



Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/ccllet

Advancements in life-on-a-chip: The impact of “Beyond Limits Manufacturing” technology



Weiwei He^{a,1}, Hongbo Zhang^{b,1}, Xudong Lin^c, Lili Zhu^a, Tingting Zheng^d, Hao Pei^d, Yang Tian^d, Min Zhang^d, Guoyue Shi^d, Lei Wu^e, Jianlong Zhao^e, Guliner Wumaier^f, Shengqing Li^f, Yufang Xu^a, Honglin Li^{a,g,h,*}, Xuhong Qian^{a,d,g,*}

^a Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

^b School of Mechanical and Power Engineering, East China University of Science and Technology, Shanghai 200237, China

^c Guangdong Provincial Key Laboratory of Sensor Technology and Biomedical Instrument, School of Biomedical Engineering, Shenzhen Campus of Sun Yat-sen University, Shenzhen 518000, China

^d Shanghai Key Laboratory of Green Chemistry and Chemical Processes, Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200241, China

^e Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences, Shanghai 200050, China

^f Department of Pulmonary and Critical Care Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China

^g Innovation Center for AI and Drug Discovery, East China Normal University, Shanghai 200062, China

^h Lingang Laboratory, Shanghai 200031, China

ARTICLE INFO

Article history:

Received 10 August 2023

Revised 8 September 2023

Accepted 11 September 2023

Available online 21 September 2023

Keywords:

Beyond Limit Manufacturing

Model-organism-on-a-chip

Organ-on-a-chip

ABSTRACT

This review explores the concept of life-on-a-chip, which involves the creation of miniaturized biological systems, such as organs, tissues, and model organisms, on microscale platforms called microfluidic chips. These chips consist of intricately etched channels, wells, and chambers that enable precise control and observation of fluids, cells, and biochemical reactions, facilitating the simulation of various aspects of human or animal physiology and the study of responses to different stimuli, drugs, or disease conditions. The review highlights the application of a novel technology, “Beyond Limit Manufacturing” (BLM), in the development of sophisticated three-dimensional cell models and model organism microchips. Model-organism-on-a-chip and organ-on-a-chip (OoC) are among the thriving developments in the field of microfluidics, allowing for the reconstruction of living microenvironments and implementation of multiple stimuli. The review discusses the latest advancements in life-on-a-chip technology using BLM and outlines potential future research directions, emphasizing the significant role of these chips in studying complex biological processes in a controlled and scalable manner.

© 2024 Published by Elsevier B.V. on behalf of Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences.

1. Introduction

The global market for three-dimensional (3D) cell culture was valued at \$1.66 billion in 2021 and is predicted to reach \$6.46 billion by 2030, with a compound annual growth rate (CAGR) of 16.3% [1]. This growth has been further amplified by the recent legislation signed by President Joe Biden in late December 2022, which allows new medicines to bypass animal testing for Food and Drug Administration (FDA) approval. This development has significantly

boosted the demand for 3D cell models. In particular, microfluidic technology has emerged as an ideal method to better mimic the microenvironment of 3D cell models, offering advantages such as a similar size to the vasculature system, flexible geometry formation, and easy integration, especially for high throughput applications.

The advancement of microfluidic technology has led to the promising concept of life-on-a-chip, which shows great potential in replacing animal tests and finds diverse applications in drug development, pathologies, and precision medicine [2]. However, despite these advancements, the manufacturing techniques for microchips have not kept pace. Currently, microchips are primarily made by molding polydimethylsiloxane (PDMS), which hampers their free-forming capability, necessitating complex processes for their production. Moreover, PDMS has its limitations, including hydrophobic properties, contamination risk from small molecules, and low

* Corresponding authors at: Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China.

E-mail addresses: hlli@ecust.edu.cn (H. Li), xhqian@ecnu.edu.cn (X. Qian).

¹ These authors contributed equally to this work.

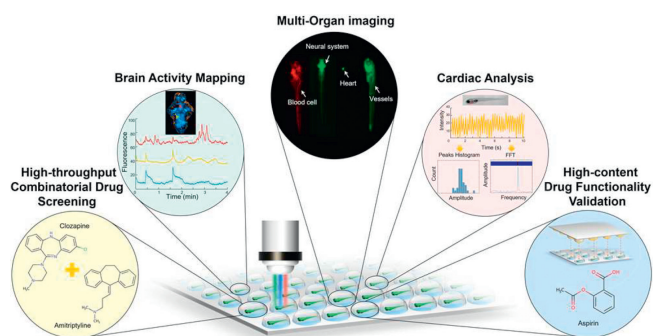


Fig. 1. High-throughput, high-content, multifunctional screens enabled by the ZoC technology. Reproduced with permission [7]. Copyright 2022, Wiley Publishing Group.

light transmission compared to polymethylmethacrylate (PMMA). As a result, new technological innovations are needed to advance the life-on-a-chip field and realize its full potential.

In 2018, Cheng & Qian *et al.* introduced the concept of "Beyond Limit Manufacturing" (BLM) technology [3]. The research scope of BLM covers the flexible design and rapid prototyping of 3D micro/nano devices, including reactors, separators, mixers, crystallizers, and extractors. Additionally, the evaluation of reaction performances in terms of throughput, selectivity, purity, and waste production is also an integral part of the research [3]. Notably, the ultrafast laser microfabrication (ULM) method, also known as ultrafast laser-assisted chemical etching, offers distinct advantages for advanced glass material micro-processing due to its ultrashort pulse widths and remarkably high peak intensities. These capabilities enable the creation of 3D volume micro/nanostructures with diverse geometries and allow for multifunctional monolithic integration, such as 3D photonic, optofluidic, and electrofluidic components [4–6]. When applied to life-on-a-chip technology, BLM shows evident advantages in manufacturing microchips with innovative evaluation readouts, advanced dynamic fluid control, and higher complexity in structures and scales. As a result, BLM-produced life-on-a-chip devices offer enhanced performance and potential for diverse applications.

2. Life-on-a-chip made by BLM

2.1. Model-organism-on-a-chip

Zebrafish, a widely used vertebrate model, offers various advantages for genetics and drug screening due to its small size, transparency, ease of culture, and amenability to gene manipulation. However, high-content screening for specific organs traditionally involves laborious manual manipulation in agarose, hindering high-throughput applications. Recent advancements in micro/nano-fabrication techniques, with a particular focus on BLM technology, have paved the way for the development of zebrafish-on-chips (ZoC) microfluidic systems (Fig. 1). For instance, the "Fish-Trap" microfluidic array system enables real-time monitoring of brain activities and has been modified for evaluating other organ systems such as heart [7]. Additionally, optofluidic platforms allow precise chemical delivery and recording of whole-brain neuronal activities in larval zebrafish [8]. Combining microfluidics with light-sheet micro-imaging, all-glass microfluidic devices have been realized through femtosecond laser processing for high-resolution imaging [9]. Such innovative systems seamlessly integrate pharmaceutical treatments with real-time multispectral microscopic imaging, eliminating the need for complex pipetting and allowing for parallel operations. By harnessing the power of machine learning algorithms, researchers conducted efficient screening and analysis

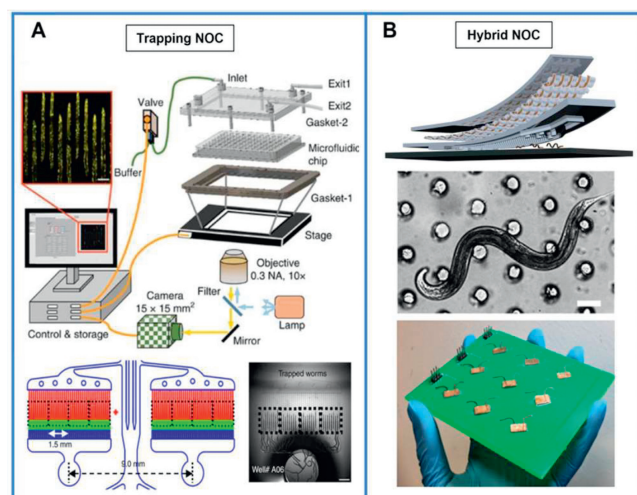


Fig. 2. NoC technologies enabled high-throughput screening of *Caenorhabditis elegans*. (A) NoC systems with terminal narrowing designs. Copied with permission [10]. Copyright 2016, Nature Publishing Group. (B) Hybrid NoC systems. Copied with permission [13]. Copyright 2023, ACS publications.

of a small library of compounds. The versatility of this approach extends its applicability to various fields, proving particularly valuable in the exploration of combinatorial drugs when sample quantities and resources are limited. With its potential to expedite the identification of novel therapeutics for precision medicine, the ZoC technology holds great promise.

Recently, microfluidic systems for behavioral monitoring of animals, such as the nematode *Caenorhabditis elegans*, have been developed using different designs, including terminal narrowing designs (to physically trap nematodes, Fig. 2A) [10], chamber designs (for long-term culture in specific spaces) [11], and multi-layer and actuator-integrated designs (to constrain nematodes between different chip layers) [12]. In contrast to the laborious and costly traditional behavioral phenotyping strategies, a more efficient hybrid nematode-on-chips (NoC) technology (Fig. 2B) that utilizes BLM technology has been successfully designed [13]. This approach converts animals' behaviors into friction deformation, resulting in a contact-separation motion between two triboelectric layers that generates electrical outputs [13]. These signals can be detected using a homemade multichannel recording system or a commercial oscilloscope, eliminating the need for microscopes. This high-throughput, scalable, and cost-effective system provides information-rich electrical readouts, which have proven sufficient to predict drug identities successfully.

The application of BLM technology in model-organism-on-a-chip is in its early stages. However, we can envision the development of model-organism-on-a-chip, including ZoC and NoC microfluidic systems with more complex microstructures using femtosecond laser micromachining. These systems might offer precise spatiotemporal control of various stimuli for deeply analyzing the physiological responses of the animals. Advancements in lasers and 3D printing may also further enable the integration of micro/nano-interfaces, sensors, and circuits into advanced model-organism-on-a-chip systems.

2.2. Organ-on-a-chip (OoC) and multifunctional automation system

The BLM technology has further expanded its applications by developing renal proximal tubule-on-a-chip (RPToC), skin-on-a-chip (SoC), 3D-vessel-on-a-chip (VoC), and tumor-on-a-chip (ToC) [14–17]. These innovative platforms serve for toxicity evaluation and personalized medicine.

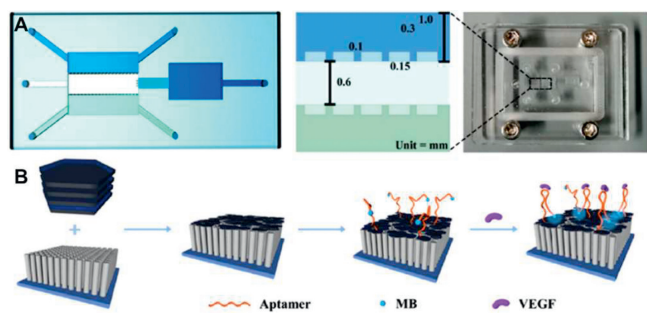


Fig. 3. The structure of IGoC. (A) Structure of multifunctional microfluidic chip IGoC. (B) Schematic illustration of the sensing mechanism of MB@TiO₂/Nb₂C. Copied with permission [23]. Copyright 2023, ELSEVIER.

In the case of RPToC, patient-specific alveolar epithelial cells were successfully isolated from lung adenocarcinoma samples. Zhang *et al.* accomplished this by using an inertial microfluidic chip to remove blood cells from lung adenocarcinoma samples, followed by the separation of patient-specific alveolar epithelial cells using a Dielectrophoresis (DEP)-based microfluidic chip [15]. This study holds great promise for advancing medical research and treatment strategies, particularly in the context of combining immunotherapy and chemotherapy to improve outcomes in the future.

In another notable development, BLM technology has been used to produce microrelief skin samples to assess cosmetic efficacy. These skin samples, incorporating microrelief, offer a convenient means of evaluating the performance of cosmetic products, such as powders. Notably, microrelief patterns undergo changes throughout the aging process. This particular attribute could be leveraged to create tailored and age-specific skin models, thereby facilitating the appraisal of anti-aging products. Both experimental results and computational simulations have demonstrated that microrelief plays a role in enhancing the skin's resilience against the effects of aging by adjusting its elastic modulus. Microrelief makes the skin more adaptable to the tensions that accompany aging and plays an important role in regulating epidermal modulus, a dominant factor in wrinkle formation. Furthermore, these skin samples were utilized to construct a SoC model, opening up new avenues for studying skin-related research [17].

VoC with intricate multi-level microstructures [18] can be meticulously fabricated by BLM to construct an *in vitro* artificial microvascular model. By precisely manipulating the growth microenvironment of human umbilical vein endothelial cells (HUVEC), tubular structures, which closely resemble authentic tubular structures, exhibit a certain level of permeability [19]. These VoCs have diverse applications in drug discovery, encompassing drug screening, toxicity assessment, disease modeling, and personalized medicine [20–22]. The integrated monitoring system in vascular organ chips enables real-time detection of various physiological and pathological indicators, such as oxygen levels, pH, reactive oxygen species (ROS), cholesterol, and low-density lipoprotein. The overall advancement significantly enhances analysis efficiency, expedites drug development, and reduces experimental expenses.

The construction of an integrated intracranial glioma-on-a-chip (IGoC) system through BLM technology allows for real-time monitoring of glioma invasion (Fig. 3) [23]. Surface-enhanced Raman spectroscopy (SERS) contributes exceptional sensitivity, enabling the detection of even ultra-low concentrations of patient biomarkers in fluids, leading to more accurate diagnostic outcomes. The utilization of microfluidic platforms permits the replication of intricate vascular networks and tumor microenvironments, thus facilitating the development and evaluation of strategies for drug

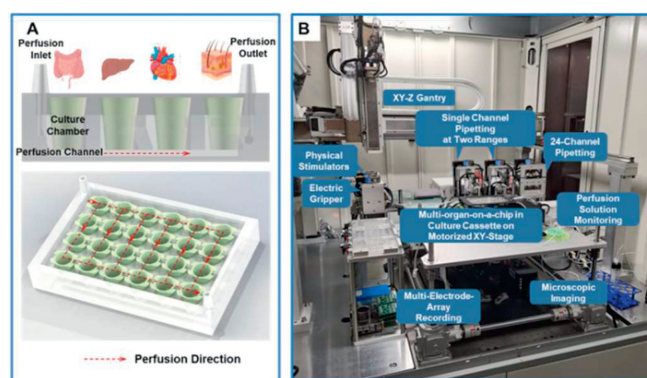


Fig. 4. A multifunctional automation system based on a multi-organ-on-a-chip. (A) The multi-organ-on-a-chip schematic diagram (Up: cross section view, Down: stereoscopic view). (B) The multifunctional automation system.

delivery. The combination of SERS technology and microfluidics makes it possible to better simulate the physiological environment and obtain quantitative measurements [24]. The research group achieved highly sensitive and quantified detection of vascular endothelial growth factor (VEGF) with a low detection limit of 3.7 pg/mL. Furthermore, TiO₂/Nb₂C was utilized to deplete ROS, which can inhibit glioma cell invasion. This innovative approach successfully combines an invasion model with SERS and microfluidic technology, allowing real-time monitoring and quantification of the invasion process by detecting VEGF secreted by glioma cells. This integration of diagnosis and treatment establishes a novel model for biomedical analysis, clinical diagnosis, and glioma treatment.

To meet the common requirements of high throughput and standardized operation in drug screening and disease detection, there is a need for the development of automation technology. A high-throughput multi-organ-on-a-chip (MOoC) has been developed alongside its versatile automation system (Fig. 4). The MOoC comprises an upper culture chamber and lower microfluidic channels, systematically arranged within a standard commercial multi-well plate. Notably, endothelial cells are cultivated on the exterior of a porous membrane at the chamber's base, giving rise to a vascular barrier. This configuration allows for the passage of medium through microfluidic channels, propelled by a peristaltic pump. Consequently, a dynamic interplay among the organoids cultured in separate chambers becomes feasible. Simultaneously, a real-time monitoring system for physiological and pathological indicators can be integrated [24], facilitating dynamic tracking of tissue growth and drug response. These integrated functionalities collectively provide precise regulation of the organoid microenvironment confined within the chip while allowing continuous monitoring of functional indicators through online assessments. This system is highly convenient for establishing diverse physiological and pathological models and offers numerous methods for drug screening. Furthermore, a modular assembly strategy utilizing porous MXene frameworks to create a diverse library of gas-sensing materials has been employed. Combined with a microchamber-hosted MF-based electronic nose, this approach enables high-discriminative pattern recognition of complex volatile organic compounds in urine. Consequently, a plug-and-play point-of-care testing platform with wireless, real-time monitoring capabilities has been developed, achieving an impressive 91.7% accuracy in the noninvasive diagnosis of multiple diseases [25]. This platform holds substantial promise for early disease detection, continuous disease monitoring, and various research applications.

3. Future perspectives

The primary challenge in fully representing the human body *in vitro* using 3D-cell-models-on-a-chip lies in effectively reconstructing the interactions between different organs in a dynamic and adaptable manner. To address this, the integration of various organs with adjustable functions becomes crucial for the successful completion of this task. A promising approach involves combining OoC technology with organoid-on-a-chip (ONoC). By leveraging the strengths of both technologies, such as the stem cell-derived nature of organoids and the spatial biomimicking of organ compartments, we may unlock the potential to create highly realistic *in vitro* human models in the future. An essential element in these microchip integrations is the use of BLM due to its advanced manufacturing approaches, facilitating the realization of these intricate setups. Through innovative advancements in this field, we are moving closer to overcoming the obstacles and achieving significant breakthroughs in the representation of human physiology on chips.

One crucial aspect to address when considering the representation of the human body *in vitro* using 3D-cell-models-on-a-chip is the matter of regulation. While there are various protocols accessible for OoC [26], the absence of standardized regulations for the processing of these microchips is a pressing concern. Therefore, it becomes imperative to establish a robust regulatory framework to govern the usage and development of microchips in this context.

4. Conclusion

BLM technology has been successfully employed to create life-on-a-chip, which included the implementation of ZoC and hybrid NoC approaches for high-throughput drug development. Additionally, various other organ-on-chip models such as RPToC, SoC, VoC, and IGoC were utilized for applications in toxicity testing and precision medicine. Furthermore, we achieved significant progress by constructing integrated systems of VoC with monitoring modules and MOoC setups.

The utilization of BLM has shown immense promise in revolutionizing life-on-a-chip, bringing us closer to the realization of replacing animal testing in the foreseeable future. The remarkable advancements made in this study hold great potential for enhancing drug development and toxicity assessment, and advancing the field of precision medicine while reducing the reliance on animal

experiments. It is our hope that these findings will contribute to the continued advancement of ethical and effective research practices in the biomedical sciences.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank the “Shanghai Beyond Limits Manufacturing Project” for supporting the realization of the BLM concept for microchip products.

References

- [1] G. Fang, Y.C. Chen, H. Lu, et al., *Adv. Funct. Mater.* 33 (2023) 2215043.
- [2] S.E. Park, A. Georgescu, D. Huh, *Science* 364 (2019) 960–965.
- [3] Y. Cheng, W. Zhu, X. Qian, *Beyond Limits manufacturing: Mass customization of Factory-on-a-Chip for Flow chemistry*, in 70 Years of excellence: ECNU's Ongoing Commitment to cutting-edge, Cross-Disciplinary Research, Science/AAAS, Washington DC, 2021, pp. 17–19. <https://www.science.org/content/resource/70-years-excellence-ecnu-ongoing-commitment-cutting-edge-cross-disciplinary-research>.
- [4] J. Xu, X. Li, Y. Zhong, et al., *Adv. Mater. Technol.* 3 (12) (2018) 1800372.
- [5] R. Hu, X. Li, J. Xu, et al., *Anal. Chem.* 95 (2023) 10422–10429.
- [6] X. Li, J. Xu, Z. Lin, et al., *Appl. Surf. Sci.* 485 (2019) 188–193.
- [7] M. Tang, X. Duan, A. Yang, et al., *Adv. Sci.* 9 (2022) 2104449.
- [8] S.K.H. Sy, D.C.W. Chan, R.C.H. Chan, et al., *Nat. Commun.* 14 (2023) 227.
- [9] K. Mattern, J.W. von Trotha, P. Erfle, et al., *Commun. Biol.* 3 (1) (2020) 311.
- [10] S. Mondal, E. Hegarty, C. Martin, et al., *Nat. Commun.* 7 (2016) 13023.
- [11] G. Aubry, M. Mišisavljević, H. Lu, *Small* 18 (17) (2022) e2200319.
- [12] J. Wan, H. Lu, *Lab Chip* 20 (24) (2020) 4528–4538.
- [13] A. Yang, X. Lin, Z. Liu, et al., *Nano Lett.* 23 (4) (2023) 1280–1288.
- [14] H.B. Zhang, L. Ma, R.X. Yin, et al., *Patent*, CN202110488516, 2021.
- [15] H.B. Zhang, K. Song, T.L. Song, et al., *Patent*, ZL202010439009.8, 2020.
- [16] M. Qiao, S. Wang, W. Fan, et al., *Mater. Express* 11 (2021) 445–451.
- [17] W. Sun, Z. Liu, J. Xu, et al., *Chin. Chem. Lett.* 34 (5) (2023) 107819.
- [18] A. Herland, A.D. Van Der Meer, et al., *PLoS One* 11 (3) (2016) e0150360.
- [19] P.H. Yang, F.Y. Zheng, Q.S. Li, et al., *Chin. J. Anal. Chem.* 50 (2) (2022) 97–101.
- [20] D. Caballero, S.M. Blackburn, M. de Pablo, et al., *Lab Chip* 17 (22) (2017) 3760–3771.
- [21] D.T.T. Phan, X. Wang, B.M. Craver, et al., *Lab Chip* 17 (2017) 511–520.
- [22] Z. Gu, M. Xie, S. Lv, et al., *Int. J. Bioprinting* 8 (2022) 619.
- [23] J. Zhao, X.Y. Liu, Y. Zhou, et al., *Chin. Chem. Lett.* 34 (2023) 107895.
- [24] Y.S. Zhang, J. Aleman, S.R. Shin, et al., *Proc. Natl. Acad. Sci. U. S. A.* 114 (12) (2017) E2293–E2302.
- [25] X. Ding, Y. Zhang, Y. Zhang, et al., *ACS Nano* 16 (10) (2022) 17376–17388.
- [26] W.L. Thompson, T. Takebe, *Methods Cell Bio.* 159 (2020) 47–68.