WHITE PAPER ON MPS

Microphysiological systems (MPS) are technological platforms that recapitulate functional units of human organs *in vitro*. There is no general unanimity in the definition of MPS and several platforms have been described as MPS technologies. Among these, are spheroids, static co-cultures, static micro patterned technologies, organ on chips (OOC), multi-organs on chips and human on chips (HOC). In this white paper we define MPS as advanced systems over the classic bi-dimensional (2D) cultures by including some of the following aspects: a three-dimensional (3D) framework (commonly referred to as scaffold) based on biomaterials of natural or synthetic origin; a 3D structure; a microfluidic counterpart; sensors to monitor MPS behaviour; an appropriate apparatus for MPS stimulation (e.g., electrical, mechanical cues); primary or stem cell-derived cells.

MPS permit the study of human physiology in an **organ-specific context**, and they can find application in several fields. For instance, MPS can 1) help to unravel phenomena involved in the regulation of disease onset and progression, 2) support the drug development pipeline, and 3) contribute to chemical risk assessment.

MPS may overcome the limits associated with traditional animal models, that are often not enough predictive of the human reality. Moreover, MPS present clear advantages in terms of cost, time, and ethical issues if compared with *in vivo*

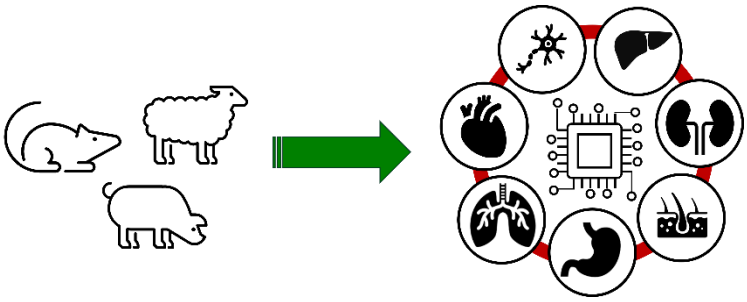

Figure 1. MPS: the emerging alternative to animal testing.

models. **MPS demonstrated to be superior** also **to traditional 2D static cell culture models,** that are not able to accurately reconstitute the *in vivo* cellular micro environment, tissue micro-architecture, and physiological functionality.
The final goal is to develop systems, which combine 3D tissue models and flow-mimetic conditions, to recapitulate *in vitro* the complexity of human patho-physiology, thus effectively reproducing human clinical responses to drugs, and other exogenous stimuli.

MPS APPLICATIONS

DRUG DEVELOPMENT

The drug development process requires both the assessment of the toxicity of a new compound and related products of its metabolism as well as the evaluation of its effects, to identify the most effective and safe candidates. The drug development pipeline is represented in figure 2. Before starting trials on humans, animal studies are required by both European Medicine Agencies (EMA) and the Food and Drug Administration (FDA). However, it has been demonstrated that animal tests cannot predict toxicity in almost 50% of the drugs between Phase I trials and pharmacovigilance stages[1]. The most common causes of drug withdrawals in the United States (US) and Europe are hepatic (21%), cardiovascular (16%), haematological (11%), neurological (9%) toxicities and carcinogenicity (8%). The introduction of alternative methods (such as MPS), which mimic human pathophysiology closer than the standard, can potentially optimize the number of *in vivo* tests, saving time and reducing consequently the cost correlated with the drug development process.
MPS have emerged over the last fifteen years as new tools to evaluate tissue and organ response to chemicals. In the US, a partnership initiative involving both a company (i.e., Emulate Inc.) and the FDA is currently active to assess~~ed~~ and validate the use of this technology for toxicology tests, mainly focusing the attention on liver and cardiac systems. Moreover, many pharmaceutical industries have started a partnership with companies that produce MPS, intending to increase the success of drug discovery and development. Roche is in partnership with Mimetas to implement new and more predictive *in vitro* models based on fluidic technology. Another example of partnership is represented by Emulate and AstraZeneca. An initial phase of their agreement is focused on the use of the Liver-Chip produced by Emulate for the drug safety test of active compounds candidates in AstraZeneca's pipeline. The results will be included within specific regulatory frameworks. Furthermore, the two companies aim to develop the Lung Tumor-Chip, Lung-Chip, and Glomerulus Kidney-Chip.
MPS have also been utilised in disease modelling, drugs screening, and identification of therapeutic targets. In recent years, the pharmaceutical industry has focused the attention on the use of MPS to test the efficacy of new drugs. For instance, Roche used a human retinal microvascular tubule-on-a-chip designed to mimic the blood-retina interaction (OrganoPlate platform, marketed by Mimetas), which can be disrupted in diabetic retinopathy and age-related macular degeneration. The model comprises a standard microtiter plate format comprising 40 to 96 chips, thus compatible with high-throughput screening. It was used in the

[1] "Limitations of animal studies for predicting toxicity in clinical trials: is it time to rethink our current approach?" J Am Coll Cardiol Basic Trans Science. 2019;4:845–854.

screening of a library of small molecules and biologics known as modulators of the Vascular Endothelial Growth Factor A (VEGFA) signalling, elucidating a number of compounds that inhibited VEGFA-induced permeability.

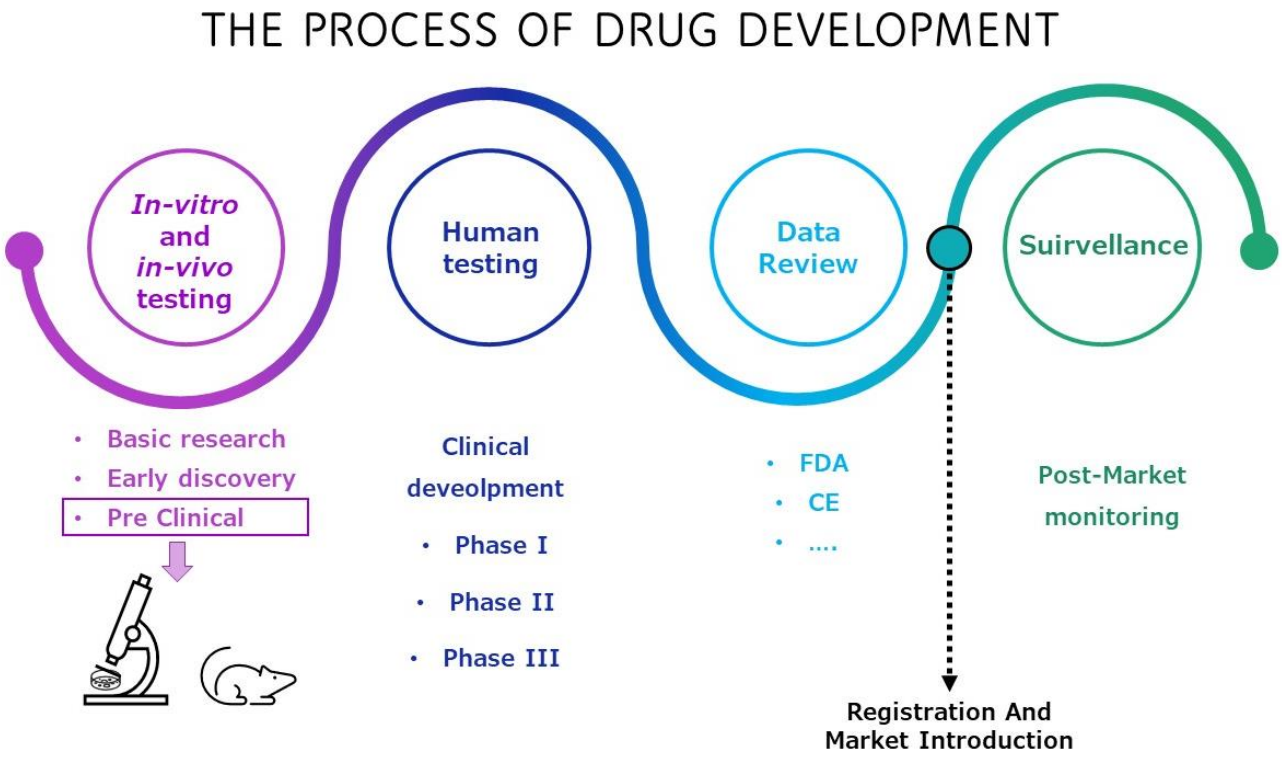

Figure 2. The drug development pipeline: from pre-clinical evaluation to post-marketing monitoring. MPS could be introduced in pre-clinical studies according to the 3R principle (Reduce, Refine, Replace)".

Roche used the OrganoPlate platform also to develop an immuno-competent intestinal model designed to mimic the impairment of the epithelial barrier due to inflammation caused due to neutrophil infiltration, subsequent to the tactivation of resident macrophages. In collaboration with GlaxoSmithKline (GSK), Tara biosystems demonstrated that their heart-on-chip replicates drug responses in humans. For instance, the system was tested with drugs for treating heart failure and chemotherapy agents. These results are encouraging towards the development of increasingly advanced MPS systems and their use in drug screening. In this context, it is worth noting that the COVID-19 pandemic has highlighted the importance of rapid drug screening platforms to accelerate the development of new therapeutics and vaccines.

MPS are also emerging as useful tools in oncology research since they can help to elucidate the complex biology of the tumor microenvironment and cancer progression. For instance, cancer on-chip (COC) can reproduce some crucial aspects of the tumor microenvironment (e.g., biochemical gradients, dynamic cell-cell and cell-matrix interactions, elaborate tissue structures). Moreover, COC systems can incorporate patient-derived tumor cells into a tissue-mimetic structure, resulting in the possibility to represent the patient-specific tumor environment closely. Different microfluidic platforms have been used to evaluate cancer treatment efficacy and/or toxicity over the last few years. COC devices have demonstrated their potential as chip, easy to reproduce, and consistent tools for a comprehensive study of mechanisms of tumor stages and the evaluation of treatment. Several researchers proved the dissimilarity between the effects of drugs on 2D and 3D cultures, showing that results obtained with COC were more similar to the one observed

in animal models. For example, higher cellular drug resistance was observed on COC devices compared to 2D cultures. Furthermore, co-cultures with liver cells allow the assessment of the systemic toxicity of drug metabolites. [2] In the future, human on chip platforms have the potential to be used in the study of metastatic spread of cancer.

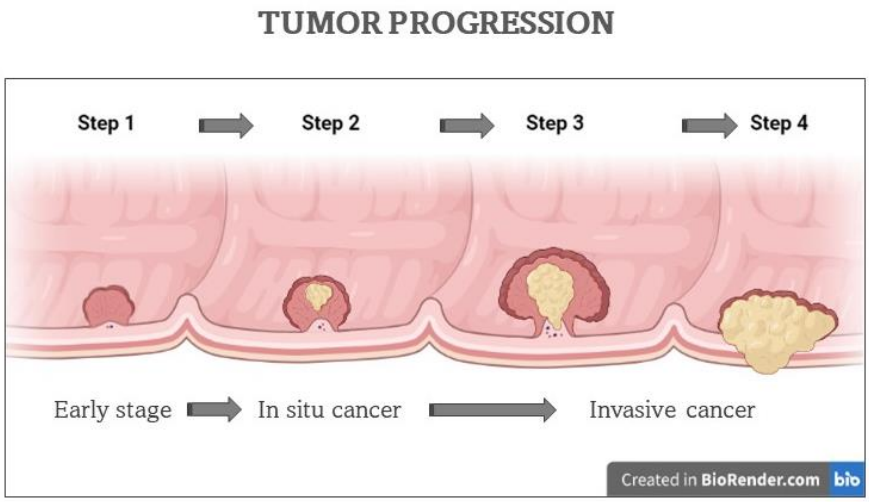

Figure 3. MPS may be used to study tumour progression, from early stage to invasive cancer.

CHEMICAL RISK ASSESSMENT

In the field of chemical safety and risk assessment, there is a growing demand for standardised devices and protocols to assess the toxicity of newly developed compounds. Also in this case, animal models often fail to reproduce specific human biological milieus, and consequently to predict the response to chemical compounds. The current concerns about the transferability of animals' results to humans and ethical issues related to animal welfare in laboratory experiments, have led to the development of New Approach Methodologies (NAMs) for chemical compound testing. Moreover, in Europe, the Directive 2010/63/EU clearly states that the "3R" Principle (Reduce, Replace and Refine) needs to be considered by the scientific community and that, whenever possible, approaches that do not use living animals should be applied. Furthermore, NAMs have also been developed to improve the concept of the Tox21-c (21st-century toxicology) program, which refers to 'the transformation underway in the tools and approaches (toxicity pathways, mechanisms/modes of action, Adverse Outcome Pathways (AOP)) used to evaluate chemical substances for possible effects on human health'. Several agencies have been created around the world (ECVAM in 1993 in Europe, ICCVAM in 1997 in the USA, JaCVAM in 2005 in Japan) to support and regulate the increased request for new regulations and methods for chemical testing. In Europe, a significant driving force in the field is represented by the REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals), which highlighted the need for increased testing, with the consequent strong suggestion to use NAMs.[3]

[2] "Organ-on-a-Chip Platforms for Drug Screening and Delivery in Tumor Cells: A Systematic Review" Cancers, 2022, 14(4), 935
[3] "New Approach Methodologies in Regulatory Science" Proceedings of a scientific workshop: Helsinki, 19-20 April 2016

Figure 4. Chemicals in the environment. Different types of chemicals (e.g., pollutants, pesticides, drugs) can be found in the environment and may affect the ecosystem and human health.

It should be observed that humans are constantly exposed to a massive amount and a variety of potentially toxic chemicals that are in the environment. One of the main classes of chemicals in this group is that of pesticides, commonly and widely used in the agricultural field. Pesticides exhibit high persistence and pervasiveness in the environment. Moreover, certain classes of drugs, such as antibiotics, parasiticides, antimycotics, and anti-cancer drugs, are questioned regarding their accumulation and potential toxicity upon accidental exposure to biota and human beings. Recent pharmacovigilance legislation in the EU acknowledges that water and soil pollution with pharmaceutical residues is an emerging environmental issue. Furthermore, the unintentional combinations of pharmaceuticals with other chemicals may result in additive and/or synergistic toxic effects. The European Commission is paying particular attention to this field by founding research centres and projects to develop and validate alternative tests to animal ones.

Major alternative methods/NAM are based on two specific categories: *in vitro* and *in silico* systems.

Among *in silico* systems the Quantitative structure-activity relationship (QSAR) technology and category approaches are approved as alternative methods to animal test. QSAR refers to the relationship between the chemical structure and the biological activity of chemicals. QSAR models can be obtained using the training dataset of tested molecules. In case of a valid correlation between structure-related properties and biological activity, the biological activity can be evaluated with QSAR models. The OECD QSAR Toolbox is a free software application that has been used by governments, chemical industry etc. for chemical hazard assessment and it makes QSAR technology easily accessible. This method is used in several voluntary and regulatory programmes: the OECD HPV Chemicals Programme, the US HPV Challenge Programme, and the EU – REACH.

Among *in vitro* systems, MPS are considered potentially game-changing technologies to reduce animal tests, alone or in combination with *in silico* models. Currently, several companies are developing MPS products and putting great efforts into validating them. MPS companies are mainly start-ups initiated by ex-academic teams. Among these, TissUse and Nortis have multi-tissue R&D activity, whereas the other companies focus their activities on the development of single-tissue. Very few companies are in the production and commercialization phase, while several are manufacturing prototypes and producing a small number of products.

MPS COMPONENTS

MPS are multidisciplinary tools in which tissue engineering is coupled with microtechnology to mimic key aspects of human physiology. Briefly, cells are cultured in a 3D matrix which resemble a specific tissue architecture. This tissue engineering construct (cellularised scaffold) is placed in a chamber/incubator, equipped with microfluidic flow. The chamber can include sensors and microscopes to properly maintain, stimulate and monitor cells cultured in the system and allowing multiple analysis. MPS may differ significantly, depending on the selection of cells, 3D matrix and stimulation. The developed artificial tissue can be analysed through classic cell culture analysis (such as western blot and ELISA), real time measurements of analytes (e.g., glucose, lactate, pH and oxygen) and with more advanced techniques. Among these, multi-omics analyses are receiving great attention since these techniques enable researchers to analyse the extracted tissue in minute detail and compare it to the *in vivo* one. The technological progress in each area involved in the design of MPS is ongoing and will progressively lead to an increasingly realistic replica of human physio-pathological conditions and improvements in their understanding.
The following chapters briefly describes the different tools and technologies used in the design of MPS and in the evaluation of their applications.

CELLULARISED SCAFFOLDS

Scaffolds are valuable supports for 3D cell culture. Due to their interconnected porosity, scaffolds facilitate oxygen, nutrients, and waste transportation. Thus, cells can proliferate and migrate within the scaffold, interact with each other, and turn into structures closed to the native tissues. A scaffold can be constituted by a **hydrogel network, a membrane, or a 3D construct**. Scaffold can be produced by using metals, glasses, and ceramics as forming materials. However, **synthetic or natural polymers** are a better choice due to the easier control of their chemical and structural surface properties. Current on-chip approaches mainly use hydrogels as scaffolding material for cell growth to better mimic the native tissue composition. Hydrogels are generally based on natural polymers, such as gelatin, alginate, hyaluronic acid, agarose, laminin, collagen, or fibrin. For example, in OrganoPlate® (Mimetas) cells are grown in an extracellular matrix (ECM) gel to support the formation of spheroids or other 3D cell interactions/networks. The ECM gel is composed of collagen and Matrigel® [i]. 3D matrices used in tissue models are typically made of bio resorbable polymers, both natural (e.g., gelatin, collagen) and synthetic (mainly polyglycolic acid, polylactic acid, poly(lactic-co-glycolic acid), polyurethanes). Emerging fabrication technologies are useful tools for obtaining miniaturized structures mimicking tissue architectures. The most relevant technologies (and associated materials) that have a significant impact on scaffold fabrication are reported in table 1.

Technology	Material
Two-photon polymerization	Photopolymers
Stereolithography	Photopolymers
Laser micromachining	Organic and inorganic materials
X-ray-based microfabrication	Metals, plastics, glasses, ceramics
Bioplotters and bioprinters (3D printer)	Biological materials, natural polymers (hydrogels)
Fused deposition modeling (3D printer)	Waves, synthetic (thermoplastic) polymers

Table 1. Common rapid manufacturing technologies in scaffold fabrication

In particular, technologies based on rapid prototyping have introduced new perspectives allowing the fabrication of highly controlled structures, which include a more effective reproduction of pore size and interconnection of native tissues, facilitating cell colonization and design reproducibility.

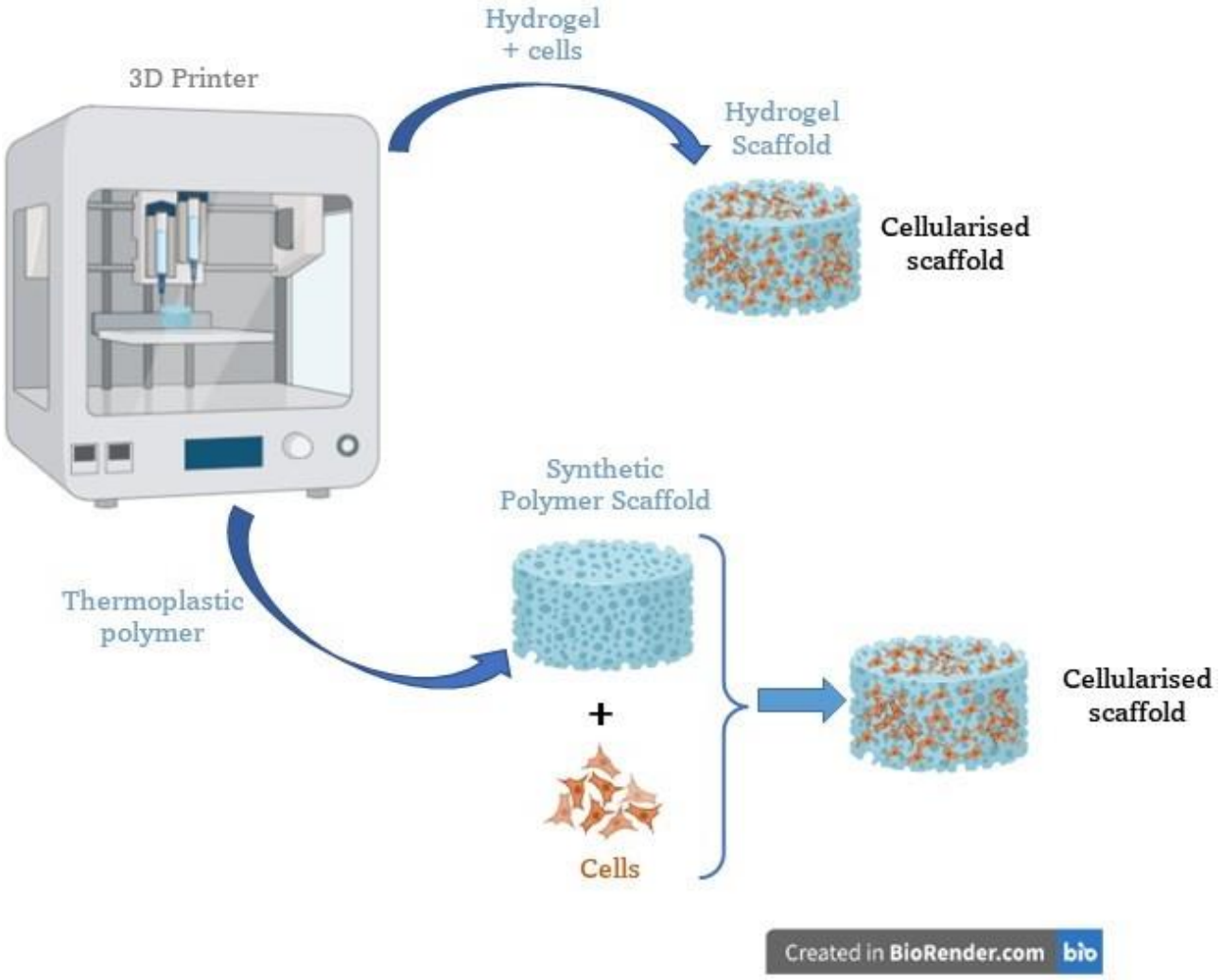

Figure 5. Example of cellularised scaffold fabricates by 3D printing

The selection of the **cell source** is a challenge in tissue-engineered model design. To obtain a proper replica of a human tissue, the best cell source would be represented by human primary cells. The main disadvantages are their limited availability and viability when extracted from their biological environment, and their limited potential for self-renewal and differentiation. Immortalized cells represent a valid alternative, but often with limited biological similarity with humans, due to changes to their genetic heritage after mutations, and sometimes contamination with more aggressive cell lines.

In the last decades, a revolution in biology was represented by **induced pluripotent stem cells (iPSCs)**. iPSCs have potentialities similar to stem cells but derive from somatic ones (such as skin fibroblasts) and not from embryos. In particular, iPSCs do not share the ethical concerns related to embryonic cells. iPSCs are generated by using a cocktail of four transcriptional factors, also known as "Yamanaka factors". Several cell models obtained with iPSC derivatives demonstrated to be similar to *in vivo* primary cell equivalent in terms of transcriptional, cellular, and functional levels whereas shown differences in terms of genetic content.

Several human **3D brain organoid microfluidic platforms** based on **iPSCs** are currently under study. For instance, iPSCs have been recently cultured into a Matrigel matrix within a microfluidic platform, *ad-hoc* engineered to allow nutrient supply and waste product removal. The use of this biomimetic micro environment to brain organoids improved their growth, survival, proliferation, and differentiation. This OOC design was used in the modelling of prenatal nicotine exposure, showing that nicotine exposure in the early stage of foetal brain development compromised neurogenesis; such result clearly correlates with the higher incidence of cognitive defects among children born from smoking mothers. The liver plays a key role in drug metabolism and detoxification, and consequently represents the main target organ for drug-induced toxicity[4]. Several research groups have developed different types of **liver-on-a-chip** to examine the toxicity induced by drugs on this human district. Latest research showed that culturing iPSC-derived hepatocytes (iPSC-HEPs) into microfluidic platforms, and/or in the form of spheroids, promotes their maturation. In another research work, the acute toxicity induced by the hepatotoxicant drug acetaminophen, was investigated showing a marked cell viability reduction, that was and both time- and dose-dependent. Recently, several approaches were developed to test drug-induced contractile cardiotoxicity and reduce failures in the latest steps of the drug development pipeline. In one of these, iPSC-derived cardiomyocytes (iPSC-CMs) seeded in a **heart-on-a-chip** device were recorded in beating rate when exposed to isoproterenol, which is a standard compound used to increase heart rate in patients. Upon isoproterenol administration, an increase in cell beating rate and a reduction in the corrected-field potential duration were observed, thus validating the platform for drug screening application. Lastly, the **gut** represents an important system to be recapitulated *in vitro* to mimic intestinal diseases, e.g., the inflammatory bowel disease and irritable bowel syndrome. Within this framework, the inflammatory bowel disease has been replicated with iPSC-derived intestinal cells in MPS by adding interferon-γ (IFN-γ) and TNF-α to iPSC-intestine cells and obtaining an inflamed intestinal epithelium like those of patients with the same disease.

[i] *Matrigel® is a mixture of ECM proteins that have been extracted from Englebreth-Holm-Swarm tumors in mice*

BIOREACTORS-MICROFLUIDIC CONNECTIONS-SENSORS

The application of MPS require a fluidic platform to impose a dynamic stimulation to the cells. In particular, this fluidic platform is based on a pump (i.e., a peristaltic or a syringe pump) which allows the flow of medium in a fluidic circuit, where the cells are cultivated, hopefully in a 3D configuration. The fluidic circuit can be composed by flexible tubes or plastic plates, where the pipes are enclosed. The chambers can be organized in 2 different categories: 1) systems that are specific for a particular tissue (e.g., PhysioMimix™, a Liver-on-a-chip by CN Bio[5] or 2) versatile products that can be used in different models (e.g. LiveBox produced by IVTech[6]). In general, a 3D *in vitro* model is characterized by 3 geometrical dimensions. However, it is possible to enrich a standard 3D model with a dynamic stimulation, represented by the flow of liquid, which acts as blood in human circulation (IVth dimension). Moreover, this exchange of medium facilitates the communication between different tissues, promoting their crosstalk and thus a cross modulation (Vth dimension). Some ALTERNATIVE partners are players in the technological arena of MPS. E.g. IVTech offers a

[4] "Induced pluripotent stem cell-based organ-on-a-chip as personalized drug screening tools: A focus on neurodegenerative disorders" Journal of Tissue Engineering, 2022;13.
[5] https://cn-bio.com/models/
[6] www.ivtech.it

ready to use kit composed by a peristaltic pump, compatible with an incubator environment, allowing for the circulation of medium in one or more interconnected chambers.

Figure 6LiveBox1 and LiveBox2 are illustrated

The cell culture chambers are transparent to permit sample real-time monitoring through an inverted microscope. Moreover, they are characterized by different cell growth area, to permit the use of standard cell culture protocols. The IVTech chambers are classified in 2 suites (Figure 6): the LiveBox1 and the LiveBox2 suites: i) LiveBox1 Suite has been developed to house 3D models in attached cell culture conditions; ii) LiveBox2 Suite has been designed to mimic barriers in a dynamic environment.

IVTech recognizes the need to control the environmental conditions, simulating the stimulation pathways of different tissues. Therefore, the attention is also focused on products such as LivePa (figure 7), a pressure modulator, which can be used to increase the pressure on a specific model. This is crucial to simulate the pathological conditions of certain diseases such as hypertension or glaucoma[7,8] . The bioreactor chamber should mimic different *in vivo* conditions such as chemical, mechanical, or electrical conditions in order to have an accurate and representative model of the biological structure and its functions. For instance, endogenous electrical fields are involved in the organization and development of tissues. The heart is the largest bioelectrical source and, therefore, it is critical to biomimic its electrical stimuli in cardiac tissue MPS models.

Figure 7. LivePa is connected to a LiveBox1 and the IVTech fluidic circuit to mimic the environmental conditions of glaucoma

The electrical stimulation system generally consists of two different elements, namely an electronic system to generate and amplify the desired electrical signals (stimuli) and a pair of conductive electrodes to apply the stimuli in the bioreactor. The electronic system should allow researchers or end-users to configure the desired signals and be able to control multiple bioreactors in parallel to fit the experimental needs. Moreover, it is key to develop very easy-to-use hardware and software to ease the use of the bioreactors in a biology lab. The electrodes may be developed using different materials and technologies. On one hand, the electrodes should be made of biocompatible, conductive materials which remain functional under *in vitro*

[7] "An Advanced In Vitro Model to Assess Glaucoma Onset" ALTEX - Alternatives to animal experimentation, 2020, 37(2), 265–274.
[8] "An Innovative In Vitro Open-Angle Glaucoma Model (IVOM) Shows Changes Induced by Increased Ocular Pressure and Oxidative Stress", Int. J. Mol. Sci. 2021, 22 (22), 12129.

physiological conditions. On the other hand, these electrodes should be integrated in the production process of the bioreactor, ensuring rapid, simple fabrication. Printed electronics can emerge as a viable alternative to develop functional, low cost, flexible printed electrodes, easily integrated in other fabrication processes such as injection moulding, casting, etc.

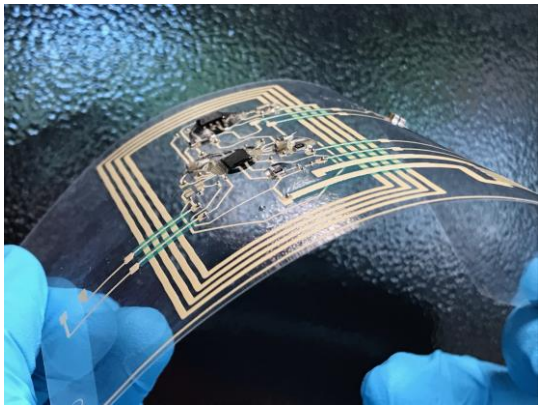

Figure 8. Picture of a printed electronic circuit using different conductive or functional inks.

Dynamic cell culture describes the *in vitro* culture of cells in the presence of applied mechanical stress. Of great emerging interest is the ability to control the flow of fluid, such as medium, over cells while they grow. Flow rate can be generated very precisely using microfluidic instruments[9] and is an ideal way to better replicate the dynamic environment that cells normally reside in. Medium microfluidic perfusion mimics the flow of blood, allowing **nutrient exchange** and the **removal of waste products**, and also adds **shear stress** to cells. A pressure-driven flow controller[10] is a smart alternative to a syringe pump. It allows a pulseless flow within a sub-second response time. It consists in using a gas input pressure within a hermetic liquid tank in order to flow liquid from the tank to your microfluidic device. A pressure controller pressurizes a reservoir, such as an Eppendorf tube, a Falcon tube, or a bottle containing the sample. The sample is then smoothly and quasi-instantly injected into your microfluidic device. When the reservoir is pressurized, the gas pushes on the fluid surface, and the fluid flows through the outlet. Thus, controlling the input gas pressure of the tank will allow controlling the liquid that flows out of the tank. Thanks to piezoelectric pressure regulation, Elvesys, one of the ALTERNATIVE partners, developed the Elveflow's systems, which are able to regulate flow within 40 ms with a 0.005 % stability. One advantage of pressure-driven flow control lies in the ability to handle fluid volumes of several hundreds of ml. You can thus turn your system into a powerful syringe pump.

[9] https://www.elveflow.com/microfluidic-products/
[10] https://www.elveflow.com/microfluidic-products/microfluidics-flow-control-systems/ob1-pressure-controller/

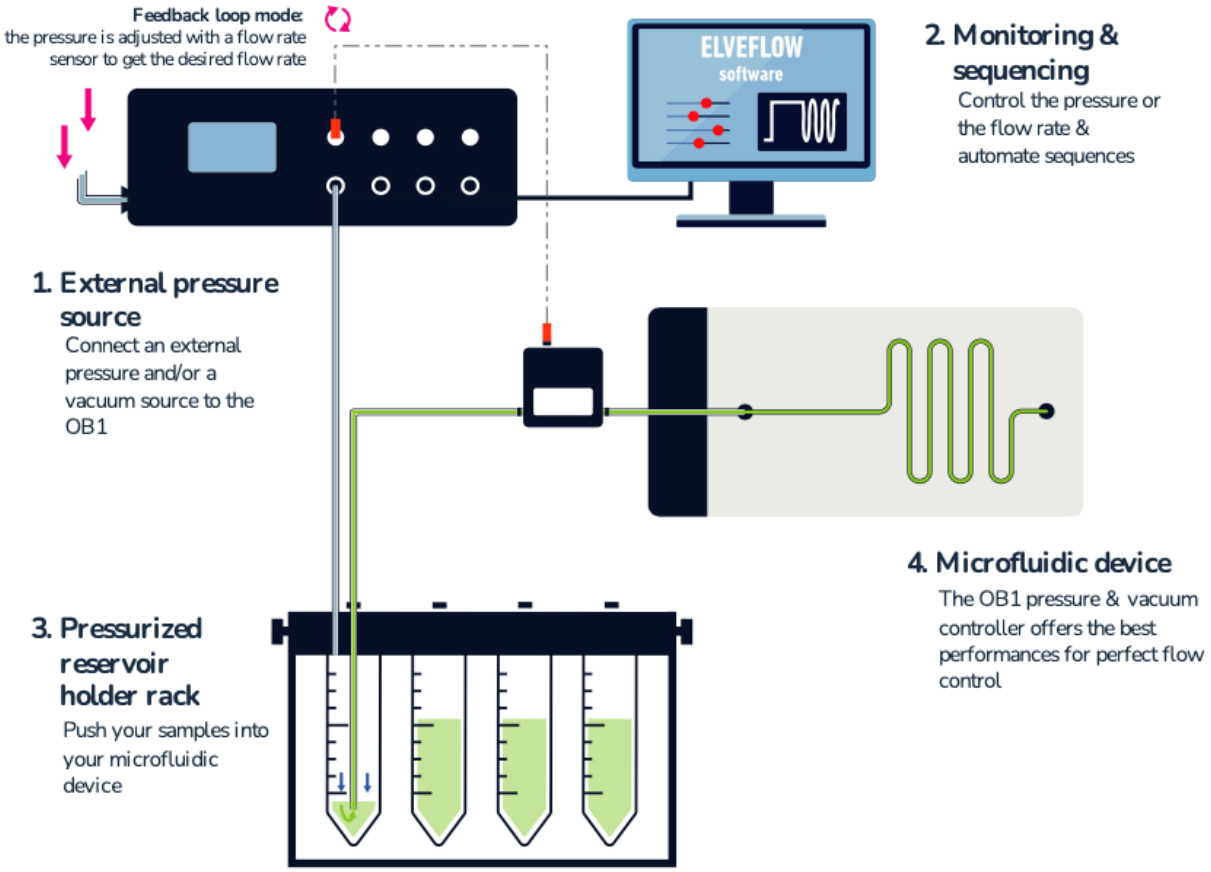

Figure 9. Basic scheme of a microfluidic setup with a pressure-driven flow control

By coupling Elveflow's pressure controller[9] with one of the flow sensors[11], you can perform ultra-precise and responsive pressure-driven flow control. You can request a flow rate value in the Elveflow Software[12] and the pressure controller will automatically adjust the pressure to reach the requested value thanks to a customizable PID feedback loop.

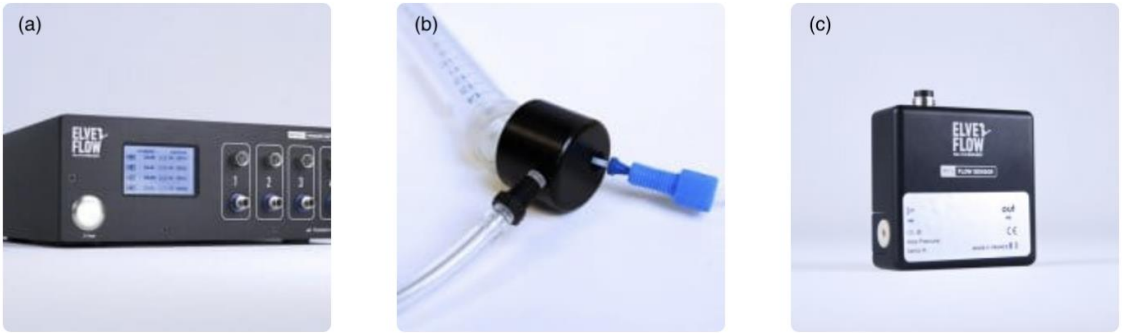

Figure 10. (a) OB1 MK3+ multi-channel pressure & vacuum microfluidic flow controller. (b) Sample falcon reservoir with standard and autoclavable microfluidic tubing, fittings and a cap allowing the pressurization of the reservoir. (c) Microfluidic flow sensor.

[11] https://www.elveflow.com/microfluidic-products/microfluidics-flow-measurement-sensors/microfluidic-liquid-mass-flow-sensors/
[12] https://support.elveflow.com/support/solutions/folders/48000517253

With the Elveflow instruments, it is also possible to **recirculate the medium** for dynamic cell culture. Thanks to the use of the OB1 pressure controller[9] you can maintain a precise flow rate of medium over cells in the perfusion chamber. The system of passive check valves enables recirculation of medium between two reservoirs **unidirectionally over your cells**. Recirculation allows **higher flow rates to be maintained for multiple days**, with medium **conditioning** and studies of **shear stress**.
The recirculation loop is composed of four passive check valves that are connected in a way that allows the flow to always be in the same direction inside the connected microfluidic chip when pressurizing the first or the second reservoir. Therefore, the principle of the recirculation using this check valve loop is to pressurize the first reservoir until it is nearly empty, then stop and start pressurizing the second reservoir. This allows the microfluidic chip to always be under unidirectional flow.

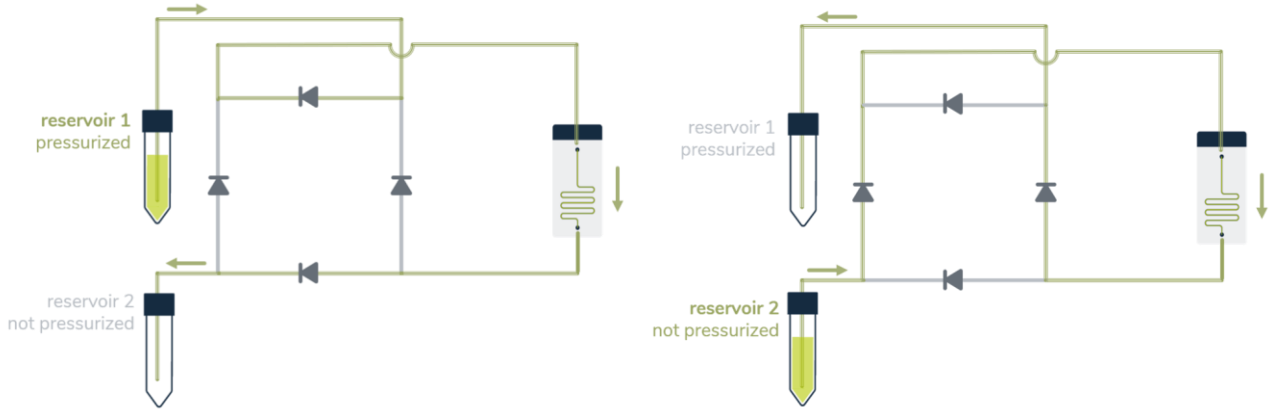

Figure 11 The principle of the medium recirculation using two reservoirs and a system of passive check valves.

Some applications of dynamic flow in cell culture include:

- Live cell imaging[13] (e.g. calcium imaging, FISH)
- Drug screening[14]
- Shear stress
- Cell rolling-adhesion assay
    - Immune response
    - Cancer invasion and metastasis
- Models of physiology and disease
    - Organs on chip[15]
    - Blood vessel formation & occlusion (atherosclerosis)
    - Bone homeostasis and disease (osteoporosis)
- And many more!

[13] https://www.elveflow.com/microfluidic-reviews/microfluidics-for-cell-biology/perfusion-for-live-cell-imaging-a-review/
[14] https://www.elveflow.com/microfluidic-reviews/organs-on-chip-3d-cell-culture/organ-on-chip-models-in-vitro-in-vivo-systems-drug-testing/
[15] https://www.elveflow.com/microfluidic-reviews/organs-on-chip-3d-cell-culture/organs-chip-review/

ANALYSIS

Figure 12. Optical microscope as an essential tool to observe cellular status.

The panel for the evaluation of the effects of chemicals and drugs on cells is wide, covering the most basic approaches related to the classic systems (ELISA assay, RT-PCR, western blotting, northern blotting, chemical assay) to the most advanced and innovative techniques (metabolomics, proteomics, transcriptomics, New Generation Sequencing, high-content imaging).

Traditional approaches can provide a fast and reliable evaluation of specific mediators of a plethora of pathways (inflammation, oxidative stress, apoptosis, etc.) in a very precise and quantitative way. These types of analyses still represent a reference for lab research. On the other side, deeper comprehension of molecular mechanisms in terms of modulation in the expression of DNA, RNA, proteins, lipids, and metabolites requests a level of investigation not possible with the standard approaches. To support this in-depth analysis, omics techniques represent an advancement of the state of the art of analytical systems, able to provide a "global picture" of molecular events associated with the administration of drugs and chemicals. These approaches can target different molecules and provide a holistic view to illustrate the different phenotypes. The use of targeted and untargeted metabolomics, lipidomics, proteomics and transcriptomics provide high-quality, high-confidence, reproducible data that could be one of the most suitable strategies to outline cellular metabolism fingerprints.

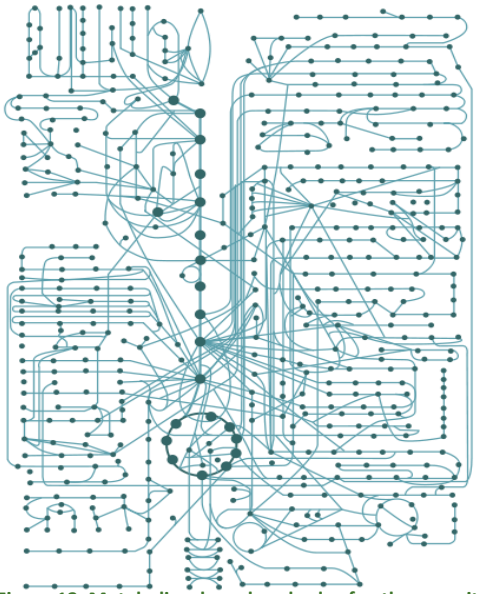

Figure 13. Metabolism have hundreds of pathways with complex crossroads.

The integration of these multi-omic layers using bioinformatics and biostatistics tools is one of the most useful approaches in the search of biomarkers, mechanism of action and interpretation of the results in complex biological systems.

This powerful strategy has demonstrated its usability in many investigations, such as the characterization of SARS-CoV-2 characteristics, the response of the host and the prediction of the disease severity, the different mechanisms of action, and the therapeutic targets to treat or prevent the Covid infection[16]. Multi-omics also has satisfactory results to assess the subtype-specific differences in immune cell composition and differential genetic/pharmacological vulnerabilities in breast cancer[17]. The application of multi-omics to explore the relation between pollution and cardiovascular diseases (CVD) has also been developed, revealing the impact on inflammation, oxidative stress and other dysfunctions that lead to CVD[18].

[16] "Multiomics integration-based molecular characterizations of COVID-19", Briefings in Bioinformatics, 2022.23, bbab485.
[17] "Multi-omics analysis identifies therapeutic vulnerabilities in triple-negative breast cancer subtypes", Nature Communications 2021, 12, 6276.
[18] "Cardiovascular effects of traffic-related air pollution: A multi-omics analysis from a randomized, crossover trial", Journal of Hazardous Materials, 2022, 435, 129031.

CONCLUSIONS

Through this white paper, the authors intend to give the readers an overview of MPS, providing increased understanding of the potential of these emerging technologies, and picturing the challenges that scientists are trying to overcome to develop innovative tools for new compound screening and advanced alternatives to animal testing.

For further information on this topic, please do not hesitate to contact the ALTERNATIVE consortium through the social media channels listed below.

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environment**AL** **T**oxicity
ch**E**mical mixtu**R**es through a**N**
innovative platform based on aged
cardiac tissue model

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