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## Microphysiological Systems: gold standard approach towards *in vitro* biomimetics and its potential to drive forward the fisheries sector to the next level

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### Abstract

High attrition rates of drug candidates in clinical trials are caused owing to the utilization of traditional cell culture and experimental animal models for preclinical drug screening. Besides, major bottlenecks affecting the crude pipeline of drug development process are time and cost involved. It takes nearly 13 to 15 years for an efficient drug to reach the market and the cost of breeding, housing and, later on disposing the experimental animal models, is extravagant. Differences between human and animal physiological conditions and signaling mechanisms compromise the drug efficiency testing protocol resulting in clinical trial failure. In this scenario, 2-D culture systems stole the limelight providing a platform for reducing drug discovery and development pipeline shortcomings, but failed in the long run. Subsequently, alternating strategy to overcome such bottlenecks has recently emerged in the name of Microphysiological Systems. These hold great potential to act as surrogates by virtue of their biomimetic attribute in providing accurate prediction of drug effects through physiologically relevant organ-organ interactions. The far reaching applications of microphysiological systems in the fields of toxicology, medicine, disease modeling and so on, have major implications in fisheries sector as well, leading to the *in vivo* modeling of two renowned fish models, zebrafish and rainbow trout, as microphysiological systems. This mini review has described different types of microphysiological systems and shed light on their immediate need in the present scenario. For the first time, current advances and applications in context to the field of fisheries have also been discussed stating that this new age technology has the potential to open new avenues to researchers, experts and scientists in fisheries sector in the ensuing years.

**Keywords:** Microphysiological Systems, Organ-on-a-chip, Biomimetics, Bioprinting, Drug discovery

### Introduction

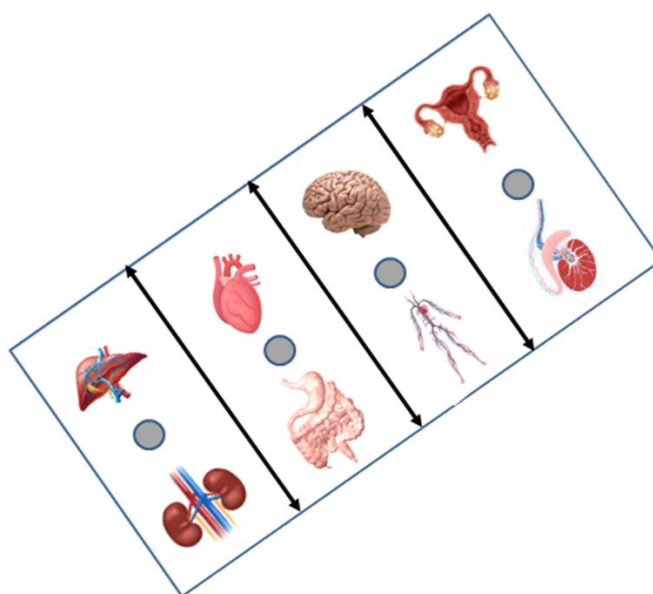
Today's era of path breaking developments and hustle of growing beyond imagination, has undoubtedly taken the mankind on a toll. Human interventions have significantly opened up new challenges to deal with countless number of diseases and thereby, survivability relies on effective drug development. In this scenario, the impeccable challenge is to reduce the due course of time a drug takes to come up in the market. On an average, the development, testing and authorization of a safe and effective drug takes 13.5 years, and many of them fail to reach the market owing to clinical trial failures<sup>[1, 2, 3, 4]</sup>. There have been series of arguments happening around the world for the use of experimental animals based on the ethical and scientific standards as well as the costs involved in breeding, housing and disposing. The findings obtained from the animal tests not translating well in the clinical trials have resulted in the conclusion that there are significant differences between the human and animal metabolic and signaling mechanisms<sup>[5, 6]</sup>. Moreover, emergence of new legislations enacted by EU to impose bans on animals testing for cosmetics and other drugs has paved the way for alternative test methods and strategies<sup>[7]</sup>. In the view of improvement and optimization in the drug development protocols, it is indispensable to reduce the false-positives and false-negatives in drug discovery as well as fatal drug interactions<sup>[8]</sup>. Henceforth, such bottlenecks in the drug discovery pipeline are being reduced with the inception of novel bioengineered

Miniature models including organ-on-chips and organ replicas, called as “Microphysiological Systems” (MPS). Novel MPS tend to create systemic models of human biology [3]. They are driven by the need to replace animal models and recapitulate human physiology favoring fast and fruitful discoveries in pharmacology and lifting the level from bench to bedside personalized medicines [9]. Such simple, reproducible and scalable platforms hold promise to improve and accelerate the overall drug discovery process [10]. Therefore, this mini review presents the scale of art of microphysiological systems, recent advancements made, upcoming challenges and the commercial outlook of the field. Besides, it also provides insight into the possible applications of this high throughput technology in the field of fisheries in the coming decade.

#### Microphysiological Systems: Clinical trials on the chip

The Microphysiological Systems (MPS) (“organs-on-chips”) are designated as miniature life supporting models comprising of single organ and multi-organ systems enabling the development of three dimensional organoids on bioengineered platforms. MPS are being immensely appreciated as they provide the minimal functional requirements of a system in order to mimic the human systemic interplay and capture more physiological complexities than standard cell cultures [11, 12, 3]. The schematic representation of MPS in terms of multi-organs-on-a-chip is illustrated in Fig1. They facilitate key organ level functions through mimicking *in vivo* tissue architecture and physiological conditions [13].

Microphysiological Systems generate high quality preclinical physiological data and thereby provide an opportunity to favor improved preclinical to clinical translation [14]. As compared to static and classical monolayer cell based assays, MPS offers advanced features enabling precise interpretation of preclinical efficacy testing, safety assessment and disease modeling [15]. These *in vitro* organs constructs, basically interconnected sets of 2-D and 3-D cellular constructs, are composed of immortal cells lines, organ specific cells, primary cells derived from humans and animals, human embryonic stem cells and induced pluripotent stem cells (iPSC) [8]. The constructs are designed in such an order so that they must recapitulate the structure and function of human or animal organ paying specific attention to the cellular heterogeneity and compartmentalization [9, 14, 16, 17]. Such mimicking of physiological conditions and provision of physiological accuracy are governed by the size of the MPS employed. Generally two types of MPS have been reported based on size differences, namely organ construct MPS and organs-on-chip MPS. Organ constructs MPS are scaled up to milihuman (mHu), basically fabricated with macroscopic characteristics, resembling engineered tissues whereas nanohuman (nHu) or microhuman (mHu) constructs, comprising of monolayers of human cells and fabricated using microfabricated devices, are referred as organs-on-chip MPS [8]. Critical steps that must be taken care before *in vitro* modeling of MPS are shown in Fig 2.



**Fig 1:** Illustration of multi-organ-on-chip based microphysiological system

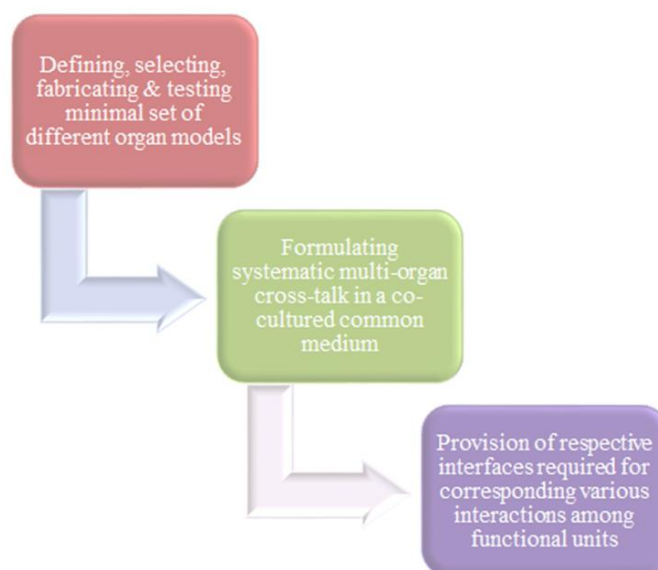
#### Historical timeline

Although, Microphysiological Systems, governing the tools of biomedical engineering in order to recapitulate the whole animal physiology on a chip, are highly acknowledged today, the roots of their evolution date back to 1940s. Sanford and colleagues came out with first publication in the year 1948 stating the first successful culture of individual mammalian cells within 6-8mm glass capillaries [4, 18]. The upscale technology forming the base for further revolution started in 1978 wherein miniaturized gas chromatograph separatory column, based on photolithography was achieved [19]. A major breakthrough was achieved when Friedrich Bonhoeffer designed soft lithographic microfabrication of microchannels in silicone, in 1987 [4]. With the passage of some years, the early and mid-90s experienced some remarkable

achievements owing to several discoveries and the year 1998 witnessed the path breaking development of PDMS soft lithography [4, 20]. This technology revolutionized the fabrication process of microfluidic devices and became the ideal substrate for microfluidic cell culture devices owing to remarkable features like biocompatibility coupled with gas permeability [4]. Generation of tissue specific microenvironmental cell specific conditions with the integration of proper fabrication and controlled conditions was evident by the beginning of 21<sup>st</sup> century. Thereafter, biomimetic technologies seeking to model *in vivo* organ tissue physiological behaviors *in vitro* including 3D bioprinting and MPS, are the current choices in circumventing the time and cost involved in the drug development pipeline. In this scenario, several advanced single organ MPS and multi-organ

MPS as microphysiological systems have been developed recently and their description in this article are worth

mentioning.



**Fig 2:** A flowchart depicting crucial steps indispensable for in vitro MPS modeling

### Single organ MPS

#### Brain-on-a-chip

Being the single most important and complex organ of the human body, brain serves as the central processing hub governing numerous biochemical and bioelectrical signals and having a centralized control over all the organs of the body [4, 16]. Owing to its complexity and importance, it is indispensable to emulate neural tissues of the brain as physiological mimic model to understand range of diseases and mechanistic studies in detail [4]. Having an insight to look into the effect of several diseases on neural tissues, soft lithographic techniques have attempted to develop models namely Alzheimer's and Parkinson's diseases [21]. Applications of MPS to model Alzheimer's disease have been extensively carried out using the continuous delivery of amyloid- $\beta$ , nutrients and oxygen [22]. A high throughput culture of 3D neuroglial networks has also been developed using advanced microfluidic chip platform [23]. From development of 3-lane microfluidic chip to have a detailed study on MSC induced neuronal differentiation [24] to assessment of three different anti-cancer pharmaceuticals on glioblastoma spheroids [25], it is evident that MPS maintained tissue viability, structure and function and also played a pioneering role in revealing several physiological aspects of brain *in vitro*. In future, MPS could open new doors for achieving greater milestone related to drug screening and tissue engineering applications as well the basic study of neuronal development in the brain [4].

#### Heart-on-a-chip

As a vital organ of the circulatory system, heart is able to pump blood by means of cross-striated muscle cells called as cardiomyocytes [4, 16]. A non-external power requiring micropump that worked for 5 days without power stimulation was developed [26]. Two of the most important characteristics of cardiomyocytes, muscular contraction and electrical activity, have been utilized to study their role *in vitro*. Two high throughput heart-on-a-chips were designed in order to measure contractility and, diastolic and systolic stresses, respectively [27, 28]. Cardiotoxicity model using human origin stem cells was developed to throw light on precise *in vivo* physiological conditions [29, 16]. Recapitulation of human heart

environment using neonatal rat and human induced pluripotent stem cell derived cardiomyocytes was carried out recently, mimicking the heart beat motion [16, 30]. Vascularized heart model using bioprinting technique and cardiac microphysiological device have been designed, respectively with the purposes of efficient drug testing and elucidating cardiovascular physiology in detail [31, 32].

#### Liver-on-a-chip

The largest organ in the abdominal cavity commonly prone to foreign metabolites, liver performs innumerable functions, from detoxification, immune regulation, and maintenance of internal homeostasis to biotransformation of exogenous substances like drugs [14, 16]. Therefore, liver is a gold standard model organ to test drug metabolism and investigate hepatotoxicity *in vitro*. Several models have been designed recently to mimic the *in vivo* physiological conditions of liver cells depending on some important factors viz., sensitivity and accuracy. A biomimetic liver model using perfusion culture conditions was proposed upon inoculating four types of hepatocytes into a porous membrane [33]. The model that could detect toxicity in hepatocytes upon chronic exposures was devised based on co-culturing of hepatic cells with non-parenchymal cells [34]. Later on, a high throughput spheroid culture model was designed in order to detect the hepatotoxicity of an anti-cancer drug namely 5-FU using HepG-2 cells [35]. Very recently, a real time based stipulation of liver injury was detected upon construction of a device using fluorescent probes [36, 37].

#### Kidney-on-a-chip

Removal of toxins and metabolites from the body and reabsorption of water and other useful substances are the responsible functions of kidneys [16]. A kidney *in vitro* microphysiological system is equivocally required to improve the predictability of nephrotoxicity as well as to have an in-depth knowledge of drug induced kidney injuries [20]. A 3-D culture system comprising of extracellular matrix in order to support the formation of cells and tubular structures was devised [38] followed by increment in the number of cells and types of different nephron segments in the chip [39, 40, 41, 42, 43].

### Eye-on-a-chip

It serves as a sensory organ of the body in detecting and processing light, with the inclusion of protective barriers in terms of epithelial tight junctions, different layers and causes a phenomenon namely biomechanical blinking for the maintenance of eye tissues [16]. In order to study various functions of eye extensively and to test number of ocular drug, limited number of studies has been reported. Using extracellular matrix substrate, collagen vitrigel, corneal microtissues were developed upon coculturing rabbit corneal endothelial cells and keratinocytes [44]. In the upcoming years, a blinking eye on a chip using 3-D printing was fabricated to reconstruct eye motion in dynamic form [45]. Therapeutic effect of bevacizumab was tested by recapitulating age related muscular degeneration condition through designing in vitro eye replica model [46]. In the meanwhile, an *in vitro* eye model was suggested based on physiological and anatomical recapitulation for subsequent ophthalmological drug development [47].

### Skin-on-a-chip

Current trends in development of skin chips are more pronounced skin being the largest organ of the human body, serves as the primary and protective barrier between the body and the environment. An integrated pump mediated continuous perfusion system was developed for skin culture system [48]. To maintain cell viability and tissue functionalities over a culture term of 28 days, a four tissue co-culture array comprising epidermal, intestinal, hepatic and renal cells was developed [49]. Furthermore, for the promotion of increased tight junction expression, co-culturing epidermal and dendritic cells gave rise to immune competent physiological model of human skin model [50]. Upon utilization of nylon wire mesh reinforce collagen matrix, a vascularized multilayered skin like tissue was engineered recently [51].

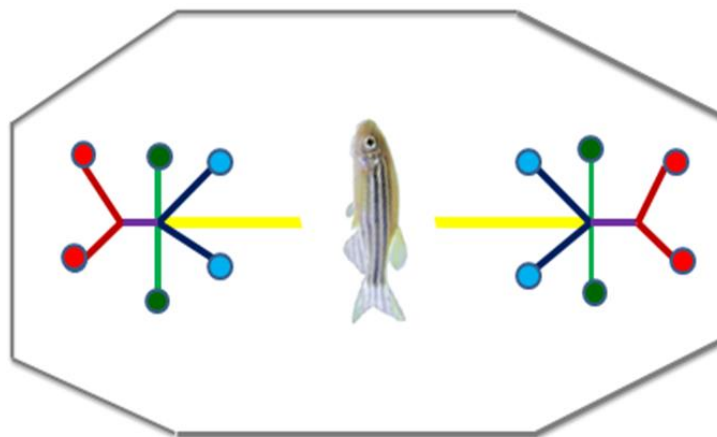
### Multi-organ-on-a-chip

Multi organ MPS have the potential to mimic the entire physiological conditions and interactions of a number of interconnected organs, thereby facilitating a wholesome response to variety of drugs and other compounds and emulating organism functionality as a whole. A schematic diagrammatic illustration on multi-organ-on-a-chip has been shown in Fig1. The first report of fabrication of interconnected multiple organ media circuit was proposed in the year 2004 [52]. The results herein revealed liver toxicity effects on multiple organs through generation of naphthalene metabolites. Interconnected liver and colorectal tumor tissues based MPS was designed and was shown that only perfused and interconnected co-culture affects the tumor growth upon treatment with cyclophosphamide prodrug [53]. Moreover, a four-organ model interconnected device comprising human intestinal model, skin biopsy model, liver spheroids model and kidney model was engineered having 28 days co-culture viability and reproducible homeostasis [54]. Recently, a multi organ MPS co-cultivating heart, neuronal liver, liver and skeletal muscle has been developed that reproduced the toxicity of five drugs using a serum free medium over 14 days [55]. A 2018 based study has engineered 4, 7 and 10- way MPS platform as a measure to implement multiple MPS approaches in drug discovery [56]. Such predefined organ systems have the

capability in answering specific questions concerning drug toxicity evaluation, drug metabolism and distribution. A synchronized MPS multi organ model is the present need that could emulate the entire intact organismal physiology and functionality, which may further surpass the shortcomings of drug development processes, including cost, time and experimental model animals.

### MPS: Whereabouts in Fisheries

As discussed in this article, MPS has far reaching applications in areas associated with drug testing, toxicology, physiology, and anatomy and henceforth, a step closer to realization of analytical automation in humans and some rodents. Fisheries sector is growing rapidly through the development of aquaculture recently, and has attracted attention as a source of good animal protein. Aquaculture accounted approximately forty percent of the total fish production in the year 2014 which is around 74 million tons [57]. Due to employment of more intensive culture methods in order to produce higher yields, the industry is facing huge loss up to 6 billion dollars owing to disease outbreak [57]. In this scenario, development of vaccines to control and prevent infectious diseases is of paramount importance. Subsequently, the idea of engineering MPS in fishes can do wonders especially in the areas of ecotoxicology and disease diagnostics. An idea of fish-on-chip has been illustrated in Fig 3. However, in fisheries, this idea is still in infancy. The sign of relief is that some noteworthy work has been initiated already. Flexible and surface microarrays of micro-electromechanical systems artificial hair cell flow system was designed in blind cave fish. They are basically termed as biological sensors as fishes are capable of performing impressive behaviors such as supermaneuverability, hydrodynamic flow 'vision' and object localization [58]. These self-powered, miniaturized, lightweight, low-cost and robust artificial lateral-line systems could improve the capabilities of underwater vehicles [58]. A multilayer microfluidic system for automated and high efficiency trapping of living zebrafish embryos has been established. The outline design is a critical milestone in studying drug toxicity parameters at a large scale [59]. Another microfluidic based lab on a chip technology was validated in zebrafish embryos for automation of toxicity test [60]. Very recently, the first of its kind fish-gut-on-a-chip model was proposed in rainbow trout for a better understanding of basic fish physiology, for the refinement of fish feed in aquaculture and for predicting chemical uptake and bioaccumulation in fish for environmental risk assessment [61]. Apart from fishes, gelatin cantilever substrate based MPS has been fabricated very recently using mussels in order to measure cardiac contractility [62]. Fishes are diverse group of organisms exhibiting varied patterns of physiology depending on their habitat conditions, food and feeding habit, and sexual orientation. The idea of MPS in fisheries can be of immense help in elucidating every bit of detail regarding entire physiological aspects in order to accurately predict the occurrence of deadly diseases and efficient drug testing in this scenario. *In vitro* modifications of physiological conditions in fishes on chips can open new doors of opportunities in order to increase the global fish production by combating deadly disease outbreaks.



**Fig 3:** A diagrammatic representation of fish-on-a-chip

### Challenges and Future Perspective

Although, MPS have evolved recently in combination of 3-D *in vitro* cell cultures in microfluidic devices using modern mechanical or electrical actuators and sensors, there are challenges needed to be strictly paid attention. The challenging and limiting factors include the appropriate size, correct relationship between the fluid and tissue volume, relevant size relationship maintenance between different tissues, development of a common suitable media, development of more robust culture systems, and increasing technology throughput [3,10,63]. These systems containing single and multi-organ devices have already shown major improvements which are physiologically relevant in terms of more accurate prediction of drug mediated organ toxicity. The number of high-tech companies governing MPS technology has become almost doubled since 2012 and the future is promising as this technology is justifiable for venture investment for feasible applications in drug discovery [10].

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