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Developing organ-on-a-chip concepts using bio-mechatronic design methodology

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Abstract

Mechatronic design is an engineering methodology for conceiving, configuring, and optimising the design of a technical device or product to the needs and requirements of the final user. In this article, we show how the basic principles of this methodology can be exploited for *in vitro* cell cultures - often referred to as organ-on-a-chip devices. Due to the key role of the biological cells, we have introduced the term bio-mechatronic design, to highlight the complexity of designing a system that should integrate biology, mechanics and electronics in the same device structure. The strength of the mechatronic design is to match the needs of the potential users to a systematic evaluation of overall functional design alternative. It may be especially attractive for organs-on-chips where biological constituents such as cells and tissues in 3D settings and in a fluidic environment should be compared, screened and selected. Through this approach, design solutions ranked to customer needs are generated according to specified criteria, thereby defining the key constraints of the fabrication.

As an example, the bio-mechatronic methodology is applied to a liver-on-a-chip based on information extrapolated from previous theoretical and experimental knowledge. It is concluded that the methodology can generate new fabrication solutions for devices, as well as efficient guidelines for refining the design and fabrication of many of today's organ-on-a-chip devices.

Keywords: Conceptual design, design optimization, organ-on-a-chip, physiological tissue models, microfluidics

Introduction

Design of microfluidic systems with living cells, capable of mimicking the functions of *in vivo* organs in order to perform pharmacological and toxicological evaluations is a demanding bioengineering

challenge^{1,2}. Even more, if such advanced platforms will be marketed for commercial use, they must be developed with considerable attention to the needs of the end-users³.

Mechatronic design methodology provides an efficient tool in the early formulation of new ideas for the development of a product with strong focus on its functions. The purpose of mechatronic design is to let the user's needs drive the development, in contrast to design methodologies driven by the technology at hand. With this prerequisite, the goal of the mechatronic design is to achieve product solutions with a high-level description of a product that has been thoroughly evaluated and compared to a variety of alternatives.

Mechanical and electrical engineers have since decades used mechatronic design for the development of mechatronic products such as coffee machines, digital copiers, and other examples where mechanics and electronics are merged into a product⁴⁻⁶. The methodology was later applied in other areas such as chemical engineering^{7,8} and information technology⁹. In recent years, mechatronic design of products related to biotechnology and bioengineering, referred to as bio-mechatronic design, have been demonstrated for bioreactors, bioprocesses and biomedical devices¹⁰⁻¹⁷. Here, we extend the opportunities with bio-mechatronic design to an emerging category of cell culture based biotechnology products.

Organs-on-chips are miniaturised devices with living cells, often combined with microfluidic perfusion, that mimic functional parts of tissues and organs¹. Compared to conventional static 2D cell culture, organs-on-chips are more physiologically relevant as they provide an *in vivo*-like environment for the cells. This may include constant supply of nutrients and gasses, exertion of shear stress, co-culture of supportive cells and improved cell-to-cell communication due to a 3D cell construct. A challenge for organs-on-chips is to handle the trade-off between the complexity of the device and the possibility for commercial applications¹⁸. Ultimately, the organ-on-a-chip technology evolves to couple several devices modelling different organs to create a human-on-a-chip. In such a system, each individual device must concede to global properties such as scaling of organ size and surface-to-volume ratios, and shear stress induced by media perfusion^{19,20}. Moreover, different cells usually need different cell culture medium. The devices must either incorporate cells that can be adjusted to a common culture medium²¹ or find ways to create local environments with specific growth factors within each device²².

Previous reports on the construction of organ-on-a-chip devices approach the design mostly from technology viewpoints²³⁻³⁰. However, the user's requirements are significantly intertwined with other conditions than those related to the technology; for example, the input and output of the biological information of the system, the management and computation of data, the supply of cells and their

maintenance, and conditions related to operators and patients. A bio-mechatronic design methodology, however, can appropriately address these demands of the users and, by that, more efficiently transfer the organ-on-a-chip technology from the research and development stage to commercial products of biological and toxicological evaluations needed in the pharmaceutical and chemical industry.

As an example, we apply bio-mechatronic design methodology to the liver, as it is one of the most important drug metabolising organs in the body. During pharmaceutical development, detailed attention is given to the effects of the drug on the liver as hepatotoxicity and other related toxicity effects are major reasons for drug withdrawal ³¹. Several liver-on-a-chip applications have recently been described ³²⁻⁴³. Among these, a few major design strategies can be identified and divided into four groups: 1) the origin of the cells used – cell lines, primary cells or stem cell derived cells, 2) the type of cells incorporated – monocultures, co-cultures or liver slice tissues, 3) the spatial orientation of the cells - 2D (monolayers or sandwich cultures) or 3D (scaffold based systems, scaffold free systems or self-assembled spheroids) and 4) whether or not an oxygen gradient is established and actively monitored. The livers-on-chips have in common that they provide a constant supply of nutrients and gasses either from adjacent micro channels or through the cell compartment. Convincing evidence support the hypothesis that *in vitro* cell culture devices benefit from establishing a 3D environment for the cells as well as the importance of co-cultures to support the hepatocytes ^{44,45}. Hence, the complex cellular micro-architecture of the canaliculi network of liver tissue is an eminent challenge to mimic in the microfluidic construct of *ex vivo* livers-on-chips. The goal of these microfluidic liver models is to analyse *e.g.* pharmacological and pharmacokinetic events that occur during drug metabolism in order to obtain results that would match the results given by *in vivo* trials.

In this article, we demonstrate how the bio-mechatronic design methodology can be exploited for efficient high-quality design of organ-on-a-chip cell cultures. We describe the complete conceptual design process from ideas to concepts and possible design solutions for researchers in the fields of microfluidic cell culture techniques. The concepts are generated from recent advances in organ-on-chip and biofabrication technology. The biological properties and constraints in combination with basic microfluidic technology direct the final structure of the product solutions. Moreover, other distinct requirements decisive for successful application and practical use of the devices are also considered. Here, we focus on the microfluidic design of a liver model. However, the procedure is applicable to other organ or and tissue models where the user's needs can be accurately defined and targeted. The background knowledge about properties of liver cells and organs, *in vitro* assay techniques, chip fabrication and micro-scale fluidic systems was acquired from pre-existing literature and other

information sources as well as our own experimental experience of working with microfluidics and cell cultures.

Bio-mechatronic design methodology

The bio-mechatronic design methodology uses an iterative working procedure based on a set of charts and tables that structure the design process (Fig. 1). The first step is to set the design mission, *i.e.* to define the goals and the constraints of the final product. The needs of the user are then identified and defined as targets with quantitative or qualitative values. Using these targets, a flow chart of the transformation process (TrP) from input to output, together with the different functions needed to accomplish the TrP are outlined in a so called Hubka-Eder map. For applications in biotechnology, these functions include biological systems (cell types, nutrients, drug metabolism) and technical systems (cell containment, liquid transportation, monitoring of the environment).

However, other important functions are also required for the transformation, such as human systems (operators, technicians, patients, researchers), information systems (signal information, software interface, statistics) and management and goal systems (operator actions, controller actions, instructions, data interpretations). Furthermore, one should also take into account the potential influences of an active and unforeseeable environment that may interfere with the design product (bio-variability, risk for infections, other unpredictable variations).

The next step is to evaluate how functions and systems affect each other. This is done in a function-interaction matrix where the strengths of the relationships between the functions are scored in order to highlight important and critical interactions. With the interactions between different functions in mind, a concept generation chart is created. Using so-called basic concept elements, as many design alternatives as possible are generated without letting any preconceptions about common or conventional design solutions interfere with the thought process (we refer to the alternatives as “permutations”). To achieve this, it is important to treat the functions on a conceptual level and not to go into details about actual physical objects. To illustrate this fundamental step in the design, a few examples of differences between concept elements and physical objects in relation to the user’s needs are presented (Table 1).

The concept permutations are scored on how well they meet the target specification values resulting in the selection of a few alternatives. At this point, real objects, *e.g.* cells, material, instruments and other equipment, are introduced to replace the concept elements in an anatomical chart. The final design solution is selected from the chart and that choice is, as a consequence of the systematic procedure, distinctly within the boundaries of the target specification. However, at this stage it is

possible, if required, to further restrict the design solution with additional targets, such as cost, feasibility to produce and estimated performance.

In this article, we applied the bio-mechatronic design methodology to microfluidic cell culture devices, also known as organs-on-chips. Common methods to fabricate microfluidic devices and organs-on-chips include microcontact printing ⁴⁶, photo- and soft lithography ⁴⁷, micromachining ⁴⁸, micro injection moulding ⁴⁹, hot embossing ⁵⁰ and 3D printing ⁵¹. For cell culture, it is important to use a material that is biocompatible, possible to sterilise, optically transparent and, while the material should be robust, it should still allow for flexibility in the design. The devices have ways to enclose cells in a defined area with channels for the supply of nutrients and other factors needed for cell survival. Additional equipment required in a microfluidic cell culture setup are pumps, valves, electrodes, sensors, microscopes and instruments for offline analysis, that concertedly are used to maintain and assess the cells and the cell environment. Their placement can be either directly on the chip or in a close proximity, connected with tubing or electrical wiring. To achieve a higher throughput, the devices are often used in parallel to include controls during the same experiment.

Results and discussion

The bio-mechatronic design methodology described above was applied to the design of a liver-on-a-chip, which is commonly used for toxicity screening, drug assessment or pharmacodynamical modelling. Below follows a detailed description of the design based on the sequential procedure presented in Figure 1.

Design mission and constraints

The mission of the design of the liver-on-a-chip was to recreate the smallest functional unit of the human liver. This liver model should be able to mirror key physiological events of the *in vivo* liver that the user wants to investigate *in vitro*. The design should be constrained to operate at the microfluidic scale using common microfluidic techniques and auxiliary equipment.

User's needs and target specification

In order to define the user's needs and set target specifications for the design of a liver-on-a-chip, critical functional aspects of the liver needed to be taken into consideration. At a functional level, the liver is built up by numerous liver lobules, with a radius of about 500 μm or 20 hepatocytes⁵². At one end, the lobule receives de-oxygenated blood from the hepatic portal vein and oxygen-rich blood from the hepatic artery for transport through the lobule and detoxification before reaching the central vein⁵². The total blood flow through the liver is approximately 100-130 ml/min/100 g of liver⁵³. During blood transportation, a drop in oxygen concentration occurs (from 85 μM to 35 μM) that has led to a specialisation of hepatocyte function depending on its localisation in the oxygen gradient⁴⁵. For example, bile and albumin formation is mostly produced close to the hepatic artery while cytochrome P450 (CYP450) concentration in hepatocytes is higher at zones close to the central vein⁵⁴. In addition to hepatocytes, the liver also contains sinusoidal endothelial cells, Kupffer cells, stellate cells and pit cells. These cell types may be required in the device to ensure a more *in vivo*-like condition.

In Table 2, the user's critical needs and desirable design targets for a liver-on-a-chip are listed. In order to recapitulate the liver at an anatomical and a functional level, the architecture of a relevant cell niche with co-culture of cells supporting the hepatocytes must be established. The functionality and the vitality of the cells should be possible to monitor either online or offline using bioanalytical assays. Common markers of phase I and phase II activity as well as markers affected by cell functionality and vitality should be available for detection in the cell culture medium or by *in situ* microscopy. An important design issue for both online and offline analysis of biomarkers is the ratio between the number of cells and the volume of the cell culture medium. While it is advantageous from the perspectives of parallelization and cost to make small culture chambers, it is necessary to acquire

sufficient molar amounts of sample molecules to supersede the detection limit of the assays. A sufficient and balanced supply of nutrients and oxygen should be provided to the cell compartment and, for hepatocyte zonation, an oxygen gradient needs to be established. Relevant *in vitro* cells should reflect the targeted disease. Alternative cell types include engineered cell lines, primary cells and stem cell derived cells. A robust *in vitro* functionality of the cells should be maintained over a period of several weeks. To accomplish this, it is important that the drug concentration administered to the cells is constant and that the potential absorption by the fabrication materials in the device and auxiliary equipment is low. A common problem in microfluidics is the formation of air bubbles that obstruct or destroy cell structures. The device should therefore be designed in a way that minimises the occurrence of air bubbles. For long-term studies with minimal manual interference, the device should be continuously perfused by cell culture medium both for the supply of nutrients but also for removal of toxic wastes. However, although the total flow through the cell compartment must be appropriate, the shear stress caused by the perfusion must be controlled.

A reasonable unit cost per device is of vital interest to the user but difficult to assess on a conceptual level. The tolerance of the unit cost is application dependent, but in general it can be claimed that the cost of each assay should be lower than comparable alternatives. For organs-on-chips with the aim to replace expensive animal experiments, a relatively high cost is acceptable. However, when successful organ-on-a-chip products start competing with each other, the fabrication cost of each device and auxiliary equipment will become a major challenge.

Functional mapping of the system

The identified needs, with their specifications, were used to create a Hubka-Eder map that included the TrP in the device and the functions required for that transformation (Fig. 2). The TrP for the liver-on-a-chip concerned metabolic degradation of drugs or other compounds that were added to the system. The TrP required provision of cells and nutrients (primary inputs) and should result in readouts or other outputs available for interpretation or assessment of the cellular state (primary outputs). The TrP was also dependent on secondary inputs and outputs that, for example, include standard lab equipment, project scheduling and the cost of running the facilities. In order to structure the TrP of the liver-on-a-chip it was divided into three phases: 1) the preparation phase where input components were prepared and conditioned, 2) the execution phase where the experiments were carried out, and 3) the finishing phase where results were compiled and evaluated. To perform the TrP, five groups of functional systems were required: 1) the biological systems (ΣBioS), 2) the technical systems (ΣTS), 3) the information systems (ΣIS), 4) the management and goal systems ($\Sigma\text{M\&GS}$) and, 5) the human

systems (Σ HuS). Furthermore, the active environment (AEnv) that indirectly affects the TrP and the systems was also included in the map.

Analysis of the interactions between functions of the map

The interactions within and between the Σ BioS functions, the Σ TS functions and the Σ IS functions of the TrP were scored in a function-interaction matrix (Fig. 3). Here, the strength of the interactions was given a score between 1 and 3, where 3 reflected the strongest relationship. As shown in Fig. 3, the majority of strong interactions were found within the Σ BioS system and by the Σ TS affecting the Σ BioS. One of the most important user's needs, the need to establish an environment with metabolically active cells, was found to be influenced by many functions both from Σ BioS and from Σ TS, especially cell organization, nutrients, co-culture, oxygen gradient and temperature.

Identification of functional elements

Basic functional elements for the liver-on-a-chip were identified (Fig. 4). These elements were combined and permuted to generate a permutation variety of conceptual design alternatives. The functional elements were collated in five groups, each having a fundamental role in the TrP of the liver-on-a-chip design (Fig. 5). These groups were: 1) Automation and handling – including the way fluids and cells should be transported to, through and out of the device; 2) Parallelisation and high throughput – the device could contain functional components to realise more efficient screenings by including, *e.g.* parallel cell compartments, multiple assays or a concentration gradient of drug supplemented medium; 3) Cell microenvironment – visualising different ways the cells could be contained in the device; 4) Detection and analysis – related to alternatives for analysis; 5) Part localisation – the position of the elements of the liver chip which could be situated either on or off the chip.

Permutation of functional elements

By permuting the basic functional element groups, a number of conceptual designs of the devices were established. A few of these configurations could be related to previously reported microfluidic liver models (Fig. 6A-D) ^{33,34,40,43}. These configurations were selected for further evaluation together with a, to our knowledge, unreported liver model (Fig. 6E). The permutation in Fig. 6A shows a device which is automatically supplied with nutrients, infused with a toxicant and provided with an assay procedure in the cell compartment and in which subsequently samples are collected for analysis. Cells are manually seeded in the device but are only let into the cell compartment at certain instances. Toxicants

can be added through a concentration gradient for increased throughput. In the cell containment, a cluster of cells is formed that is protected from shear forces by a porous barrier. The cells are monitored by optical visualization. In the system shown in Fig. 6B the nutrients and the toxicant are supplied from a reservoir on the chip and automatically perfused through the device for analysis off the chip. The fluid passes through a co-culture of cells seeded on or into a scaffold in a 3D environment from which samples can manually be collected for analysis. A different approach is outlined in Fig. 6C. Here, cells are seeded in a defined pattern on a flat substrate under static conditions. Multiple assays can be performed by different analytical methods, on and off the chip. A similar concept with a static cell culture is shown in Fig. 6D. Here, cell clusters reside in wells on the bottom of the chamber. The permutations can also lead to a device with an oxygen gradient within the cell culture chamber. Clusters of co-cultured cells can be seeded on a scaffold in the oxygen gradient and supplied with nutrients by continuous perfusion (Fig. 6E).

The five permutations were evaluated as listed in Table 2 by assessing how well they accomplished the needs in Table 3. Scores were allotted to each need at three levels (0 for no effect at all, + for modest effect and ++ for substantial effect).

Screening the permutations

The permutations were assessed based on their ability to incorporate and maintain multicellular constructs in a 3D configuration (Table 3). As recent research indicates, elevated levels of phase I and phase II activity in *in vitro* models can be achieved either by a mixture of relevant cell types³³ or a 3D cell organization³⁴. A device that can combine these two user's needs was therefore expected to be a good platform for liver cell metabolism. Of the generated permutations, four alternatives contained multicellular constructs (Perm. 2-5). Four of the permutations were intended for cells in 3D (Perm. 1, 2, 4 and 5). For long-term studies, the device should contain functional cells throughout the experiment. Cell functionality of liver cells is supported both by a 3D cellular state³⁴ and by additional cell types³³, but also by perfusion and shear stress⁴³. It was therefore considered advantageous if the permutation included those functionalities. The perfusion also made some permutations preferable due to the continuous supply of nutrients and growth and differentiation factors, the removal of waste as well as the possibility to control the supply of oxygen in the cell culture medium. For on-chip analysis the 2D alternative was considered to better fulfil the user's need than the other alternatives. Higher scoring was also given to the devices not including a scaffold as it can obstruct visual monitoring. High scores for off-chip analysis were given to the alternatives that could include both a large number of cells and provide sample volumes in the quantitative measurement range for offline instruments. The complexity of the device was noted by the user's need of having a short time for the preparation of

the device. Here, permutation 3 and 4 were considered to be easier to use as they included cells in 2D or premade 3D cell clusters for direct loading. On the other hand, the operability was considered to be better for perfusion alternatives as it demanded less hands-on work during the experiments. Automation techniques using robotics to maintain standard well-plate formats could also be considered for users and customers in larger industries, but was left out in this example. Without any physical components implemented, the cost of fabrication is difficult to assess at this conceptual stage. However, as an estimation of the cost, configurations comprising of several functional elements would likely become more expensive products than those with just a few. The highest ranked alternative, permutation 5, was considered to be the alternative that most satisfactorily fulfilled the majority of the user's needs. The advantage of this permutation was the close resemblance to the *in vivo* liver.

Generation of anatomical blueprint from the selected permutation

To create an anatomical blueprint of the device, the basic functional elements were examined and transformed into different choices of components *i.e.* physical design objects. To guide the choice of anatomical components, a few possible alternatives of physical objects were again screened towards the user's needs. Some of the user's needs were not relevant at an anatomical level and therefore left out, while a few new aspects that could be considered were added (Table 4). For permutation number 5 the functional components that could be altered were the construction material of the device, the type of barrier to separate the cells from the perfusion of fluids, the method of on-chip detection, how the oxygen gradient could be generated and how to establish the internal fluidic flow.

There were several options when it came to the choice of the construction material of the microfluidic device⁵⁵. The most commonly used material in microfluidics is polydimethylsiloxane (PDMS) due to its simplicity for moulding. However, this material needs to be reconsidered due to its tendency to absorb hydrophobic drugs and the difficulties for use in mass production⁵⁶. Other commonly used materials for cell culture devices are polystyrene and quartz. The scoring between the three material alternatives resulted in a high score for polystyrene. The robustness and the manufacturability of this plastic material is very favourable from a commercial point of view, however, the PDMS alternative can still be considered for prototyping. The cells should be maintained in a 3D state having access to nutrients and gasses while being protected from too high shear stress. Here, we chose to consider three methods common in microfluidics with cell cultures; micropillars, scaffolds and polymeric membranes. Pillars and membranes support the integrity of the cells in 3D by hindering them from escaping a confined area as well as screening the cells from direct flow contact. Scaffolds on the other hand, directly maintain the 3D cell configuration by being a support throughout the whole construct. The major problem with scaffolds and porous membranes are the risk for degradation of the scaffold during long-

term experiments, as well as the risk that membranes become clogged by proteins and other macromolecules. The seeding of cells in a pillar-encaged compartment was considered to be the most wasteful method for precious cell types as they can escape between the large gaps in the barrier resulting in a longer filling time.

Two methods for creating the oxygen gradient in the device were considered. The first consisted of an on-chip gradient network, similar to previously reported concentration gradients for pharmaceuticals⁵⁷, where high and low concentrations of oxygen supplemented in the cell culture medium are mixed at different ratios before reaching the cells. An alternative is to supply the cell chamber with a known amount of oxygen and rely on the diffusion of gas through the material during transportation through the cell construct. In this case, the on-chip gradient was considered to be more reliable and suitable for parallelization compared to the alternative.

The flow of cell culture medium through a microfluidic device can be performed in several different ways. A common method is to use a syringe pump that, connected via tubing, easily can control the flow rate through the device. A drawback is that the amount of tubing rapidly increases as the device becomes more complicated, especially when using high-throughput applications. Furthermore, the need to fill the tubing prior to the cell containment chamber leads to an undesired dead volume in the system. There is also an inherent start and stop delay of the flow using syringe pumps that could limit its use with a concentration gradient generator. Fluid transport can also be performed passively by capillary force or gravity directly on the chip. These are easy methods to ensure that the cell culture does not dry out but it is more difficult to dynamically control the flow rate making it hard to create a reliable oxygen concentration gradient. A fourth alternative that is more difficult to prototype but potentially could be very suitable for high-throughput applications is the use of electroosmotic pumps⁵⁸. The high control over the local cell environment could be very useful in a liver-on-a-chip application.

From this discussion, a blueprint for a prototype of a liver-on-a-chip was created that, to a large extent, fulfils the user's needs (Fig. 7). In the device, cells are encapsulated in a scaffold on top of an oxygen sensor. Cell culture medium with different concentrations of oxygen and drugs is supplied through the cell containment by electroosmotic pumps. For analysis, the oxygen sensitive sensor detects the concentration of oxygen present in the device, both for validating the functionality of the pumps as well as quantifying the metabolism performed by the cells. In-line monitoring of cells is performed by *in situ* holographic microscopy to assess cell morphology and cell alignment in the construct. Finally, the cell culture medium is continuously sampled for off-line analysis using mass spectrometry or other advanced high performance instrumentation.

Conclusion

In this study, we have applied a bio-mechatronic design methodology to develop organs-on-a-chip concepts, specifically, livers-on-chips, with a strong emphasis on the needs of the user. The design criterion of this liver model was to perform bioanalytical assays of drug metabolism in order to evaluate drug efficacy or toxicity. The user's needs were defined and targeted based on the knowledge about the function of the liver and the procedures of bioanalytical assays. Furthermore, a critical examination of previous publications on *in vitro* liver devices and microfluidics was performed in order to establish some general design principles. The functions necessary to transform inputs into the required outputs were outlined and integrated in a transformation process map. A function-interaction matrix was established that guided the conceptual thought process in order to better understand interactions between functions. Basic concept elements used in organs-on-chips and microfluidic devices were identified, categorised and permuted to create alternatives of the device on a conceptual level. The alternatives were screened based on their possibility to fulfil the user's needs. One of the permutations was further evaluated on an anatomical level with different physical object alternatives for the conceptual elements. Finally, a blueprint of such a device, with the anatomical components best fulfilling the user's needs and the design mission, was suggested.

We would like to recommend this approach at an early stage of the design work of organ-on-a-chip devices in general. However, it is important to note that the user needs should be configured and redefined depending on the intended application. The needs of the user might change not only between different types of organs, but also between different types of tasks or assays within the same organ. When applied in a careful and open-minded manner, this methodology gives a thorough understanding of the design assignment and hopefully also a new way of creative and useful design solutions for microfluidic devices.

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Figure Captions

Figure 1. The process of the conceptual design work-flow. The design mission and its constraints are defined followed by an outline of user needs and target specifications. This information is used to create a Hubka-Eder map giving an overview of the transformation process and the different supporting functions. The interactions between functions are assessed to guide the concept generation using concept elements. By permuting the concept elements, numerous configurations, more or less realistic, are obtained. The configurations are screened and assessed based on how well they fulfil the user's needs. The highest ranked configurations are transformed into physical objects in anatomical charts resulting in the selection of a final design solution.

Figure 2. A Hubka-Eder map representing the TrP and the functional systems that are necessary to convert the primary inputs (e.g. cells, nutrients and drugs) into primary outputs (e.g. results and used reagents).

Figure 3. In the Function – Interaction matrix, the strength of the relationship between different functions is indicated by a number from 1 (none or low) to 3 (high). The sum sign (Σ) of the systems is a representation of the several functions each system includes. The matrix is outlined as how the vertical functions (y) affects the horizontal functions (x).

Figure 4. Basic functional elements used in the conceptual design of a liver-on-a-chip, collated in five groups typical for the design and use of microfluidic cell cultures.

Figure 5. Examples of functional element groups of a liver-on-a-chip device. Permutations were obtained by combining one or several elements from each group.

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Figure 7. Blueprint of a single unit of the proposed set-up for a liver-on-a-chip. Several cell compartments can be connected via the cell and scaffold in- and outlets for parallelisation.

Figure 1

Figure 2

Main systems		ΣBioS						ΣTS							ΣIS		
	<div><div>Subsystems</div><div><div></div><div></div></div></div>	Cell organization	Cell metabolism	Bio scaffold/ECM	Nutrients	Co-culture	Oxygen gradient	Perfusion	Scaffold	Temperature	Barrier	Shear stress	Device geometry/size	On-chip analysis	Off-chip analysis	Controller software	Image analysis
ΣBioS	Cell organization		3	3	1	3	1	1	3	1	1	1	1	1	1	1	1
	Cell metabolism	1		1	2	1	3	1	1	1	1	1	1	1	1	1	1
	Bio scaffold/ECM	3	2		1	1	2	2	2	1	1	1	1	2	1	1	3
	Nutrients	1	3	1		3	1	1	1	1	1	1	1	1	1	1	1
	Co-culture	3	3	3	1		1	1	3	1	1	1	1	1	1	1	2
	Oxygen gradient	3	3	2	1	1		1	1	1	1	1	1	1	1	3	1
ΣTS	Perfusion	1	2	1	3	1	3		1	1	1	3	1	3	1	3	1
	Scaffold	3	2	3	1	3	1	1		1	1	2	1	1	1	1	3
	Temperature	1	3	1	1	3	1	1	1		1	1	1	2	1	3	1
	Barrier	3	1	1	1	3	2	2	1	1		2	1	1	1	1	1
	Shear stress	2	2	1	1	2	1	1	1	1	1		2	2	1	1	1
	Device geometry/size	3	1	3	2	1	3	2	2	1	1	3		3	3	1	3
	On-chip analysis	1	1	1	1	1	1	1	1	2	1	1	1		1	1	3
	Off-chip analysis	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1
ΣIS	Controller software	1	1	1	1	1	3	3	1	3	1	3	1	3	1		1
	Image analysis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	

Figure 3

Inputs and outputs

Cell containment

Analysis

Protocols and instructions

Figure 4

Figure 5

Figure 6

Figure 7

Table 1

User's need	Concept element	Physical object
Biologic material	Cell monolayer Cell cluster Co-culture	Cell type Liver tissue slice Supporting cell type
Cell containment	Barrier Cell supportive matrix Cell separation	Wells Scaffold Membrane
Cell environment monitoring	Optical inspection In-line sensor Offline instrument	Microscope Oxygen sensor Mass spectrometer
Delivery of nutrients	Fluid transportation	Peristaltic pump Gravity based pump Electroosmotic flow
Fluid behaviour	Fluid manipulation	Solenoid valve PDMS valve Concentration gradient
Physiological temperature	Temperature control	Incubator Hot-plate Peltier heater

Table 2

User need	Variables/Units	Target metrics
CELL BIOLOGY		
Multicellular reconstruction	Cell type and co-cultures	Hepatocytes (60-65%), Sinusoidal endothelial cells (10-20%), Kupffer cells (8-12%), Stellate cells (3-8%), Pit cells (<2%)
Cells in 3D	Diameter	> 50 μm
Metabolically active cells	Phase I activity	CYP expression
	Phase II activity	UGT expression GST expression
Functional and viable cells	Markers	Albumin, urea, bile
Nutrient supply	Y/N	Yes
Oxygen supply	Y/N	Yes
Oxygen gradient established	Concentration	85 μM periportal to 45 μM pericentral
TOXICOLOGY		
Disease specific cells	iPSCs, primary cells, engineered cell lines	Characterization
Allow long term studies	Time period	2 weeks
Predictability with <i>in vivo</i> data	Correlation coefficient	$R^2 > 0.8$
Reproducibility	Variance	< $\pm 10\%$
TECHNICAL ASPECTS		
Predictable drug concentrations	Absorption/adsorption	< 0.5%
Low risk of bubble formation	Bubble incidents	< 1%
On-chip analysis	Number of methods for analysis (e.g. sensors, optical, imaging)	At least 1
Off-chip analysis	Volume of the culture medium possible to collect	At least 500 μl
Continuous perfusion	Shear stress	Low to moderate
Short time for preparation of the device	Time	< 1 h
CONVENIENCE OF OPERATION		
Parallelization	Number of parallel devices	At least 2
Operability	Time for hands-on work during experiment	< 10 min/day
MANUFACTURABILITY		
Cost per device	Application dependent	Lower than comparable alternatives

Cost auxiliary platform	Application dependent	Lower than comparable alternatives
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Table 3

User need	Perm. 1	Perm. 2	Perm. 3	Perm. 4	Perm. 5
Multicellular reconstruction	0	++	++	0	++
Cells in 3D	++	++	0	++	++
Phase I and phase II activity	+	++	+	+	++
Functional and viable cells	+	++	+	+	++
Supply of nutrients and factors	++	++	+	+	++
Controlled supply of oxygen	++	+	0	0	++
Oxygen gradient	0	0	0	0	++
Disease specific cells	0	0	0	0	++
Allow long term studies	++	++	+	+	++
Predictability with <i>in vivo</i> data	+	++	+	+	++
Reproducibility	+	+	++	+	+
Predictable drug concentrations	+	++	++	++	+
Low risk of bubble formation	0	+	++	++	+
Continuous perfusion	++	++	0	0	++
On-chip analysis	+	0	++	+	+
Off-chip analysis	++	++	0	+	++
Short time for preparation of the device	0	0	+	++	0
Parallelization	+	0	++	++	+
Operability	++	+	++	+	+
Cost (device and equipment)	0	++	+	+	0
Score	21	26	21	20	30

Table 4

User need	Material			Barrier			Oxygen gradient		Optical monitoring			Internal fluidic flow			
	Elastomer	Polystyrene	Quartz	Pillars	Scaffold	Polymeric membrane	Built in network	External generator	Movable CCD device	Docking to confocal microscope	Holography in situ	Syringe pump	Capillary force transport	Electroosmotic flow	Gravity pump
Multicellular reconstruction	+	++	+	+	0	++			0	++	+				
Cells in 3D	+	+	+	0	++	0			0	++	+				
Oxygen gradient							++	++							
Allow long term studies	+	+	++	++	+	+	++	++	++	0	++	++	+	++	+
Supply of nutrients and factors												++	+	++	+
Controlled supply of oxygen	0	++	++	++	++	+	++	+				++	0	++	0
On-chip analysis	+	+	++	++	0	+	++	++	+	++	++				
Off-chip analysis	+	++	++									++	+	+	++
Low adhesion of compounds	+	++	++												
Short time for preparation of the device				0	++	++						+	++	0	++
Parallelization	++	++	0				++	0	++	0	++	+	++	++	+
Operability							++	+	++	+	++	+	+	++	+
Ease of prototyping	++	0	0									+	+	0	+
Manufacturability	+	++	0				++	++				+	++	++	+
Low cost (device and equipment)	++	++	0	++	++	++	++	0	++	0	+	0	++	0	++
Score	13	17	12	9	9	9	16	10	9	7	11	13	13	13	12

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Table 1. Examples of concept elements and physical objects and their relation to user’s needs

Table 2. User needs and targets for a liver-on-a-chip

Table 3. Screening permutations A-E towards the defined user needs

Table 4. Assessment of anatomical components for the blueprinting of design solutions