) 2606–2613; https://doi.org/10.1016/j.mex.2019.10.038

Electrochemical assays are carried out using thin-film electrodes-based setups to detect a broad spectrum of analytes and toxins, biomarkers, biological contaminants. Furthermore, monitoring of cell culture with biosensors for amperometeric, voltametric and impedance measurement and separation techniques such as dielectrophoresis can be carried out. Nowadays, thin-film electrodes can be fabricated onto a variety of different flexible and long-living materials such as glass, silicon and polymers. Nevertheless, thin-film electrodes fabricated onto porous polymeric membranes, which are used in biochips and organ-on-a-chip, are quite a challenge and often suffer from poor adhesion and limited resolution. Here an optimized bi-layer lift-off method for fabricating thin-film electrodes on commercially available porous polymeric membranes is presented by using a combination of LOR3A and AZ521E photoresists to overcome the limitations of micro-structuring. Trans epithelial electric resistance was measured in a tetrapolar biosensing setup to remove artificial resistance of the porous membranes. The on-chip setup and the integration of the membrane-based thin-film electrodes was achieved because of the manufacturing, assembly and sealing with the inclusion of biocompatible adhesives. The new established setup was also used to quantify the barrier resistance of BeWo trophoblast epithelial cells and was compared to a standard Transwell assay measured with an EVOM2 volt-ohm meter.

Author contributions: S.R.A. Kratz. conceived and performed the experiments and established the manufacturing of the on-chip set up.

V. A lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells

Patrick Schuller, Mario Rothbauer, Sebastian Rudi Adam Kratz, Gregor Höll, Philipp Taus, Markus Schinnerl, Jakob Genser, Neus Bastus, Oscar H. Moriones, Victor Puntes, Berthold Huppertz, Monika Siwetz, Heinz Wanzenboeck, Peter Ertl

Sensors & Actuators: B. Chemical 312 (2020) 127946;
https://doi.org/10.1016/j.snb.2020.127946

The human placenta is a unique organ that undergoes constant change to maintain pregnancy and fetal health. Its key function is to mediate the exchange of different endogenous as well as exogenous substances and gases between the mother and fetus during pregnancy. Besides regulating the exchange of essential substances, the placental barrier protects the fetus from a broad spectrum of harmful substances found in the maternal blood such as environmental toxins as well as viral and bacterial infections. Due to the increasing presence of man-made nanoparticles in our environment, placenta research nowadays investigates the impact of those engineered nanoparticles on the placenta barrier and their capability to cross the barrier. Here a placenta-on-a-chip system with embedded thin-film electrodes on porous PET membranes is manufactured, assembled and sealed through the inclusion of biocompatible adhesives. To measure continuously and label-free barrier integrity of trophoblast-derived BeWo cells under the impact of titanium dioxide (TiO2), silicon dioxide (SiO2), and zinc oxide (ZnO) nanoparticles, trans-epithelial electrical resistance (TEER) is quantified.

Author contributions: S.R.A. Kratz performed experiments together with P. Schuller, M. Rothbauer, G. Höll, P. Taus, M. Schinnerl and J. Genser. S.R.A. Kratz visualized the graphs and figures with P. Schuller and M.R. Rothbauer. S.R.A. Kratz co-wrote and corrected the manuscript with P. Schuller, M. Rothbauer, G. Höll, P. Taus, M. Schinnerl, J. Genser, N. Bastus, O. H. Moriones, V. Puntes, B. Huppertz, M. Siwetz, H. Wanzenboeck and P. Ertl.

VI. Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing

Mario Rothbauer, Gregor Höll, Christoph Eilenberger, Sebastian Rudi Adam Kratz, Bilal Farooq, Patrick Schuller, Isabel Olmos Calvo, Ruth A. Byrne, Brigitte Meyer, Birgit Niederreiter, Seta Küpcü, Florian Sevelda, Johannes Holinka, Oliver Hayden, Sandro F. Tedde, Hans P. Kiener and Peter Ertl

Lab on a Chip 20 (2020) 1461; https://doi.org/10.1039/c9lc01097a

Rheumatoid arthritis causes chronic joint damage because of inflammation caused by an autoimmune response. Even though the investigation and use of anti-TNFα as therapeutic agent therapies are not capable of introducing disease remission. Understanding the mechanisms of destructive inflammatory process of rheumatoid arthritis is essential to develop and understand novel therapeutic approaches. Here a three-dimensional synovium-on-a-chip, manufactured, assembled and sealed through biocompatible adhesives, is introduced to investigate pathogenesis and development of inflammatory synovial tissue. Primary synovial organoids derived from patients are cultured for over a week, with and without tumor-necrosis-factor. Through the integration of label-free, non-invasive optical light-scattering biosensors the inflammation induced three-dimensional tissue change can already be measured after two days. The inflammatory process causes a change in the level of the light-scattering of the three-dimensional tissue. The combination of the lab-on-a-chip setup with complex human synovial organ cultures creates a synovial-on-a-chip device to investigate systemic stress factor effects on synovial tissue.

Author contributions: S.R.A. Kratz performed experiments and analyzed data together with M. Rothbauer, G. Höll, C. Eilenberger, S. Küpcü, R. A. Byrne, B. Farooq, P. Schuller, I. O. Calvo, B Meyer and B. Niederreiter. All authors co-wrote the manuscript.

5. Conclusion – Scientific value of the Dissertation

The goal of the work presented in this thesis was to develop tools and strategies that can enable rapid prototyping and manufacturing of multi-material and sensor integrated devices for maintenance and monitoring of new *in vitro* micro physiological models.

First the recent progress in biosensor integrated micro physiological organs-on-a-chip and multi-organs-on-a-chip systems were reviewed to evaluate the current status of sensor integrated systems. The review showed that only a small spectrum of biosensing strategies are established. New methods for creating those systems are needed to improve the sensor variety for probing and monitoring representative organ characteristics. This task is getting more complex in terms of multi-organs-on-a-chip, where every organ and tissue model requires an individual biosensing strategy. Then a new method is developed for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems based on functional biocompatible pressure-sensitive adhesives with matching, necessary requirements such as optical transparency, oxygen permeability, vapor permeability, bonding properties (height, tensile and shear strength), resolution in micro structuring and biocompatibility. Furthermore, the adhesives allow rapid prototyping within one hour, one-step manufacturing as well as easy realization of sensor integration and multi-material composition through a biocompatible pressure sensitive adhesive. To evaluate the capability of manufacturing multi-material composed systems, a compression transmission device for the quantification of bonding strength of biocompatible microfluidic and biochip materials and systems is developed. The manufacturing of multi-material composed devices is quantified in regard to the bonding strength of material and bonding procedures commonly used for cell-based lab-on-a-chip and organ- on-a-chip systems. To demonstrate the establishment of the new rapid prototyping and manufacturing methods, the development and realization of systems based on functional biocompatible pressure-sensitive adhesives are presented. To optimize plasma-assisted bi-layer photoresist fabrication for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes, the new presented rapid prototyping procedure is used to realize the first on chip concept as a proof of principle. Based on these outcomes and the presented manufacturing method, a cell-based lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells is established. To demonstrate additional

capabilities of the new developed manufacturing procedure, a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing for monitoring tissue-level remodeling during inflammatory arthritis is presented.

Besides the work presented here in this thesis, recent publications show even further establishments of the newly developed methods. A combination of *in vitro* and in silico approaches, to describe shear-force dependent uptake of nanoparticles in microfluidic vascular models, was carried out on a tape based microfluidic chip. The set up showed that it is possible to estimate flow-dependent nanomaterial uptake of dynamic cultured endothelial cells based on the determination of the critical shear force parameter [89]. A set up based on the methods presented in this thesis was used to downscale a multifunctional bioreactor array on-chip for screening yeast cultures. The microreactor on the chip can be used to optimize and speed up lactic acid bioproduction of yeast [90]. Furthermore, non-invasive real-time oxygen biosensing in two- and three-dimensional microfluidic cell models was established in tape-based devices relying on the here presented methods [48]. Besides the enormous advantage of very fast rapid prototyping, a low-cost microphysiological system feasibility study of a tape-based barrier-on-chip for small intestine modeling was carried out. Based on the one-step manufacturing procedure presented in this thesis, a multilayered device was realized to demonstrate mimicking tissue barriers and highlighting the inexpensive advantages of tape-based organ- on-a-chip systems [91].

Along with the here presented progress for rapid prototyping, manufacturing and application of organ-on-a-chip and cell-based lab-on-a-chip systems, the steady progress in microfluidic, biosensing and stem cell biology research additionally enables physiological relevant modelling of disease and infection as well as personalized medicine. Therefore, insights in human physiology and drug response can be gained to develop new therapies for regeneration.

In addition to the scientific approach to overcome the limitation of static cell culture and animal testing, political institutions like the European Commission and National Institute of Health in America as well as governments all over the globe invest to support and provide funding to develop accessible, reliable and affordable *in vitro* physiological models [20]. Furthermore the technology opens a field for a new product and service generating new business opportunities spanning from small start-ups to global life-science enterprises [32, 44, 92]. All these efforts aim to establish organ-on-a-chip and cell-based lab-on-a-chip systems as a new standard for *in vitro* modelling of human physiology.

Although organs-on-a-chip can never truly mimic an entire human body and the technology is still in its infancy, models are capable to recapitulate key aspects of organs. However, organ-on-a-chip technology is a promising way to tackle the still remaining lack of clarity in the field of medicine and human biology. Medical research is mostly based on male models of cells, tissues, animals and humans regardless of the influence of sex in molecular and cellular biology. The serious variations in the physiology of men and women have a significant effect in etiopathology, toxic impact of compounds and the potency of pharmaceuticals. Traditional *in vitro* approaches are highly restricted in the possibilities of mimicking the female physiology in regard to the dynamics sex steroids secretion and the concentration of these as well as the complicated interrelationships of the organs of the female reproductive system [93]. Organ-on-a-chip technology is now capable of tackling these limitations and hopefully of helping to understand the physiology of all human beings

Results – Publications

I. Latest Trends in Biosensing for Microphysiological Organs-on-a-Chip and Body-on-a-Chip Systems

II. Characterization of four functional biocompatible pressure-sensitive adhesives for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems

III. A compression transmission device for the evaluation of bonding strength of biocompatible microfluidic and biochip materials and systems

IV. Optimized plasma-assisted bi-layer photoresist fabrication protocol for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes

V. A lab-on-a-chip system with an embedded porous membrane-based T impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells

VI. Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing

I. Latest Trends in Biosensing for Microphysiological Organs-on-a-Chip and Body-on-a-Chip Systems

Authors:

Sebastian Rudi Adam Kratz, Gregor Höll, Patrick Schuller, Peter Ertl and Mario Rothbauer

Journal:

Biosensors 9, 110; (2019) doi:10.3390/bios9030110

II. Characterization of four functional biocompatible pressure-sensitive adhesives for rapid prototyping of cell-based lab-on-a-chip and organ- on-a-chip systems

Authors:

Sebastian Rudi Adam Kratz, Christoph Eilenberger, Patrick Schuller, Barbara Bachmann, Sarah Spitz, Peter Ertl & Mario Rothbauer

Journal:

Scientific reports 9.1 (2019): 1-12; https://doi.org/10.1038/s41598-019-45633-x

III. A compression transmission device for the evaluation of bonding strength of biocompatible microfluidic and biochip materials and systems

Authors:

Sebastian Rudi Adam Kratz, Barbara Bachmann, Sarah Spitz, Gregor Höll, Christoph Eilenberger, Hannah Goeritz, Peter Ertl & Mario Rothbauer

Journal:

Scientific reports 10.1 (2020): 1-13; https://doi.org/10.1038/s41598-020-58373-0

IV. Optimized plasma-assisted bi-layer photoresist fabrication protocol for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes

Authors:

Patrick Schuller, Mario Rothbauer, Christoph Eilenberger, Sebastian Rudi Adam Kratz, Gregor Höll, Philipp Taus, Markus Schinnerl, Jakob Genser, Peter Ertl, Heinz Wanzenboeck

Journal:

MethodsX 6 (2019) 2606–2613; https://doi.org/10.1016/j.mex.2019.10.038

V. A lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells

Authors:

Patrick Schuller, Mario Rothbauer, Sebastian Rudi Adam Kratz, Gregor Höll, Philipp Taus, Markus Schinnerl, Jakob Genser, Neus Bastus, Oscar H. Moriones, Victor Puntes, Berthold Huppertz, Monika Siwetz, Heinz Wanzenboeck, Peter Ertl

Journal:

Sensors & Actuators: B. Chemical 312 (2020) 127946;

https://doi.org/10.1016/j.snb.2020.127946

VI. Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing

Authors:

Mario Rothbauer, Gregor Höll, Christoph Eilenberger, Sebastian Rudi Adam Kratz, Bilal Farooq, Patrick Schuller, Isabel Olmos Calvo, Ruth A. Byrne, Brigitte Meyer, Birgit Niederreiter, Seta Küpcü, Florian Sevelda, Johannes Holinka, Oliver Hayden, Sandro F. Tedde, Hans P. Kiener and Peter Ertl

Journal:

Lab Chip 20 (2020), 1461; https://doi.org/10.1039/c9lc01097a

References

1. Heylman, C., et al., *A strategy for integrating essential three-dimensional microphysiological systems of human organs for realistic anticancer drug screening.* Experimental biology and medicine, 2014. **239**(9): p. 1240-1254.
2. Wu, Q., et al., *Organ-on-a-chip: recent breakthroughs and future prospects.* BioMedical Engineering OnLine, 2020. **19**(1): p. 9.
3. Paul, S.M., et al., *How to improve R&D productivity: the pharmaceutical industry's grand challenge.* Nature reviews Drug discovery, 2010. **9**(3): p. 203-214.
4. Greek, R. and A. Menache, *Systematic reviews of animal models: methodology versus epistemology.* International journal of medical sciences, 2013. **10**(3): p. 206.
5. Doke, S.K. and S.C. Dhawale, *Alternatives to animal testing: A review.* Saudi Pharmaceutical Journal, 2015. **23**(3): p. 223-229.
6. Paguirigan, A.L. and D.J. Beebe, *Microfluidics meet cell biology: bridging the gap by validation and application of microscale techniques for cell biological assays.* BioEssays, 2008. **30**(9): p. 811-821.
7. Mittal, R., et al., *Organ-on-chip models: Implications in drug discovery and clinical applications.* Journal of cellular physiology, 2019. **234**(6): p. 8352-8380.
8. van der Meer, A.D. and A. van den Berg, *Organs-on-chips: breaking the in vitro impasse.* Integrative Biology, 2012. **4**(5): p. 461-470.
9. Langhans, S.A., *Three-dimensional in vitro cell culture models in drug discovery and drug repositioning.* Frontiers in pharmacology, 2018. **9**: p. 6.
10. Chen, Y., et al., *ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors.* Science, 2006. **314**(5806): p. 1792-1795.
11. Haycock, J.W., *3D cell culture: a review of current approaches and techniques*, in *3D cell culture*. 2011, Springer. p. 1-15.
12. Ionescu-Zanetti, C., et al., *Mammalian electrophysiology on a microfluidic platform.* Proceedings of the National Academy of Sciences, 2005. **102**(26): p. 9112-9117.
13. Gravesen, P., J. Branebjerg, and O.S. Jensen, *Microfluidics-a review.* Journal of micromechanics and microengineering, 1993. **3**(4): p. 168.
14. Huh, D., G.A. Hamilton, and D.E. Ingber, *From 3D cell culture to organs-on-chips.* Trends in cell biology, 2011. **21**(12): p. 745-754.
15. Sosa-Hernández, J.E., et al., *Organs-on-a-chip module: a review from the development and applications perspective.* Micromachines, 2018. **9**(10): p. 536.
16. Young, E.W. and D.J. Beebe, *Fundamentals of microfluidic cell culture in controlled microenvironments.* Chemical Society Reviews, 2010. **39**(3): p. 1036-1048.
17. Shafiee, A., et al., *Biosensing technologies for medical applications, manufacturing, and regenerative medicine.* Current Stem Cell Reports, 2018. **4**(2): p. 105-115.
18. Rothbauer, M., H. Zirath, and P. Ertl, *Recent advances in microfluidic technologies for cell-to-cell interaction studies.* Lab on a Chip, 2018. **18**(2): p. 249-270.
19. Huh, D., et al., *Reconstituting organ-level lung functions on a chip.* Science, 2010. **328**(5986): p. 1662-1668.
20. Zheng, F., et al., *Organ-on-a-Chip Systems: microengineering to biomimic living systems.* Small, 2016. **12**(17): p. 2253-2282.

21. Yum, K., et al., *Physiologically relevant organs on chips.* Biotechnology journal, 2014. **9**(1): p. 16-27.
22. Rothbauer, M., et al., *Tomorrow today: organ-on-a-chip advances towards clinically relevant pharmaceutical and medical in vitro models.* Current opinion in biotechnology, 2019. **55**: p. 81-86.
23. Günther, A., et al., *A microfluidic platform for probing small artery structure and function.* Lab on a Chip, 2010. **10**(18): p. 2341-2349.
24. Blundell, C., et al., *A microphysiological model of the human placental barrier.* Lab on a Chip, 2016. **16**(16): p. 3065-3073.
25. Griep, L.M., et al., *BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function.* Biomedical microdevices, 2013. **15**(1): p. 145-150.
26. Grosberg, A., et al., *Muscle on a chip: in vitro contractility assays for smooth and striated muscle.* Journal of pharmacological and toxicological methods, 2012. **65**(3): p. 126-135.
27. Uzel, S.G., et al., *Microfluidic device for the formation of optically excitable, three-dimensional, compartmentalized motor units.* Science advances, 2016. **2**(8): p. e1501429.
28. Grosberg, A., et al., *Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip.* Lab on a chip, 2011. **11**(24): p. 4165-4173.
29. Polacheck, W.J., et al., *Microfluidic platforms for mechanobiology.* Lab on a Chip, 2013. **13**(12): p. 2252-2267.
30. Park, S.-H., et al., *Chip-based comparison of the osteogenesis of human bone marrow-and adipose tissue-derived mesenchymal stem cells under mechanical stimulation.* PloS one, 2012. **7**(9): p. e46689.
31. Kaarj, K. and J.-Y. Yoon, *Methods of delivering mechanical stimuli to Organ-on-a-Chip.* Micromachines, 2019. **10**(10): p. 700.
32. Zhang, B., et al., *Advances in organ-on-a-chip engineering.* Nature Reviews Materials, 2018. **3**(8): p. 257-278.
33. Geraili, A., et al., *Controlling Differentiation of Stem Cells for Developing Personalized Organ-on-Chip Platforms.* Advanced healthcare materials, 2018. **7**(2): p. 1700426.
34. Rothbauer, M., et al., *A comparative study of five physiological key parameters between four different human trophoblast-derived cell lines.* Scientific reports, 2017. **7**(1): p. 1-11.
35. Baharvand, H., et al., *Differentiation of human embryonic stem cells into hepatocytes in 2D and 3D culture systems in vitro.* International Journal of Developmental Biology, 2004. **50**(7): p. 645-652.
36. Ju, X., et al., *Hepatogenic differentiation of mesenchymal stem cells using microfluidic chips.* Biotechnology Journal: Healthcare Nutrition Technology, 2008. **3**(3): p. 383-391.
37. Ho, C.-T., et al., *Liver-cell patterning lab chip: mimicking the morphology of liver lobule tissue.* Lab on a Chip, 2013. **13**(18): p. 3578-3587.
38. Ertl, P., et al., *Lab-on-a-chip technologies for stem cell analysis.* Trends in biotechnology, 2014. **32**(5): p. 245-253.
39. Vatine, G.D., et al., *Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications.* Cell stem cell, 2019. **24**(6): p. 995-1005. e6.

40. Vunjak-Novakovic, G., et al., *HeLiVa platform: integrated heart-liver-vascular systems for drug testing in human health and disease.* Stem cell research & therapy, 2013. **4**(1): p. 1-6.
41. Skardal, A., T. Shupe, and A. Atala, *Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling.* Drug discovery today, 2016. **21**(9): p. 1399-1411.
42. Rosenthal, S.B., K.T. Bush, and S.K. Nigam, *A network of SLC and ABC transporter and DME genes involved in remote sensing and signaling in the gut-liver-kidney axis.* Scientific reports, 2019. **9**(1): p. 1-19.
43. Deng, J., et al., *Recent organ-on-a-chip advances toward drug toxicity testing.* development, 2018. **19**: p. 20.
44. Ribas, J., J. Pawlikowska, and J. Rouwkema, *Microphysiological systems: Analysis of the current status, challenges and commercial future.* Microphysiol. Syst, 2018. **2**(10): p. 10.21037.
45. Cui, P. and S. Wang, *Application of microfluidic chip technology in pharmaceutical analysis: A review.* Journal of Pharmaceutical Analysis, 2019. **9**(4): p. 238-247.
46. Edmondson, R., et al., *Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors.* Assay and drug development technologies, 2014. **12**(4): p. 207-218.
47. Pires, N.M.M., et al., *Recent developments in optical detection technologies in lab-on-a-chip devices for biosensing applications.* Sensors, 2014. **14**(8): p. 15458-15479.
48. Zirath, H., et al., *Every breath you take: non-invasive real-time oxygen biosensing in two-and three-dimensional microfluidic cell models.* Frontiers in physiology, 2018. **9**: p. 815.
49. Yin, M.-j., et al., *Optical fiber LPG biosensor integrated microfluidic chip for ultrasensitive glucose detection.* Biomedical optics express, 2016. **7**(5): p. 2067-2077.
50. Zhang, Y.S., et al., *Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors.* Proceedings of the National Academy of Sciences, 2017. **114**(12): p. E2293-E2302.
51. Osaki, T., S.G. Uzel, and R.D. Kamm, *On-chip 3D neuromuscular model for drug screening and precision medicine in neuromuscular disease.* Nature protocols, 2020. **15**(2): p. 421-449.
52. Lai, B.F.L., et al., *InVADE: integrated vasculature for assessing dynamic events.* Advanced Functional Materials, 2017. **27**(46): p. 1703524.
53. Riahi, R., et al., *Automated microfluidic platform of bead-based electrochemical immunosensor integrated with bioreactor for continual monitoring of cell secreted biomarkers.* Scientific Reports, 2016. **6**(1): p. 24598.
54. Shin, S.R., et al., *Aptamer-based microfluidic electrochemical biosensor for monitoring cell-secreted trace cardiac biomarkers.* Analytical chemistry, 2016. **88**(20): p. 10019-10027.
55. Mermoud, Y., et al., *Microimpedance tomography system to monitor cell activity and membrane movements in a breathing lung-on-chip.* Sensors and Actuators B: Chemical, 2018. **255**: p. 3647-3653.
56. Inácio, P.M., et al., *Bioelectrical signal detection using conducting polymer electrodes and the displacement current method.* IEEE Sensors Journal, 2017. **17**(13): p. 3961-3966.

57. Perrier, R., et al., *Bioelectronic organ-based sensor for microfluidic real-time analysis of the demand in insulin.* Biosensors and Bioelectronics, 2018. **117**: p. 253-259.
58. Koutsouras, D.A., et al., *Simultaneous monitoring of single cell and of micro-organ activity by PEDOT: PSS covered multi-electrode arrays.* Materials Science and Engineering: C, 2017. **81**: p. 84-89.
59. Oleaga, C., et al., *Long-term electrical and mechanical function monitoring of a human-on-a-chip system.* Advanced Functional Materials, 2019. **29**(8): p. 1805792.
60. Xu, Y., et al., *A review of impedance measurements of whole cells.* Biosensors and Bioelectronics, 2016. **77**: p. 824-836.
61. Ramadan, Q. and F.C.W. Ting, *In vitro micro-physiological immune-competent model of the human skin.* Lab on a Chip, 2016. **16**(10): p. 1899-1908.
62. Henry, O.Y., et al., *Organs-on-chips with integrated electrodes for trans-epithelial electrical resistance (TEER) measurements of human epithelial barrier function.* Lab on a Chip, 2017. **17**(13): p. 2264-2271.
63. Kratz, S.R.A., et al., *Latest Trends in Biosensing for Microphysiological Organs-on-a-Chip and Body-on-a-Chip Systems.* Biosensors, 2019. **9**(3): p. 110.
64. Lorenz, H., et al., *SU-8: a low-cost negative resist for MEMS.* Journal of Micromechanics and Microengineering, 1997. **7**(3): p. 121.
65. Xia, Y. and G.M. Whitesides, *Soft lithography.* Annual review of materials science, 1998. **28**(1): p. 153-184.
66. Bhattacharya, S., et al., *Studies on surface wettability of poly (dimethyl) siloxane (PDMS) and glass under oxygen-plasma treatment and correlation with bond strength.* Journal of microelectromechanical systems, 2005. **14**(3): p. 590-597.
67. Bhattacharjee, N., et al., *Desktop-Stereolithography 3D-Printing of a Poly (dimethylsiloxane)-Based Material with Sylgard-184 Properties.* Advanced Materials, 2018. **30**(22): p. 1800001.
68. Toepke, M.W. and D.J. Beebe, *PDMS absorption of small molecules and consequences in microfluidic applications.* Lab on a Chip, 2006. **6**(12): p. 1484-1486.
69. Uhl, C., W. Shi, and Y. Liu, *Organ-on-Chip Devices Toward Applications in Drug Development and Screening.* Journal of Medical Devices, 2018. **12**(4).
70. Sharma, V., et al., *Surface characterization of plasma-treated and PEG-grafted PDMS for micro fluidic applications.* Vacuum, 2007. **81**(9): p. 1094-1100.
71. Berthier, E., E.W. Young, and D. Beebe, *Engineers are from PDMS-land, Biologists are from Polystyrenia.* Lab on a Chip, 2012. **12**(7): p. 1224-1237.
72. Ahadian, S., et al., *Organ-on-a-chip platforms: a convergence of advanced materials, cells, and microscale technologies.* Advanced healthcare materials, 2018. **7**(2): p. 1700506.
73. Shanti, A., et al., *Multi-Compartment 3D-Cultured Organ-on-a-Chip: Towards a Biomimetic Lymph Node for Drug Development.* Pharmaceutics, 2020. **12**(5): p. 464.
74. Lee, H. and D.-W. Cho, *One-step fabrication of an organ-on-a-chip with spatial heterogeneity using a 3D bioprinting technology.* Lab on a Chip, 2016. **16**(14): p. 2618-2625.
75. Miri, A.K., et al., *Bioprinters for organs-on-chips.* Biofabrication, 2019. **11**(4): p. 042002.
76. Bartholomeusz, D.A., R.W. Boutté, and J.D. Andrade, *Xurography: rapid prototyping of microstructures using a cutting plotter.* Journal of Microelectromechanical systems, 2005. **14**(6): p. 1364-1374.

77. Chow, W.W.Y., et al., *Microfluidic channel fabrication by PDMS-interface bonding.* Smart materials and structures, 2005. **15**(1): p. S112.
78. Arık, Y.B., et al., *Barriers-on-chips: Measurement of barrier function of tissues in organs-on-chips.* Biomicrofluidics, 2018. **12**(4): p. 042218.
79. Tang, L. and N.Y. Lee, *A facile route for irreversible bonding of plastic-PDMS hybrid microdevices at room temperature.* Lab on a Chip, 2010. **10**(10): p. 1274-1280.
80. Sunkara, V., et al., *Simple room temperature bonding of thermoplastics and poly (dimethylsiloxane).* Lab on a Chip, 2011. **11**(5): p. 962-965.
81. Xu, B.-Y., et al., *One step high quality poly (dimethylsiloxane)-hydrocarbon plastics bonding.* Biomicrofluidics, 2012. **6**(1): p. 016507.
82. Cortese, B., M.C. Mowlem, and H. Morgan, *Characterisation of an irreversible bonding process for COC–COC and COC–PDMS–COC sandwich structures and application to microvalves.* Sensors and Actuators B: Chemical, 2011. **160**(1): p. 1473-1480.
83. Gu, P., et al., *Chemical-assisted bonding of thermoplastics/elastomer for fabricating microfluidic valves.* Analytical chemistry, 2011. **83**(1): p. 446-452.
84. Sticker, D., et al., *Oxygen management at the microscale: a functional biochip material with long-lasting and tunable oxygen scavenging properties for cell culture applications.* ACS applied materials & interfaces, 2019. **11**(10): p. 9730-9739.
85. Sticker, D., et al., *Microfluidic migration and wound healing assay based on mechanically induced injuries of defined and highly reproducible areas.* Analytical chemistry, 2017. **89**(4): p. 2326-2333.
86. Ding, C., et al., *Biomedical Application of Functional Materials in Organ-on-a-Chip.* Frontiers in Bioengineering and Biotechnology, 2020. **8**: p. 823.
87. Schizas, C. and D. Karalekas, *Mechanical characteristics of an Ormocomp® biocompatible hybrid photopolymer.* Journal of the mechanical behavior of biomedical materials, 2011. **4**(1): p. 99-106.
88. ávan der Meer, A.D., et al., *Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems.* Lab on a Chip, 2015. **15**(3): p. 745-752.
89. Charwat, V., et al., *Combinatorial in Vitro and in silico approach to describe shear-force dependent uptake of nanoparticles in microfluidic vascular models.* Analytical chemistry, 2018. **90**(6): p. 3651-3655.
90. Totaro, D., et al., *Downscaling screening cultures in a multifunctional bioreactor array-on-a-chip for speeding up optimization of yeast-based lactic acid bioproduction.* Biotechnology and Bioengineering, 2020.
91. Winkler, T.E., et al., *Low-cost microphysiological systems: feasibility study of a tape-based barrier-on-chip for small intestine modeling.* Lab on a Chip, 2020. **20**(7): p. 1212-1226.
92. van de Burgwal, L.H., et al., *Hybrid business models for 'Organ-on-a-Chip'technology: The best of both worlds.* PharmaNutrition, 2018. **6**(2): p. 55-63.
93. Nawroth, J., et al., *Organ-on-a-Chip Systems for Women's Health Applications.* Advanced healthcare materials, 2018. **7**(2): p. 1700550.