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3D Bioprinitng technologies for the enhancement and application of functional lung organoid models

Running title: 3D bioprinted multicellular lung organoids

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Abstract

Respiratory diseases, ranging from minor infections to serious chronic diseases and malignancies, negatively affect the respiratory system and are influenced by various environmental factors such as air pollution, occupational hazards, and tobacco smoke, as well as lifestyle, genetic causes, and infectious agents. The prevalence and severity of respiratory diseases requires the development of advanced models to better understand their pathophysiology and develop effective treatments. In this context, 3D bioprinting technology is emerging as an innovative tool to create functional lung organoid models. The use of induced pluripotent stem cells (iPSCs) and extracellular matrix (ECM) in bioprinting enables the development of organoids that closely mimic human lung tissue. Bioprinting-based organoids can better replicate the dynamic environment of the human lung, facilitating more accurate disease modeling and drug testing. In this review, we highlight the potential of bioprinted lung organoids in understanding the mechanisms of chronic respiratory diseases, testing the efficacy and safety of new drugs, and exploring regenerative medicine approaches. The integration of advanced bioprinting and organoid technologies is a promising field in respiratory disease research and treatment, offering new hope for patients suffering from lung diseases.

Keywords: Alveolar organoid; Chronic respiratory disease; Bioprinting; Extracellular matrix; Induced pluripotent stem cells

1. Introduction

1-1. Traditional animal models in pulmonary disease research and limitation

Respiratory diseases constitute a significant component of global health challenges, encompassing a wide range of conditions that adversely impact the respiratory system [1]. These conditions vary from mild infections to severe chronic disorders and malignancies, which significantly affect the quality of life and, in many instances, lead to considerable morbidity and mortality [2]. In the realm of drug development and respiratory research, creating and utilizing accurate disease models is crucial for understanding disease mechanisms and testing potential treatments. To replicate actual respiratory diseases, various agents including bleomycin (BLM), particulate matter (PM2.5), tobacco smoke, and porcine pancreatic elastase (PPE) have long been fundamental tools in the study of pulmonary diseases [3-6]. While the BLM model is widely used to study pulmonary fibrosis, it primarily induces acute lung injury, which typically resolves over time in mice [7]. Models utilizing tobacco smoke, PM2.5, and PPE exposure are essential for studying diseases like COPD [8]. In addition, lung cancer mouse models induced by gene mutations such as EGFR and P53 are also significant in research [9]. However, these models present substantial limitations that can hinder the translation of research findings to human applications. The rapid clearance mechanisms in mice contrast with the slower, more cumulative effects observed in human lungs, leading to differences in disease manifestation and severity. The differences in metabolism and immune responses between mice and humans can also result in variations in drug processing and side effects [10, 11]. Compounds metabolized differently can demonstrate efficacy or toxicity in mice that do not translate to humans [12]. This discrepancy poses a significant challenge to the predictive value of mouse models for human pharmacology. The use of animals in research, especially for conditions that could be studied through alternative methods, also raises ethical concerns. Furthermore, maintaining animal facilities is costly and resource-intensive, which could otherwise be allocated towards developing and refining alternative models. Given these limitations, there is an increasing emphasis on developing alternative models that can better mimic human lung diseases. Advances in organ-on-a-chip technology, 3D bioprinting, and ex vivo human lung tissue cultures offer

promising avenues. These technologies not only replicate human tissue architecture and physiological responses more accurately but also allow for the study of human-specific disease processes and treatment responses [13-15]. The shift towards these innovative models could significantly improve the predictive accuracy of preclinical trials and enhance the development of more effective therapies.

In conclusion, while traditional in vivo mouse models have provided foundational insights into pulmonary diseases, their limitations in translating findings to human conditions necessitate the development of advanced, human-relevant models. The integration of these new technologies into respiratory research and drug development is crucial for making significant strides in treating lung diseases.

1-2. The emergence of bioprinting technology for lung models

Previous studies have used either 2D cell culture methods or 3D constructs to create in vitro alveolar models. 2D cell culture methods involve growing cells in a flat, two-dimensional layer, which allows for easy observation and manipulation of cells [16]. These methods are cost-effective and relatively simple, making them a popular choice for initial studies. However, 2D cultures fail to replicate the three-dimensional environment of tissues, leading to differences in cell morphology, polarization, and function compared to in vivo conditions. This limitation hampers the study of cell-cell and cell-matrix interactions, which are crucial for understanding cellular behavior in a more physiologically relevant context [17]. On the other hand, 3D constructs provide a three-dimensional scaffold that supports more complex cell growth, allowing cells to interact with their surroundings in a manner more akin to natural tissues. These constructs can be created using a variety of materials, including hydrogels, which mimic the extracellular matrix (ECM) and provide biochemical cues to the cells [18]. 3D culture systems can better simulate the physical and biochemical environment of tissues, resulting in more accurate representations of cellular functions, differentiation patterns, and responses to stimuli. However, these approaches still face limitations, such as the difficulty in precisely controlling the spatial distribution of cells and materials, as well as the challenge of replicating the intricate architecture of native tissues at a high resolution [19]. To address these limitations, 3D bioprinting technology has emerged. This

technology allows for precise control over the spatial distribution of materials and cells, enabling the recreation of complex tissue structures using a variety of materials, including hydrogels and polymers [20]. Consequently, 3D bioprinting offers significant advantages for the development of in vitro alveolar models by better replicating the structural complexity of alveoli and enhancing the cellular environment. This is crucial for accurately representing cell growth, differentiation, and native physiological conditions, which are essential for applications such as drug screening and disease research [21].

This review focuses on the application of bioprinting technologies in modeling lung disease. Firstly, we examine organoids for disease modeling in the lung, discussing their advantages and limitations. Following this, we explore research involving 3D bioprinting technology, proposing strategies to overcome the aforementioned challenges. Despite the promise of these technologies, there are inherent limitations that need to be addressed. Finally, we delve into bioprinting-based lung organoid disease modeling techniques and strategies, discussing their potential and the future directions for this field.

2. Organoid

2-1. Lung structure and cellular function

The lungs are intricately structured and play a crucial role in the gas exchange processes essential to our body [22]. This vital function is enabled by the unique anatomical architecture and complex cellular composition of the lungs, which are divided into distinct parts: airways, alveoli, and blood vessels, with each component made up of highly specialized cells [23]. The primary gas exchange occurs in the alveoli, where a variety of cells and blood vessels, such as alveolar type I (AT1) cells, alveolar type II (AT2) cells, fibroblasts, and vascular endothelial cells (EC) perform essential roles [24].

AT1 cells cover most of the alveolar wall and are flat and thin, maximizing the efficiency of gas exchange [25]. Their extremely thin cell membranes allow oxygen and carbon dioxide to move rapidly between the blood and the alveoli [26]. While AT1 cells are essential for efficient gas exchange, they have limited regenerative capabilities [27]. Therefore, recovery from damage to these cells can be slow and sometimes incomplete, complicating lung repair processes [28]. AT2 cells play a critical role in regulating the microenvironment within the alveoli and are primarily responsible for the production and secretion of surfactant, which reduces surface tension within the alveoli to prevent collapse [29]. In addition, AT2 cells have the potential to differentiate into type I cells when necessary, thereby playing a critical role in lung tissue repair and regeneration [30]. The surfactant production and regenerative capabilities of AT2 cells are essential for maintaining overall lung health (Fig. 1A) [31].

Fibroblasts provide structural support to lung tissue by producing fibrous proteins such as collagen and elastin, and are crucial for maintaining the elasticity and structural integrity of the lungs [32]. However, excessive activation or abnormal proliferation of fibroblasts can lead to diseases such as pulmonary fibrosis [33]. Pulmonary fibrosis involves the abnormal hardening and functional degradation of lung tissue, primarily caused by the overactivity of fibroblasts [34]. ECs are essential for maintaining lung function by optimizing gas exchange in the lungs, regulating the transfer of substances between blood and tissue, and modulating inflammatory responses and blood clotting (Fig. 1B) [35]. In particular, disruption of the blood-air barrier due to damage or dysfunction of endothelial cells can be associated with a variety of pathological conditions, including various inflammatory lung diseases such

as pulmonary hypertension and pulmonary edema [36, 37]. The interactions among these various cell types help maintain proper lung functions and enable recovery from environmental damage. A deep understanding of the lungs' anatomical structure and the functions of these cells provides foundational knowledge that is crucial for the treatment and management of respiratory diseases [38]. This comprehensive understanding is essential for optimizing clinical interventions and developing more effective treatments for specific respiratory conditions.

2-2. Definition and description of organoid

An organoid is an assembly of cells cultured in three-dimensional environments to mimic the structure and function of a human organ or tissue [39]. This advanced technology is useful in various fields such as regenerative medicine, disease modeling, and drug development [40]. Traditional animal disease modeling and two-dimensional cell cultures are limited in their ability to accurately mimic human organs or disease pathogenesis. For example, lung diseases in humans are often mostly irreversible, whereas mouse animal models have a rapid recovery, which limits their ability to simulate diseases caused by cellular senescence and viral infections [41-43]. In addition, due to differences in gene and protein structures from humans, it is difficult to apply therapeutic strategies from animal disease models to humans due to clear differences in mechanisms [44]. Traditional two-dimensional cell cultures are grown as monolayers of cells on flat surfaces, which limits cell-cell interactions and spatial arrangements compared to real human organs and tissue. In contrast, organoids use real human cells, so they have the same genes as humans, and the tissue environment they create can mimic disease more accurately, which can be more precise in predicting how they will respond in the organ [45, 46]. They also use complex culture media, including extracellular matrix, to simulate the cells' natural environment. This not only provides physical support for the cells but also a variety of biochemical signals that regulate their differentiation and function [47].

2-3. Advantages and Limitation of lung organoid 3D modeling

Lung organoids are primarily generated using human pluripotent stem cells (hPSCs), which form the various cell types that compose lung tissue [48, 49]. During this process, they use the extracellular matrix to form three-dimensional structures, which can recreate various functions of the lungs. For example, the development of lung alveolar organoids can simulate the structure of air sacs in the lungs by inducing differentiation into AT1 cells for gas exchange and AT2 cells for repairing AT1 cells [50, 51]. In other studies, organoid models have also been reported to identify fibroblast activation, which is important for lung tissue repair and wound healing [52, 53]. Therefore, the main challenge in developing lung organoids is to simulate the developmental process and complex structure and function of the real human lung. In addition, bronchial organoids can be differentiated into various epithelial cells, including club cells, basal cells, and other cell types that exist in the real bronchi, to reproduce the secretion and function of mucus in the real bronchi [54, 55]. Recently, they have also been used to simulate lung development to study the mechanisms of genetic and infantile lung diseases such as bronchopulmonary dysplasia [56-58].

Currently, researchers are trying to overcome this challenge by optimizing the type of cells, three-dimensional culture conditions, and composition of the extracellular matrix. However, there are several limitations of current studies. First, there are limitations in perfectly recreating the structure of alveoli and bronchioles. For example, the significantly higher proportion of AT2/AT1 cells in alveolar organoids compared to human lung tissue indicates that organoids are not yet perfect mimics. This is probably because most organoids are made by inducing a certain amount of differentiation of AT2 into AT1 cells [59-61]. Second, lung organoids are mainly composed of cells derived from specific stem cells. Therefore, it is difficult to differentiate all cell types present in the lungs from stem cells. For example, ECs are key regulators of interleukins, which are important for homeostasis and inflammation [62-64]. In addition, immune cells such as macrophages regulate the lung microenvironment through the release of pro-inflammatory cytokines and chemokines [65-67]. The absence of these cells can lead to inaccurate results in drug efficacy testing and disease mechanism studies using organoids. Therefore, increasing the similarity of lung organoids to actual lung tissue is essential to enhance their potential.

In addition, developing lung organoid models for reproducible organoid production and mass production for high-throughput drug efficacy validation systems will improve their utility [68].

Recently, several studies have been reported to overcome these challenges. To overcome the limitation of alveolar organoids consisting of only AT1 and AT2 cells, Nádia et al cultured Wharton's jelly MSCs using sodium alginate and gelatin matrix bioprinting, and confirmed their differentiation into ciliated and goblet cells [69]. There is also active research to validate alveolar organoids to mimic the tumor microenvironment (TME). Krijn et al. co-cultured PBMCs from peripheral blood with patient-derived NSCLC organoids and demonstrated tumor suppression by T-cell responses [70]. The potential of lung organoids depends on their similarity to real lung tissue, and the composition of the microenvironment must continue to be studied.

3. Bioprinting Techniques for Lung Model Fabrication

Additive Manufacturing (AM), also known as 3D printing, involves creating components by layering materials [71]. AM systems are categorized into seven distinct processes based on how material layers are formed: Material Extrusion (MEX), where material is selectively dispensed through a nozzle or orifice; Vat Photopolymerization (VPP), which uses a vat of liquid photopolymer selectively cured by light-activated polymerization; Powder Bed Fusion (PBF), where thermal energy selectively fuses regions of a powder bed; Binder Jetting (BJT), in which a liquid binder is selectively deposited to bind powder materials; Material Jetting (MJT), where droplets of build material are selectively deposited using an inkjet print head; Directed Energy Deposition (DED), which utilizes focused thermal energy to fuse materials by melting them as they are being deposited; and Sheet Lamination (SHL), where sheets of material are bonded together to form a part. Among these seven methods, those applicable to bioprinting technology using living cells are MEX, VPP, and MJT. 3D printing and bioprinting, while similar in their layer-by-layer fabrication approach, differ significantly in their applications and materials used. 3D printing is primarily used for creating non-biological objects from materials such as plastics, metals, and ceramics, with common applications in manufacturing, prototyping, and the creation of complex mechanical parts [72]. It utilizes materials that are often rigid and non-living,

focusing on the structural integrity and functional aspects of the printed object. In contrast, bioprinting is specifically designed for creating biological constructs, including tissues and organs, using bioinks composed of living cells and biomaterials [73-75]. It aims to replicate the complex architecture of biological tissues, facilitating cell growth and function, with applications predominantly in the field of medicine and biological research, such as tissue engineering, regenerative medicine, and the development of in vitro models for drug testing. Bioprinting technology can be broadly categorized into three main types: extrusion based bioprinting (EBB), inkjet based bioprinting (IBB), and laser based bioprinting (LBB). These three bioprinting methods each have unique advantages and are chosen based on the specific requirements of the tissue engineering application (Fig. 2). These technologies holds significant potential for advancing tissue engineering, as it enables the creation of complex structures that closely mimic the natural architecture of biological tissues, such as lung tissue [76].

3-1. Extrusion based bioprinting

Extrusion-based bioprinting is one of the most commonly employed methods in the field of bioprinting due to its versatility and ability to handle a broad range of biomaterials, including hydrogels, biopolymers, and cell-laden bioinks [19, 77]. This technique operates by the controlled extrusion of bioink through a nozzle, which deposits material layer by layer to construct three-dimensional structures [78]. The continuous filament formation under regulated pressure allows EBB to effectively create scaffolds embedded with cells, resulting in complex tissue constructs [75].

For instance, Miller et al. demonstrated the use of EBB to fabricate a lung model integrated with a perfusable vascular network [79]. This development is critical for replicating the oxygenation functions of the lungs, as the vascular network facilitates efficient nutrient and oxygen delivery throughout the tissue. Additionally, extrusion-based bioprinting supports the integration of high cell densities and various biomaterials, making it suitable for the fabrication of large and intricate structures [80, 81]. It also allows for the incorporation of mechanical and biochemical cues within the printed scaffolds [82]. The capability to incorporate mechanical and biochemical cues within printed scaffolds further enhances the potential of EBB in tissue engineering applications.

Despite its advantages, EBB typically produces structures with lower resolution compared to other bioprinting techniques. he necessity for high-viscosity bioinks, which are required to maintain the structure's integrity during printing, can lead to challenges such as nozzle clogging and uneven cell distribution [78]. Moreover, the precision of cell placement and the fine resolution of the printed constructs are often compromised, which can affect the overall functionality and replicability of the bioprinted tissue.

3-2. Inkjet based bioprinting

Inkjet based bioprinting utilizes droplets of bioink ejected from a printhead to build structures layer by layer [83]. This method is known for its high resolution and ability to deposit small volumes of bioink with precise control [19]. Also, this technique uses thermal or piezoelectric actuators to generate droplets of bioink, which are then ejected onto a substrate in a controlled manner [84]. Inkjet bioprinting is particularly suitable for creating detailed tissue constructs and high-throughput applications [85].

Researchers have utilized inkjet bioprinting to create high-resolution lung models with precise cell placement. Dayoon Kang et al. developed a 3D pulmonary fibrosis model using inkjet bioprinting [86]. They layered endothelial cells, type I collagen, fibroblasts, AT1 and AT2 cells using inkjet bioprinting technology. In addition, they achieved high precision in cell placement and tissue architecture by creating microfabricated lung models with inkjet bioprinting. They reported that this layered structure mimics the alveolar barrier model and can easily induce epithelial-mesenchymal transition, an important pathogenesis in pulmonary fibrosis, and identify biomarker expression, allowing for quick and effective simulation of pulmonary fibrosis therapeutics.

Inkjet bioprinting offers high precision and resolution, making it suitable for fabricating intricate tissue structures [87]. It also allows for the use of low-viscosity bioinks, which can improve cell viability and functionality [85]. However, the low viscosity requirement limits the range of usable bioinks, and the shear forces generated during droplet ejection can potentially damage cells [83]. In addition, the technique is generally limited to producing relatively thin layers of tissue.

3-3. Laser based bioprinting

Laser based bioprinting, including laser-induced forward transfer (LIFT) and stereolithography (SLA), uses laser energy to pattern bioinks with high precision [88]. These methods leverage laser energy to pattern bioinks with high precision and resolution, making them particularly suitable for creating complex and detailed tissue constructs [89]. Laser-based bioprinting offers unparalleled control over the microarchitecture of printed tissues, which is essential for replicating the intricate structures of lung tissue [90]. LIFT is a non-contact bioprinting method that uses a pulsed laser to transfer bioink from a donor substrate to a receiver substrate. The laser energy creates a focused microbubble at the interface of the bioink and the donor substrate, propelling a droplet of bioink towards the receiver substrate [81]. This technique allows for high precision in droplet placement and minimal damage to cells due to the gentle transfer process. SLA is a photopolymerization-based bioprinting technique that uses a laser to selectively cure photo-crosslinkable bioinks layer by layer [88]. This method is highly precise and can produce structures with intricate details and smooth surfaces. SLA is particularly advantageous for fabricating scaffolds with complex geometries and internal features that are challenging to achieve with other bioprinting methods. Guillotin et al. demonstrated the use of LIFT to create high-resolution alveolar structures [91]. The precise control offered by LIFT allowed for the deposition of alveolar epithelial cells in defined patterns, closely mimicking the native architecture of lung alveoli. This approach enabled the formation of functional alveolar units with enhanced gas exchange capabilities. Zhu et al. utilized stereolithography to print lung models with detailed vascular networks [92]. By carefully controlling the laser parameters and the properties of the photo-crosslinkable bioinks, they were able to create microvascular structures that supported perfusion and enhanced the functionality of the printed lung tissue. These vascular networks are essential for providing nutrients and oxygen to the cells, thereby improving cell viability and tissue integration.

Laser-based bioprinting offers high precision and resolution, enabling the creation of intricate and detailed tissue structures [93]. The non-contact nature of LIFT minimizes cell damage, while the layer-by-layer approach of SLA allows for the fabrication of complex geometries [89]. These techniques also support the use of photo-crosslinkable bioinks, which can enhance the stability and functionality of the

printed constructs [91]. However, the requirement for photo-crosslinkable materials in SLA limits the range of usable bioinks [83]. In addition, the high cost and complexity of laser-based systems can be a barrier to widespread adoption [94]. The scalability of these methods for large-scale tissue production also remains a challenge [93].

3-4. 3D lung bioprinting and its considerations

When developing a lung model using 3D bioprinting, several critical considerations must be taken into account to ensure the model's functionality and relevance. One of the primary considerations is the selection of bioink, which must be carefully chosen to support cell viability, proliferation, and differentiation [19, 73]. The bioink should closely mimic the extracellular matrix (ECM) of lung tissue, providing the necessary biochemical and mechanical cues for appropriate cell behavior, including attachment, migration, and differentiation into specific lung cell types. The bioink composition often includes natural polymers like collagen, gelatin, and hyaluronic acid, which are known for their biocompatibility and ability to promote cell growth. The lung's complex and hierarchical structure, with its branched airways and alveoli, poses a significant challenge for bioprinting. Precise control over the bioprinting process is essential to ensure that the printed structures accurately replicate the native tissue's intricate geometry and functionality. This involves optimizing the printing resolution and layer thickness to create detailed and functional lung models. Techniques such as multi-material bioprinting can be employed to print different cell types and ECM components in a spatially controlled manner, closely mimicking the heterogeneous composition of lung tissue [74, 95]. Another important consideration is the mechanical properties of the printed lung model. The lung tissue is subjected to cyclic stretching and relaxation during respiration, so the printed structures must exhibit similar mechanical behavior to native lung tissue. This requires careful tuning of the bioink formulation and scaffold design to achieve the appropriate elasticity and strength. Incorporating materials that can undergo dynamic mechanical stimulation can help in replicating the physiological conditions of the lung, thereby enhancing the functionality and longevity of the bioprinted lung model. Furthermore, vascularization is a critical aspect of lung tissue engineering. The printed lung model must include a

perfusable vascular network to ensure the delivery of nutrients and oxygen to the cells, as well as the removal of metabolic waste. This can be achieved through advanced bioprinting techniques that allow for the incorporation of endothelial cells and the creation of microvascular structures within the lung model. The inclusion of vascular networks not only improves cell viability but also enhances the overall functionality of the bioprinted lung tissue. To facilitate widespread adoption and practical application, the bioprinting process must be both scalable and reproducible. This entails standardizing bioink formulations, printing parameters, and post-processing steps to ensure consistent and reliable results across different batches. Developing robust protocols for bioink preparation, bioprinting, and subsequent tissue maturation is crucial for achieving reproducibility. Additionally, integrating automated systems and real-time monitoring during the bioprinting process can further enhance the precision and consistency of the printed lung models [75, 96, 97].

4. Research on bioprinting-based 3D organoid and tissue modeling

Recently, there has been significant progress in utilizing 3D bioprinting technology to replicate the structure and function of lungs, thereby enhancing the ease of organoid creation. Agnieszka et al used extrusion-based bioprinting (EBB) to develop a lab-on-a-chip device that simulates the mechanical and biological environment of the lung. The researchers created six different hydrogel inks by varying the proportions of sodium alginate, agar, chitosan, gelatin, and methylcellulose, and optimized them with different bioprinting parameters. The hydrogel-based lab-on-chip (LOC) composed of 3% sodium alginate, 7% gelatin, and 90% NaCl showed the highest cell viability and had similar elasticity modulus values to biological tissues (0.060–0.512 MPa) at 37 °C conditions [98]. While bioprinting has not yet fully simulated lung structure, scaffolds have been developed that are conducive to cell culture for use in in vitro studies. Amparo Guerrero et al. created a hydrogel mixture by combining Matrigel, porcine skin gelatin, and sodium alginate. To conduct Three-Dimensional Bioprinting of Organoid-Based Scaffolds (OBST), the layered structure was based on 10% porcine skin gelatin and 10% Matrigel, with variable proportions of sodium alginate. Considering the viscosity to maintain cell comfort, the sodium alginate concentration was fixed at 2% at 35 °C and the extrusion speed at 13 mm/s. The hydrogel

mixture was then combined with Calu-3 cells, a human lung adenocarcinoma cell line, and subsequently bioprinted. They reported that bioprinted Calu-3 cells treated with colloidal toxic silver nanoparticles (AgNPs), known for their potential antitumor properties, exhibited IC 50 values similar to toxicological studies conducted in mice [99]. Anna Urociuolo et al. demonstrated that a photosensitive polymer, 7-hydroxycoumarin-3-carboxylic acid (HCCA)-gelatin, can be added to Matrigel and subsequently cross-linked during cell culture using two-photon mediated bioprinting [100]. The researchers added liquid HCC-Gelatin to the existing matrigel and incubated it for 15 minutes before photocrosslinking using bioprinting. The cross-linked hydrogel maintained structural integrity after incubating the matrigel droplets in DMEM for 2 days and showed accuracy with minimal linewidth (1.5 ± 0.8 μm), even when multiple hydrogel parallelepipeds of different heights were fabricated and then compared. This technique can be utilized to cross-link tissue constructs in cell culture to mimic geometric constraints such as cell migration or structural features in bud-tip real-time as the lung develops (Fig.4C). Choi et al. incorporated patient-derived lung cancer organoids with porcine lung-derived decellularized extracellular matrix (LudECM) hydrogel to establish a lung cancer microenvironment and provide physical and biochemical signals [101]. In this particular study, bioprinting demonstrated a new potential to overcome the absence of blood vessels, which is a typical limitation of lung organoids (Fig.4D). Resistance to targeted anti-cancer drugs in lung cancer organoids was significantly higher in LudECM compared to conventional Matrigel. In addition, they demonstrated the creation a lung model with a perfusable vascular network by use of EBB. This model is crucial for mimicking the oxygenation function of the lungs, as it allows for the integration of a functional blood supply within the organoid. Chambers for culturing lung cancer organoids (LCOs), IPF-derived lung fibroblasts (ILFs), and ECs were printed in PEVA Blood vessel constructs were then printed in gelatin, after which the ILF bioink was treated and the gelatin was removed. These ILFs were cultured in LudECM for one day and then further cultured with ECs. Finally, LCOs were printed in the fibroblast area in the blood vessels. The resulting bioprinting-based vascular constructs were stable in shear recovery evaluation. This study highlights the potential of 3D bioprinting in creating complex, multi-cellular structures that are essential for accurately replicating lung physiology [102]. The field of 3D bioprinting for organoid and tissue

modeling is poised for remarkable advancements, driven by ongoing research and technological innovation. One promising direction is the integration of advanced bioinks, which can provide better support for cell viability and functionality. These bioinks, enriched with growth factors, extracellular matrix components, and other biomolecules, could significantly enhance the complexity and functionality of bioprinted tissues [103, 104]. In addition, the development of more sophisticated bioprinting techniques, such as light-assisted bioprinting [105], volumetric bioprinting [106], and microfluidics-assisted bioprinting [107] can improve the precision and resolution of tissue structures, enabling the reproduction of complex vascular networks and organ-specific microstructures. These advancements are crucial for overcoming current limitations in vascularization and tissue complexity. We have summarized the various bioprinting-based 3D lung modeling approaches in Table 1, Fig. 3 and Fig. 4.

Despite these advancements, several limitations and challenges remain in the development of vascularized lung organoid models. Replicating the complex branching and hierarchical structure of native blood vessels is particularly challenging. Current bioprinting techniques often struggle to achieve the fine resolution required to accurately recreate these intricate networks. Maintaining perfusion and ensuring the long-term culture of vascularized lung organoids are additional challenges [108]. The development of bioreactors and dynamic culture systems that can provide a continuous supply of nutrients and oxygen is essential for addressing these issues. Collaboration between biologists, engineers, and clinicians will be crucial for translating bioprinting advancements into clinical settings. Regulatory frameworks and ethical guidelines will need to evolve in tandem with technological progress to ensure the safe and effective use of bioprinted tissues in regenerative medicine, drug testing, and disease modeling [109].

5. Conclusion

In summary, organoid and tissue modeling research using 3D bioprinting has shown significant progress in recreating complex tissue structures and modeling disease environments. By applying bioprinting technologies, these studies overcome the limitations of conventional 3D tissue modeling and pave the way for future innovations toward the bioprinting of therapeutic functional organs [110]. However, many challenges remain, such as improving the similarity and stability of bioprinted structures and integrating functional vascular networks [111]. In addition, significant improvements are needed in achieving uniform cell distribution and vascularization within the printed structures. Continued research and collaboration between biomedical engineering, materials science, and clinical disciplines is essential to resolve these challenges and fully realize the potential of bioprinting in medical science.

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Conflict of interest

The authors declare they have no competing interests

Author contributions

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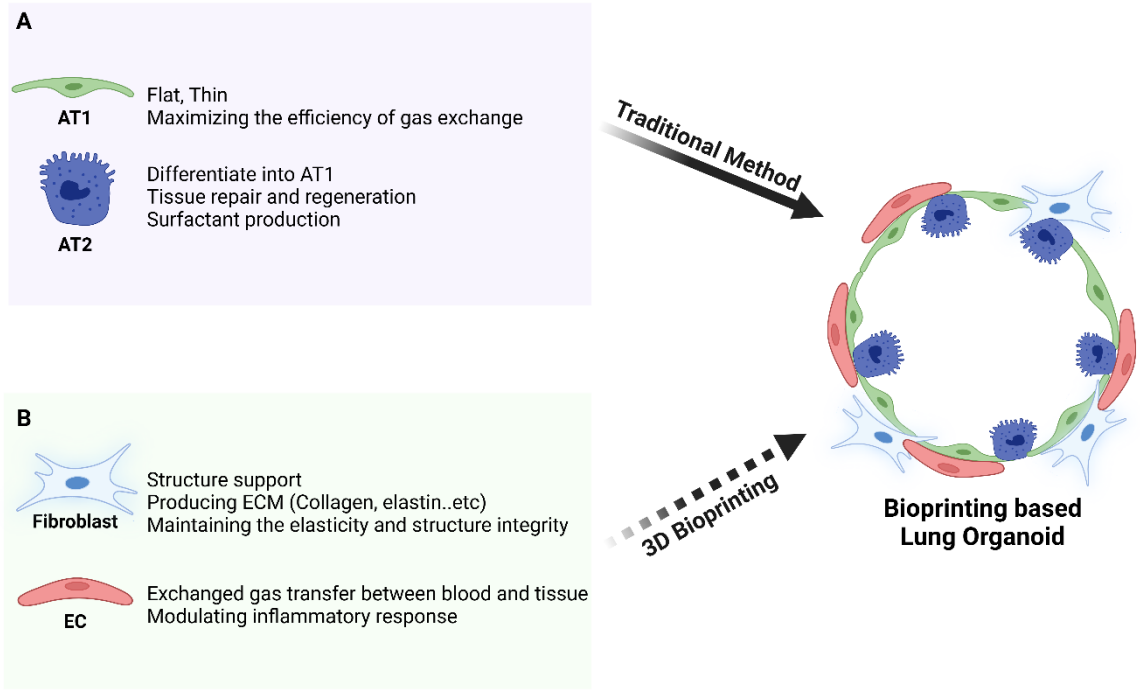

Figure 1. Schematic of lung cellular function and Lung organoid. The structure of the lung is largely composed of AT1, AT2, fibroblasts, and ECs. Each cell has a unique function and regulates the microenvironment within the lung parenchyma. Generally, lung organoids are developed using the differentiation of only AT1 and AT2, however organoids containing fibroblasts and ECs are being studied by applying 3D bioprinting.

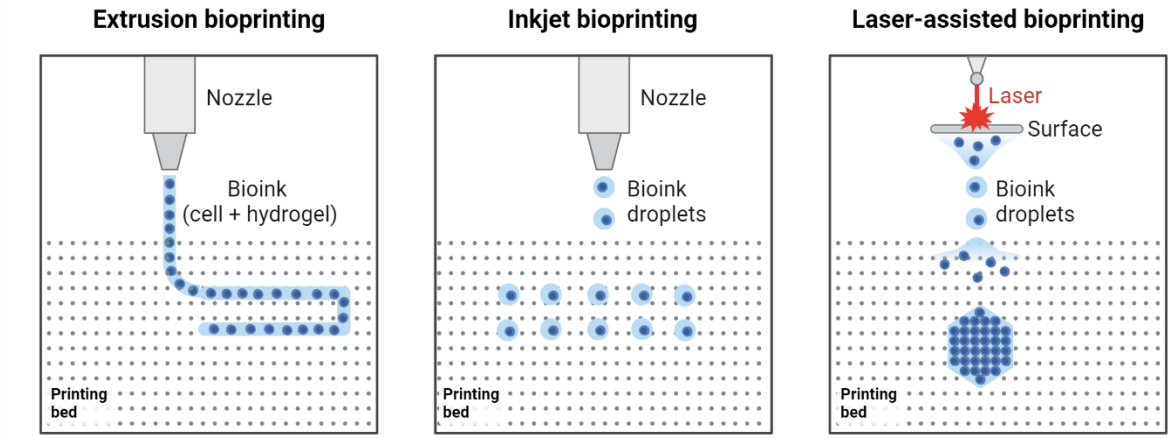

Figure 2. Schematic of three main bioprinting techniques. There are three main bioprinting techniques including extrusion-based, inkjet-based, and laser-based bio-printings. This figure adapted from "Types of Additive Manufacturing in Bioprinting Processes", by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates.

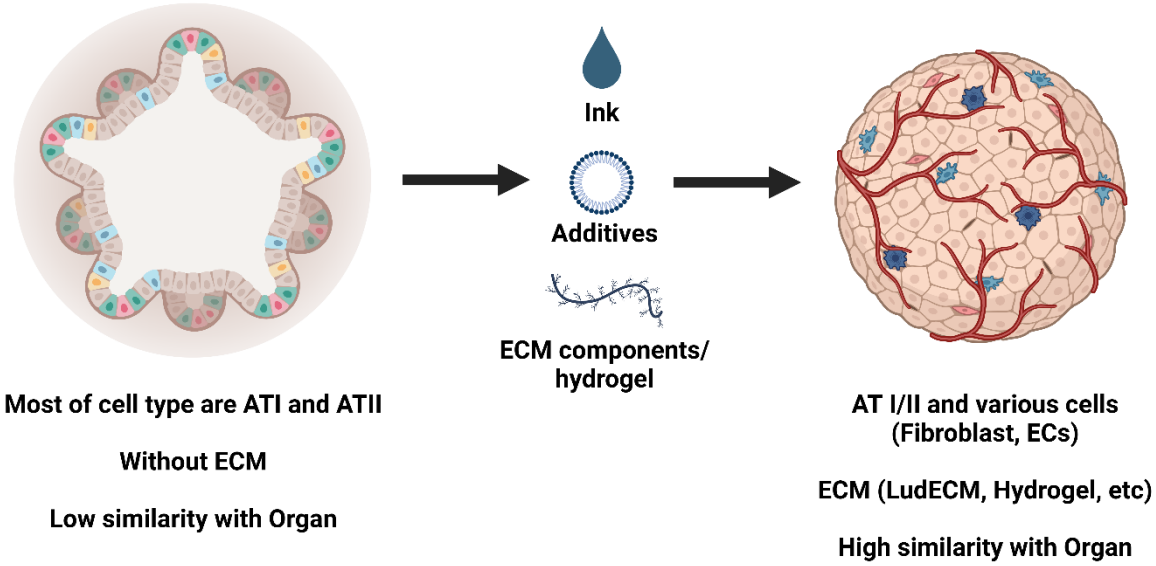

Figure 3. Improved structure of a lung organoid using 3D bioprinting. Traditional organoids generally consist of only AT1 and 2 cells without ECM, resulting in a low similarity to the lung. In the other hand, 3D bioprinting-based lung organoid contains various cell types (Fibroblast, vascular ECs) and ECM, improving the similarity to organ.

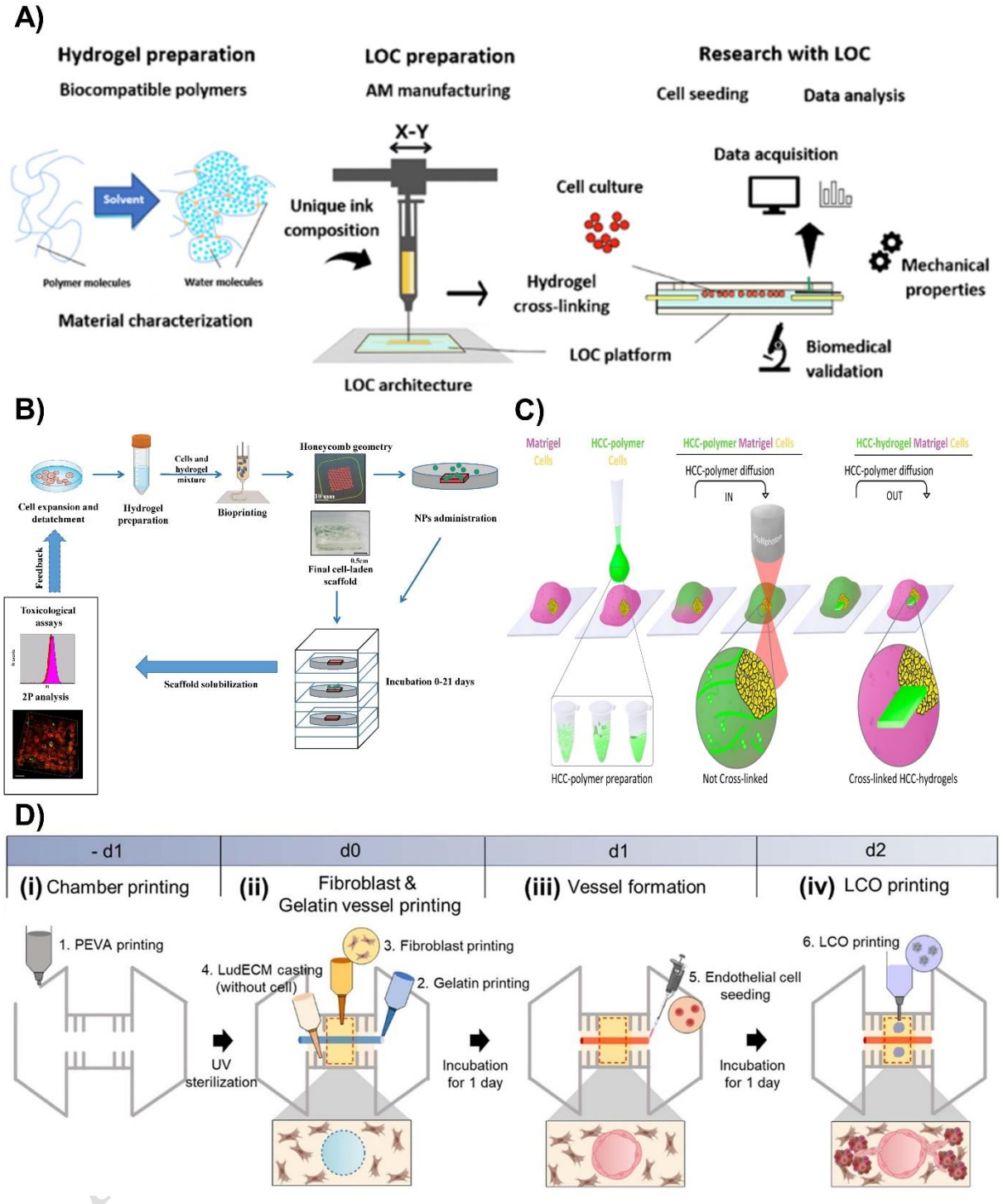

Figure 4. Researches for the production of lung 3D models. A) Schematic overview of the study showing the development of a LOC platform using six hydrogel inks [98]. B) Protocol for the study of silver nanoparticle(AgNPs) toxicology using organoid-based scaffold [99]. C) Method for hydrogel-in-hydrogel live bioprinting using two-photon (2P) bioprinting [100]. D) Schematic of fabrication of vascularized LCO models including lung tissue-specific ECM components and LCOs, stroma cells, and vascular [101]. © IOP Publishing. Reproduced with permission. All rights reserved.

Table 1. Bioprinting-based 3D lung modeling

Bio printing	Cell or Material	Printing parameters	Mimic or Improvement	Results	Ref
IBB	Epithelial, Endothelial, Collagen1, Fibroblasts	80-µm-sized (piezoelectric nozzle) 3 m s^{-1} (jet speed) 10 µs (rise, fall, dwell time) ±80 V (peak drive voltage)	Alveolar barrier models	Easily induce epithelial-mesenchymal transition	[86]
EBB	Sodium alginate Agar Chitosan Gelatin Methylcellulose NaCl	28 °C (Ink) 13-15 mm/s 10-12 kPa 27 °C (table) 60 °C (head)	Lab-on-a-chip (LOC)	Lung cancer based LOC analysis	[98]
EBB	Hydrogel (Calu-3 cells, AgNPs)	10% porcine skin gelatin 10% Matrigel 2% Sodium alginate 35 °C (Ink) 13 mm/s	Technology for cell culture	Long-term culturing of cell lines due to the reduction in oxidative stress	[99]
LBB	Hydrogel (HCCA-gelatin-Matrigel crosslinked)	1 mW (laser power) 800 nm (wavelength)	Lung development (Bud tip)	Dynamic fabrication, Live modulation	[100]
EBB	PEVA LudECM	25 G metal nozzle, 500 kPA (PEVA Chamber) 5 to 12 kPa (1% w/v LudECM) within the iLFs region	Vascularized lung cancer	Anti-cancer drug evaluation	[101]
LBB	Hydrogel (water and poly-ethylene glycol-diacrylate)	20 wt % 6-kDa PEGDA 5 pl (voxel resolution) 1 h (print time)	Vascularized alveolar model topology	Control over tissue architecture and design of regenerative tissues	[105]