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REVIEW ARTICLE

Multiscale 3D bioprinting for the recapitulation of lung tissue

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Abstract

Lung tissue engineering (LTE) has gained significant attention as a highly promising and innovative strategy to tackle the formidable obstacles posed by lung-related diseases and the lack of compatible donor organs availability. In the realm of groundbreaking advancements in tissue engineering (TE), one particular technology that has emerged as a game-changer is three-dimensional (3D) bioprinting. It distinguishes itself by offering a potent and versatile approach to constructing intricate structures while opening up new horizons for TE and regenerative medicine (RM). This review focuses on the application of multiscale 3D bioprinting techniques in LTE and the reconstitution of lung tissue *in vitro*. We analyzed the key aspects such as bioink formulations and printing strategies utilized from macroscale 3D bioprinting to micro/nanoscale 3D bioprinting. Additionally, we evaluated the potential of multiscale bioprinting to replicate the complex architecture of the lung, ranging from macrostructures to micro/nanoscale features. We discussed the challenges and future directions in biofabrication approaches for LTE. Furthermore, we highlight the current progress and future perspectives in tissue reconstitution of lung *in vitro*, considering factors such as cell source, functionalization, and integration of physiological cues. Overall, multiscale 3D bioprinting offers exciting possibilities for the development of functional lung tissues, enabling disease modeling, new drug screening, and personalized regenerative therapies.

Keywords: Multiscale 3D bioprinting; Lung tissue engineering; Biofabrication; Tissue reconstitution *in vitro*

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1. Introduction

Lung tissue engineering (LTE) represents a dynamically progressing domain, with continuous research endeavors directed toward surmounting the obstacles linked to the

expansion of functional lung tissue production, enhancing vascularization approaches, and ensuring sustained long-term functionality[1,2]. Conditions such as chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and lung cancer pose significant challenges to global health, severely impacting patients' quality of life and overall prognosis[3,4]. LTE is of paramount importance in the context of lung diseases, as it holds great potential for revolutionizing innovative strategies to replace or repair damaged lung tissue by leveraging the principles of biology, materials science, and engineering[5]. The ultimate goal is to provide effective treatments and potential cures for regenerative medicine (RM), offering new possibilities for patients requiring respiratory interventions. Especially, LTE combined with three-dimensional (3D) bioprinting holds great promise for advancing our understanding of lung diseases, developing new therapies, and potentially providing transplantable lung tissue in the future.

Bioprinting, as defined by the American Society for Testing Materials (ASTM), is a specific method used to 3D-print biomaterials into various structures. 3D bioprinting is a specific technique used within the broader field of tissue engineering (TE), to precisely deposit cells, biomaterials, and growth factors in a 3D manner to create complex structures[6,7]. The common strategies of 3D bioprinting include inkjet-based bioprinting, extrusion-based bioprinting, and laser-assisted (e.g., stereolithography) bioprinting (Figure 1)[8]. Over the past few decades, the field of 3D bioprinting has experienced significant advancements in terms of the types of tissue models that can be constructed, including cancer[9], blood vessels[10,11], heart[12], and lungs[13,14]. Indeed, 3D bioprinting has the potential to offer various benefits and applications beyond just lung transplantation. 3D bioprinting allows the creation of patient-specific tissues and organs, tailored to individual needs. Additionally, researchers can create disease-specific models using bioprinting, allowing them to study the effects of drugs on specific tissues or organs without endangering patients[9,15,16]. Although this technology is still in its early stages, researchers have made progress in generating small, simplified organs like liver patches, kidney tissues, and more[17]. Therefore, the ability of 3D bioplotting to recreate the lung tissue allows researchers to investigate disease progression, cellular interactions, and responses to different drugs or treatments[18]. 3D bioprinting has emerged as a transformative tool that enables the creation of intricate 3D structures across different scales, ranging from macroscale to microscale and even nanoscale.

Considering the complex structure and dynamic characteristics of lung, researchers have made various summaries[19,20]. 3D bioprinting enables the construction of artificial lung tissues or lung organs at the macroscale, precise tissue compositions at the microscale, and even cell and molecular compositions at the nanoscale. Although extensive work has been done on the 3D bioprinting for LTE, there, to the best of our knowledge, appears no review paper in this field. In this review, we present a comprehensive overview of the principles and recent advancements in 3D bioprinting for LTE. We proceed to explore crucial elements such as the composition of bioink and the printing methodologies employed, and explore the potential of multiscale bioprinting to faithfully reproduce the intricate architecture of the lung, ranging from macrostructures to nanoscale features. Furthermore, we emphasized the current progress and future perspectives in the *in vitro* reconstitution of lung tissue, covering crucial considerations like cell sourcing, functionalization, and integration of physiological cues. With these groundbreaking techniques, a new era is dawning in the realm of lung tissue development, opening doors to functional and biologically accurate constructs. This remarkable progress promises to revolutionize disease modeling, drug screening, and RM for lung conditions.

2. Material inks for lung tissue fabrication

As the native extracellular matrix (ECM) can offer structural support to tissues, it is important to find an engineered ECM that can serve the same purpose. Material inks play a crucial role in TE as they provide scaffolds for cell growth and differentiation, facilitating the formation of functional tissues[21]. In the context of lung fabrication, several biomaterials are being explored for creating lung tissue *in vitro*. For example, hydrogels (including alginate, collagen, gelatin, and fibrin) are water-based materials that can mimic pulmonary ECM[18]. They provide a 3D environment for cells to grow and can be engineered to have specific mechanical properties and biochemical cues. Additionally, biocompatible and biodegradable synthetic polymers like poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and polyethylene glycol (PEG) are commonly used in 3D printing or melded into the scaffolds. In addition, decellularized ECM (dECM) encompasses the characteristics of an ideal tissue scaffold: complex composition, vascular networks, and unique tissue-specific architecture[22,23]. Therefore, dECM has emerged as a potential biomaterial ink with tissue-specific composition for LTE[24]. During the fabrication process, microcarrier inks, which are small, spherical particles, play a role in carrying and protecting cells. Furthermore, the incorporation of nanofibers and nanoparticles can enhance the mechanical properties and surface area of the nanoscale lung scaffolds. To achieve lung-like structures with appropriate architecture and functionality,

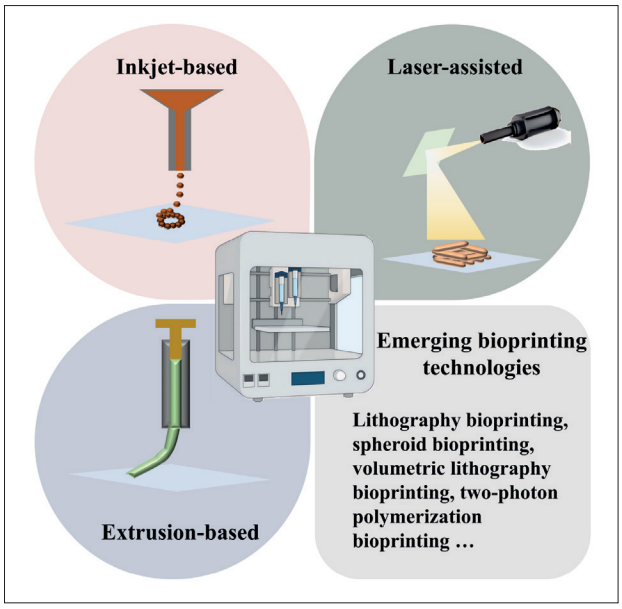

Figure 1. Types of 3D bioprinting, including inkjet-based bioprinting, extrusion-based bioprinting, laser-assisted bioprinting, and other emerging technologies. Created with BioRender (www.biorender.com).

a combination of these biomaterials with advanced 3D bioprinting techniques proves highly promising.

3. Macroscale 3D bioprinting

3.1. Macroscale 3D bioprinting techniques

Macrobioprinting application addresses the medical requirements to develop transplantable tissue structures that meet feasible anatomical dimensions on a macroscale[25]. Macroscale 3D bioprinting focuses on creating larger and more complex tissue constructs (e.g., artificial trachea) that closely resemble natural tissues in terms of their macroscopic features[26]. Macroscale bioprinting materials and strategies have shown significant progress in TE, encompassing the overall shape, structure, and volume of printed biological tissues[27]. Macroscale 3D bioprinting involves the use of larger bioink formulations and printing strategies to create 3D structures[28]. These bioink materials need to possess biocompatibility, biodegradability, bioabsorbability, and printability. The bioink also incorporates biomaterials, which act as a support structure and provide cues for cell growth and tissue formation. Commonly used biomaterials in macroscale bioprinting include hydrogels (e.g., alginate, gelatin, or collagen) or synthetic polymers[29]. In addition to biomaterials, macroscale bioprinting allows for the printing of various cell types, including stem cells, differentiated cells, or a combination of multiple cell types[30]. Macroscale bioprinting employs different strategies to deposit bioink and build 3D structures. The most common methods include extrusion-based bioprinting, where bioink is extruded through a nozzle or a syringe, lithography bioprinting, and inkjet-based bioprinting, where small droplets of bioink are deposited layer by layer. To achieve the desired tissue morphology (especially the complex respiratory system) in macroscale bioprinting, various factors need to be considered, including the biomaterial inks, the design of printing patterns, and the optimization of printing parameters.

Macroscale 3D bioprinting for lung tissue involves the fabrication of larger-scale structures that mimic the architecture and functionality of native lung tissue[31]. By accurately depositing material inks and creating appropriate scaffolds, researchers can recreate the structural organization necessary for proper lung function (Figure 2A). The construction of macroscale lung tissue can be deposited layer by layer using techniques like 3D bioprinting or assembled into larger structures to mimic the desired lung tissue architecture[32]. This technology facilitates precise deposition of biomaterials, such as hydrogels, in a controlled manner, enabling the construction of intricate structures that mimic the native macroscale tissues[33]. Macroscale 3D bioprinting for lung tissue holds promise for various applications and potentially transplantation in the future.

3.2. Macroscale 3D bioprinting for lung tissue recapitulation and application

3D spheroid bioprinting technology has the potential to create human lung tissues on a macroscale, utilizing biomaterial scaffolds, which can include the intricate

network of artificial trachea, blood vessels, and alveoli. Researchers designed and developed a tissue-specific photo-crosslinked bioink, and applied 3D bioprinting technology to construct a bionic trachea with alternate cartilage-vascularized fibrous tissue (Figure 2B)[34]. Multiscale vascularization remains a critical challenge in LTE. In order to study the lung tissue with natural morphological structure, scientists attempted to incorporate the structural attributes of the natural distal lung into a bioinspired model that mimicked the alveolar morphology and facilitated oxygen transport[35]. They used a hydrogel that can support mechanical stretching during the process of collecting air in the small airways' circulation, and the size of the 3D lung model printed is like a coin. Furthermore, researchers successfully prepared a human alveolar lung model *in vitro* through macroscale 3D bioprinting[36]. This lung model has collagen matrix, alveolar lung epithelium, endothelium, and fibroblasts, and maintains high cell vitality, proliferation, and viability in this printed structure. Moreover, to reproduce the 3D pulmonary cyst-like architecture, particularly alveoli epithelial side, researchers have successfully generated epithelial cysts utilizing the macroscale 3D bioprinting[37]. By incorporating epithelial cysts as a cellular component within material inks, it becomes feasible to hierarchically structure them through bioprinting, ultimately leading to the creation of constructs that closely resemble alveoli.

The trachea-like engineered lung tissues can serve as models for studying lung diseases like tracheal stenosis[38]. In addition, through the incorporation of advanced imaging techniques, computer-aided design models, and

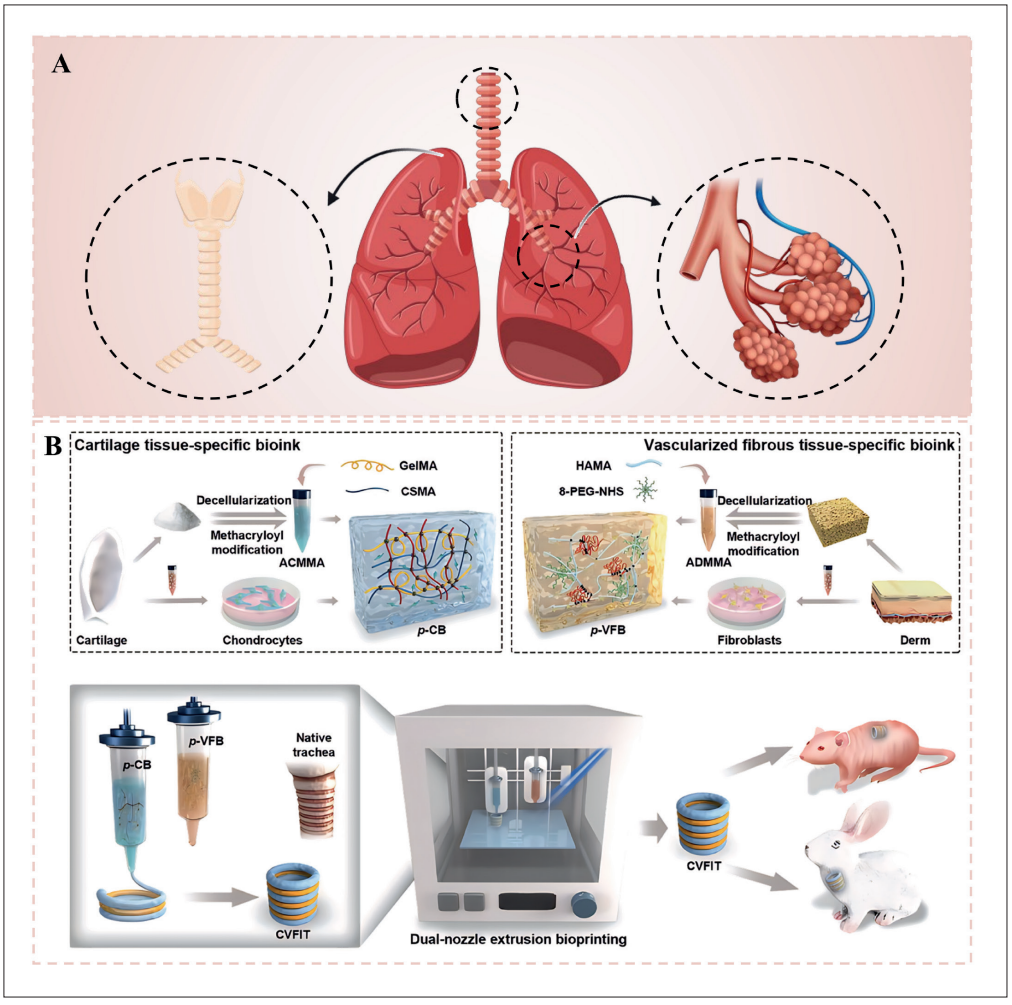

Figure 2. Macroscale 3D bioprinting for lung tissue. (A) Schematic diagram of pulmonary macroscale structure. Created with BioRender (www.biorender.com). (B) Schematic illustration of the designs of cartilage tissue-specific and vascularized fibrous tissue-specific bioinks and the 3D-bioprinted CVFIT for trachea regeneration in nude mice and *in situ* trachea reconstruction of rabbits[34]. Reprinted (and adapted) with permission from John Wiley and Sons. Copyright © *2022 The Authors. Advanced Science published by Wiley-VCH GmbH.* Abbreviations: CVFIT, cartilage-vascularized fibrous tissue-integrated trachea; *p*-CB, photo-crosslinkable cartilage-specific bioink; *p*-VFB: photo-crosslinkable-vascularized fibrous tissue-specific bioink.

precise robotic control systems, macroscale bioprinting facilitates the fabrication of scaffold-free isogenic artificial tracheas, which can be utilized as tracheal grafts in rats[39]. Researchers have demonstrated the transplantation of a macroscale 3D-printed trachea that mimics the natural trachea into a rabbit model to enhance the regeneration of tracheal mucosa and cartilage[40]. In another study, a macroscopic structure composed of lung epithelial cells printed on the basis of primary lung fibroblasts and monocyte cells was used to reconstruct alveolar model (about 7 mm long) *in vitro* to detect influenza virus infection[41]. These constructs can be used to study lung development, investigate disease mechanisms, and develop new therapies.

4. Microscale 3D bioprinting

4.1. Microscale 3D bioprinting techniques

Research on microscale systems to reconstruct local microenvironmental cues and microscale characteristics is also worthy of attention for realizing pulmonary structure functions *in vitro*[42]. Within the living organism, cells reside in a complex microenvironment consisting of diverse biophysical and biochemical cues[43,44]. Microscale bioprinting refers to the fabrication of structures at a smaller scale, typically in the range of micrometers[45,46]. Microscale 3D bioprinting constructs aim to replicate the complex biochemical and biophysical processes that occur within and between cells in living tissues[47,48]. This approach offers several advantages, including enhanced precision, increased resolution, and improved control over cell placement, which are crucial for mimicking the natural cellular composition and organization found in native tissues and organs[49,50]. Commonly used strategies are laser-assisted bioprinting and inkjet-based bioprinting. Laser-assisted bioprinting realizes the printing of photosensitive bioink by using plane projection, while inkjet-based bioprinting uses a piezoelectric printhead to deposit droplets of bioink onto a substrate. Such strategies enable us to create complex microscale tissue structures. In microscale bioprinting, bioinks must possess specific properties, such as shear-thinning behavior (to enable extrusion), biocompatibility, and appropriate rheological properties (to simulate lung stretching) for precise printing. The bioprinted cells can interact with the surrounding ECM or biomaterials[51,52]. By controlling the printing parameters and the composition of the bioink, researchers can achieve desired mechanical properties, cell densities, and functionalities within the printed microscale constructs[53,54]. For example, hydrogels made from human lung dECM can resemble the biophysical traits of native lung tissue[55].

Micro lung structure refers to the detailed anatomical components and organization of the lung at the microscopic level. It involves the study and understanding of the intricate structures within the lung tissue, such as the alveoli, bronchioles, capillaries, and various types of cells[56]. The small airways also play a crucial role in the microstructure of the lung and distribute air to the alveoli and help regulate airflow within the lungs. The alveoli are surrounded by a network of capillaries, allowing for efficient exchange of air between the alveoli and the blood (Figure 3A). In the blood–gas barrier, the proximity between an alveolus and a capillary is approximately 0.5 μm, facilitating gas exchange through the process of diffusion[57]. Continued research and advancements in microscale bioprinting hold huge promise for the advancements of functional lung tissues as well as lung tissue recapitulation and application in future.

4.2. Microscale 3D bioprinting for lung tissue recapitulation and application

Microscale bioprinting materials and strategies offer precise control over the fabrication of lung tissue constructs at a smaller scale, which enable the creation of intricate structures, mimic the native lung microenvironment, and promote cell viability and functionality[58,59]. Using 3D bioprinter with a printing resolution in the micrometer range, researchers printed a complex engineering microscale 3D air–blood tissue barrier for safety assessment and drug efficacy testing (Figure 3B)[60]. This development is expected to pave the way for high-throughput drug screening *in vitro*. Due to the impossibility of a single material ink to establish a "synthetic" microenvironment that accurately simulates the *in vivo* conditions, there has been a growing emphasis on multimaterial bioprinting[61]. Researchers developed a groundbreaking material ink by combining alginate with dECM, showcasing its remarkable ability to maintain biological activity during the entire process of 3D-bioprinting intricate and mechanically resilient tissues, both during and after printing (Figure 3C)[62]. Through their research, it was discovered that the enhanced bioink, enriched with lung dECM, exhibited remarkable potential for 3D bioprinting of subsegmental human bronchus. This bioink consisted of primary human lung smooth muscle cells and primary airway epithelial progenitor cells, which possessed the capacity to differentiate into diverse cell types typically found in the airway.

The progress of microscale 3D bioprinting has significantly advanced the simulation of lung diseases *in vitro*. In a recent and influential study, scientists made a significant breakthrough by 3D bioprinting, which has proven to be a valuable model for investigating influenza infection within the lung[63]. Additionally, studies have demonstrated the feasibility of bioprinting microscale lung using acellular porcine lung hydrogel without external crosslinking, using

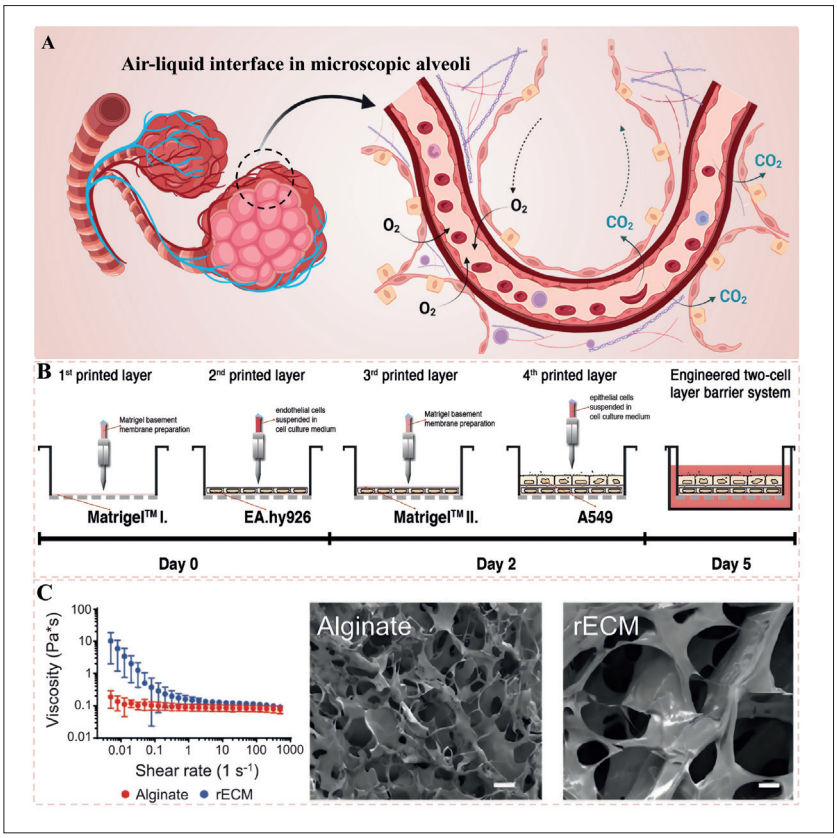

Figure 3. Microscale 3D bioprinting for lung tissue. (A) Schematic diagram of pulmonary microscale structure. Created with BioRender (www.biorender.com). (B) Schematic of the timeline for bioprinting the two cell-layer barrier system[60]. Reprinted (and adapted) with permission from Springer Nature. Copyright © *2015, The Author(s).* (C) Biophysical characteristics of reinforced ECM (rECM) for microscale bioprinting and observation under scanning electron microscope (scale bars = 50 μm)[62]. Reprinted (and adapted) with permission from John Wiley and Sons. Copyright © *2022 The Authors. Advanced Science published by Wiley-VCH GmbH.*

microscale 3D bioprinting technology[64]. For instance, a thrombosis chip was constructed, offering insights into effective treatment strategies for pulmonary embolism[65]. In a separate investigation, researchers employed a bioink composed of gelatin-sodium alginate blended with a suspension of lung cancer cells A549/95-D. By utilizing 3D bioprinting technology, they successfully created a tumor-like lung cancer model[66]. However, the field still faces numerous challenges that must be addressed, especially concerning the development of materials and techniques specifically tailored for lung cells. One of the key objectives is to enable the formation of nanoscale architecture within the air–blood barrier, necessitating further research and innovation.

5. Nanoscale 3D bioprinting

5.1. Nanoscale 3D bioprinting techniques

Nanoscale microenvironment features, such as ridges, steps, and grooves, have a significant impact on cell attachment, proliferation, and cytoskeletal assembly[67]. With the advancing integration of bioprinting and nanomaterials, engineered tissues are expected to achieve higher levels of complexity and functionality, gradually approaching the level of complete organ replicas[68,69]. Nanocomposite material inks have caught scientists' attention, considering the pulmonary complexity[70,71]. Over the years, the convergence of 3D bioprinting with nanotechnology in lung reconstruction *in vitro* has gained increasing attention. Nanoscale 3D bioprinting entails the precise positioning of biomaterials, cells, and nanoparticles at the nanoscale resolution[72]. Nanoscale 3D bioprinting encompasses bioprinting of cells in precise distribution and arrangement, thereby facilitating their interaction with the matrix, optimizing cell density in tissues, and orchestrating biochemical and biophysical processes within and between cells[73]. Nanoscale 3D bioprinting techniques, such as two-photon polymerization or laser-assisted forward transfer (LIFT), enable precise layer-by-layer deposition of the bioink, facilitating the construction of desired structures at the nanoscale. Nanoscale bioprinting takes this process a step further by integrating nanomaterials, nanoparticles,

or nanofibers into the material inks, thereby providing enhanced control over the cellular microenvironment and facilitating tissue development (Figure 4A). The process of nanoscale 3D bioprinting typically starts with the formulation of a bioink, which is a specialized material comprising living cells and biomaterials. Nanocomposite materials find widespread use due to their ability to enhance the mechanical traits of hybrid organic/inorganic composites[74]. Engineered nanofiber networks play a crucial role in promoting cellular growth and regulating cellular behaviors in a manner that closely emulates physiological conditions[75]. The application of nanotechnologies has been instrumental in engineering nanofibrous and nanocomposite structures, as well as nanoscale surface topographies and networks within scaffolds. These advancements effectively replicate the intricate nanoscale structure of various tissue types, including lung tissue. A remarkable advancement in research is the development of a transparent biomimetic nanoscale fibrillar matrix gel, offering flexibility in choosing bioink materials[76].

Nanoscale structure refers to the detailed anatomical components and organization of the lung at the nanoscale level[77]. It involves the study and understanding of the structures and processes that occur at the nanoscale within the lung tissue. The alveoli, which are the smallest pulmonary functional units, have complex nanostructures. The walls of the alveoli are extremely thin, facilitating efficient gas exchange[78]. The alveoli are lined with a surfactant layer, which is composed of lipids and proteins. These surfactant monolayers play a critical role in reducing surface tension within the alveoli, preventing their collapse during exhalation and promoting efficient gas exchange. Nanoparticles and gases can diffuse across the alveolar epithelium, enabling the exchange of oxygen and carbon dioxide between the air in the alveoli and the adjacent capillaries. Additionally, nanoscale vesicles and exosomes play a role in cellular communication. The surface of lung epithelial cells is lined with specialized nanostructures, such as microvilli or cilia, which aid in functions like absorption or mucociliary clearance. Understanding the nanoscale structure of the lung is crucial for comprehending respiratory diseases and the effects of nanoscale interactions on lung health.

5.2. Nanoscale 3D bioprinting for lung tissue recapitulation and application

Nanoscale 3D bioprinting technology enables the precise arrangement in 3D structures, mimicking the complexity and functionality of lung tissues. Researchers print nanoscale ECM hydrogels by extruding cellular and acellular gels into stacked cell ring structures, which has the potential to study lung nanostructures[79]. By accurately positioning these cells within the 3D structure, nanoscale bioprinting can promote the formation of functional lung tissue and facilitate gas exchange. A bioink-containing nanofibrils have been developed for nanoscale 3D-printing lung tissue scaffolds (Figure 4B)[80]. One of the primary challenges in 3D bioprinting is to achieve precise control over the nanoscale architecture while ensuring compatibility with living cells. Nanoforms play a crucial role in promoting cell survival, growth, and differentiation, enabling cells to assume the necessary functions for tissue regeneration and repair.

Concerning efficient gas exchange, it is crucial to accurately manufacture the thin air–blood barrier via LTE. Advanced lung tissue models in the field are highly sought-after, aiming to achieve both biomimetic structural properties and the ability to precisely regulate cell behavior. The researchers prepared a three-organ chip composed of liver, heart, and lung through 3D nanobioprinting, and evaluated its physiological response to drugs and toxic substances[81]. The nanofibrous structure, resembling the morphology of the ECM, promotes cell attachment and enhances nutrition and oxygen transport due to its high surface area and interconnectivity[82]. The submicrometer pore structure and pore size can be controlled between 1000 μm and 10 nm, and its excellent adsorption performance is beneficial to the 3D culture of cells (Figure 4C)[83]. Nanoscale structural elements in 3D bioprinting can be effective in the promotion of cell distribution and new tissue formation. Furthermore, 3D bioprinting has emerged as a valuable tool in the design and development of disease models, including infectious diseases like COVID-19. At the nanoscale, 3D bioprinting allows for the creation of realistic disease models that can be used for studying pathogenesis, drug discovery, and personalized medicine[84]. Continued advancements in nanomaterials and nanoscale fabrication techniques will further contribute to the advancements in respiratory disease research.

6. Challenges and future directions

3D bioprinting is a promising technology with vast potential in tissue engineering, although it is still in its early stages of development. Several technical challenges must be addressed, particularly achieving high-resolution cell patterning and distribution. Current techniques like material extrusion have several drawbacks, including low cell viability, resolution, and working speed, which hinder the fabrication of submicroscale and nanoscale structures. To enable the production of macroscale tissues for clinical applications, printing capabilities and speed must be improved[85]. In addition, for successful *in vivo* transplantation, the printed tissue must possess appropriate

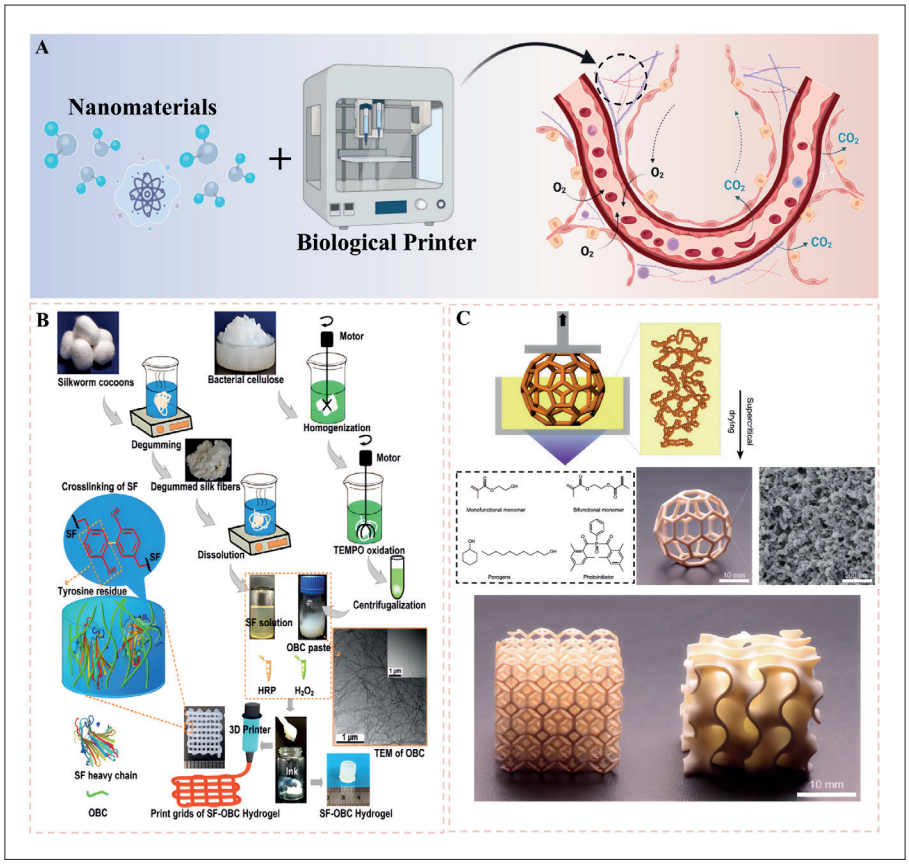

Figure 4. Nanoscale 3D bioprinting for lung tissue. (A) Nanomaterials-assisted bioprinting to simulate alveolar nanostructures. Created with BioRender (www.biorender.com). (B) Process of fabricating silk fibroin (SF) solution, oxidized bacterial cellulose (OBC) paste, SF-OBC composite ink, and SF-OBC nanofibrils hydrogel for the proliferation of lung epithelial stem cells[80]. Reprinted (and adapted) with permission from Springer Nature. Copyright © *2020, Springer Nature B.V.* (C) 3D objects with nanoscale porous structures are manufactured by using digital light processing printing technology[83]. Reprinted (and adapted) with permission from Springer Nature. Copyright © *2021, The Author(s).*

mechanical traits to facilitate suturing with the host circulation and withstand the rhythmic pulsations of blood flow[86]. To realize the objective of bioprinting functional tissues, it is crucial to foster collaboration and integrate expertise from diverse fields, such as manufacturing, material science, biology, and medicine. By bringing together experts from different fields, we can collectively tackle these obstacles and pave the way for groundbreaking advancements in the field of bioprinting[87,88]. In summary, while 3D bioprinting holds great potential for fabricating functional tissues, it requires concerted efforts and interdisciplinary collaborations to overcome technical challenges and advance the field toward clinical translation.

The complex multiscale structure of organs and tissues presents a significant challenge for replication using a single program or tool[89,90]. Future development will focus on multisize, multimaterial, and multicell bioprinting, integrating precise modeling of cell–cell interactions and segregation at the intratissue level, combined with architectural control at the macroscale (Figure 5). This approach shows promise for engineering tissue constructs that closely resemble native tissues in their morphometric features[91]. While bioprinted constructs have demonstrated high cell viability and specific functions in laboratory research, they are still in the early stages of development and not ready for clinical applications[92,93]. Except for advanced structural design, it is important to determine the role of mechanical cues in lung-related research as to design, develop, and apply suitable material inks[94,95]. With every breath, lung cells are subjected to dynamic or continuous mechanical loads, including tension, compression, and shear stress. These mechanical forces serve as vital signals for maintaining the steady state, remodeling, and optimal functioning of lung tissue. By simulating physiological respiratory movement through cyclic mechanical stretch, more realistic lung models can be achieved[96]. Considering the mechanical cues and their associated signaling pathways in the design of material inks for bioprinting lung tissue can help us build a more realistic functional model. By incorporating these mechanical regulatory factors into

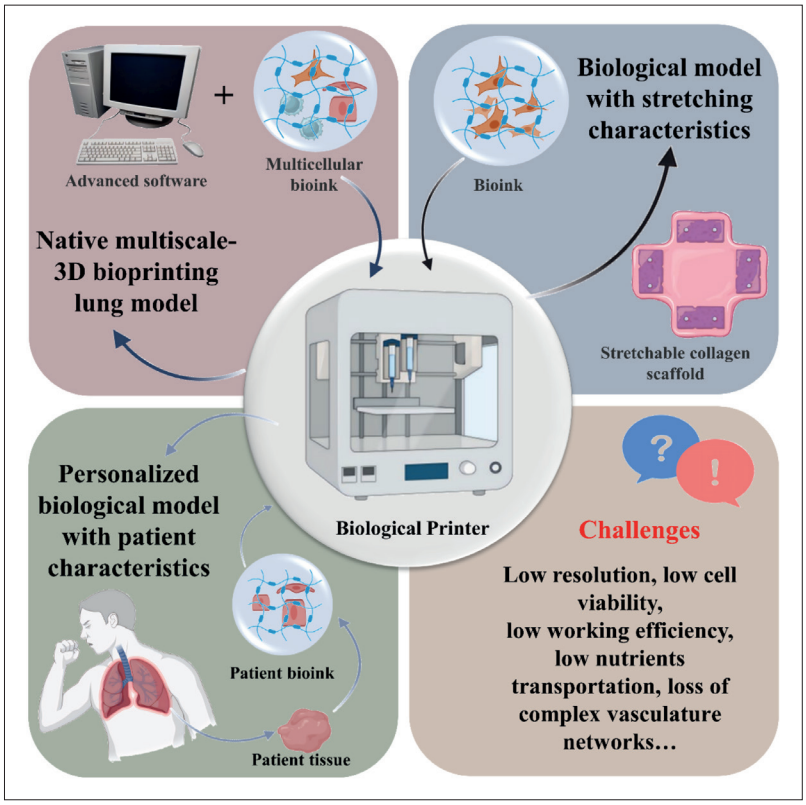

Figure 5. The future and challenges of multiscale 3D bioprinting of lung tissue. In the future, advancements in technology will bring forth an array of enhanced features in the realm of 3D bioprinting. These improvements will include more life-like multiscale lung models, biological models possessing stretching characteristics, and personalized pathological models. However, it is important to acknowledge that numerous challenges still lie ahead. Created with BioRender (www.biorender.com).

the bioprinting process, researchers can create material inks that mimic the mechanical microenvironment of the lung, promoting cell behavior and tissue development that closely resemble native lung tissue.

Despite being in its early stages of development, 3D bioprinting is showing great promise in the field of TE[97]. Beyond organ printing, 3D bioprinting has multifaceted applications in medicine, including testing drug delivery systems and advancing lung disease treatments. Through the simulation of lung tissue characteristics, it enables more accurate assessments of drug delivery and release efficiency for treating lung diseases[98]. Moreover, by replicating patients' lung tissues using 3D bioprinting, researchers gain valuable insights into the underlying causes of diverse lung diseases, significantly improving disease understanding and fostering the development of novel treatments and medications. An additional benefit of 3D bioprinting lies in its ability to create artificial lung tissue that precisely matches a patient's unique anatomical structure. This advancement is invaluable in predicting organ compatibility and evaluating potential outcomes before actual transplantation takes place. By conducting pretrials with artificial lung tissue, the risk of organ transplantation failure is reduced, and tailored treatment plans can be devised, ultimately improving patient outcomes. The continuous advancements of 3D bioprinting research further expand its application in RM, drug discovery, and personalized healthcare. With further developments and refinement, the field of medicine has the potential to undergo a transformative revolution with the advent of 3D bioprinting.

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Conflict of interest

The authors declare no conflicts of interests.

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Writing – review & editing: Han Liu, Jiansheng Li, Qingzhen Yang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Hwang KS, Seo EU, Choi N, *et al.*, 2023, 3D engineered tissue models for studying human-specific infectious viral diseases. *Bioact Mater*, 21: 576–594.

   http://doi.org/10.1016/j.bioactmat.2022.09.010

2. Margolis EA, Friend NE, Rolle MW, *et al.*, 2023, Manufacturing the multiscale vascular hierarchy: Progress toward solving the grand challenge of tissue engineering. *Trends Biotechnol,* S0167-7799(23): 00124–5.

   http://doi.org/10.1016/j.tibtech.2023.04.003

3. Christenson SA, Smith BM, Bafadhel M, *et al.*, 2022, Chronic obstructive pulmonary disease. *Lancet*, 399(10342): 2227–2242.

   http://doi.org/10.1016/S0140-6736(22)00470-6

4. Pugashetti JV, Adegunsoye A, Wu Z, *et al.*, 2023, Validation of proposed criteria for progressive pulmonary fibrosis. *Am J Respir Crit Care Med*, 207(1): 69–76.

   http://doi.org/10.1164/rccm.202201-0124OC

5. Bailey KE, Floren ML, D'Ovidio TJ, *et al.*, 2019, Tissue-informed engineering strategies for modeling human pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol*, 316(2): L303–L320.

   http://doi.org/10.1152/ajplung.00353.2018

6. Heinrich MA, Liu W, Jimenez A, *et al.*, 2019, 3D Bioprinting: From benches to translational applications. *Small*, 15(23): e1805510.

   http://doi.org/10.1002/smll.201805510

7. Daly AC, Prendergast ME, Hughes AJ, *et al.*, 2021, Bioprinting for the biologist. *Cell*, 184(1): 18–32.

   http://doi.org/10.1016/j.cell.2020.12.002

8. Moroni L, Burdick JA, Highley C, *et al.*, 2018, Biofabrication strategies for 3D in vitro models and regenerative medicine. *Nat Rev Mater*, 3(5): 21–37.

   http://doi.org/10.1038/s41578-018-0006-y

9. Fang L, Liu Y, Qiu J, *et al.*, 2022, Bioprinting and its use in tumor-on-a-chip technology for cancer drug screening: A review. *Int J Bioprint*, 8(4): 603.

   http://doi.org/10.18063/ijb.v8i4.603

10. Foresti R, Rossi S, Pinelli S, *et al.*, 2020, In-vivo vascular application via ultra-fast bioprinting for future 5D personalised nanomedicine. *Sci Rep*, 10(1): 3205.

    http://doi.org/10.1038/s41598-020-60196-y

11. Foresti R, Rossi S, Pinelli S, *et al.*, 2020, Highly-defined bioprinting of long-term vascularized scaffolds with Bio-Trap: Complex geometry functionalization and process parameters with computer aided tissue engineering. *Materialia*, 9: 100560.

    http://doi.org/10.1016/j.mtla.2019.100560

12. Yang Q, Xiao Z, Lv X, *et al.*, 2021, Fabrication and biomedical applications of heart-on-a-chip. *Int J Bioprint*, 7(3): 370.

    http://doi.org/10.18063/ijb.v7i3.370

13. Ma X, Liu J, Zhu W, *et al.*, 2018, 3D bioprinting of functional tissue models for personalized drug screening and in vitro disease modeling. *Adv Drug Delivery Rev*, 132: 235–251.

    http://doi.org/10.1016/j.addr.2018.06.011

14. Matai I, Kaur G, Seyedsalehi A, *et al.*, 2020, Progress in 3D bioprinting technology for tissue/organ regenerative engineering. *Biomaterials*, 226: 119536.

    http://doi.org/10.1016/j.biomaterials.2019.119536

15. Liu Y, Yang Q, Zhang H, *et al.*, 2021, Construction of cancer-on-a-chip for drug screening. *Drug Discov Today*, 26(8): 1875–1890.

    http://doi.org/10.1016/j.drudis.2021.03.006

16. Knowlton S, Joshi A, Yenilmez B, *et al.*, 2016, Advancing cancer research using bioprinting for tumor-on-a-chip platforms. *Int J Bioprint*, 2(2): 3–8.

    http://doi.org/10.18063/ijb.2016.02.003

17. Liu F, Liu C, Chen Q, *et al.*, 2018, Progress in organ 3D bioprinting. *Int J Bioprint*, 4(1): 128.

    http://doi.org/10.18063/IJB.v4i1.128

18. Pan T, Song W, Cao X, *et al.*, 2016, 3D bioplotting of gelatin/alginate scaffolds for tissue engineering: Influence of crosslinking degree and pore architecture on physicochemical properties. *J Mater Sci Technol*, 32(9): 889–900.

http://doi.org/https://doi.org/10.1016/j.jmst.2016.01.007

19. Dabaghi M, Carpio MB, Moran-Mirabal JM, *et al.*, 2023, 3D (bio) printing of lungs: Past, present, and future. *Eur Respir J*, 61(1): 2200417.

http://doi.org/10.1183/13993003.00417-2022

20. Shakir S, Hackett TL, Mostaço-Guidolin LB, 2022, Bioengineering lungs: An overview of current methods, requirements, and challenges for constructing scaffolds. *Front Bioeng Biotechnol*, 10: 1011800.

http://doi.org/10.3389/fbioe.2022.1011800

21. Liu S, Yu J-M, Gan Y-C, *et al.*, 2023, Biomimetic natural biomaterials for tissue engineering and regenerative medicine: New biosynthesis methods, recent advances, and emerging applications. *Mil Med Res*, 10(1): 16.

http://doi.org/10.1186/s40779-023-00448-w

22. Yang J, Dang H, Xu Y, 2022, Recent advancement of decellularization extracellular matrix for tissue engineering and biomedical application. *Artif Organs*, 46(4): 549–567.

http://doi.org/10.1111/aor.14126

23. Zhang H, Wang Y, Zheng Z, *et al.*, 2023, Strategies for improving the 3D printability of decellularized extracellular matrix bioink. *Theranostics*, 13(8): 2562.

http://doi.org/10.1039/D2BM01273A

24. Kort-Mascort J, Flores-Torres S, Chavez OP, *et al.*, 2023, Decellularized ECM hydrogels: prior use considerations, applications, and opportunities in tissue engineering and biofabrication. *Biomater Sci*, 11(2): 400–431.

http://doi.org/10.1039/d2bm01273a

25. Bock S, Rades T, Rantanen J, *et al.*, 2022, Additive manufacturing in respiratory sciences-current applications and future prospects. *Adv Drug Delivery Rev*, 186: 114341.

http://doi.org/10.1016/j.addr.2022.114341

26. Cui H, Nowicki M, Fisher JP, *et al.*, 2017, 3D bioprinting for organ regeneration. *Adv Healthc Mater*, 6(1): 1601118.

http://doi.org/10.1002/adhm.201601118

27. Murphy SV, De Coppi P, Atala A, 2020, Opportunities and challenges of translational 3D bioprinting. *Nat Biomed Eng*, 4(4): 370–380.

http://doi.org/10.1038/s41551-019-0471-7

28. Yu C, Schimelman J, Wang P, *et al.*, 2020, Photopolymerizable biomaterials and light-based 3D printing strategies for biomedical applications. *Chem Rev*, 120(19): 10695–10743.

http://doi.org/10.1021/acs.chemrev.9b00810

29. Jammalamadaka U, Tappa K, 2018, Recent advances in biomaterials for 3D printing and tissue engineering. *J Funct Biomater*, 9(1): 22.

http://doi.org/10.3390/jfb9010022

30. Brassard JA, Nikolaev M, Hübscher T, *et al.*, 2021, Recapitulating macro-scale tissue self-organization through organoid bioprinting. *Nat Mater*, 20(1): 22–29.

http://doi.org/10.1038/s41563-020-00803-5

31. Raman R, Bashir R, 2017, Biomimicry, biofabrication, and biohybrid systems: The emergence and evolution of biological design. *Adv Healthcare Mater*, 6(20): 1700496.

http://doi.org/10.1002/adhm.201700496

32. Levato R, Jungst T, Scheuring RG, *et al.*, 2020, From shape to function: The next step in bioprinting. *Adv Mat*, 32(12): e1906423.

http://doi.org/10.1002/adma.201906423

33. Bittner S M, Guo J L, Melchiorri A, *et al.*, 2018, Three-dimensional printing of multilayered tissue engineering scaffolds. *Mater Today*, 21(8): 861–874.

http://doi.org/10.1016/j.mattod.2018.02.006

34. Huo Y, Xu Y, Wu X, *et al.*, 2022, Functional trachea reconstruction using 3D-bioprinted native-like tissue architecture based on designable tissue-specific bioinks. *Adv Sci*, 9(29): 2202181.

http://doi.org/https://doi.org/10.1002/advs.202202181

35. Grigoryan B, Paulsen SJ, Corbett DC, *et al.*, 2019, Multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science*, 364(6439): 458–464.

http://doi.org/10.1126/science.aav9750

36. Ng WL, Ayi TC, Liu Y-C, *et al.*, 2021, Fabrication and characterization of 3D bioprinted triple-layered human alveolar lung models. *Int J Bioprint*, 7(2): 332.

http://doi.org/10.18063/ijb.v7i2.332

37. Lewis KJR, Tibbitt MW, Zhao Y, *et al.*, 2015, In vitro model alveoli from photodegradable microsphere templates. *Biomater Sci*, 3(6): 821–832.

http://doi.org/10.1039/c5bm00034c

38. Machino R, Matsumoto K, Taniguchi D, *et al.*, 2019, Replacement of rat tracheas by layered, trachea-like, scaffold-free structures of human cells using a Bio-3D printing system. *Adv Healthc Mater*, 8(7): e1800983.

http://doi.org/10.1002/adhm.201800983

39. Taniguchi D, Matsumoto K, Tsuchiya T, *et al.*, 2018, Scaffold-free trachea regeneration by tissue engineering with bio-3D printing. *Interact Cardiovasc Thorac Surg*, 26(5): 745–752.

http://doi.org/10.1093/icvts/ivx444

40. Kim IG, Park SA, Lee S-H, *et al.*, 2020, Transplantation of a 3D-printed tracheal graft combined with iPS cell-derived MSCs and chondrocytes. *Sci Rep*, 10(1): 4326.

http://doi.org/10.1038/s41598-020-61405-4

41. Berg J, Weber Z, Fechler-Bitteti M, *et al.*, 2021, Bioprinted multi-cell type lung model for the study of viral inhibitors. *Viruses*, 13(8): 1590.

http://doi.org/10.3390/v13081590

42. Wanczyk H, Jensen T, Weiss DJ, *et al.*, 2021, Advanced single-cell technologies to guide the development of bioengineered lungs. *Am J Physiol Lung Cell Mol Physiol*, 320(6): L1101–L1117.

http://doi.org/10.1152/ajplung.00089.2021

43. Liu H, Wu M, Jia YB, *et al.*, 2020, Control of fibroblast shape in sequentially formed 3D hybrid hydrogels regulates cellular responses to microenvironmental cues. *NPG Asia Mater*, 12(1): 45.

http://doi.org/10.1038/s41427-020-0226-7

44. Xu F, Wu J, Wang S, *et al.*, 2011, Microengineering methods for cell-based microarrays and high-throughput drug-screening applications. *Biofabrication*, 3(3): 034101.

http://doi.org/10.1088/1758-5082/3/3/034101

45. Wang L, Qiu M, Yang Q, *et al.*, 2015, Fabrication of microscale hydrogels with tailored microstructures based on liquid bridge phenomenon. *ACS Appl Mater Interfaces*, 7(21): 11134–11140.

http://doi.org/10.1021/acsami.5b00081

46. Dong Y, Jin G, Hong Y, *et al.*, 2018, Engineering the cell microenvironment using novel photoresponsive hydrogels. *ACS Appl Mater Interfaces*, 10(15): 12374–12389.

http://doi.org/10.1021/acsami.7b17751

47. Liu H, Li M, Huang G, *et al.*, 2021, Bioinspired microstructure platform for modular cell-laden microgel fabrication. *Macromol Biosci*, 21(9): e2100110.

http://doi.org/10.1002/mabi.202100110

48. Ma Y, Ji Y, Huang G, *et al.*, 2015, Bioprinting 3D cell-laden hydrogel microarray for screening human periodontal ligament stem cell response to extracellular matrix. *Biofabrication*, 7(4): 044105.

http://doi.org/10.1088/1758-5090/7/4/044105

49. Ravanbakhsh H, Karamzadeh V, Bao G, *et al.*, 2021, Emerging technologies in multi-material bioprinting. *Adv Mater*, 33(49): e2104730.

http://doi.org/10.1002/adma.202104730

50. Han YL, Wang W, Hu J, *et al.*, 2013, Benchtop fabrication of three-dimensional reconfigurable microfluidic devices from paper-polymer composite. *Lab Chip*, 13(24): 4745–4749.

http://doi.org/10.1039/c3lc50919b

51. Ferreira LP, Gaspar VM, Mano JF, 2020, Decellularized extracellular matrix for bioengineering physiomimetic 3D in vitro tumor models. *Trends Biotechnol*, 38(12): 1397–1414.

http://doi.org/10.1016/j.tibtech.2020.04.006

52. Huang G, Li F, Zhao X, *et al.*, 2017, Functional and biomimetic materials for engineering of the three-dimensional cell microenvironment. *Chem Rev*, 117(20): 12764–12850.

http://doi.org/10.1021/acs.chemrev.7b00094

53. Hölzl K, Lin S, Tytgat L, *et al.*, 2016, Bioink properties before, during and after 3D bioprinting. *Biofabrication*, 8(3): 032002.

http://doi.org/10.1088/1758-5090/8/3/032002

54. Qing H, Ji Y, Li W, *et al.*, 2020, Microfluidic printing of three-dimensional graphene electroactive microfibrous scaffolds. *ACS Appl Mater Interfaces*, 12(2): 2049–2058.

http://doi.org/10.1021/acsami.9b17948

55. de Hilster RHJ, Sharma PK, Jonker MR, *et al.*, 2020, Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue. *Am J Physiol Lung Cell Mol Physiol*, 318(4): L698–L704.

http://doi.org/10.1152/ajplung.00451.2019

56. Zepp JA, Morrisey EE, 2019, Cellular crosstalk in the development and regeneration of the respiratory system. *Nat Rev Mol Cell Biol*, 20(9): 551–566.

http://doi.org/10.1038/s41580-019-0141-3

57. Knudsen L, Ochs M, 2018, The micromechanics of lung alveoli: Structure and function of surfactant and tissue components. *Histochem Cell Biol*, 150(6): 661–676.

http://doi.org/10.1007/s00418-018-1747-9

58. Bertassoni LE, Cecconi M, Manoharan V, *et al.*, 2014, Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip*, 14(13): 2202–2211.

http://doi.org/10.1039/c4lc00030g

59. Kang D, Park JA, Kim W, *et al.*, 2021, All-inkjet-printed 3D alveolar barrier model with physiologically relevant microarchitecture. *Adv Sci (Weinh)*, 8(10): 2004990.

http://doi.org/10.1002/advs.202004990

60. Horváth L, Umehara Y, Jud C, *et al.*, 2015, Engineering an in vitro air-blood barrier by 3D bioprinting. *Sci Rep*, 5: 7974.

http://doi.org/10.1038/srep07974

61. Gu Z, Fu J, Lin H, *et al.*, 2020, Development of 3D bioprinting: From printing methods to biomedical applications. *Asian J Pharm Sci*, 15(5): 529–557.

http://doi.org/10.1016/j.ajps.2019.11.003

62. De Santis MM, Alsafadi HN, Tas S, *et al.*, 2021, Extracellular-matrix-reinforced bioinks for 3D bioprinting human tissue. *Adv Mat*, 33(3): e2005476.

http://doi.org/10.1002/adma.202005476

63. Berg J, Hiller T, Kissner MS, *et al.*, 2018, Optimization of cell-laden bioinks for 3D bioprinting and efficient infection with influenza A virus. *Sci Rep*, 8(1): 13877.

http://doi.org/10.1038/s41598-018-31880-x

64. Falcones B, Sanz-Fraile H, Marhuenda E, *et al.*, 2021, Bioprintable lung extracellular matrix hydrogel scaffolds for 3D culture of mesenchymal stromal cells. *Polymers (Basel)*, 13(14): 2350.

http://doi.org/10.3390/polym13142350

65. Zhang YS, Davoudi F, Walch P, *et al.*, 2016, Bioprinted thrombosis-on-a-chip. *Lab Chip*, 16(21): 4097–4105.

http://doi.org/10.1039/c6lc00380j

66. Wang X, Zhang X, Dai X, *et al.*, 2018, Tumor-like lung cancer model based on 3D bioprinting. *Biotech*, 8(12): 501.

http://doi.org/10.1007/s13205-018-1519-1

67. Teixeira AI, McKie GA, Foley JD, *et al.*, 2006, The effect of environmental factors on the response of human corneal epithelial cells to nanoscale substrate topography. *Biomaterials*, 27(21): 3945–3954.

http://doi.org/10.1016/j.biomaterials.2006.01.044

68. Melchor-Martínez EM, Torres Castillo NE, Macias-Garbett R, *et al.*, 2021, Modern world applications for nano-bio materials: Tissue engineering and COVID-19. *Front Bioeng Biotechnol*, 9: 597958.

http://doi.org/10.3389/fbioe.2021.597958

69. Xu F, Inci F, Mullick O, *et al.*, 2012, Release of magnetic nanoparticles from cell-encapsulating biodegradable nanobiomaterials. *ACS Nano*, 6(8): 6640–6649.

http://doi.org/10.1021/nn300902w

70. Jia Y, Wang Y, Niu L, *et al.*, 2021, The plasticity of nanofibrous matrix regulates fibroblast activation in fibrosis. *Adv Healthc Mater*, 10(8): e2001856.

http://doi.org/10.1002/adhm.202001856

71. Liu H, Du C, Liao L, *et al.*, 2022, Approaching intrinsic dynamics of MXenes hybrid hydrogel for 3D printed multimodal intelligent devices with ultrahigh superelasticity and temperature sensitivity. *Nat Commun*, 13(1): 3420.

http://doi.org/10.1038/s41467-022-31051-7

72. Malda J, Visser J, Melchels FP, *et al.*, 2013, 25th anniversary article: Engineering hydrogels for biofabrication. *Adv Mater*, 25(36): 5011–5028.

http://doi.org/10.1002/adma.201302042

73. Hollister SJ, 2005, Porous scaffold design for tissue engineering. *Nat Mater*, 4(7): 518–524.

http://doi.org/10.1038/nmat1421

74. Alarçin E, Guan X, Kashaf SS, *et al.*, 2016, Recreating composition, structure, functionalities of tissues at nanoscale for regenerative medicine. *Regen Med*, 11(8): 849–858.

http://doi.org/10.2217/rme-2016-0120

75. Liu W, Thomopoulos S, Xia Y, 2012, Electrospun nanofibers for regenerative medicine. *Adv Healthc Mater*, 1(1): 10–25.

http://doi.org/10.1002/adhm.201100021

76. Bakht SM, Gomez-Florit M, Lamers T, *et al.*, 2021, 3D bioprinting of miniaturized tissues embedded in self-assembled nanoparticle-based fibrillar platforms. *Adv Funct Mater*, 31(46): 2104245.

http://doi.org/10.1002/adfm.202104245

77. Halappanavar S, Nymark P, Krug HF, *et al.*, 2021, Non-animal strategies for toxicity assessment of nanoscale materials: Role of adverse outcome pathways in the selection of endpoints. *Small*, 17(15): e2007628.

http://doi.org/10.1002/smll.202007628

78. Sisler JD, Pirela SV, Friend S, *et al.*, 2015, Small airway epithelial cells exposure to printer-emitted engineered nanoparticles induces cellular effects on human microvascular endothelial cells in an alveolar-capillary co-culture model. *Nanotoxicology*, 9(6): 769–779.

http://doi.org/10.3109/17435390.2014.976603

79. Skardal A, Zhang J, McCoard L, *et al.*, 2010, Dynamically crosslinked gold nanoparticle - hyaluronan hydrogels. *Adv Mater*, 22(42): 4736–4740.

http://doi.org/10.1002/adma.201001436

80. Huang L, Yuan W, Hong Y, *et al.*, 2021, 3D printed hydrogels with oxidized cellulose nanofibers and silk fibroin for the proliferation of lung epithelial stem cells. *Cellulose (Lond)*, 28(1): 241–257.

http://doi.org/10.1007/s10570-020-03526-7

81. Skardal A, Murphy SV, Devarasetty M, *et al.*, 2017, Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Sci Rep*, 7(1): 8837.

http://doi.org/10.1038/s41598-017-08879-x

82. Bhattacharyya A, Janarthanan G, Noh I, 2021, Nano-biomaterials for designing functional bioinks towards complex tissue and organ regeneration in 3D bioprinting. *Addit Manuf*, 37: 101639.

http://doi.org/10.1016/j.addma.2020.101639

83. Dong Z, Cui H, Zhang H, *et al.*, 2021, 3D printing of inherently nanoporous polymers via polymerization-induced phase separation. *Nat Commun*, 12(1): 247.

http://doi.org/10.1038/s41467-020-20498-1

84. Pérez RA, Won J-E, Knowles JC, *et al.*, 2013, Naturally and synthetic smart composite biomaterials for tissue regeneration. *Adv Drug Delivery Rev*, 65(4): 471–496.

http://doi.org/10.1016/j.addr.2012.03.009

85. Gao B, Yang Q, Zhao X, *et al.*, 2016, 4D bioprinting for biomedical applications. *Trends Biotechnol*, 34(9): 746–756.

http://doi.org/10.1016/j.tibtech.2016.03.004

86. Choi Y-M, Lee H, Ann M, *et al.*, 2023, 3D bioprinted vascularized lung cancer organoid models with underlying disease capable of more precise drug evaluation. *Biofabrication*, 15(3): 034104.

http://doi.org/10.1088/1758-5090/acd95f

87. Liberti DC, Morrisey EE, 2021, Organoid models: Assessing lung cell fate decisions and disease responses. *Trends Mol Med*, 27(12): 1159–1174.

http://doi.org/10.1016/j.molmed.2021.09.008

88. Valdoz JC, Franks NA, Cribbs CG, *et al.*, 2022, Soluble ECM promotes organotypic formation in lung alveolar model. *Biomaterials*, 283: 121464.

http://doi.org/10.1016/j.biomaterials.2022.121464

89. Kim S, Uroz M, Bays JL, *et al.*, 2021, Harnessing mechanobiology for tissue engineering. *Dev Cell*, 56(2): 180–191.

http://doi.org/10.1016/j.devcel.2020.12.017

90. Tan Q, Choi KM, Sicard D, *et al.*, 2017, Human airway organoid engineering as a step toward lung regeneration and disease modeling. *Biomaterials*, 113: 118–132.

http://doi.org/10.1016/j.biomaterials.2016.10.046

91. Ji Y, Yang Q, Huang G, *et al.*, 2019, Improved resolution and fidelity of droplet-based bioprinting by upward ejection. *ACS Biomater Sci Eng*, 5(8): 4112–4121.

http://doi.org/10.1021/acsbiomaterials.9b00400

92. Li M, Yang Q, Liu H, *et al.*, 2016, Capillary origami inspired fabrication of complex 3D hydrogel constructs. *Small*, 12(33): 4492–4500.

http://doi.org/10.1002/smll.201601147

93. Yang Q, Gao B, Xu F, 2020, Recent advances in 4D bioprinting. *Biotechnol J*, 15(1): e1900086.

http://doi.org/10.1002/biot.201900086

94. Yang Y, Jia Y, Yang Q, *et al.*, 2023, Engineering bio-inks for 3D bioprinting cell mechanical microenvironment. *Int J Bioprint*, 9(1): 632.

http://doi.org/10.18063/ijb.v9i1.632

95. Huang G, Wang L, Wang S, *et al.*, 2012, Engineering three-dimensional cell mechanical microenvironment with hydrogels. *Biofabrication*, 4(4): 042001.

http://doi.org/10.1088/1758-5082/4/4/042001

96. Huh D, Matthews BD, Mammoto A, *et al.*, 2010, Reconstituting organ-level lung functions on a chip. *Science*, 328(5986): 1662–1668.

http://doi.org/10.1126/science.1188302

97. Doryab A, Tas S, Taskin MB, *et al.*, 2019, Evolution of bioengineered lung models: recent advances and challenges in tissue mimicry for studying the role of mechanical forces in cell biology. *Adv Funct Mater*, 29(39): 1903114.

http://doi.org/10.1002/adfm.201903114

98. Wang J, Zhang Y, Aghda NH, *et al.*, 2021, Emerging 3D printing technologies for drug delivery devices: Current status and future perspective. *Adv Drug Delivery Rev*, 174: 294–316.

http://doi.org/10.1016/j.addr.2021.04.019