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"REVOLUTIONIZING PRECLINICAL DRUG DISCOVERY: THE PROMISE OF MICROFLUIDIC ORGAN-ON-CHIP TECHNOLOGY".

1Parth P. Rana, 2Dr. Ajay I. Patel, 3Dr. Amit J. Vyas, 4Dr. Nilesh K. Patel

1Department of Pharmaceutical Quality Assurance, 2Department of Pharmaceutical Quality Assurance, 3Department of Pharmaceutical Quality Assurance, 4Department of Pharmaceutical Quality Assurance

1B. K. Mody Government Pharmacy College, Polytechnic campus, Near Aji dam, Rajkot - 360003, Gujarat, India,

2B. K. Mody Government Pharmacy College, Polytechnic campus, Near Aji dam, Rajkot - 360003, Gujarat, India. ,

3B. K. Mody Government Pharmacy College, Polytechnic campus, Near Aji dam, Rajkot - 360003, Gujarat, India. ,

4B. K. Mody Government Pharmacy College, Polytechnic campus, Near Aji dam, Rajkot - 360003, Gujarat, India.

1.Introduction:

Modern drug discovery faces significant challenges in identifying and optimizing new drug candidates. [1,2] Pre clinical studies are crucial for drug development, evaluating safety and efficacy. Traditional models like 2D culture and animal models are insufficient due to their limitations in mimicking human tissue physiology. This resulted in biased information and inability to accurately predict in-vivo drug responses. Additionally, the complex physiology of animal models makes it difficult to distinguish causal relationships.[3] Conventional in-vitro platforms study signal molecules related to physiological processes, but they don't accurately mimic cell-to-cell interactions or extracellular mechanisms.[5] Artificial intelligence can improve studies by providing accurate data through preclinical chips, which are microscale designs that replicate the microenvironment of specific human organs or tissues.[4] The pharmaceutical industry is striving for an efficient R&D framework for drug discovery, but current platforms for cell culture and animal experimentation are insufficient for accurate preclinical evaluations.[6,7] Around 40% of early drugs failed clinical trials due to variable responses and unexpected toxicity in humans, even after completing preclinical evaluation with animal models.[8] Advances in microfluidics-based Organ-on-a-Chip technique, mimicking human organ functionality on a chip, could revolutionize drug development and personalized medicine by replacing animal testing.[9] In vitro models can predict in-vivo toxicity more accurately than animal models, reducing drug failure rates and

eliminating species-specific differences between animal and human clinical trials.[10] Single-organ chip assays identify biological mechanisms and test drug efficiency and toxicity in target organs during preclinical development, providing a reliable reference for clinical trials. Multi-organ chips integrate multiple organ units, enabling more comprehensive studies, such as gut, liver, and kidney compartments, in a single chip.[11] The 'Body-on-a-Chip' or 'Human-on-a-Chip' is being developed to provide a single platform for drug pharmacokinetic and pharmacodynamic analyses of the entire human body.[12] Preclinical drug development relies on 2D or 3D cell cultures and in-vivo animal models. However, these methods lack a 3D physiological tissue environment, and animal models are complex and species-specific. Microfluidic Organ-on-Chip platforms offer a simple and physiologically relevant platform for controlling cell culture within an organotypic microarchitectural environment.[4]

2.Types of Preclinical Chips:

2.1 Liver-on-a-Chip:

Liver is the major organ of drug metabolism and thereby a primary target of drug-induced toxicity. In-vivo toxicity is one of the major reasons for the failure of ≈ 90% of the drugs after costly Phase I clinical trials.[13] To achieve this goal, engineering hepatic culture systems that optimally mimic in-vivo conditions to evaluate drug metabolism and toxicity is highly desirable. Static culture systems, such as culture flasks and multi-well plates, are not ideal as they lack continuous perfusion and have non-physiological medium-to-cell ratio with cells grown in 2D monolayers.[10]

Researchers have used microfluidic devices to study the human liver's responses in drug testing. They cultured primary hepatocytes in these devices, demonstrating the potential of PDMS-made channels for mimicking liver anatomy. The microdevices were made using epoxy-based negative photoresist (SU-8) for silicone replicate moulding with photo-lithography. The devices showed extensive cell-cell contact, continuous nutrient exchange, and culturing of primary hepatocytes for over 7 days without decreasing viability. However, the lack of waste and toxicity experiments is a disadvantage.[14]

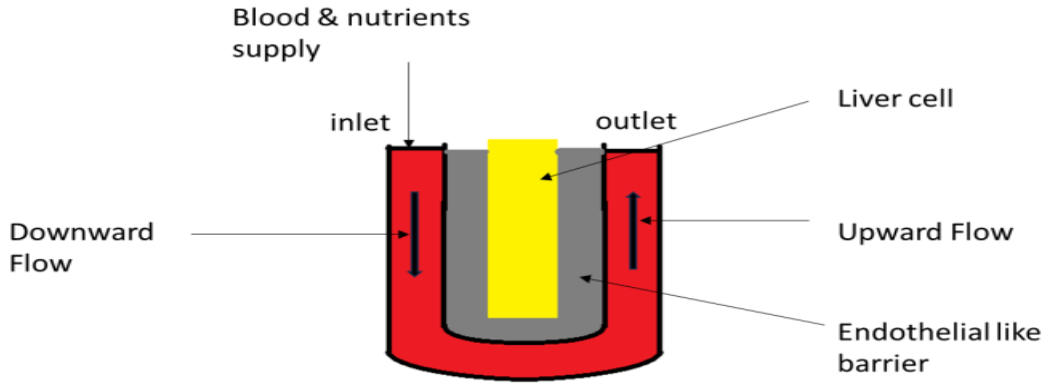

Figure 1 Liver on a chip [15]

Recent studies have developed liver-on-chip microdevices to investigate drug and treatment adverse effects. Bhushan and colleagues created a microfluidic construct mimicking human liver tissue, containing hepatocytes, stellate cells, Kupffer cells, and endothelial cells. The chip generates biochemical and metabolic information and features fluorescent markers to detect drug-induced liver injury. This data can be used to create predictive models of human hepatotoxicity. [16]

2.2 Heart-on-a-Chip:

A method to construct heart models *in-vitro*, has attracted considerable attention. Various fabrication methods have been developed and different applications, for example, drug screening, physiology study and disease modelling, have been proposed [18]. Integration of multiple experimental factors such as cell attachment, elastic properties, microscale topography, flow rate, electrical and biochemical regimes are critical to effectively imitate the cardiovascular tissue on a small chip. Many groups have produced macroscale dynamic cell culture systems and tissue bioreactors [19,20]. One of the most important aspects for microfluidic devices is that the traditional materials used in the fabrication of these devices, such as PDMS, do not necessarily have the biomimetic matrix elasticity and stiffness to facilitate cell attachment [21]. The cellular uptake and release of calcium ions are some of the main functional features of the heart cells, which can be affected during acute hypoxic processes. Changes in calcium dynamics are related to arrhythmias, ischemia and heart failure [22].

2.3 Lung-on-a-Chip:

The alveolar-capillary membrane, the smallest lung's structural unit, is a bilayer interface made up of alveolar epithelial and microvascular endothelial cells. It facilitates gas exchange, prevents pathogens from entering the pulmonary circulatory system, and produces antimicrobial reagents and anti-inflammatory signaling molecules. This membrane also acts as a physical barrier, attracting immune cells to infected areas [23,26] .In order to mimic part of this interface, that is, the alveolar epithelium, reports have focused on the use of a single alveolar epithelial cell line as a lung tissue model for drug delivery studies [24,25].

Device	Fabrication Materials	Fabrication Techniques	Features
Pulmonary edema-On a chip	PDMS	Soft Lithography	-Can replicate some of the physiological consequences of pulmonary edema -Reliable for drug efficacy & toxicity tests
Lung airway on a chip	PMMA	Micro milling	-Open microfluidic Device to suspend hydrogel -To study SMCs-ECs Interaction
Airway on a chip	PCL & PDMS	3D Bioprinting	-Formed an interface of vascular networks

Table 1: Lung-on-a-chip device fabrication techniques [27].

Several key aspects of the mechanism by which IL-2 induces pulmonary edema were found:

1. a vascular leakage results from intercellular gap formation in both the epithelium and endothelium;
2. mechanical breathing motions play a major role in IL-2--induced edema; and
3. the circulating cells of the immune system are not necessarily responsible for the onset and progression of pulmonary edema [28].

2.4 Kidney-on-a-Chip:

The kidney is a major organ for drug clearance from the body, playing an important role in the biotransformation of xenobiotics, and is one of the main targets of drug toxicity studies [29]. Integrated porous membranes with several types of pores into a microfluidic system. Human kidney proximal tubule epithelial cells and primary renal proximal tubule epithelial cells grew as a confluent layer on the membrane and expressed biomarkers of differentiated epithelia, that is, of a re-absorptive renal epithelial barrier responsive to mechanical stimulation. Cisplatin-induced proximal tubule nephrotoxicity was demonstrated in an analogue system by Jang et al [30,31].

2.5 Brain-on-a-Chip:

The blood-brain barrier (BBB) is a unique blood vessel in the brain that regulates nutrient and waste transport, preventing pathogens and neurotoxicants. A porous membrane-based BoC mimics the BBB structure, allowing for cytokine-mediated communication and establishing a three-dimensional cell-culture environment. A spinal cord-on-a-chip has been developed, using induced pluripotent stem cell-derived ventral spinal neurons and brain endothelial cells in the porous-membrane-based BoC.

2.6 Intestine-on-a-Chip:

This text discusses the design and fabrication of an intestine-on-a-chip, its essential elements, and its application in biomedical research. It discusses the impact of physical factors, microbiota, and tumour cells on the intestine-on-a-chip, and reviews analytical methods like MS, electrochemistry, gene sequencing, and fluorescence imaging. The text also discusses current challenges and future perspectives for intestine-on-a-chip models.[32]

The creation of intestine-on-a-chips with suitable microstructures for intestinal cell growth is crucial for creating an intact epithelium and studying the intestinal microenvironment. Coculture of living cells and controlling gas and dynamic flow is essential.[32] Laminar flow in an intestine chip enhances tissue oxygen exchange, ECM remodelling, basement membrane production, mucus and microvilli production, tight junction expression, cytoskeleton generation, and vacuolization formation, forming intestinal villus structures and intestinal barrier integrity.[33] The human intestine contains around 1,000 bacteria species, including obligate, facultative, and aerobes. To accommodate complex gut microbiota and co-culture, an anaerobic gradient should be created in the intestine chip.[34]

2.7 Skin-on-a-Chip:

Organ-on-chip technologies offer advantages and require reliable skin models for drug and cosmetic testing. Skin-on-a-chip microfluidic devices allow tissue culture under control of physical and biochemical parameters. Classifying devices based on skin generation is challenging due to differences in fabrication process, materials, and tissue maintenance. Two main approaches have been developed for designing microfluidic chips for skin modelling: introducing skin fragments directly from a biopsy or HSE in the chip (transferred skin-on-a-chip) and in-situ tissue generation directly on the chip (in situ skin-on-a-chip).[35]

2.8 Multi-Organ Chips:

A human-on-a-chip platform is being developed to simulate and predict whole-body drug responses by integrating multiple organ units in a single microfluidic system.[36] A study by van Midwood et al. regulated bile acid homeostasis by placing rat intestinal and liver slices in a microfluidic chip. The liver metabolized

metabolites from the intestinal construct, emulating in vivo processes. The study also investigated liver metabolites' effects on adipose tissue in a bioreactor system composed of several compartments for culturing primary hepatocytes and adipose cells from rats and by focusing on liver metabolism of urea synthesis, albumin, glycerol, free fatty acid, and glucose [38].

2.9 Disease-Specific Chips**:**

Chips mimic diseases like cancer and Alzheimer's, aiding disease modelling, drug screening, and understanding disease mechanisms. Tumour microenvironment involves heterogenic, dynamic interactions between cancer cells.[39] A microfluidic concentration gradient chip was developed to analyse apoptosis in human uterine cervix cancer cells. It tested two anticancer drugs, 5-fluorouracil, and cyclophosphamide, using a stepwise concentration gradient generator. This method provides a more physiological environment than static culture platforms.[40] Recent microfluidic chips have been used for cancer drug testing, specifically in gastric cancer epigenetic therapy. Chip-on-chip analysis evaluates microRNA levels and cytotoxicity on tumour spheroids.[41] Further, a device that could provide a convenient in situ assay tool to assess the cytotoxicity of anticancer drugs on tumour spheroids was developed [42].

2.10 Customized Chips:

Researchers can design customized chips to replicate specific organs, tissues, or disease models not covered by standard preclinical chip types. Used for specialized research needs. These are just some examples of preclinical chip types. The field of preclinical chips is dynamic, with ongoing advancements and innovations leading to the development of new chip models and applications. Researchers and organizations often choose specific chip types based on their research goals and the physiological systems they aim to replicate for drug development, disease modelling, or toxicity testing [10].

3.Technology and Design:

Instead, this technique aims to mimic the key organotypic cellular architecture and functionality, 3D extracellular matrix (ECM), biochemical factors, and biophysical cues at a smaller scale, which serves the purpose for disease modelling and drug screening. As a type of microfluidic device, Organ-on-a-Chip is fabricated with the silicon-based organic polymer polydimethylsiloxane (PDMS) using the standard soft lithography technique; as such [43]**.**

3.1Microfluidics:

Microfluidics, a powerful tool for high-sensitivity, high-speed, high-throughput, and low-cost analysis, uses microfabricated channel and chamber structures to precisely pattern cells and manipulate fluidic and chemical parameters. such as flow rate, pressure, oxygen, and pH, providing controllable culture conditions It also has rapid growing implementations in both sophisticated chemical/biological analysis and low-cost point-of-care assays [44]. Microchips, designed for efficient mixing, are made from various materials like polydimethylsiloxane, silicon, glass, quartz, polycarbonate, and PMMA. They consist of a reagent inlet, sample inlet, valves, grooves, drainage system, and sensor part. The flow of fluids is regulated by valves and the drainage system removes waste material [45].

3.1.1Droplet-based microfluidics (DBM) chips:

Droplet-based microfluidics (DBM) systems utilize immiscible fluid volumes for analysis. Droplet formation involves two phases: continuous and dispersed. Methods include active and passive flow methods. Passive methods, which do not require external energy, produce simplified results. Droplet formation operates under

low Reynolds numbers for laminar liquid flow. Surfactants stabilize microdroplets by decreasing interfacial tension [45].

3.1.2Microfluidic chip integrated with 3D culture technique:

A microfluidic chip integrated with 3D culture technique has been developed for drug sensitivity testing of anticancer drugs. This chip is designed with polydimethylsiloxane polymer and consists of a reservoir, concentration-gradient generator, and three chambers of cell culture. The gradient concentration generator (CGG) is used to maintain an equal flow rate between drug and medium inputs. This design allows for the culture and testing of dissimilar cells and drugs for any disease condition [45].

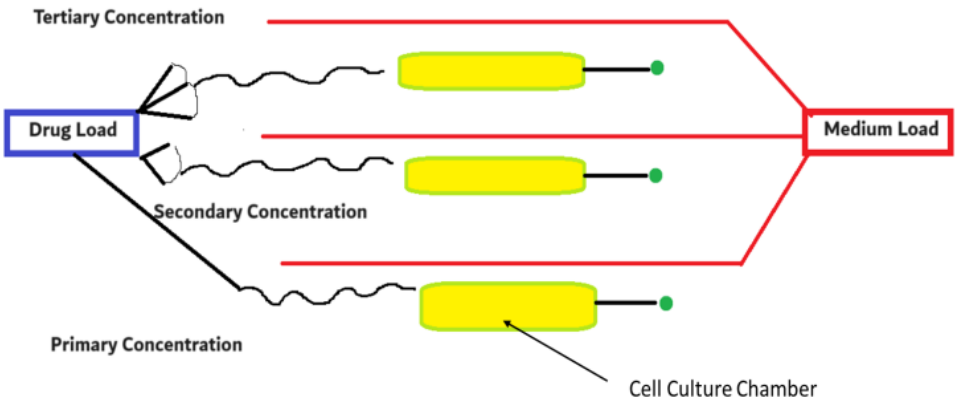

Figure 2 : A schematic design of a microfluidic 3D co-culture chip for cell culture and medication testing [45].

3.1.3 Microfluidic hydrogel chip:

PDMS faces challenges in cell adhesion and attachment, which are crucial for cell proliferation and differentiation. To address this, microfluidic channels can be coated with a cell-compatible hydrogel layer inside a PDMS-layered device. Natural polymers include agarose, alginate, collagen, dextran, fibrin, and laminin, whereas synthetic polymers include polyethylene glycol (PEG) and PEG diacrylate (PEG-DA). Hydrogels, composed of natural or synthetic polymers, are used for encapsulation of cells and barrier establishment.

3D hydrogel-based microfluidic chips have been found to be helpful in angiogenesis and cell migration studies. Shin et al. created a platform with four gel regions and three channels for cell culture, coated with extracellular matrix proteins and poly-d-lysine hydrobromide. This allowed for time-dependent flow and concentration gradient manipulation, as well as high-resolution real-time images for observing single-cell behaviour and cell-matrix interactions. Annabi et al. developed hydrogel coated channels for cardiomyocyte culture.

Tropoelastin hydrogels show better cell attachment and high voluntary beating rates in primary cardiomyocytes cultured on them compared to photo-cross linkable gelatin. These hydrogels are suitable for fabricating OoC due to their elasticity and exposure to elastic tissues like blood vessels. The developed chips, coated with gelatin and gelatin methacrylate, are biocompatible and promote cell functionalization. They offer a promising model for studying vascularization, inflammation, tissue engineering, and drug development. However, non-swelling hydrogel-based microchips were created using di-acrylated Pluronic F127, maintaining mechanical strength and channel morphology. This technology also established a vessel-on-a-chip with higher endothelial functions.[45]

3.2 Perfusion system:

3.2.1 Theoretical Model

The fluidic perfusion system uses a single input/output configuration with two pneumatic pressure controllers, a flow rate sensor, and a micro control unit for data streaming and custom-written software control [46].

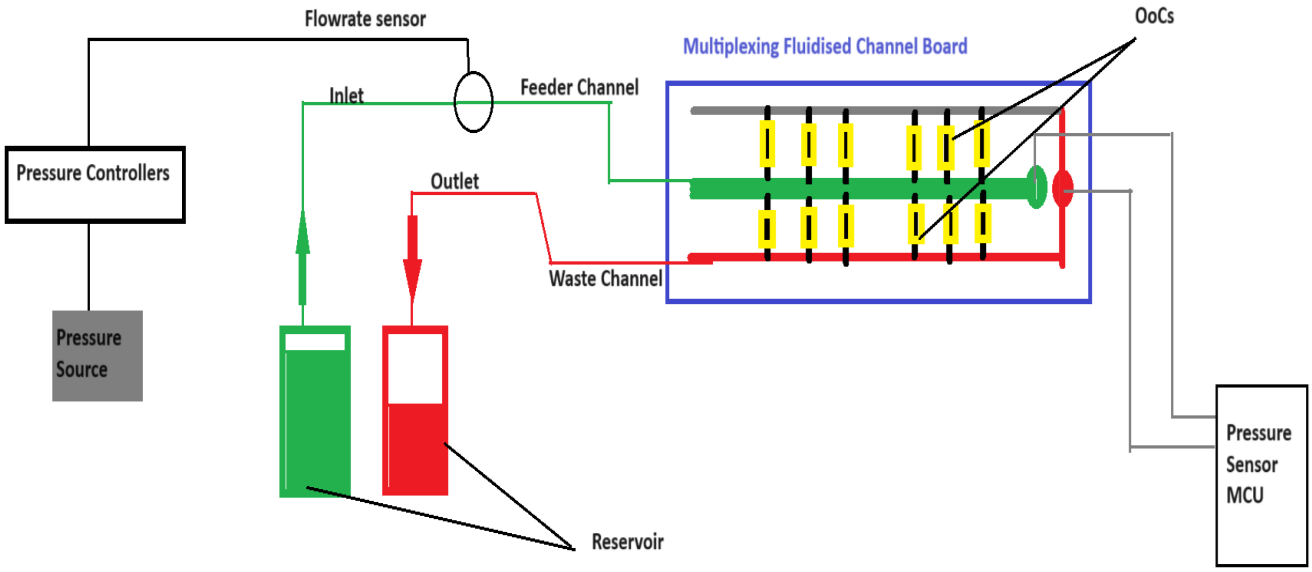

Figure 3: Overview of the perfusion system [46].

(a) Schematic of the microfluidic circuit consisting of 2 pressure controllers connected to a pressure source, additional pressure sensors (p), a passively controlled recirculation circuit and a custom-made fluidic circuit board to multiplex the perfusion to multiple samples [46].

3.2.2 Fluidic Circuit Board

A fluidic circuit board (FCB) was used for multiplex perfusion of OoC devices. The circuit consists of a feeder channel, waste channel, and pressure sensors. The FCB can connect up to four OoC devices, each with three microfluidic channels.

3.2.3 Controlled Perfusion and Recirculation

Custom PID-controller software was written in Python to maintain constant pressure difference (dP) and internal sample pressure (POoC).

Material	Qty.	Manufacturer	Supplier	Product SKU
FCB or single microfluidic device	-	Custom	-	-
Pressure cap for 15ml falcon-S(4 port)	2	Elveflow	Darwin-microfluidics	LVF-KPT-S-4
15ml reservoirs	2	Falcon	-	-
Check valves	4	Master flex	Darwin-microfluidics	MF-30505-92
Flow sensor L or XL	1 or 2	Fluigent	Fluigent	FLU-L-D-FDG
Flow EZ-line Up 345 mbar	2	Fluigent	Fluigent	LU-Fez-345
Link-up	1	Fluigent	Fluigent	LU-LNK-0002
Pressure sensor	4-6	Honeywell	Famell	MPRLS0300YG0001B

Luer to 1/16 barb	24	IDEX	Darwin-microfluidics	CIL-P-854
1/4-28-Female-to-male Luer adepter	2	IDEX	Darwin-microfluidics	CIL-P-655-01
3-way valve	2	IDEX	-	-
1/4-28-Female-to-Female Luer Lock adaptors	2 in	IDEX	Darwin-microfluidics	CIL-P-678
Y-connector	2	IDEX	Darwin-microfluidics	CIL-P-512
PTFE tubing-1/16 " OD X 1/32" ID	-	ELVEFLOW	Darwin-microfluidics	LVF-KTU-15
Printed circuit board (PCB) for pressure sensor	1	Custom	-	-
Arduino or other MCU	1	Arduiro	-	-
Ribbon wires	4-6	-	-	-
Optional:				
Microfluidic Manifold 9 Port	2	ELVEFLOW	Darwin-microfluidics	LVF-KMM-02
2-Switches	2	Fluigent	Fluigent	2SW002
Pressure source 1.2 bar	1	Fluigent	Fluigent	FLPG005
Pressure & flow rate range are setup depended	-	-	-	-

Table 2: Materials used for assembly of various fluidic circuits [46]

3.3 Microfabrication:

The term "chip" in organ-on-a-chip comes from the original manufacturing approach of photolithographic etching, used to produce computer microchips. This technology allows for control over surface features on the same scale, and is also used for patterning proteins and cells. Soft lithography, an alternative to photolithography, is often used in microfluidic culture systems for biochemistry and biology. It is cheap, simple, and can replicate patterns in biocompatible and flexible materials. Soft photolithography involves pouring a liquid polymer like poly-dimethyl siloxane (PDMS) on a silicon substrate, creating an optically transparent, rubber-like material. This process allows real-time, high-resolution imaging of cell responses to environmental cues.

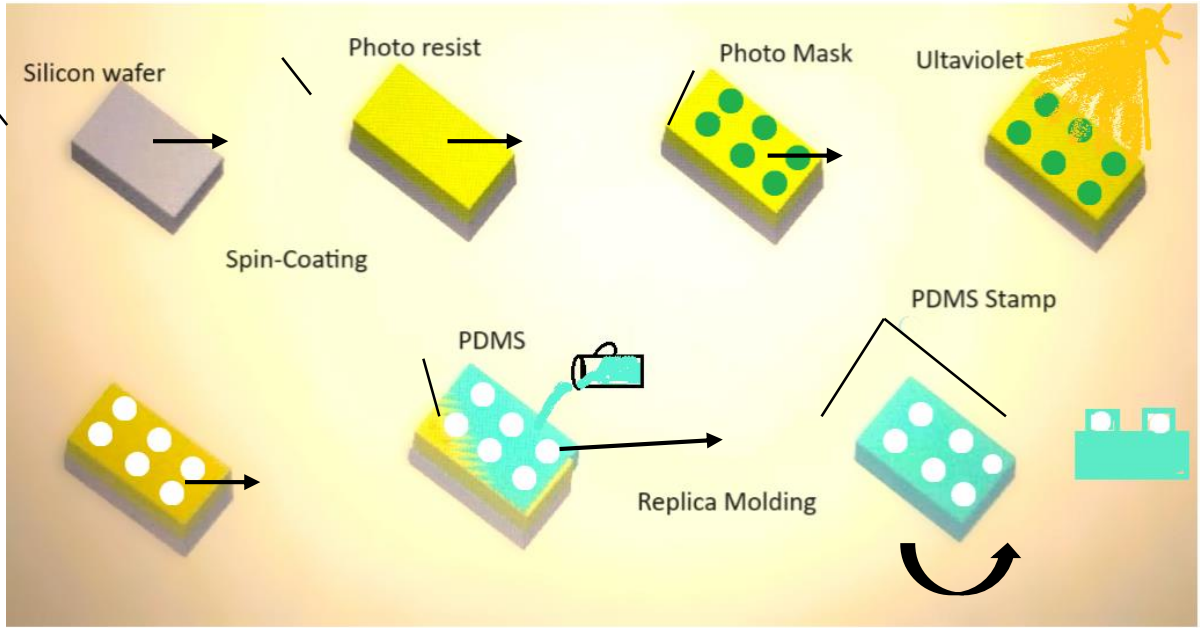

Figure 4: Fabrication methods for microfluidic chips [47].

4.Applications:

- **Drug Screening and Development:**
  Preclinical chips screen drug candidates for efficacy and safety, providing rapid and cost-effective screening in controlled environments, benefiting early-stage drug development.[47]
- **Disease Modelling:**
  Preclinical chips, including cancer-on-a-chip and Alzheimer's-on-a-chip, replicate disease-specific conditions, aiding researchers in studying disease mechanisms, progression, and potential treatments. [39]
- **Toxicology Testing:**
  Chips are utilized in toxicology studies to evaluate the safety of drugs, chemicals, and environmental toxins, enabling researchers to examine their effects on specific organs or tissues.[33]
- **Personalized Medicine:**
  Preclinical chips enable personalized medicine by incorporating patient-specific cells or genetic information, allowing researchers to test individual patient cells' responses to potential treatments.[27]
- **Understanding Disease Mechanisms:**
  These chips help unravel the underlying mechanisms of diseases by replicating physiological conditions. Researchers gain insights into disease pathways and cellular responses.
- **Pharmacokinetics and Pharmacodynamics (PK/PD) Studies:**
  Preclinical chips aid in studying drug absorption, distribution, metabolism, and elimination within specific organs, providing data on drug concentration, kinetics, and pharmacological effects.[17]
- **Organ Interaction Studies:**
  Multi-organ chips simulate interactions between organs, allowing researchers to study how drugs or diseases affect different organs and the body as a whole.[36]
- **Drug Delivery Research:**
  Preclinical chips are used to study drug delivery mechanisms, especially for targeted and controlled drug release. Researchers can optimize drug formulations and delivery methods.

- **Biological Research:**
  Beyond drug development, preclinical chips are used in fundamental biological research. They help scientists explore cell behaviour, cellular interactions, and tissue development.
- **Regulatory Testing and Validation:**
  Preclinical chips are used in regulatory testing for drug safety and efficacy evaluation, providing data that can aid in regulatory submissions.[48]
- **Environmental and Toxicity Studies:**
  Preclinical chips are applied to assess the impact of environmental factors, such as pollutants and toxins, on specific organs. These chips can simulate exposure to environmental stressors [48].
- **Educational and Training Tools:**
  Preclinical chips are utilized in educational settings to train future biomedicine researchers, offering a more efficient and physiologically relevant way to study human biology, diseases, and drug responses.

5. Advantages and Limitations:

These two methods have their own advantages and limitations. For animal model, it is different with human being in physiological conditions and organ functionalities. Thus, the results of animal experiments may not precisely predict the response and/or functionalities of humans. In addition, animal experiments have other limitations, such as time-consuming investigations, high cost, and low efficiency. Moreover, there are ethical issues to consider when conducting research. In recent years, Society for the Prevention of Cruelty to Animals has been established around the world, which strongly opposes animal experiments. Therefore, research communities are under increasing public pressure as to whether animals can be used in experiments [18].

6. Validation and Standardization:

Validation is a process to demonstrate the reproducibility and relevance of a test method. Validation is the interface between the development and optimization of a test method and its regulatory acceptance and permits a knowledge-based evaluation of the suitability of a method for a specific regulatory purpose.
Based on Hartung et al. (2004), this guidance describes a modular approach to validation defining six central modules:

1) test method definition,
2) within-laboratory reproducibility,
3) transferability,
4) between-laboratory reproducibility,
5) predictive capacity and
6) applicability domain as well as an optional definition of performance standards to facilitate the development of similar methods

Standardization of an OoC involves describing the preparation and use of an OC in such detail in an SOP that the results of the readouts are robust, reproducible, and transferable. For complex systems such as an OoC, a rigorous and strict standardization of the methods will be necessary to achieve sufficient reproducibility. Standardization also comprises computer control and measurement of flow, pH, lactate, etc. [48]

7. Current Challenges and Future Directions:

- The complexity and diversity of human physiology and pathology, such as the intricate connections and interactions in the human brain, are challenging to reproduce on a chip.
  Scalability and reproducibility of preclinical chips are influenced by fabrication methods, material quality, cell sources, culture conditions, and experimental protocols, resulting in varying organoids sizes, shapes, and functions.[49]
- Preclinical chips validation and translation involve comparing data from animal models, human clinical trials, and in vitro models, as organoids-on-chip devices may not accurately represent human drug pharmacokinetics and pharmacodynamics.[50]
- Future directions for preclinical chips include multi-organ integration, immune components, patient-specific models, advanced biomimetic materials, improved data analysis tools, regulatory acceptance, and global collaboration.
- Future Organ-on-a-Chip platforms need standardization and improvement in manufacturing processes, system designs, and interfaces. Currently, most devices are manually fabricated with PDMS, requiring a high-throughput, low-cost process. Advanced additive manufacturing methods, current materials, and a modular format with biologically inert materials should be considered.[51]

8. Conclusion:

In conclusion, preclinical chips, also known as micro physiological systems or organs-on-a-chip, represent a transformative innovation in the field of biomedical research and drug development. These microscale devices aim to bridge the gap between traditional laboratory experiments and the complex reality of the human body. Preclinical chips offer an unprecedented level of human relevance by replicating the microenvironment of specific human organs and tissues. They provide a platform to study human biology, diseases, and drug responses more accurately than traditional methods. The advantages of preclinical chips include reduced reliance on animal testing, high-throughput capabilities, precise control over experimental conditions, and the ability to monitor real-time responses. They also contribute to ethical considerations by reducing the use of animals in research. These chips find applications in drug screening and development, disease modelling, toxicity testing, personalized medicine, and understanding disease mechanisms. They serve as valuable tools for advancing biomedical research and healthcare innovations. Challenges in the field include validation and standardization, complexity in replicating human physiology, long-term culture, scalability, and addressing ethical and regulatory considerations. Ongoing research aims to address these challenges. Preclinical chips have the potential to revolutionize how we study human biology, diseases, and drug responses. While there are challenges to overcome, the field continues to advance, offering new opportunities to improve healthcare, reduce the reliance on animal testing, and contribute to a deeper understanding of human physiology and diseases.

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