ACCSCIENCE PUBLISHING

International Journal of Bioprinting

REVIEW ARTICLE

Miniature modeling while aiming for transplantation: Current challenges and future perspectives of lung bioprinting

Judit Bovari-Biri[1], Kitti Garai[1], Borbala Lovaszi[2], Zoltan Vereb[2], and Judit E. Pongracz[1]*

[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs, Pécs, Baranya, Hungary

[2]Department of Dermatology and Allergology, Albert Szentgyorgyi Clinical Centre, University of Szeged, Szeged, Hungary

(This article belongs to the Special Issue: *3D Printing for tissue engineering and regenerative medicine*)

Abstract

The development of three-dimensional (3D) lung organs or tissues using gravitational methods and bioprinting technologies displays significant promise for producing lung tissue for research, pharmaceutical, and clinical applications. The advancement of innovative technologies can improve our understanding of lung diseases and, if necessary, enable the production of replacement lungs for transplantation. The development of functional organs-on-a-chip and disease-specific lung tissues could provide a deep understanding of the molecular mechanisms underlying lung diseases and aid the identification of drug targets. This knowledge has the potential to enhance our understanding of lung tissue regeneration processes, potentially leading to the development of more effective treatments for human lung diseases. This could eliminate the need for lung transplants in most disease-induced cases, as appropriate medications could induce regeneration of the damaged organ. This review highlights the importance of using a variety of materials, preparation methods, and sizes of lung tissues in 3D bioprinting technologies to better understand lung function, facilitate drug selection during therapy, and ultimately produce transplantable organs if needed. The review also emphasizes the need for improvements in legislation and guidelines for researchers aiming to achieve quality-assured biomanufacturing.

Keywords: Lung diseases; Lung bioprinting; Lung tissue aggregates; Personalized medicine; Drug testing; Organ-on-a-chip

***Corresponding author:**
Judit E. Pongracz
(pongracz.e.judit@pte.hu)

Citation: Bovari-Biri J, Garai K, Lovaszi B, Vereb Z, Pongracz JE. Miniature modeling while aiming for transplantation: Current challenges and future perspectives of lung bioprinting. *Int J Bioprint*. 2025;11(1):30-56. doi: 10.36922/ijb.4673

Received: August 28, 2024
Revised: September 28, 2024
Accepted: October 7, 2024
Published Online: October 7, 2024

Copyright: © 2024 Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Lung diseases represent a significant global health concern (The main types of lung diseases and the applicable therapies are collected in Table 1). According to the World Health Organization, over four million people succumb to respiratory diseases annually, with nearly two million additional deaths attributed to various types of lung cancers.[1] Despite the wide prevalence of a broad range of lung diseases, public attention and health concerns are primarily focused on different diseases, including heart diseases,

Table 1. Main types of lung diseases and the applicable therapy

Main types of lung diseases	Transplantation might be necessary	Medication
Asthma	+	+
Acute respiratory distress syndrome (ARDS)	+	+
Chronic obstructive pulmonary disease (COPD)	+	+
Emphysema	+	+
Cystic fibrosis	+	+/−
Interstitial lung disease (ILD)	+	−
Infections	−	+
Infection induced fibrosis	+	−
Lung cancers	−	+

Note: "+" denotes likely medical intervention; "-" denotes unlikely medical intervention.

stroke, and cancers, which present more prominent and early symptoms. The delayed diagnosis of lung diseases highlights the crucial need to identify early diagnostic markers and therapeutic targets for timely intervention during the initial disease stages. It is evident that to address all the demands, a thorough understanding of healthy lung anatomy and function is just as crucial as understanding the diseases and therapy responses, clinical needs, and regulatory issues. Understanding the development and progression of lung diseases, identifying potential therapeutic targets, and evaluating drugs to find effective treatments or stimulate regenerative processes for damaged lung tissue present complex challenges for the research community and clinicians. While treating lung cancers require a different approach, lung modeling is equally important in lung cancer modeling as it is for other lung diseases. Additionally, in cases of severe, end-stage lung tissue damage, lung transplantation may be the only, albeit extremely challenging, option.

Bioprinting is pivotal to numerous applications in research laboratories, including understanding disease mechanisms, identifying drug targets, testing medications, and producing transplantation-ready organs. Bioprinting dates back to the late 1980s[2] and started to gain significance in the 1990s[2] when 3D bioprinting technology became associated with the broader field of tissue engineering (TE) to create increasingly complex structures. Technical approaches were broad and included inkjet-based bioprinting, extrusion-based bioprinting, and laser-assisted (e.g., stereolithography) bioprinting[3] mostly to create tissue models for understanding organogenesis, diseases, and drug reactions, as well as organ regeneration processes. With technological advancements, the creation of patient-specific tissues and organs tailored to individual needs became possible. 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 normal proximal and distal lung tissue assembly, blood vessel infiltration into the tissue, and lung disease modeling (e.g., cancer, chronic obstructive pulmonary disease [COPD], cystic fibrosis), which offer various benefits and applications beyond lung transplantation. Considering the complex structure and dynamic characteristics of the lung, various reviews have described the construction of artificial lung tissues, mostly focusing on specific lung tissue segments, mostly on the microscale, and discussed precise tissue compositions at the macroscale. Although extensive work has been done on portraying lung bioprinting, our review is the first one to associate the challenges posed by clinical procedures and the application of model tissues that aid the clinical application of microscale lung printing. While numerous reviews have delved into the details of printing technologies and the myriad applications currently available, our review uniquely focuses on the obstacles still hindering the integration of lung tissue printing into clinical practice. Moreover, we draw attention to the overlooked aspect of lung transplantation, noting the absence of bioprinting technologies in understanding the clinical requirements necessary for making bioprinted tissues or organs viable for surgical manipulation. Consequently, we offer a summary of the current challenges in clinically applicable bioprinting, diverging from the usual summaries that tend towards an optimistic portrayal of solutions for the hurdles encountered in the development of printing processes. Public expectations often lean towards a swift readiness of sophisticated solutions, particularly the bioprinting of soft tissues like the lung, anticipated to supply donor organs within a few years. Despite the enthusiasm from the scientific community to foster such expectations, the

reality falls short. This is partly due to unresolved issues such as the sourcing of the trillions of cells necessary for bioprinted lung tissue, the regulation of cellular subtype differentiation, the integration of functional capabilities, and the quality control of transplantation-ready tissues that are safe for surgical handling. In addition to technical and biological challenges, we also highlight another critical facet often sidelined—the regulatory and legislative framework governing bioprinting. These regulatory aspects, crucial for the advancement of bioprinting applications, are not keeping pace with the technological and procedural innovations in the field. While bioprinting of solid scaffold-based tissue seems to have achieved a level of clinical applicability, recent discoveries point to emerging challenges. For instance, different scaffold materials, pore sizes, growth and differentiation factors, and cell sources are necessitated for the regeneration of lung tissues—a nuance that may not hold significant importance in a research lab but is of utmost relevance from the perspectives of clinicians and patients alike. Accordingly, we have divided the present manuscript into two main areas:

(i) Bioprinting macro-sized, transplantation-ready lung tissue, addressing the challenges and recent discoveries necessary before printing full transplantable lungs.

(ii) Bioprinting micro-sized lung tissue for research and clinical applications to evaluate personalized therapy effectiveness as well as for pharmaceutical applications to test drug candidates for toxicology, drug–drug interactions, and efficacy.

Finally, we highlight the prospects for *in vitro* production of lung tissue and the incorporation of all knowledge obtained from bioprinted lung tissue models into personalized clinical applications.

2. Transplantation-ready—macroscale-lung tissue

2.1. Lung transplantation

Lung transplantation is only considered for patients who have advanced and progressively declining lung function (e.g., interstitial lung disease) and those with no other treatment options available. Candidates typically experience symptoms during their daily activities and have a limited life expectancy. Ideally, they should not have any other organ dysfunctions and preferably no other underlying health conditions.[4,5] In recent years, there has been an increased demand for lung transplants due to severe fibrosis resulting from Coronavirus disease 2019 (COVID-19) infections. This has led to more potential recipients without a proportional increase in available transplantable organs, boosting the interest in lung bioprinting. Theoretically, organs, including the lungs, can be printed *in vitro*. However, several factors must be considered when creating a functional organ.

The anatomical structure of the lung poses the first challenge for clinical application. The airways (proximal lung)[6] begin with the trachea, which bifurcates into the main bronchi and finally into bronchioles. The trachea and upper airways are lined with columnar ciliated and mucous secretory cells. These cells coordinate to trap inhaled particles in mucus, which is then expelled by the cilia. Additionally, cuboidal secretory club cells can be found in the bronchioles producing mucus. They secrete various proteins to protect the bronchiolar epithelium. The transition from terminal bronchioles to the alveoli marks the beginning of the distal respiratory zone (Figure 1).

The distal airways of a healthy human lung have around 300–700 million alveoli,[7] and each alveolus is surrounded by a homogenous mesh of capillary vessels, forming the vast surface area of gas exchange. An alveolar septum separates each alveolus. Previously, it was believed that there were only three main cell types in alveoli: type 1 pneumocytes (AT1), type 2 pneumocytes (AT2), and alveolar macrophages. In recent years, single-cell sequencing identified dozens more subtypes of cells in the gas exchange area.[8] In general, AT1 cells cover 70%–95% of the internal surface of each alveolus as thin, squamous cells with surfactant proteins to decrease surface tension and form an air-blood barrier for gas exchange. They are connected via tight junctions, creating an almost impermeable barrier to fluid infiltration into the alveoli. The cuboidal AT2 cells cover 7% of the internal surface of each alveolus and have apical microvilli and characteristic lamellar bodies. AT2 cells produce surfactant proteins to decrease alveolar surface tension and serve as tissue-specific progenitor cells to trans-differentiate into AT1 cells when regeneration of the gas-exchange surface is necessary following tissue damage.[9] Recently, it was discovered that the alveolar capillary network is composed of two intermingled, stable cell types, the general capillary cells and the aCap (aerocytes) cells.[10] The aCap cells are complex, large cells (diameter: >100 μm; mean volume: 21 $\times 10^3$ μm^3) with ramified extensions surrounding the pores (mean of six pores per cell). The cells have a variety of sizes and shapes, and a single cell frequently spans multiple alveoli to increase the efficiency of gas exchange. A similar morphological complexity has also been described for AT1 cells.[11] Alveolar macrophages are mononuclear phagocytes resident in the alveoli and play a key role in protecting the distal lung tissue from infections.[12]

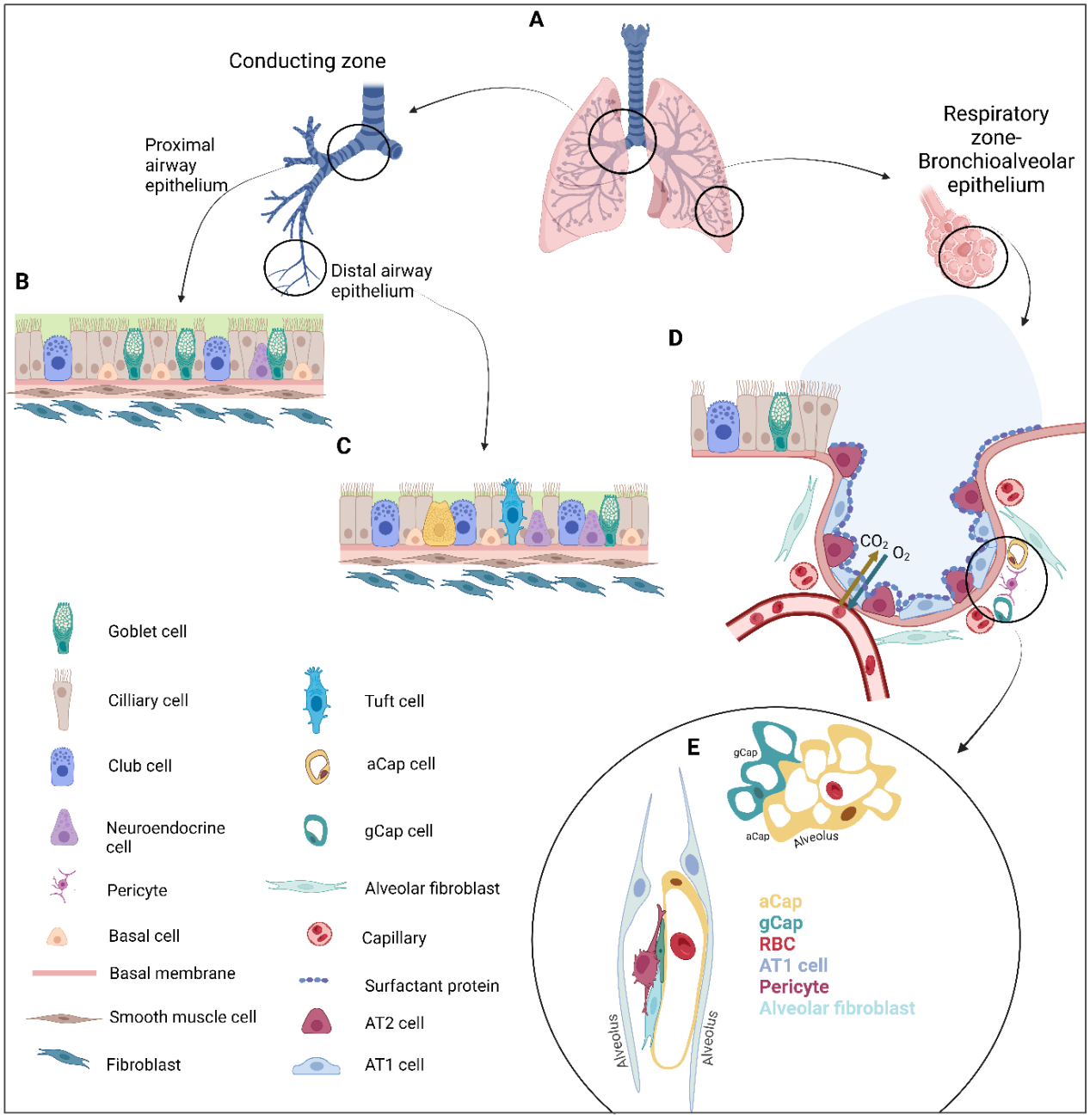

Figure 1. Anatomical characterization of the different tissue types of the lungs. (A) The main functional roles of the lung tissue are conducting and respiratory zones. (B) Characteristic cell types of the proximal airway epithelium. (C) Characteristic cell types of the distal airway epithelium. (D) Characteristic cell types of the bronchioalveolar epithelium. (E) The relative position of the different types of capillary cells. Abbreviations: gCap, general capillary cells; aCap, aerocyte capillary cells; RBC, red blood cell; AT1, alveolar epithelial cell type 1; AT2, alveolar epithelial cell type 2.

The total thickness of the interstitium together with an ultrathin basement membrane (BM) is about 1 μm, while the BM is less than 100 nm.[13] The BM is elastic and porous, allowing for up to 10% linear strain under physiological conditions, and has Young's modulus of 3–7 kPa,[14] making the lung tissue resilient to constant inflation and deflation.

In vitro production of the lung presents unique challenges. Preparing lung tissues for transplantation includes dealing with large tissue sizes spanning from dozens of centimeters (trachea) to nanometers (alveoli) and establishing a complex vascular network for such a large organ to maintain the appropriate and coordinated blood supply.

Without computer-aided design (CAD) and computer-aided manufacturing (CAM),[15] it would not be possible to create human-sized tissues with the complexity required for organs such as the lungs. The CAD/CAM technology and the development of degradable scaffolds and bioinks are crucial for advancing lung bioprinting. This involves using medical imaging and anatomical knowledge (Figure 1) to identify tissue types and translate scanned frameworks into suitable computer programs for printing.

2.2. Criteria for bioprinting lung and tracheal constructs

Recently, several reviews have summarized the technical issues associated with lung tissue bioprinting,[16,17] including cell sources, cell cultures, growth and differentiation factors and markers, and the various printing methodologies (e.g., extrusion-based, inkjet-based, pressure-assisted, and laser-assisted bioprinting).[18–22] Based on existing knowledge, the challenges of printing a transplant-ready lung include (Figure 2):

(i) The massive number of cells required. Therefore, the number and the origin of the cells are essential (primary human organ-specific tissue cells, stem cells, tissue-specific progenitor cells, induced pluripotent stem cells [iPSCs]).

(ii) The required cell types must be able to differentiate reliably and be scalable for further differentiation within the appropriate microenvironment (including scaffold material, other cells, secured nutrition, and oxygen supply). It poses an additional challenge if the interactions between cells do not initiate the differentiation process and the organ produced *in vitro* requires additional proteins (growth and differentiation factors) at the appropriate concentration and timing (Figure 3).

(iii) The cells must differentiate into the required and appropriate cell subtypes. Suitable mechanical parameters and an air–liquid interface are required for complete cellular differentiation and polarization.

(iv) Ability to print a nanoscale scaffold with appropriate grooves where the cells attach, proliferate, and create the necessary cytoskeleton.

(v) The matrices where the cells are grown and then printed must be reproducible and adjustable.

(vi) The bioprinter should be suitable for printing structures as small as an alveolus (with a diameter of 200 μm) and as large as a full lung (with three lobes, approximately 1.3 kg, and a total respiratory surface area of about 70–100 m^2).

(vii) Time is crucial—the tissue needs to be printed and matured at an accelerated pace.

(viii) The tissue needs to be maintained in a differentiated, oxygenated, and fully formed state *in vitro* under sterile conditions to make it suitable to be considered for human surgery.

(ix) The organ designated for transplantation must undergo a quality assurance test to rapidly determine its clinical suitability.

(x) The *in vitro*-produced organ being considered for transplant must meet all legal requirements.

It is important to emphasize that generating a healthy clone of a damaged lung for transplantation is not achievable with a small number of stem cells alone. Growing a fully functional organ from such a limited starting point would require several months or even years. Given the urgent demand for the organ and the absence of a defined timeline, it is crucial to explore alternative solutions.

2.3. Sources of cells

Human-sized organs necessitate an astounding number of cells, hovering around the trillion mark. To bioprint a complete lung, approximately 11×10^9 cells are required, and these cells must be healthy and capable of proliferation and differentiation into dozens of subtypes necessary to produce the functional organ. While many lung tissue printing techniques are tested using cancer cell lines, determining how applicable these techniques are for primary tissue is challenging.

Primary cells can be forced to proliferate, but only a limited number of replication cycles can be expected, while genetic changes cannot be ruled out, and the ability to re-differentiate is questionable. The best source to achieve the required trillions of cells for a full organ transplant appears to be iPSCs, preferentially created from isolated progenitor cells. Any kind of tissue replacement therapy could use iPSCs for either autologous or carefully tested allogeneic applications. While the creation of iPSCs sounds like an easy and straightforward process based on the protocol created by Yamanaka et al.,[23] who identified the transcription factors (Oct3/4, Sox2, Klf4, c-Myc) that play a vital role in the creation of iPSCs, several factors need to be considered to use human donor cells for making a transplantation-ready iPSC-based organ. Even for iPSC, the quality of the donated cells is essential. Quality assurance of iPSCs includes observation of iPSC colony morphology, cell surface and internal protein immunochemistry-based markers, metabolic function testing, karyotyping chromosome anomalies, and endotoxin testing. Nevertheless, the question remains: how many of the

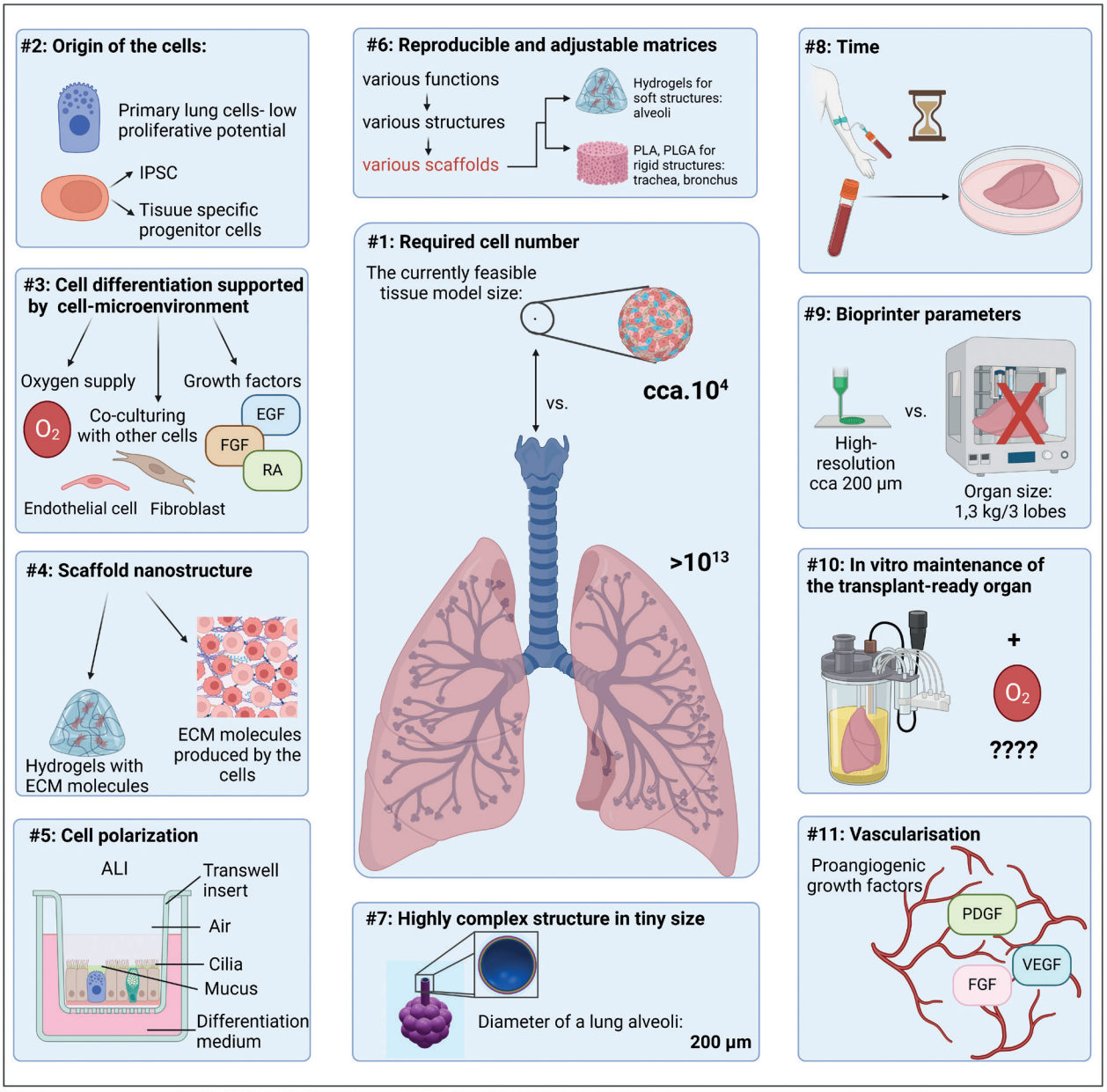

Figure 2. Summary of the challenges of transplant-ready lung tissue bioprinting. #1 Required cell number: The size of the currently feasible and reproducible tissue model is only a fraction of the size of transplant-ready lung tissue. #2 Origin of the cells: The proliferation of the primary cells is slow and limited; induced pluripotent stem cells (IPSCs) are genetically modified cells and may not be suitable for transplantation; tissue-specific progenitor cells are difficult to isolate in large quantities, especially from a diseased lung. #3 Cell differentiation supported by cell-microenvironment: Cell differentiation is influenced by various microenvironmental factors, such as other co-cultured cell types, growth factors, and oxygenation. #4 Scaffold nanostructure: Extracellular matrix molecules characteristic of the lungs must be applied during tissue culturing, or these molecules need to be produced by the cultured and printed cells themselves. #5 Cell polarization: Cell polarization of the epithelial cells in various airway sections is crucial for transplant-ready lung tissue; the air-liquid interface (ALI) promotes cell differentiation and cell polarization. #6 Reproducible and adjustable matrices: Various regions of lung tissue have different functions and structures, necessitating the use of various scaffold materials for tissue engineering; e.g., hydrogels in the case of lung alveoli, poly(lactic-co-glycolic) acid (PLGA) or poly-lactic acid (PLA) in the case of the conducting zone. #7 Highly complex structure in tiny size: To reproduce the highly complex, three-layered structure of the lung alveoli in a relatively tiny size (200 µm) is also challenging. #8 Time: One of the biggest challenges in creating a transplant-ready lung model is the time-consuming nature of the process; the upscaling of the isolated autologous cells itself would take an invaluable amount of time, if feasible at all, not to mention the additional time required for the printing process. #9 Bioprinter parameters: Printing a transplant-ready lung model would require a 3D bioprinter with high-resolution capabilities that can also print large tissue models; such a printer is currently not available on the market. #10 In vitro maintenance of the transplant-ready organ: Researchers have to solve the maintenance problem of transplant-ready lung tissue, i.e., nutrition and oxygenation. #11 Vascularization: Vascularization is also a crucial point in tissue engineering protocols, while complex tissues in a larger size start to necrotize without proper vascularization.

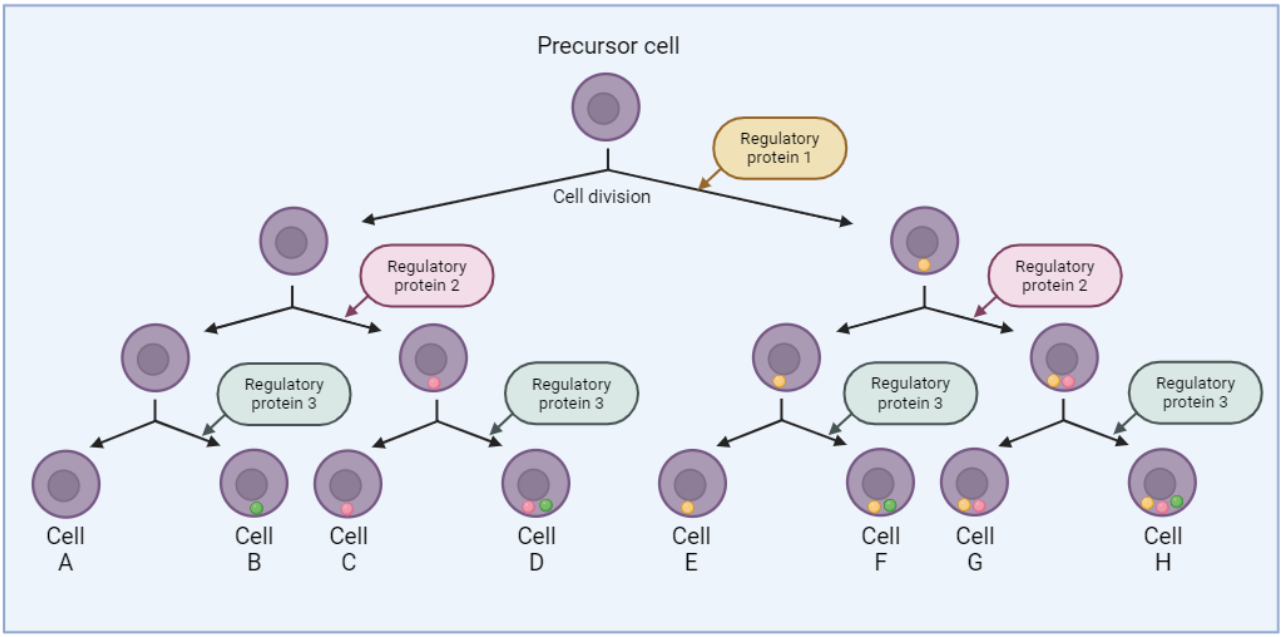

Figure 3. Exposure to various combinations of regulatory factors changes the direction of cellular subtype differentiation.

iPSCs can be printed directly on scaffolds, and how many pre-differentiation steps need to be performed to speed up functional organ production? How can we ensure that no de-differentiation will occur in the transplant-ready tissue? Additionally, the dedifferentiation of reprogrammed cells may not be the sole issue in the clinical use of iPSCs. The potential risk factors associated with iPSC-based applications include the cell source, the process of *in vitro* culturing, and the reprogramming method. A major risk is the teratoma-forming capability of the undifferentiated and pluripotent cells of the iPSC-derived donor organ.[24,25] To produce safe iPSC-derived transplant organs, quantifying undifferentiated iPSCs with a real-time monitoring system is necessary. The genetic instability of the iPSCs can be traced back to the reprogramming method or to the extended culturing of the cells *in vitro*.[26] The gene transfer of the reprogramming factors can result in the accumulation of pre-existing mutations, reprogramming factor integration-induced mutagenesis, and the resetting of the epigenetic codes of the genome.[27] A further problem is the spontaneous reactivation of reprogramming factors in the daughter cells of differentiated cells, which can result in tumor generation. The origin of the reprogrammed cell is also a crucial risk factor (Figure 4). In animal research, the transplanted, autologous murine embryonic stem (ES) cells induced teratocarcinoma formation, while xenotransplantation of ES cells did not induce tumor growth. The patient's reprogrammed cells can also carry disease-causing genes with expanded repeat regions, that can cause further genetic instability.[28] Hence, careful characterization of the donor cells is essential before any further steps are taken. From a clinical perspective, the origin and cell number remain unresolved issues for producing human transplantation—ready lung tissue.

2.4. Matrices

When the problem of cell numbers is resolved, it is essential to use degradable scaffolds and bioinks with the right biochemical and mechanical properties to support region-specific lung cells and the hierarchical lung structure.

In the proximal conducting airways, the trachea is the major airway that connects the larynx and bronchi, allowing air to enter and exit the lungs. Although the trachea is an airway, it is morphologically different from the distal airways of the lung. The trachea is composed of 16–20 cartilage rings that prevent it from collapsing. These rings are surrounded by smooth muscle and connective tissue.[29] Natural proteins, like collagen, elastin, fibronectin, and laminin, are commonly used in TE and regenerative medicine. Scaffolds and bioinks must be biocompatible and promote cell adhesion, proliferation, and differentiation.[30–32] Several scaffolds have been implemented to print a trachea, such as biodegradable reticular polycaprolactone (PCL), chitosan-based nanofiber membrane (CHIM), or a 1-day cell self-aggregation technique using a synthesized zwitterionic polymer.[33–35] The trachea scaffold has been suspended or immersed in human bronchial epithelial cells (hBECs), iPSC-derived mesenchymal stem cells (iPSC-MSCs), and iPSC-derived chondrocytes (iPSC-Chds)

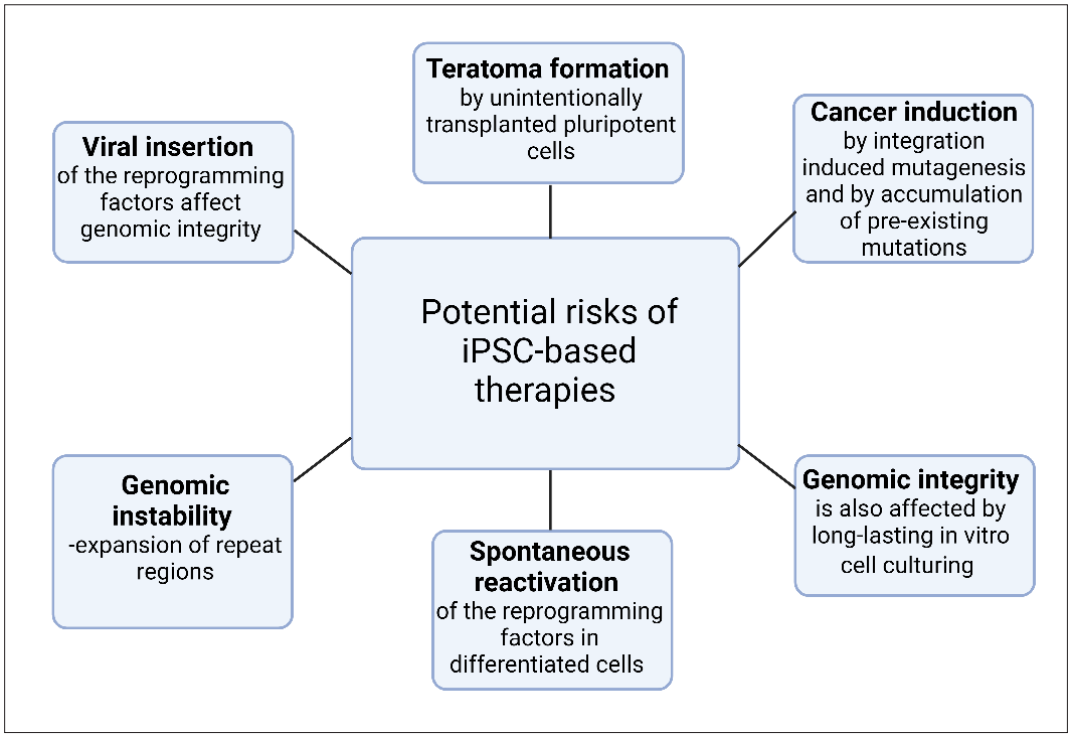

Figure 4. Overview of potential risks of reprogrammed stem cell-based therapies. Abbreviation: iPSC, induced pluripotent stem cell.

to maximize the regeneration of tracheal mucosa and cartilage cells to create the suitable tissue that is functional *in vivo*. Similarly to trachea printing, PCL and chitosan were combined with hydrogels to print bronchi and to create a lung-like structure.[36] Differentiation of the cells on the scaffolds also requires mechanical stimuli. Hence, the cells on the inner surface, for example, on the bronchi, must be exposed to air to initiate ciliated differentiation and mucus production. Practically, creating a tissue-size air–liquid interface[37] is a technique frequently used in the *in vitro* modeling of lung tissues for experimentation.

The distal respiratory airways provide different challenges for bioprinting. There are multiple modalities, but the two main categories are (i) acellular scaffold printing with subsequent recellularization and (ii) direct printing with cells suspended in a "bioink." Bioinks directly printed for the final shape with or without live cells, often include additional biomaterials, including growth factors (Figure 3), chemicals, microfactors, etc., that are mixed into hydrogels. While these hydrogels are generally soft due to naturally high water content, they can be gelled or reinforced to create functional scaffolds.[38] Hydrogels, mechanically weaker than conventional 3D-printed substrates, allow bioprinters to incorporate cells into the construct. Hydrogels are often used with the supportive scaffold, which is also necessary for producing a human-size, functional lung. The hydrogels are polymers and are made from natural or synthetic components. Components of natural polymers are designed to mimic the extracellular matrix (ECM) and include gelatin, collagen, laminin, and fibronectin, and other natural polymers such as alginate, chitosan, silk fibrin, and hyaluronic acid (HA).[39] When used alone, the hydrogels lack the mechanical strength required as bioinks, particularly for stereolithography bioprinting,[40,41] which is gaining recognition in lung bioprinting. Chemical modifications are often performed to make bioinks suitable for printing specific tissues. The mechanical properties of bioprinted constructs directly depend on the chemical composition of the bioink. HA is a widely used material that is present in the ECM and provides mechanical support and is also prone to undergo fairly rapid degradation, which might leave the maturing tissue construct without structural support before the cells can build their matrixes. Methacrylated HA (MeHA) is a chemically modified HA that can be further crosslinked by adding a photoinitiator under ultraviolet (UV) light exposure.[42] By chemically modifying gelatin with unsaturated methacrylamide groups, a crosslinkable polymer, gelatin-methacrylamide (GelMA), is created that can then be tailored for various mechanical properties and can encapsulate cells and drugs for TE.[43,44] GelMA has been used as the primary bioink to print human blood-derived endothelial colony-forming cells (ECFCs) and MSCs,

which could form a vascular network.[45] Hybrid bioinks are designed to improve the individual materials' disadvantages and desired properties. The hybrid bioink containing HA and gelatin can mimic ECM and its role in the mechanical support of native human tissues. A crosslinkable hybrid hydrogel containing GelMA and MeHA can be tailored for different TE applications.[46,47] By changing the crosslinkers and their concentrations, the storage modulus could be shifted from 100 Pa to 20 kPa, making such hybrid bioink suitable for printing several tissues, including the lung. The aforementioned matrices, cell types, and ECM molecules are summarized in Table 2.

2.5. Vascularization

The main challenge in human lung bioprinting is the development of vasculature, essential for the *in vitro*-produced lung to fulfill its primary function of gas exchange. Vasculature formation involves advancing bioinks and printing techniques to construct a viable vascular network within an engineered construct. The vasculature in the lung construct plays a crucial role in oxygen and nutrient delivery, as well as waste product transportation during maturation until the organ achieves functionality. Physiologically, cells must not be farther than 200 μm[48–50] from capillary blood vessels. The primary challenge lies in reliably printing capillaries. Capillary-sized networks have not yet been successfully printed, and there have been limitations in printing blood vessels smaller than 6 mm in diameter.[51] To address this issue, the microextrusion technique,[52] in combination with innovative and established methodologies, appears to be the most promising direction for resolving challenges in capillary network generation. Despite advancements in creating blood vessels, the techniques are slow to produce a functional vascular network and have several limitations, such as restricted sizes, shapes, and stable bifurcations.[53,54] To ensure cell survival in this environment, cellular metabolism needs to be slowed down by adding adenosine[55,56] and/or extra oxygen supplied transiently using particulate oxygen generators within the construct. However, achieving a safe and fully functional vascularization solution remains unrealized. Additionally, tissue vascularization is another challenge that should be addressed.[57–59] During the bioprinting process, the use of scaffolds and bioinks is inevitable. The goal is for the implanted scaffolds to degrade into non-toxic components, as high concentrations of waste components can be detrimental to cellular health, creating necrotic zones within the bioprinted organ, thus failing quality assurance. The extensive blood vessel network is paramount for the lung, and until this issue is resolved, clinically applicable transplant-ready lungs cannot be produced.[57,58,60–63]

2.6. Surgical suitability

While the technical challenges of creating laboratory-based organs are significant, additional requirements must be met for transplant-ready printed organs to be suitable for handling in the operating theatre (Figure 5). Therefore, additional factors must be considered alongside the challenges associated with producing a surgery-ready, transplantable lung:

(i) The tissue must be suitable for surgical procedures, e.g., suitable for suturing.

(ii) The lung must have connectable airways (bronchi).

(iii) The lung must be connectable to appropriately sized blood vessels (pulmonary artery and vein); therefore, a transplantable organ ready for surgery should also be designed and printed, bearing the above steps in mind.

After the organ is printed, the transplantation procedure will follow the standard protocol for any transplant surgery. Lung transplantation surgery typically lasts 3–8 h under

Table 2. Matrices of the airways

Airway	Airway section	Tissue-type	ECM	Scaffold-types	Cell-types
Proximal conducting airway	Trachea	Cartilage, smooth muscle, connective tissue, ciliated epithelium	Fibronectin, elastin, collagen, laminin	PCL, CHIM, CAT	hBECs, iPSC-MSCs, iPSC-Chds
	Larynx				
	Bronchi			PCL, CHIM, CAT, PCL+chitosan	
Distal respiratory airway	Bronchioles	Smooth muscle, connective tissue, ciliated epithelium	Gelatin, collagen, laminin, fibronectin, alginate, chitosan, silk fibrin, hyaluronic acid	HA or meHA hydrogels+ supportive scaffold (optional); GelMA	ECFC, MSCs
	Alveoli	Squamous epithelium, connective tissue			

Abbreviations: ECM, extracellular matrix; PCL, polycaprolactone; CHIM, chitosan-based nanofiber membrane; CAT, catalase chitosan; hBEC, human bronchial epithelial cell; iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cells; Chds, chitosan derivatives; HA, hyaluronic acid; meHA, Methacrylated HA; GelMA, gelatin-methacrylamide; ECFC, endothelial colony-forming cells.

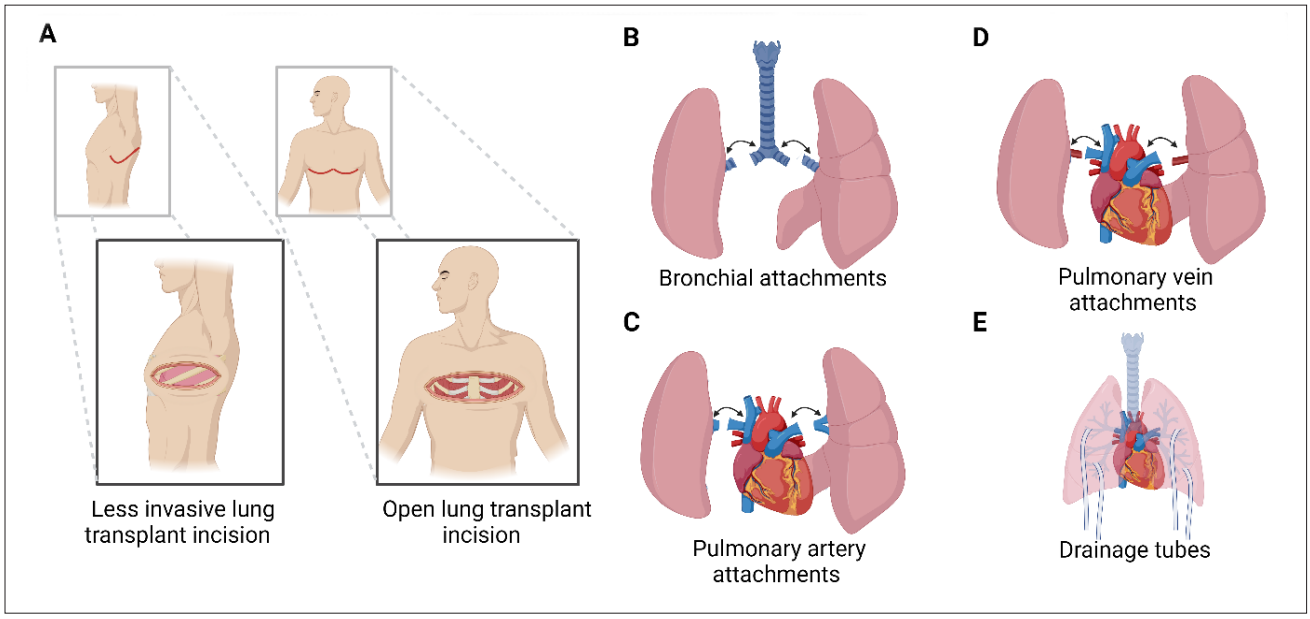

Figure 5. The process of a lung transplantation surgery. (A) Various types of lung transplant incisions. (B) The surgeon attaches the bronchial section of the donor's lung and the recipient's bronchial stub is attached by the surgeon. (C and D) The surgeon also connects the pulmonary artery (C) and veins (D) of the donor and recipient. (E) Drainage tubes are placed in the transplanted lungs.

general anesthesia, depending on the number of lung lobes being transplanted.

During the surgery, the damaged lung's bronchi and pulmonary blood vessels are cut and removed. Then, a healthy lung from a donor or, in the present case, from the tissue bioreactor is inserted. The new lung must have strong and fully formed airways and blood vessels, like those of a healthy human lung, to be connected to the recipient's airways and blood vessels. The surgery is completed by closing the incision. While all surgeries have risks, organ transplant surgeries carry high risks of infection, bleeding, and death. Even if a well-prepared and tested lung transplant is created using the patient's cells, there is still a potential risk shared with donated lungs. This risk is primary graft dysfunction, which is the most common cause of death after lung transplantation. Recent data from the International Society for Heart and Lung Transplantation Registry revealed that adult lung transplant recipients since 2010 have a 1-year survival rate of 85% and a 5-year survival rate of 59%. Despite ongoing advancements in patient and donor selection, surgical techniques, and pre- and postoperative care, lung transplantation survival rates still fall short of expectations.[64] The main reason why the development of macroscale printed organs is pursued, apart from the shortages of transplantable organs, is to avoid the additional risks associated with organ transplantation. If the printed organ is created from the recipient's cells, donor lung rejection is not expected, eliminating the need for immunosuppressant medications. Consequently, complications after surgery (e.g., kidney damage[65] or increased cancer and infection risks[66]) can be avoided.

2.7. Quality assurance

When printing a therapy-ready and clinical-grade organ, quality assurance must begin by ensuring the quality of the cell source, testing the donor and the harvested cells, and then scaling up the process to increase the cell numbers. First, the patients or donors must be screened for health and cleared to undergo the biopsy in a suitable operating room. Documentation of the entire process must follow each step. Biopsies have to be transported to a manufacturing facility for cell extraction. Using an iPSC-based therapy, somatic cell isolation will be followed by cell expansion to initiate the reprogramming procedure for the iPSC line. The entire process should utilize good manufacturing practice (GMP)-grade reagents, consumables, and facilities to ensure clinical compliance. GMP is critical for cell therapy, as it ensures the consistency, safety, and quality of therapeutic products. By adhering to GMP standards, risks such as contamination, variability, and errors in production are minimized, which is essential for patient safety and the efficacy of cell-based treatments. Compliance with GMP also meets regulatory requirements, facilitating the approval process and ensuring that the cell therapy product can be reliably and safely scaled for clinical use.

Once the organ is printed and matured, quality assurance of the functionality of the printed organ would be essential before even attempting surgical application of the *in vitro*-prepared lung.[67,68] Due to the unresolved challenges of biomanufacturing transplant-ready organs, relatively little attention is given to quality assurance in biomanufacturing. Quality assurance must be an independent field that develops in parallel with transplantation ready organs and rigorously scrutinizes every aspect of organ production, function, and transplant surgery. The *in vitro*-prepared lung tissue must have all the functional cell types, cultured most likely from iPSCs to complete organ cultures; cells and tissues must be infection-free (including mycoplasma) throughout the preparation. The final tissue must have a tight capillary network, culminating in pulmonary blood vessels. Test methods for building such organs and testing the complete biostructure before surgery require further development of bioreactors, allowing continuous oxygen and nutrition supply until the organ is mature enough to be used in surgery. Hence, the engineering requirements of *in vitro* organ production have to be developed in parallel with TE, focusing on the final product. Currently, we can only anticipate the primary requirements for quality assurance. Firstly, preliminary biocompatibility and safety testing will be required on animals to ensure that the 3D-bioprinted materials are not harmful and that the printed lung functions effectively, essentially validating that the protocol to print a functional lung is potentially suitable for human lung bioprinting. Thereafter, the printed organs will need to undergo clinical trials with human subjects, provided that the quality assurance protocol for each newly produced lung proves that the organ is functional. The key question is how the quality assurance will be carried out. As there is currently no fully functional bioprinted human lung, a standard quality assurance methodology has not yet been developed, hindering bioprinted lung tissue production and its clinical application.

2.8. Legislative regulation

The primary principle to conduct organ production for human beings involves the clear determination of ethical principles. Countries have developed ethical guidelines for conducting and evaluating research involving human participants, and many of these are necessary for organ production. The main guidelines are outlined in the World Medical Association's Declaration of Helsinki[69] and the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Health-Related Research Involving Humans.[70]

In most countries, the country-specific biomedical research councils have adopted the CIOMS guidelines,[70] regulating the collection, storage, and use of biological materials and related data. Additionally, in the European Union (EU), the EU Tissues, and Cells Directive[71] sets quality and safety standards for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells. In the United Kingdom (UK), the Human Tissue Act 2004[72] regulates the application of human cells and tissues; in the United States of America (USA), this is governed by the Code of Federal Regulations[73]; and in Australia, the guidelines are covered by the National Health and Medical Research Council.[74] Human biospecimens in laboratory-based research or transplantation are well-established. However, the challenge of personalized tissue production for patients in need of *in vitro*-made transplantable organs is compounded by the lack of well-defined administrative regulations for *in vitro*-produced, complex, functional tissues. To streamline regulatory pathways, it is crucial to establish clear and expeditious legislation for this relatively novel biomanufacturing process. The laws should facilitate the rapid implementation of organ production methods that can be brought to the market. Furthermore, the biotech industry requires support to navigate the regulatory framework and to scale up from research to biomanufacturing.[75] Although attention is given to the issue in the EU,[76] the series of overarching recommendations do not yet provide clear, step-by-step protocols for the research and biomanufacturing community to follow and implement the legal and ethical aspects of their work. Certainly, ethical and legal issues must be addressed, and solutions must be clearly communicated to the research and biomanufacturing community. This community is working on establishing regulations for biomanufacturing and approving biomanufactured products, especially when creating complex organs like the lung. In the USA, permissions are granted by the Institutional Review Boards; in Canada, by the Research Ethics Boards; and in Australia, by the Human Research Ethics Committees. These institutions must collaborate with administrative agencies for approval of drugs, devices, or diagnostic tools for sale or use in clinical settings, which is overseen by national drug and therapeutic goods regulators such as the US Food and Drug Administration (FDA) or the Australian Therapeutic Goods Administration. In Europe, the EU Medical Device Regulations also apply; in China, several agencies control the regulatory field.[77] It is important to note that not only the procedures but also the tools used in the process need to comply with standards and regulations. Currently, the biggest challenge in creating engineered tissues is that neither bioprinters nor bioreactors have medical device

Conformité Européenne (CE) certification (CE, EU) or GMP certification.

3. Aggregates and organoids for *in vitro* microscale lung tissue for clinical applications

It is crucial to study cell–cell interactions and differentiation processes in the normal lung to comprehend deviations from health and to gain insights into the development and progression of lung diseases. These studies can aid in creating lung tissue models that replicate lung diseases and in testing drugs for safety and efficacy. Furthermore, these investigations offer the opportunity to explore differences in therapy responses among various populations and to tailor medicine accordingly. Understanding the regenerative potential of specific tissues is also vital for promoting the generation of healthy, self-derived cells in the regenerative process, thereby reducing the reliance on complex medication and surgery as therapy (Figure 6). Hence, microscale lung tissue models can be just as clinically relevant as the transplantation-ready, functional lung.

3.1. Normal physiology

Based on the knowledge of lung anatomy and molecular studies, it has been realized that cell functions are governed by internal processes and various external factors, including direct and indirect interactions with neighboring cells, ECM, and soluble factors that shape and differentiate cells into specific tissues by additional physical forces.

In the initial 3D pulmonary models, a significant advancement was the use of mixed cell tissue cultures comprising primary human small airway epithelial cells (SAECs), normal human lung fibroblasts (NHLFs), and human microvascular endothelial cells (HMVECs). Together, these cells create the necessary ECM and restore specific tissue differentiation markers characteristic of the proximal or distal respiratory tract (Figure 7A), including surfactant proteins.[78] These markers are lost when lung epithelial cells are cultured in 2D environments. If the above primary cell types are simply incubated (natural gravitation) or forced (centrifuged together) to sediment in a low-affinity tissue culture plate, they form an aggregate culture within 24 h. The simplest distal airway model is

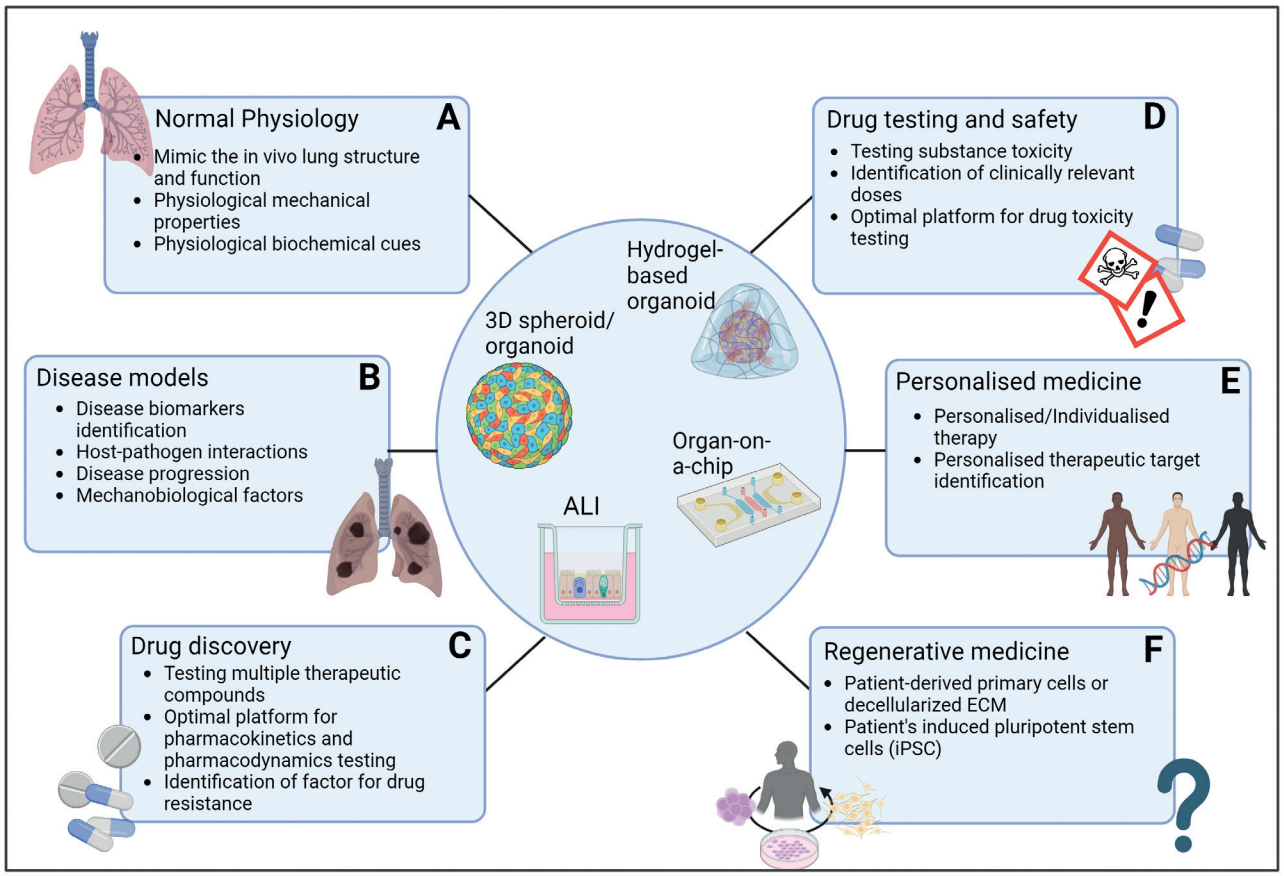

Figure 6. Summary of various applications of lung micro-tissue models. Abbreviations: ALI, air–liquid interface; ECM, extracellular matrix.

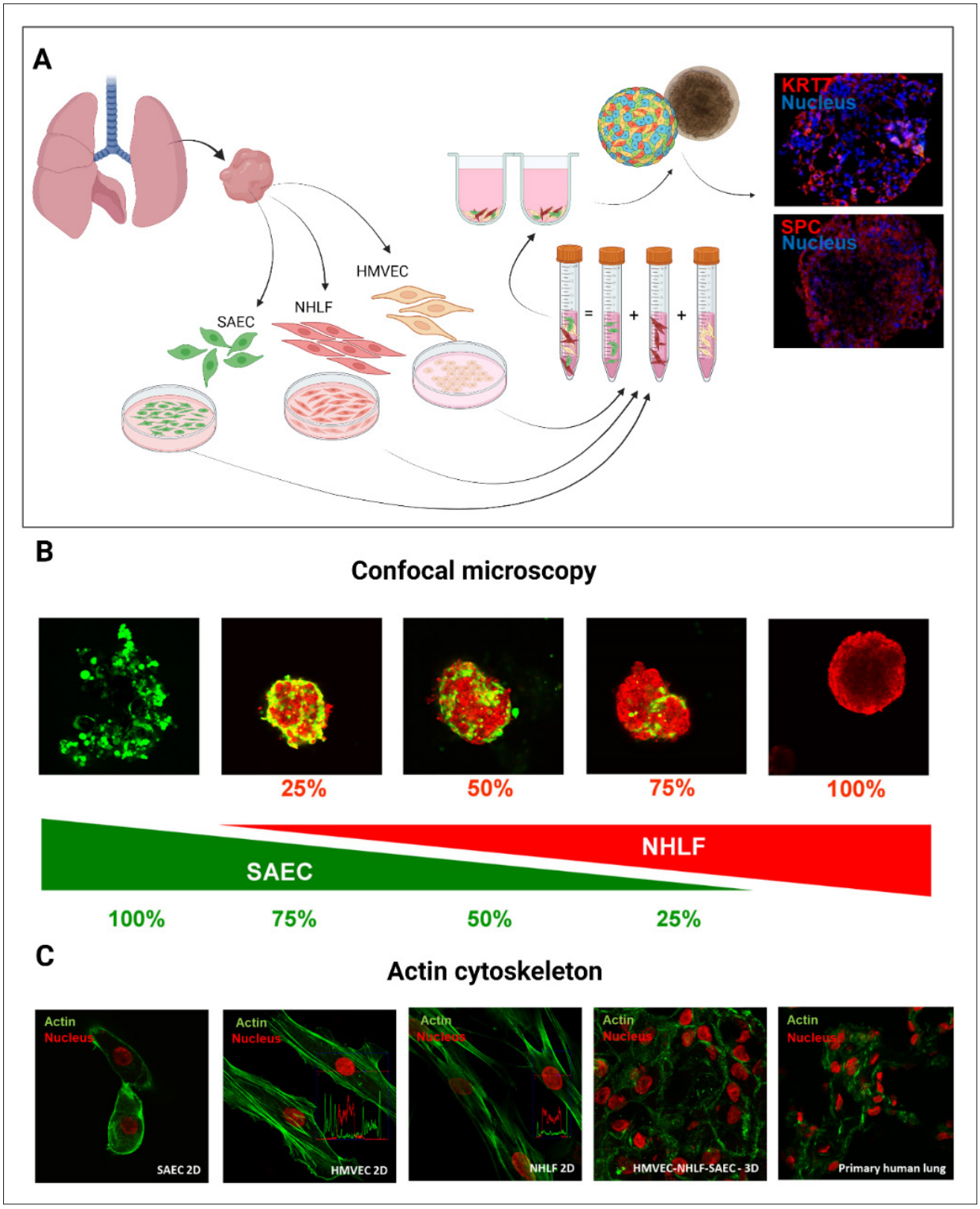

Figure 7. The production and characterization of 3D aggregation cultures. (A) The visualized protocol of the 3D aggregate models, with three different cell types. The fluorescent microscopic images display the molecular characteristics of the cells (red: KRT7 and SPC; blue: nuclear staining). (B) Confocal microscopic images of 3D aggregate models of SAEC and NHLF cells in different relative ratios. Cells in 2D monolayers were washed twice in PBS and incubated with CFSE, DiI, or DiD at the concentration of 0.5 μg/mL at 37°C for 10 min. (C) Confocal microscopic images of the actin cytoskeleton of various cell cultures versus human primary lung (green: actin; red: nuclear staining). Abbreviations: SAEC, small airway epithelial cells; NHLF, normal human lung fibroblasts; PBS, phosphate-buffered saline; CFSE, Carboxyfluorescein succinimidyl ester.

the combination of SAEC and NHLF at varying ratios, followed by the incubation of the aggregates for various lengths of time, depending on the purpose of the tissue model. The maximum size of such aggregate culture cannot exceed 500 μm in diameter and contain no more than about 30,000 cells in total. Otherwise, the inner core of the aggregate becomes necrotic due to a lack of nutrition and oxygen. The inner structure of such 3D aggregates maintains a certain arrangement due to the cell type-specific surface tension of cells.[79,80] Tracing the location of SAECs and NHLFs specifically within the aggregate, the individual 2D monocultures were first stained with fluorescent physiological dyes (carboxyfluorescein succinimidyl ester [CFSE], DiI (orange fluorescence) [DiIC18(3) or 3H-Indolium] and DiD (red fluorescence) [DiIC18(5) a longer-wavelength DiI analog], respectively). Within the aggregates, SAECs formed the outer layer on the surface of the inner NHLF core network.[81] The tissue size depends on the epithelial cell-to-fibroblast ratio and the total cell number (Figure 7). During the 24-h time frame, differentiation cannot be detected; instead, only the preferential location of each cell type in the aggregate can be observed (Figure 7B). This arrangement is based on the cell surface tension and biophysical parameters mentioned above. The arrangement of the cytoskeleton also changes depending on the type of cell culture and the cell types used creating the lung model. Adding endothelial and smooth muscle cells to the model can enhance its complexity. In 2D monolayers, the actin cytoskeleton demonstrates the stretched-out formation of the cells; in 3D aggregate cultures, the actin cytoskeleton takes on the formation that is highly similar to normal primary lung tissue pattern (Figure 7C), which becomes detectable after 72 h in culture.[82] The presence of endothelial cells was also found to stimulate the expression of functional molecules such as ABC drug transporters.[83] This makes the aggregate models easy to create and suitable for investigating biochemical mechanisms and molecular regulation of healthy and diseased lung tissues.[84] Such tissue structures are also suitable for testing the functionality of the expressed molecules and drug response.

Cells in the airways are exposed to mechanical effects; hence, the most effective models for producing tissues closely resembling normal human airways are those subjected to constant dynamic stress and oxygen tension. Among the various methodologies currently available, the air–liquid interface (ALI) method stands out for its simplicity and ability to promote cell differentiation and optimize the morphological and histological characteristics of airway epithelial cells.[37]

The epithelial layer exhibits various functional phenotypes, such as tightly packed cells forming a barrier to external elements through tight intercellular junctions, cilia growth, and mucus secretion. These phenotypes depend on the tissue type and mechanical effects. This model requires exposure to air to develop these properties. The mechanical environment within the bronchial airway model, whether normal or modified (e.g., constricted airways), is distinct.[85] In order to replicate constricted airways, we need to apply lateral compressive strain to the model, inducing buckling folds similar to those observed *in vivo*. This compression can lead to matrix deformation affecting hBECs and human fetal fibroblasts (HLFs), which are in direct communication via the ECM. It is crucial to analyze the system's response to mechanical strain and the interaction between different cell types sharing the same ECM. This investigation can shed light on normal development, the effects of mechanical strains, disease development, or any other stimuli and associated molecular changes.

The 3D lung models previously mentioned generate their own ECM. However, in creating intricate tissue models, hydrogels[37] are frequently used. Hydrogels are gel-like materials containing networks of interconnected polymers that are swollen with water. They are available in various forms and are employed to mimic the functions of the ECM, which surrounds and sustains cells in our tissues. Hydrogels can help create complex tissues and provide experimental systems to modify molecules that affect tissue differentiation and natural physiology. This is important for gaining a better understanding of normal human lung physiology and for creating controls for disease models.

3.2. Disease models

Understanding the mechanisms that drive human disease pathologies is essential for developing effective treatments. However, this is a complex task due to the lack of biologically relevant disease models. Rodents have been extensively used to attempt to replicate human diseases for many years, owing to their status as mammalian models that lend themselves to experimental assessment and genetic engineering. The fundamental interspecies differences render it impossible for rodent models to precisely mirror or fully reproduce human clinical pathophysiology.[86]

Several companies offer the same cell types (SAEC, NHLF, HMVC, etc.) isolated from patients suffering from lung complications (e.g., asthma and COPD). By applying the same model system for the controls as for the donated cells, and using the same growth and air–liquid interface media, researchers can create disease models that reflect the great variety of human lung diseases using the techniques mentioned in the previous section. However, primary cell types are not as readily available, which may

limit the cell numbers available to generate the required quantity of tissue aggregates. As a result, more tests and analyses could be conducted using recently developed methods involving iPSCs derived from patients suffering from various lung diseases.

Human iPSCs (hiPSCs) have emerged as an attractive platform for overcoming the conventional limitations of animal models and primary lung cells from patients with lung diseases. The simple aggregate or hydrogel models using iPSCs are suitable for studying a great variety of lung diseases, gaining spatial and temporal data on tissue morphology, and providing the opportunity to identify disease biomarkers and factors that induce disease progression. The aggregate and hydrogel cultures described above are easy to create and use in the laboratory, while the whole arsenal of molecular technology[87] and clinical and open databases support the analysis of disease progression.

For example, hydrogels could provide the necessary binding capacity[88] and a more comprehensive *in vitro* model of the airway wall as a dynamic system. Due to its ability to support multiple cell types without contracting the matrix, this model is suitable for observing and understanding the long-term effects of factors, such as inflammation and mechanical stress, on remodeling. It is especially relevant for studying remodeling events associated with asthma.[89] The development of appropriate disease models, such as ALI, for investigating asthma and identifying drug targets is essential to streamline the drug discovery and test process. As the models work similarly to the human body, the developed medications are likely to be more effective with fewer adverse reactions. It is essential to have non-invasive methods for measuring cellular functionality and maturation in tissue model systems (e.g., ALI) to ensure their reliability. A commonly used technique for this purpose is trans-epithelial electrical resistance, which assesses the permeability of the cell or tissue layer to monitor barrier integrity. Preserving intact barriers is crucial for evaluating the transport of small molecules or drugs across the barrier in these model systems.[90]

3.3. Drug discovery

Understanding and confirming potential drug targets involves delving into the intricate mechanisms and specific points where intervention can be effective for a particular disease or condition. It is essential to ascertain that a potential target plays a pivotal role in initiating and perpetuating the disease. According to the hypothesis, there are diverse potential targets to consider for intervention, including genes, epigenetic factors, proteins, RNA, miRNA, and other components involved in the disease's pathogenesis that could be targeted for treatment or modulation.

Despite a surge in investments in drug research, most drugs fail during clinical stages due to various reasons. One significant factor contributing to this translational gap is the heavy reliance on animal-derived data[91,92] to make decisions about advancing a drug candidate into clinical trials. Consequently, biomedical research is currently undergoing a significant shift toward approaches centered on human disease models, including micro-tissue lung models, such as organoids, functionality models, and the cutting-edge technology of organs-on-chips (OOCs).[93] This paradigm shift has been fueled by the notably high failure rates of the current drug development process.

Organ-on-a-chip (OOC) microfluidic cultures represent a groundbreaking advancement in human tissue mimicry, simulating the intricate cellular interactions, molecular regulations, and mechanical influences that cells experience within real organs (Figure 8A). This innovative technology is revolutionizing our ability to replicate organ-level functions and responses, offering a more accurate model of physiological systems. On a chip, the microfluidic form of a microphysiological system can be of different sizes and shapes, but all contain hollow channels lined with living cells and tissues cultured under dynamic fluid flow (Figure 8B). Several different fluid systems have been built from suitable materials and miniature pumps and the application of microphysical forces to move fluids and attached detection systems.[94] While microfluidic systems involve tiny volumes, they also ensure fast interactions between fluids and cells. The pulmonary alveolus model was created using a soft lithography-based[95,96] manufacturing approach, adapted from the microchip industry. The microfluidic lung alveolar chip device was made from optically clear, elastomeric polydimethylsiloxane material[97] that contains two parallel hollow channels separated initially by a porous membrane,[98] which was eventually replaced by an ECM gel to support tissue ECM formation.[99,100] The channels coated with ECM and lined with epithelial (SAECs differentiated into AT1) cells on one side and endothelial cells (HMVEC-L) on the other to mimic the alveolar–capillary interface. The suitable culture medium is perfused through the endothelium-lined channel to mimic vascular perfusion, while air is introduced into the epithelial channel to mimic the air–liquid interface in the lung, promoting polarization, and differentiation of the epithelial cell lining. It also instigates cyclic aspiration on the hollow side chambers, allowing cyclic tissue deformation to occur at the flexible tissue–tissue interface, and mimicking respiratory movement.

Human-based models are invaluable tools in every stage of the drug development process, including high-throughput screening to identify target or lead molecules, efficacy and safety testing in preclinical stages, and

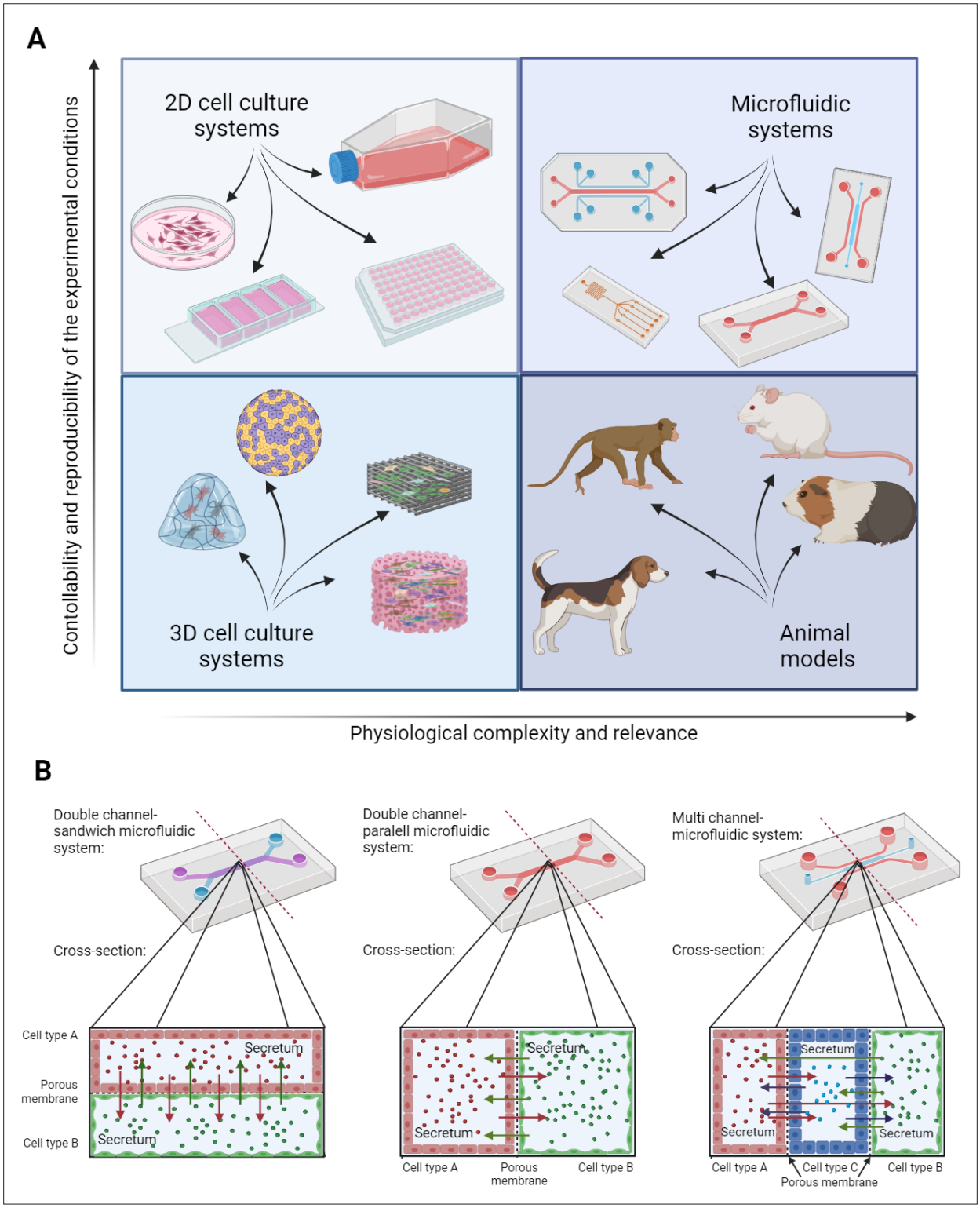

Figure 8. Complexity and reproducibility of disease models. (A) *In vitro* and *in vivo* techniques used in the pharmaceutical industry for testing toxicology and efficacy. (B) Various complexities of microchips using fully human tissue mimicking microfluidic systems for reproducible testing.

implementation in clinical trials and decision-making. Rational drug design and development require a proper understanding of the underlying disease mechanism. The need for *in vitro* human lung modeling became apparent during the COVID-19 pandemic. For instance, central disease mechanisms, such as endothelial dysfunction, the "cytokine storm," and the variability of infection susceptibility between cells, were identified using organoids and lung-on-a-chip models.[101–104] It was discovered that exposure to Sars-CoV-2 (Severe acute respiratory syndrome coronavirus 2) significantly reduces endothelial barrier function, leading to increased release of pro-inflammatory cytokines. Endothelial dysfunction is aggravated upon the introduction of peripheral blood mononuclear cells, resulting in excessive inflammation. Organoids also exhibit inefficient interferon responses after COVID-19 infection, highlighting the value and predictivity of human micro-tissue models. Organoids of the lung and other tissues, such as the brain, kidneys, and heart, have facilitated the identification of SARS-CoV-2 tropism[105] and receptor angiotensin-converting enzyme 2 preferences, making it easier to identify the most likely target organs. Similar examples include allergic asthma[106] and influenza. It was found that organoids can be supplemented with primary immune cells to closely mimic the host microenvironment following infection, aiding the understanding of disease mechanisms and drug target identification. The 3D human cell-based infection models were extremely valuable in identifying new antiviral treatments and repurposing drugs. Over a thousand drugs were tested using high-throughput screening against COVID-19, and some have been approved by the United States (US) FDA. Interestingly, when physiological breathing mechanics were applied in alveoli-on-chip models, it was found that mechanical stimuli increased the expression of interferon-related genes and improved the host's defense, indicating antiviral activity.[107]

Significant progress has been made in the fight against cancer through the use of human disease models. In human-derived organoids, MCLA-158, a bispecific antibody that targets the epidermal growth factor receptor (EGFR) and leucine-rich repeat-containing G-protein-coupled receptor 5, emerged as the most effective antibody against wild-type and KRAS (Kirsten rat sarcoma virus)-mutant colorectal cancer (CRC) after screening a large biobank of patient-derived CRC organoids; the research results have reached clinical application within 3 years.[108] A similar result was achieved in the treatment of non-small cell lung cancer (NSCLC), where the efficacy of amivantamab, a bispecific antibody against EGFR and the mesenchymal-epithelial transition receptor, was preclinically tested in patient-derived cells and organoids with EGFR Exon20ins mutations.[109] Patients with this mutation have an extremely poor prognosis because standard tyrosine kinase inhibitors are not effective in therapy. These results were validated in clinical trials, leading to the approval of amivantamab by the European Medicines Agency for the treatment of EGFR mutant NSCLC.

3.4. Drug testing and safety

Pharmaceutical applications of human tissue models were considered early on when the 3Rs (Replacement, Reduction, and Refinement) of animal testing were introduced as a concept over 60 years ago.[110,111]

Due to strict regulations in the pharmaceutical industry, implementation remains difficult despite billions of dollars being spent on drugs, vaccines, and advanced therapeutic products that fail in humans after successful pre-clinical animal testing. Some fail to demonstrate efficacy, like the tuberculosis drug MV85a[112]; while others, like the monoclonal antibody Hu5c8 (CD40L), exhibit life-threatening toxicity[113,114] and unexpected thrombotic complications. Researchers are working relentlessly to make human tissue systems available for human disease research and drug testing.[115] Certainly, the USA has recently taken a bold step forward, evidenced by the US FDA Modernization Act of 2021[116] and the Humane Research and Testing Act (HR 1744).[117,118] Unfortunately, neither Act that was passed by the 117th Congress makes *in vitro* drug testing compulsory for human drugs before clinical trials commence.

Nevertheless, researchers are working to develop a great variety of pre-clinical *in vitro* models to address the challenges posed by the safety and efficacy testing of human medications. The use of 3D cultures in drug target identification, drug efficacy testing, and toxicity assessment is high on the agenda, as selecting the most effective compound for clinical trials is in the interest of the pharmaceutical industry.

The recently developed multicompartment nasal-bronchial-acinar-on-chip has demonstrated cytokine responses in lower lung compartments upon administration of an aerosolized virus to the nasal chamber.[45] However, this study has not applied cell stretch, which could enhance aerosolized nanoparticles' cellular uptake and transport.[46] The development of complex *in vitro* models to emulate the interplay between various lung compartments with aerosolized drug delivery could improve the predictive capacity of lung models to study the fate of aerosol inhalation.

The microfluidic-based platform for lungs suffers from a lack of high throughput, which, albeit not harmful during the initial drug discovery phase, poses a severe challenge

in the later stages of drug development. Adapting the outcome of current microphysiological systems to the drug development pipeline is challenging due to the inaccurate extrapolation and scaling-up of data to the *in vivo* level. Potential strategies would be cell assay standardization, scaling up the microvolume size of these platforms using parallel cell culture chambers,[47] or applying milli-scale systems, such as the newly developed mini-lung model.[36]

The establishment of healthy/disease *in vitro* models based on isolated human primary cells or hiPSCs using organoid technology[57–59] or organotypic cells from patients (disease-specific) could enhance the mimicry of the models, such as cell–cell interactions and cellular responses. It could also improve the prediction of drug pharmacodynamics and pharmacokinetics, thereby facilitating *in vitro–in vivo* translation. It has been reported that the culture of ATII cell-derived organoids under physiologic stretch conditions (10% strain) using alveolus-on-chip could preserve the ATII phenotype (such as supported by the expression of SPC [Surfactant Protein C]).[60] Likewise, a lower expression level of vimentin as an epithelial-mesenchymal transition-related marker was observed compared to the static Transwell inserts. Hence, combining the lung-on-chip models with primary cell-derived organoids[61,62] could enhance the predictive capacity of the preclinical models for at least some clinically relevant aspects to levels higher than those obtained with animal models.

The developed systems involve aggregate cultures (often called spheroids or organoids) as well as OOCs or body-on-a-chip (BOCs) microfluidic cultures by fluidically coupling two or more organ chips.[119] A microfluidic lung model was used, for example, to investigate drug-induced pulmonary edema[120] by mimicking the physiology of the distal lung as OOC.

The advances in stem-cell technology have enabled the cultivation of personalized stem cells that can now be integrated into OOCs for studying disease progression, identifying drug targets, and testing developed drugs for safety and efficacy.[121]

As the sophistication of the models increases, advancements in OOC technology can shift the focus to complex human tissue tests instead of animal testing before clinical trials in the future.[122] This shift is important in personalized medicine, as the need for personalized therapy is highlighted by inter-racial disparities in drug response, stressing the importance of considering diverse genetic factors in optimizing treatment outcomes.[123]

3.5. Personalized therapy selection

A broad variation in individual response to the same therapy can be caused by differences in the molecular microenvironment within the affected tissue, personal variability in the patient's genetics, environmental effects, etc., influencing the treatment selection and therapy outcome.

Currently, the most widely studied therapy response is investigated in cancer therapy, where a well-identifiable altered tissue can be removed from the affected person to identify drug sensitivity, drug administration sequence, and selection of the best sequence of combination therapy.[124,125]

By preserving or recreating the individual tumor microenvironment *in vitro*, we can test drug responses and identify the most effective drug combinations for therapy. Creating these test systems is easy to understand, as they are similar to the methods used to identify specific antibiotics for microbial infections. Just as antimicrobial resistance remains the world's most urgent public health concern,[126] cancer cells can also inherently possess or develop drug resistance.[127]

In clinical applications, the use of 3D lung micro-tissues serves as groundbreaking test systems for the precise selection of cancer therapy. This innovative approach promises to revolutionize cancer treatment by providing a more tailored and effective strategy. For example, in NSCLC,[128] a suspension of solid cancer tissue is created, which is then reaggregated and exposed to recommended chemotherapeutic drugs and combinations; the results can be compared with patient therapy responses. The importance of personalized drug response testing became evident through an analysis conducted by the Evidence-based Synthesis Program, which reviewed over 820 trials and published meta-analyses.[129–132] The conclusions were ambiguous and determined mostly what was already known; doublet chemotherapy with a platinum agent has a slight advantage over non-platinum doublets[133]; cisplatin combinations may have a slight advantage over carboplatin combinations in terms of survival and response rate.[134] However, carboplatin generally has a milder toxicity than cisplatin.[135] Triplet cytotoxic therapy might have some slight advantages in terms of response rate but at an increased risk of toxicity; doublet chemotherapy probably has slight benefits in terms of survival compared to singlet therapy but causes more toxicity. The question remains: why are the drugs not chosen more effectively? According to general treatment guidelines (www.cancer.org), in NSCLC, the most commonly used chemotherapeutic drugs include cisplatin, carboplatin, paclitaxel, albumin-bound paclitaxel, docetaxel, gemcitabine, vinorelbine, irinotecan, etoposide, vinblastine, and pemetrexed.[136] The 11 different kinds of chemotherapeutic drugs are most often applied as a combination, typically involving two selected drugs—either two chemotherapeutic drugs or in combination

with targeted therapy drugs, such as afatinib, gefitinib,[137] crizotinib, certinib,[138] bevacizumab,[139] cetuximab, and ramucirumab.[140] These therapies target specific small molecules in signaling pathways or the immune system, including nivolumab and ipilimumab.[141]

The unpredictable efficacy of therapy is not surprising, as over 140 driver mutations[142–145] and an unknown number of passenger mutations[146–148] have been identified, both of which may impact carcinogenesis. Effectively, at least two to eight driver mutations per cancer sample regulate

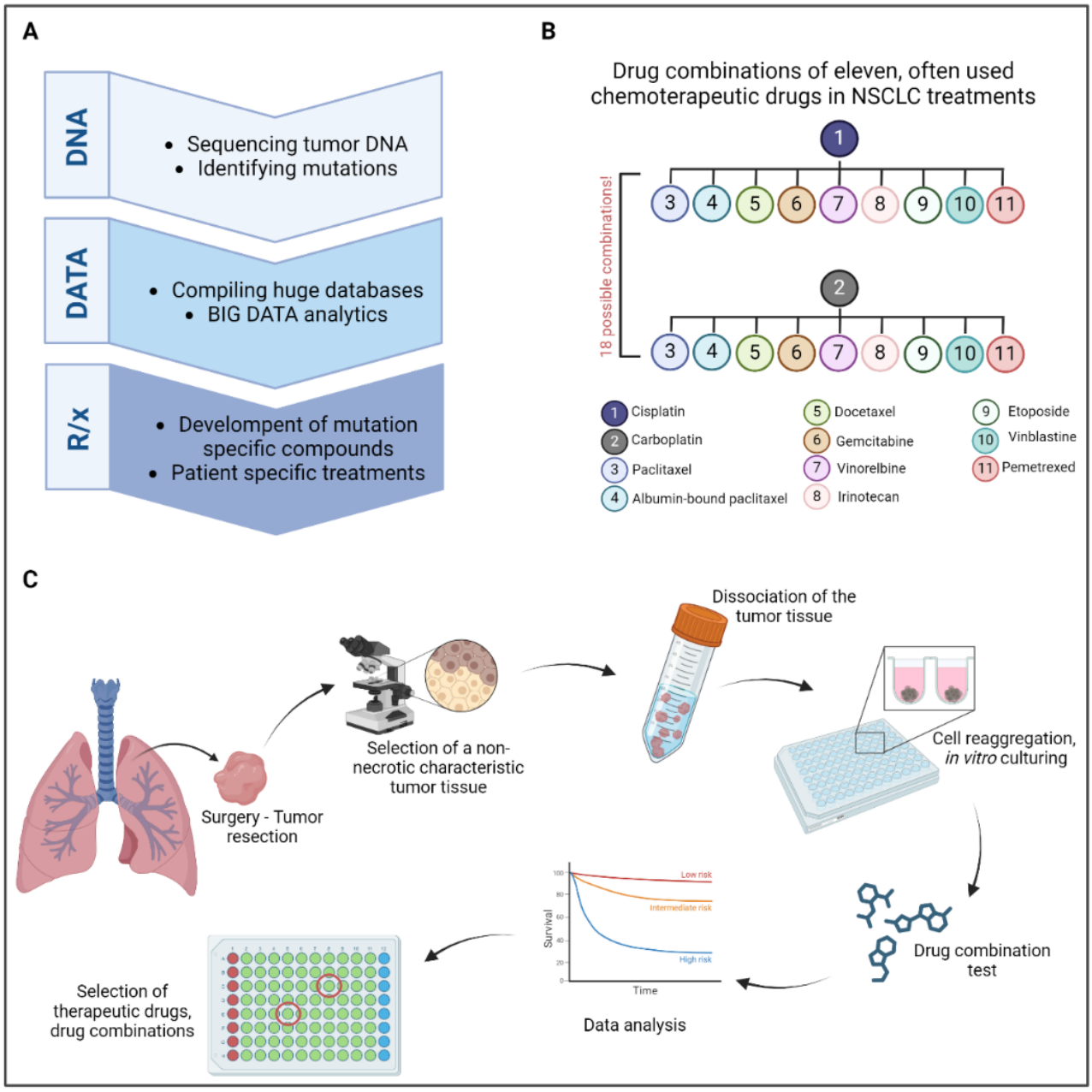

Figure 9. Lung models in personalized medicine. (A) The response of the biotech industry to the challenges of cancer genetics. Analyzing sequenced tumor DNAs and identified mutations can form the basis of personalized medicine. (B) The combinational options of different chemotherapeutic drugs used in the treatment of NSCLC. The listed drugs can be applied in 18 different combinations to treat the disease. (C) Resected dissociated, reaggregated, and *in vitro* cultured tumor cells can provide a useful platform for finding the best combination of chemotherapeutic drugs to treat cancer in a personalized way. Abbreviation: NSCLC, non-small cell lung cancer.

cancer progression. Additionally, non-mutation-induced metabolic changes and personalized environmental factors can also modulate therapy response, making therapy selection extremely challenging for the clinical team (Figure 9).

Hence, clinicians could determine the best combination of drugs—those with the most efficacy and the least adverse effects—on an individual level if the potential drug combinations were tested on the tumor tissue *in vitro* after surgery and before therapy selection (Figure 9), while the patient recovers from surgery and becomes ready for chemotherapy, targeted therapy, or immunotherapy.[149]

3.6. Lung tissue regeneration

The key therapeutic promise of investigating lung tissue regeneration is its potential to cure acute lung failure and reduce the need for lung transplantation surgery. Lung tissue possesses a natural regenerative ability, initiating repair processes following injury. Under controlled conditions, lung tissue can fully restore its normal structure and function. Disruptions in this process can lead to respiratory system failure and even death. The primary types of respiratory diseases include COPD, idiopathic pulmonary fibrosis, and acute respiratory distress syndrome. Various cells, such as lung epithelial cells, endothelial cells, fibroblasts, and immune cells, are involved in regulating lung injury repair. Although the regulatory mechanism of lung repair has not yet been fully elucidated, clinical trials are targeting different cells and signaling pathways to achieve therapeutic effects. For example, primary lung stem cell-like ATII cells were induced to transdifferentiate into ATI (AT1-like) cells[9] by changing the wingless (WNT) ligand microenvironment in 3D lung microtissues. While WNT4 and WNT5A downregulated ATII-type characteristics, the presence of WNT7A strengthened ATI-like markers, including aquaporin 5 (AQ5). Identification of all the markers, cytokines, miRNAs, and other factors involved in the process of lung tissue regeneration is still in its early stages. However, the establishment of healthy and disease *in vitro* models based on isolated human primary cells or hiPSCs using 3D lung microtissue technology enhances the mimicry of the models, such as the cell–cell interactions and cellular responses. This approach can facilitate combination therapies for faster recovery of lung tissue after disease-induced damage. Most available *in vitro* lung models investigating regenerative processes rely on primary or iPSCs to mimic proximal or distal pulmonary cells during tissue damage. These models also help identify the best combinations of factors for tissue regeneration.

4. Conclusion

The intricate 3D structure of the lung tissue, characterized by thin boundaries and interconnected pores, presents significant challenges for bioprinting. Technical limitations and unresolved ethical and legal issues complicate the process. While research laboratories are making progress in overcoming these technical challenges, the prospect of a transplant-ready organ currently remains distant from clinical reality. However, researchers and engineers in the TE sector are relentlessly advancing lung bioprinting, committed to developing fully functional, transplantation-ready lungs. They are also determined to overcome the significant challenges, while fully recognizing that achieving this milestone may take several decades. Nonetheless, at present, the growing understanding of lung tissue complexity suggests that regenerative therapy could become a more effective and safer alternative for specific disease types.

Meanwhile, advances in lung bioprinting technologies and discoveries are enhancing our understanding of molecular interactions in healthy lungs. Disease-specific lung models are crucial for identifying new drug targets, improving our understanding of metabolic changes, and offering robust systems to test new drug candidates' safety and efficacy. These accomplishments are driving drug development and regenerative therapy forward. Additionally, further research in various fields, including bioink development, chemistry, materials science, bioprinter, and bioreactor engineering, is crucial to effectively model the lung for individual clinical applications. The most promising human-based preclinical *in vitro* lung models with high clinical translational capacity include detailed, complex tissue models derived from patient-specific cells, and leading *in vitro* platforms that mimic more physiological and pathological conditions through non-invasive functional analysis. There is significant potential in modern fabrication technology and approaches to achieve truly biomimetic material designs, integrating tissue-specific multicellularity with structural and mechanical biomimicry. This approach can produce biologically functional and realistic *in vitro* lung tissue models suitable for clinical applications, ultimately improving the health of individuals with lung diseases.

Acknowledgments

None.

Funding

This work was funded by a grant from the University of Pécs (grant number POC/2024).

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Judit E Pongracz
Visualization: Judit Bovari-Biri, Borbala Lovaszi
Writing–original draft: Judit Bovari-Biri, Judit E Pongracz
Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

Further disclosure

Part of or the entire set of findings has been presented at a conference (The role of 3D bioprinting in drug testing (in Hungarian). 2018, Partner Day Conference, Pécs, Hungary).

References

1. Barnes-Harris M, Allingham S, Morgan D, et al. Comparing functional decline and distress from symptoms in people with thoracic life-limiting illnesses: lung cancers and non-malignant end-stage respiratory diseases. *Thorax.* 2021;76:989-995. doi: 10.1136/thoraxjnl-2020-216039.
2. Dey M, Ozbolat IT. 3D bioprinting of cells, tissues and organs. *Sci Rep.* 2020;10:14023. doi: 10.1038/s41598-020-70086-y
3. Derakhshanfar S, Mbeleck R, Xu K, Zhang X, Zhong W, Xing M. 3D bioprinting for biomedical devices and tissue engineering: a review of recent trends and advances. *Bioact Mater.* 2018;3:144-156. doi: 10.1016/j.bioactmat.2017.11.008
4. Tuppin MP, Paratz JD, Chang AT, et al. Predictive utility of the 6-minute walk distance on survival in patients awaiting lung transplantation. *J Heart Lung Transplant.* 2008;27:729-734. doi: 10.1016/j.healun.2008.03.017
5. Nathan SD. Lung transplantation: disease-specific considerations for referral. *Chest.* 2005;127;1006-1016. doi: 10.1378/chest.127.3.1006
6. Van Scott MR, Chandler J, Olmstead S, Brown JM, Mannie M. Airway anatomy, physiology, and inflammation. In: Weggs WJ, ed. The Toxicant Induction of Irritant Asthma, Rhinitis, and Related Conditions; 2013:19–61. doi: 10.1007/978-1-4614-9044-9_2
7. Ochs M, Nyengaard JR, Jung A, et al. The number of alveoli in the human lung. *Am J Respir Crit Care Med.* 2004;169:120-124. doi: 10.1164/rccm.200308-1107OC
8. Travaglini KJ, Nabhan AN, Penland L, et al. A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature.* 2020;587:619-625. doi: 10.1038/s41586-020-2922-4
9. Abdelwahab EMM, Rapp J, Feller D, et al. Wnt signaling regulates trans-differentiation of stem cell like type 2 alveolar epithelial cells to type 1 epithelial cells. *Respir Res.* 2019;20:204. doi: 10.1186/s12931-019-1176-x
10. Gillich A, Zhang F, Farmer CG, et al. Capillary cell-type specialization in the alveolus. *Nature.* 2020;586:785-789. doi: 10.1038/s41586-020-2822-7
11. Kadur Lakshminarasimha Murthy P, Sontake V, Tata A, et al. Human distal lung maps and lineage hierarchies reveal a bipotent progenitor. *Nature.* 2022;604:111-119. doi: 10.1038/s41586-022-04541-3
12. Woo YD, Jeong D, Chung DH. Development and functions of alveolar macrophages. *Mol Cells.* 2021;44:292-300. doi: 10.14348/molcells.2021.0058
13. Weibel ER. What makes a good lung? *Swiss Med Wkly.* 2009;139:375-386. doi: 10.4414/smw.2009.12270
14. Doryab A, Tas S, Taskin MB, et al. 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*. 2019;29:1903114. doi: 10.1002/adfm.201903114
15. Bonassar LJ, Vacanti CA. Tissue engineering: the first decade and beyond. *J Cell Biochem.* 1998;72(Suppl 30–31):297-303. doi:10.1002/(SICI)1097-4644(1998)72:30/31+<297::AID-JCB36>3.0.CO;2-6
16. Fan P, Jin F, Qin Y, et al. Multiscale 3D bioprinting for the recapitulation of lung tissue. *IJB.* 2023;9:1166. doi: 10.36922/ijb.1166
17. Mahfouzi SH, Hamid S, Ghassem, A. 3D bioprinting for lung and tracheal tissue engineering: criteria, advances, challenges, and future directions. *Bioprinting.* 2021;21:e00124. doi: 10.1016/j.bprint.2020.e00124
18. Papaioannou TG, Manolesou D, Dimakakos E, Tsoucalas G, Vavuranakis M, Tousoulis D. 3D bioprinting methods and techniques: applications on artificial blood vessel fabrication. *Acta Cardiol Sin.* 2019;35:284-289. doi: 10.6515/ACS.201905_35(3).20181115A
19. Roche CD, Brereton RJL, Ashton AW, Jackson C, Gentile C. Current challenges in three-dimensional bioprinting

heart tissues for cardiac surgery. *Eur J Cardiothorac Surg.* 2020;58:500-510.
doi: 10.1093/ejcts/ezaa093

20. Li J, Chen M, Fan X, Zhou H. Recent advances in bioprinting techniques: approaches, applications and future prospects. *J Transl Med.* 2016;14:271.
doi: 10.1186/s12967-016-1028-0

21. Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet-based bioprinting: past, present and future. *Biomaterials.* 2016;102:20-42.
doi: 10.1016/j.biomaterials.2016.06.012

22. Hinton TJ, Jallerat Q, Palchesko RN, et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci Adv.* 2015;1:e1500758.
doi: 10.1126/sciadv.1500758

23. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126:663-676.
doi: 10.1016/j.cell.2006.07.024

24. Ben-David U, Nudel N, Benvenisty N. Immunologic and chemical targeting of the tight-junction protein Claudin-6 eliminates tumorigenic human pluripotent stem cells. *Nat Commun.* 2013;4:1992.
doi: 10.1038/ncomms2992

25. Cunningham JJ, Ulbright TM, Pera MF, Looijenga LH. Lessons from human teratomas to guide development of safe stem cell therapies. *Nat Biotechnol.* 2012;30:849-857.
doi: 10.1038/nbt.2329

26. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. *J Transl Med.* 2011;9:29.
doi: 10.1186/1479-5876-9-29

27. Blasco MA, Serrano M, Fernandez-Capetillo O. Genomic instability in iPS: time for a break. *EMBO J.* 2011;30:991-993.
doi: 10.1038/emboj.2011.50

28. Haston KM, Finkbeiner S. Clinical trials in a dish: the potential of pluripotent stem cells to develop therapies for neurodegenerative diseases. *Annu Rev Pharmacol Toxicol.* 2016;56:489-510.
doi: 10.1146/annurev-pharmtox-010715-103548

29. Holbert JM, Strollo DC. Imaging of the normal trachea. *J Thorac Imaging.* 1995;10:171-179.
doi: 10.1097/00005382-199522000-00003

30. Xing H, Lee H, Luo L, Kyriakides TR. Extracellular matrix-derived biomaterials in engineering cell function. *Biotechnol Adv.* 2020;42:107421.
doi: 10.1016/j.biotechadv.2019.107421

31. Morwood AJ, El-Karim IA, Clarke SA, Lundy FT. The role of extracellular matrix (ECM) adhesion motifs in functionalised hydrogels. *Molecules.* 2023;28(12):4616.
doi: 10.3390/molecules28124616

32. Noro J, Vilaça-Faria H, Reis RL, Pirraco RP. Extracellular matrix-derived materials for tissue engineering and regenerative medicine: a journey from isolation to characterization and application. *Bioact Mater.* 2024;34:494-519.
doi: 10.1016/j.bioactmat.2024.01.004

33. Gao M, Zhang H, Dong W, et al. Tissue-engineered trachea from a 3D-printed scaffold enhances whole-segment tracheal repair. *Sci Rep.* 2017;7:5246.
doi: 10.1038/s41598-017-05518-3

34. Kim H, Lee JY, Han H, et al. Improved chondrogenic performance with protective tracheal design of Chitosan membrane surrounding 3D-printed trachea. *Sci Rep.* 2021;11:9258.
doi: 10.1038/s41598-021-88830-3

35. Hiwatashi S, Iwai R, Nakayama Y, Moriwaki T, Okuyama H. Successful tracheal regeneration using biofabricated autologous analogues without artificial supports. *Sci Rep.* 2022;12:20279.
doi: 10.1038/s41598-022-24798-y

36. Rezaei FS, Khorshidian A, Beram FM, et al. 3D printed chitosan/polycaprolactone scaffold for lung tissue engineering: hope to be useful for COVID-19 studies. *RSC Adv.* 2021;11:19508-19520.
doi: 10.1039/d1ra03410c

37. Silva S, Bicker J, Falcão A, Fortuna A. Air-liquid interface (ALI) impact on different respiratory cell cultures. *Eur J Pharm Biopharm.* 2023;184:62-82.
doi: 10.1016/j.ejpb.2023.01.013

38. Sears NA, Seshadri DR, Dhavalikar PS, Cosgriff-Hernandez E. A review of three-dimensional printing in tissue engineering. *Tissue Eng Part B Rev.* 2016;22:298-310.
doi: 10.1089/ten.TEB.2015.0464

39. Aazmi A, Zhang D, Mazzaglia C, et al. Biofabrication methods for reconstructing extracellular matrix mimetics. *Bioact Mater.* 2024;31:475-496.
doi: 10.1016/j.bioactmat.2023.08.018

40. Kumar H, Kim K. Stereolithography 3D bioprinting. *Methods Mol Biol.* 2020;2140:93-108.
doi: 10.1007/978-1-0716-0520-2_6

41. Li W, Wang M, Ma H, Chapa-Villarreal FA, Lobo AO, Zhang YS. Stereolithography apparatus and digital light processing-based 3D bioprinting for tissue fabrication. *iScience.* 2023;26:106039.
doi: 10.1016/j.isci.2023.106039

42. GhavamiNejad A, Ashammakhi N, Wu XY, Khademhosseini A. Crosslinking strategies for 3D bioprinting of polymeric hydrogels. *Small.* 2020;16:e2002931.
doi: 10.1002/smll.202002931

43. Schuurman W, Levett PA, Pot MW, et al. Gelatin-methacrylamide hydrogels as potential biomaterials for fabrication of tissue-engineered cartilage constructs. *Macromol Biosci.* 2013;13:551-561.

doi: 10.1002/mabi.201200471

44. Galliger Z, Vogt CD, Panoskaltsis-Mortari A. 3D bioprinting for lungs and hollow organs. *Transl Res.* 2019;211:19-34. doi: 10.1016/j.trsl.2019.05.001

45. Chen YC, Lin RZ, Qi H, et al. Functional human vascular network generated in photocrosslinkable gelatin methacrylate hydrogels. *Adv Funct Mater.* 2012;22: 2027-2039. doi: 10.1002/adfm.201101662

46. Camci-Unal G, Cuttica D, Annabi N, Demarchi D, Khademhosseini A. Synthesis and characterization of hybrid hyaluronic acid-gelatin hydrogels. *Biomacromolecules.* 2013;14:1085-1092. doi: 10.1021/bm3019856

47. Skardal A, Devarasetty M, Kang HW, et al. A hydrogel bioink toolkit for mimicking native tissue biochemical and mechanical properties in bioprinted tissue constructs. *Acta Biomater.* 2015;25:24-34. doi: 10.1016/j.actbio.2015.07.030

48. Kaully T, Kaufman-Francis K, Lesman A, Levenberg S. Vascularization--the conduit to viable engineered tissues. *Tissue Eng Part B Rev.* 2009;15:159-169. doi: 10.1089/ten.teb.2008.0193

49. Bertassoni LE, Cecconi M, Manoharan V, et al. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip.* 2014;14:2202-2211. doi: 10.1039/c4lc00030g

50. Nashimoto Y, Hayashi T, Kunita I, et al. Integrating perfusable vascular networks with a three-dimensional tissue in a microfluidic device. *Integr Biol (Camb.)* 2017;9:506-518. doi: 10.1039/c7ib00024c

51. L'Heureux N, McAllister TN, de la Fuente LM. Tissue-engineered blood vessel for adult arterial revascularization. *N Engl J Med.* 2007;357:1451-1453. doi: 10.1056/NEJMc071536

52. Eduardo DT, Ana SE, José BF. A micro-extrusion 3D printing platform for fabrication of orodispersible printlets for pediatric use. *Int J Pharm.* 2021;605:120854. doi: 10.1016/j.ijpharm.2021.120854

53. Bos GW, Poot AA, Beugeling T, van Aken WG, Feijen J. Small-diameter vascular graft prostheses: current status. *Arch Physiol Biochem.* 1998;106:100-115. doi: 10.1076/apab.106.2.100.4384

54. Seifu DG, Purnama A, Mequanint K, Mantovani D. Small-diameter vascular tissue engineering. *Nat Rev Cardiol.* 2013;10:410-421. doi: 10.1038/nrcardio.2013.77

55. Boison D, Yegutkin GG. Adenosine metabolism: emerging concepts for cancer therapy. *Cancer Cell.* 2019;36:582-596. doi: 10.1016/j.ccell.2019.10.007

56. Wirth AN, Tsutsui K, Maltsev VA, Lakatta EG. Adenosine reduces sinoatrial node cell action potential firing rate by uncoupling its membrane and calcium clocks. *Front Physiol.* 2022;13;977807. doi: 10.3389/fphys.2022.977807

57. Dall'Olmo L, Zanusso I, Di Liddo R, et al. Blood vessel-derived acellular matrix for vascular graft application. *Biomed Res Int.* 2014;2014:685426. doi: 10.1155/2014/685426

58. L'Heureux N, Pâquet S, Labbé R, Germain L, Auger FA. A completely biological tissue-engineered human blood vessel. *FASEB J.* 1998;12:47-56. doi: 10.1096/fasebj.12.1.47

59. McAllister TN, Maruszewski M, Garrido SA, et al. Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study. *Lancet.* 2009;373:1440-1446. doi: 10.1016/S0140-6736(09)60248-8

60. Gao G, Lee JH, Jang J, et al. Tissue engineered bio-blood-vessels constructed using a tissue-specific bioink and 3d coaxial cell printing technique: a novel therapy for ischemic disease. *Adv Funct Mater.* 2017;27:1700798. doi: 10.1002/adfm.201700798

61. Ju YM, Choi JS, Atala A, Yoo JJ, Lee SJ. Bilayered scaffold for engineering cellularized blood vessels. *Biomaterials.* 2010;31:4313-4321. doi: 10.1016/j.biomaterials.2010.02.002

62. Assmann A, Delfs C, Munakata H, et al. Acceleration of autologous in vivo recellularization of decellularized aortic conduits by fibronectin surface coating. *Biomaterials.* 2013;34:6015-6026. doi: 10.1016/j.biomaterials.2013.04.037

63. Jung Y, Ji H, Chen Z, et al. Scaffold-free, human mesenchymal stem cell-based tissue engineered blood vessels. *Sci Rep.* 2015;5:15116. doi: 10.1038/srep15116

64. Bos S, Vos R, Van Raemdonck DE, Verleden GM. Survival in adult lung transplantation: where are we in 2020? *Curr Opin Organ Transplant.* 2020;25:268-273. doi: 10.1097/MOT.0000000000000753

65. Kędzierska K, Sindrewicz K, Sporniak-Tutak K, et al. Does immunosuppressive therapy affect markers of kidney damage? *Ann Transplant.* 2016;21:137-144. doi: 10.12659/aot.895275

66. Engels EA, Pfeiffer RM, Fraumeni JF, et al. Spectrum of cancer risk among US solid organ transplant recipients. *JAMA.* 2011;306;1891-1901. doi: 10.1001/jama.2011.1592

67. Kirillova A, Bushev S, Abubakirov A, Sukikh G. Bioethical and Legal Issues in 3D Bioprinting. *Int J Bioprint.* 2020;6:272. doi: 10.18063/ijb.v6i3.272

68. Ricci G, Gibelli F, Sirignano A. Three-dimensional bioprinting of human organs and tissues: bioethical and medico-legal implications examined through a scoping review. *Bioengineering (Basel).* 2023;10:1052.

doi: 10.3390/bioengineering10091052

69. Association WM. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310:2191-2194. doi: 10.1001/jama.2013.281053

70. van Delden JJ, van der Graaf R. Revised CIOMS International ethical guidelines for health-related research involving humans. *JAMA*. 2017;317:135-136. doi: 10.1001/jama.2016.18977

71. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on Setting Standards of Quality and Safety for the Donation, Procurement, Testing, Processing, Preservation, Storage and Distribution of Human Tissues and Cells; 2004.

72. Human Tissue Act; 2004.

73. Code of Federal Regulations. *Part 1271: Human Cells, Tissues, and Cellular and Tissue-Based Products*; 2024.

74. National Health and Medical Research Council. Australian Research Council and Universities Australia (2007, Updated 2018) National Statement on Ethical Conduct in Human Research; 2018.

75. Garielchyk T. Biotech Boost without Extra Money, Vol. 23, *Summer ed.*; 2024.

76. Kritikos M. 3D Bio-Printing for Medical and Enhancement Purposes: Legal and Ethical Aspects; 2018. doi: 10.2861/923327

77. Li Y, Verter F, Wang B, Gu N. Regulations on cell therapy products in China: a brief history and current status. *Regen Med.* 2019;14:791-803. doi: 10.2217/rme-2019-0069

78. Kovacs T, Csongei V, Feller D, et al. Alteration in the Wnt microenvironment directly regulates molecular events leading to pulmonary senescence. *Aging Cell.* 2014;13:838-849. doi: 10.1111/acel.12240

79. Foty RA, Pfleger CM, Forgacs G, Steinberg MS. Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development.* 1996;122:1611-1620. doi: 10.1242/dev.122.5.1611

80. Forgacs G. Surface tension and viscoelastic properties of embryonic tissues depend on the cytoskeleton. *Biol Bull.* 1998;194:328-329; discussion 329-330. doi: 10.2307/1543103

81. JE, Pongracz, 2009. Unpublished observations

82. JE, Pongracz, 2016. Unpublished observations

83. Vesel M, Rapp J, Feller D, et al. ABCB1 and ABCG2 drug transporters are differentially expressed in non-small cell lung cancers (NSCLC) and expression is modified by cisplatin treatment via altered Wnt signaling. *Respir Res.* 2017;18:52. doi: 10.1186/s12931-017-0537-6

84. Rapp J, Kiss E, Meggyes M, et al. Increased Wnt5a in squamous cell lung carcinoma inhibits endothelial cell motility. *BMC Cancer.* 2016;16:915. doi: 10.1186/s12885-016-2943-4

85. Kilic O, Yoon A, Shah SR, et al. A microphysiological model of the bronchial airways reveals the interplay of mechanical and biochemical signals in bronchospasm. *Nat Biomed Eng.* 2019;3:532-544. doi: 10.1038/s41551-019-0366-7

86. Sayed N, Liu C, Wu JC. Translation of human-induced pluripotent stem cells: from clinical trial in a dish to precision medicine. *J Am Coll Cardiol.* 2016;67: 2161-2176. doi: 10.1016/j.jacc.2016.01.083

87. Baxter A, Thain S, Banerjee A, et al. Targeted omics analyses, and metabolic enzyme activity assays demonstrate maintenance of key mucociliary characteristics in long term cultures of reconstituted human airway epithelia. *Toxicol In Vitro.* 2015;29:864-875. doi: 10.1016/j.tiv.2015.03.004

88. Shafiee A, Norotte C, Elham G. Cellular bioink surface tension: a tunable biophysical parameter for faster maturation of bioprinted tissue. *Bioprinting.* 2017;8:13-21. doi: 10.1016/j.bprint.2017.10.001

89. Nizamoglu M, Joglekar MM, Almeida CR, et al. Innovative three-dimensional models for understanding mechanisms underlying lung diseases: powerful tools for translational research. *Eur Respir Rev.* 2023;32(169):230042. doi: 10.1183/16000617.0042-2023.

90. Felix K, Tobias S, Jan H, Nicolas S, Michael M. Measurements of transepithelial electrical resistance (TEER) are affected by junctional length in immature epithelial monolayers. *Histochem Cell Biol.* 2021;156:609-616. doi: 10.1007/s00418-021-02026-4

91. Golding H, Khurana S, Zaitseva M. What is the predictive value of animal models for vaccine efficacy in humans? The importance of bridging studies and species-independent correlates of protection. *Cold Spring Harb Perspect Biol.* 2018;10(4):a028902. doi: 10.1101/cshperspect.a028902

92. Franco R, Cedazo-Minguez A. Successful therapies for Alzheimer's disease: why so many in animal models and none in humans? *Front Pharmacol.* 2014;5:146. doi: 10.3389/fphar.2014.00146

93. Si L, Bai H, Rodas M, et al. A human-airway-on-a-chip for the rapid identification of candidate antiviral therapeutics and prophylactics. *Nat Biomed Eng.* 2021;5:815-829. doi: 10.1038/s41551-021-00718-9

94. Iakovlev AP, Erofeev AS, Gorelkin PV. Novel pumping methods for microfluidic devices: a comprehensive review. *Biosensors (Basel).* 2022;12(11):956. doi: 10.3390/bios12110956.

95. Xia Y, Whitesides GM. Soft lithography. *Angew Chem Int Ed Engl.* 1998;37:550-575. doi: 10.1002/(SICI)1521-3773(19980316)37:5<550::AID-ANIE550>3.0.CO;2-G

96. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. *Nat Biotechnol.* 2014;32:760-772. doi: 10.1038/nbt.2989

97. Miranda I, Souza A, Sousa P, et al. Properties and applications of pdms for biomedical engineering: a review. *J Funct Biomater.* 2021;13(1):2. doi: 10.3390/jfb13010002

98. Huh D, Matthews BD, Mammoto A, et al. Reconstituting organ-level lung functions on a chip. *Science.* 2010;328:1662-1668. doi: 10.1126/science.1188302

99. Saldin LT, Cramer MC, Velankar SS, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: structure and function. *Acta Biomater.* 2017;49:1-15. doi: 10.1016/j.actbio.2016.11.068

100. Pouliot RA, Link PA, Mikhaiel NS, et al. Development and characterization of a naturally derived lung extracellular matrix hydrogel. *J Biomed Mater Res A*. 2016;104:1922-1935. doi: 10.1002/jbm.a.35726

101. Zhang M, Wang P, Luo R, et al. Biomimetic human disease model of SARS-CoV-2-induced lung injury and immune responses on organ chip system. *Adv Sci (Weinh).* 2021;8:2002928. doi: 10.1002/advs.202002928

102. Katsura H, Sontake V, Tata A, et al. Human lung stem cell-based alveolospheres provide insights into SARS-CoV-2-mediated interferon responses and pneumocyte dysfunction. *Cell Stem Cell.* 2020;27(6):890-904.e898. doi: 10.1016/j.stem.2020.10.005

103. Lamers MM, van der Vaart J, Knoops K, et al. An organoid-derived bronchioalveolar model for SARS-CoV-2 infection of human alveolar type II-like cells. *EMBO J.* 2021;40:e105912. doi: 10.15252/embj.2020105912

104. Thacker VV, Sharma K, Dhar N, Mancini GF, Sordet-Dessimoz J, McKinney JD. Rapid endotheliitis and vascular damage characterize SARS-CoV-2 infection in a human lung-on-chip model. *EMBO Rep.* 2021;22:e52744. doi: 10.15252/embr.202152744

105. Ramani A, Müller L, Ostermann PN, et al. SARS-CoV-2 targets neurons of 3D human brain organoids. *EMBO J.* 2020;39:e106230. doi: 10.15252/embj.2020106230

106. Nawroth JC, Lucchesi C, Cheng D, et al. A microengineered airway lung chip models key features of viral-induced exacerbation of asthma. *Am J Respir Cell Mol Biol.* 2020;63:591-600. doi: 10.1165/rcmb.2020-0010MA

107. Bai H, Si L, Jiang A, et al. Mechanical control of innate immune responses against viral infection revealed in a human lung alveolus chip. *Nat Commun.* 2022;13:1928. doi: 10.1038/s41467-022-29562-4

108. Herpers B, Eppink B, James MI, et al. Functional patient-derived organoid screenings identify MCLA-158 as a therapeutic EGFR × LGR5 bispecific antibody with efficacy in epithelial tumors. *Nat Cancer.* 2022;3:418-436. doi: 10.1038/s43018-022-00359-0

109. Yun J, Lee SH, Kim SY, et al. Antitumor activity of amivantamab (JNJ-61186372), an EGFR-MET bispecific antibody, in diverse models of. *Cancer Discov.* 2020;10:1194-1209. doi: 10.1158/2159-8290.CD-20-0116

110. Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. *Animals (Basel).* 2019;9(10):754. doi: 10.3390/ani9100754

111. Russel WMS, Burch RL. The Principles of Human Experimental Technique; 1992.

112. Tameris MD, Hatherill M, Landry BS, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet.* 2013;381:1021-1028. doi: 10.1016/S0140-6736(13)60177-4

113. Arpinati M, Chirumbolo G, Rondelli D. Enhancement of T cell activation by immobilized hu5C8 (anti-CD40L) monoclonal antibody. *Eur J Haematol.* 2008;80: 322-330. doi: 10.1111/j.1600-0609.2007.01015.x

114. Shock A, Burkly L, Wakefield I, et al. CDP7657, an anti-CD40L antibody lacking an Fc domain, inhibits CD40L-dependent immune responses without thrombotic complications: an in vivo study. *Arthritis Res Ther.* 2015;17:234. doi: 10.1186/s13075-015-0757-4

115. Breslin S, O'Driscoll L. Three-dimensional cell culture: the missing link in drug discovery. *Drug Discov Today.* 2013;18:240-249. doi: 10.1016/j.drudis.2012.10.003

116. Han JJ. FDA Modernization Act 2.0 allows for alternatives to animal testing. *Artif Organs*. 2023;47(3):449-450. doi: 10.1111/aor.14503

117. Administration F.a.D. HR-1744 Human Research and Testing Act of 2021 117th Congress; 2021–2022.

118. Wadman M. FDA no longer has to require animal testing for new drugs. *Science.* 2023;379:127-128. doi: 10.1126/science.adg6276

119. Kimura H, Sakai Y, Fujii T. Organ/body-on-a-chip based on microfluidic technology for drug discovery. *Drug Metab Pharmacokinet.* 2018;33:43-48. doi: 10.1016/j.dmpk.2017.11.003

120. Huh D, Leslie DC, Matthews BD, et al. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. *Sci Transl Med.* 2012;4:159ra147. doi: 10.1126/scitranslmed.3004249

121. Palasantzas VEJM, Tamargo-Rubio I, Le K, et al. iPSC-derived organ-on-a-chip models for personalized human genetics and pharmacogenomics studies. *Trends Genet.* 2023;39:268-284. doi: 10.1016/j.tig.2023.01.002

122. Ma C, Peng Y, Li H, Chen W. Organ-on-a-chip: a new paradigm for drug development. *Trends Pharmacol Sci.* 2021;42:119-133. doi: 10.1016/j.tips.2020.11.009

123. Chen ML. Ethnic or racial differences revisited: impact of dosage regimen and dosage form on pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet.* 2006;45:957-964. doi: 10.2165/00003088-200645100-00001

124. Driehuis E, Kretzschmar K, Clevers H. Establishment of patient-derived cancer organoids for drug-screening applications. *Nat Protoc.* 2020;15:3380-3409. doi: 10.1038/s41596-020-0379-4.

125. Papp E, Steib A, Abdelwahab EM, et al. Feasibility study of in vitro drug sensitivity assay of advanced non-small cell lung adenocarcinomas. *BMJ Open Respir Res.* 2020;7(1):e000505. doi: 10.1136/bmjresp-2019-000505

126. Holmes AH, Moore LS, Sundsfjord A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet.* 2016;387:176-187. doi: 10.1016/S0140-6736(15)00473-0

127. Weng WH, Wang CY, Yan ZY, Lee HT, Kao CY, Chang CW. Isolation and characterizations of multidrug-resistant human cancer cells by a biodegradable nano-sensor. *Biosens Bioelectron.* 2024;249:115985. doi: 10.1016/j.bios.2023.115985

128. Gridelli C, Rossi A, Carbone DP, et al. Non-small-cell lung cancer. *Nat Rev Dis Primers.* 2015;1:15009. doi: 10.1038/nrdp.2015.9

129. Yang X, Yang K, Kuang K. The efficacy and safety of EGFR inhibitor monotherapy in non-small cell lung cancer: a systematic review. *Curr Oncol Rep.* 2014;16:390. doi: 10.1007/s11912-014-0390-4

130. Tajarernmuang P, Aliaga F, Alwakeel AJ, et al. Accuracy of cytologic vs histologic specimens for assessment of programmed cell death Ligand-1 expression in non-small cell lung cancer: a systematic review and meta-analysis. *Chest.* 2024;165:461-474. doi: 10.1016/j.chest.2023.09.013

131. Bremnes RM, Camps C, Sirera R. Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. *Lung Cancer.* 2006;51:143-158. doi: 10.1016/j.lungcan.2005.09.005

132. Xue C, Zheng S, Dong H, et al. Association between efficacy of immune checkpoint inhibitors and sex: an updated meta-analysis on 21 trials and 12,675 non-small cell lung cancer patients. *Front Oncol.* 2021;11:627016. doi: 10.3389/fonc.2021.627016

133. Boni C, Tiseo M, Boni L, et al. Triplets versus doublets, with or without cisplatin, in the first-line treatment of stage IIIB-IV non-small cell lung cancer (NSCLC) patients: a multicenter randomised factorial trial (FAST). *Br J Cancer.* 2012;106:658-665. doi: 10.1038/bjc.2011.606

134. Griesinger F, Korol EE, Kayaniyil S, et al. Efficacy and safety of first-line carboplatin-versus cisplatin-based chemotherapy for non-small cell lung cancer: a meta-analysis. *Lung Cancer.* 2019;135:196-204. doi: 10.1016/j.lungcan.2019.07.010

135. Rajeswaran A, Trojan A, Burnand B, Giannelli M. Efficacy and side effects of cisplatin- and carboplatin-based doublet chemotherapeutic regimens versus non-platinum-based doublet chemotherapeutic regimens as first line treatment of metastatic non-small cell lung carcinoma: a systematic review of randomized controlled trials. *Lung Cancer.* 2008;59:1-11. doi: 10.1016/j.lungcan.2007.07.012

136. Tsvetkova D, Ivanova S. Application of approved cisplatin derivatives in combination therapy against different cancer diseases. *Molecules.* 2022;27(8):2466. doi: 10.3390/molecules27082466

137. van der Wekken AJ, Saber A, Hiltermann TJ, Kok K, van den Berg A, Groen HJ. Resistance mechanisms after tyrosine kinase inhibitors afatinib and crizotinib in non-small cell lung cancer, a review of the literature. *Crit Rev Oncol Hematol.* 2016;100:107-116. doi: 10.1016/j.critrevonc.2016.01.024

138. Li J, Knoll S, Bocharova I, Tang W, Signorovitch J. Comparative efficacy of first-line ceritinib and crizotinib in advanced or metastatic anaplastic lymphoma kinase-positive non-small cell lung cancer: an adjusted indirect comparison with external controls. *Curr Med Res Opin.* 2019;35:105-111. doi: 10.1080/03007995.2018.1541443

139. Socinski MA, Nishio M, Jotte RM, et al. IMpower150 final overall survival analyses for atezolizumab plus bevacizumab and chemotherapy in first-line metastatic nonsquamous NSCLC. *J Thorac Oncol.* 2021; 16:1909-1924. doi: 10.1016/j.jtho.2021.07.009

140. Vacchelli E, Pol J, Bloy N, et al. Trial watch: tumor-targeting monoclonal antibodies for oncological indications. *Oncoimmunology.* 2015;4:e985940. doi: 10.4161/2162402X.2014.985940

141. Stoff R, Grynberg S, Asher N, et al. Efficacy and toxicity of Ipilimumab-Nivolumab combination therapy in

elderly metastatic melanoma patients. *Front Oncol.* 2022;12:1020058.
doi: 10.3389/fonc.2022.1020058

142. Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J Clin Oncol.* 2022;40:611-625.
doi: 10.1200/JCO.21.01626

143. Harrison PT, Vyse S, Huang PH. Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. *Semin Cancer Biol.* 2020;61:167-179.
doi: 10.1016/j.semcancer.2019.09.015

144. Lim TKH, Skoulidis F, Kerr KM, et al. KRAS G12C in advanced NSCLC: prevalence, co-mutations, and testing. *Lung Cancer.* 2023;184:107293.
doi: 10.1016/j.lungcan.2023.107293

145. Wu J, Lin Z. Non-small cell lung cancer targeted therapy: drugs and mechanisms of drug resistance. *Int J Mol Sci.* 2022;23:15056.
doi: 10.3390/ijms232315056

146. Daniels MG, Bowman RV, Yang IA, Govindan R, Fong KM. An emerging place for lung cancer genomics in 2013. *J Thorac Dis.* 2013;5(Suppl 5):S491-497.
doi: 10.3978/j.issn.2072-1439.2013.10.06

147. Merlotti A, Sadacca B, Arribas YA, et al. Noncanonical splicing junctions between exons and transposable elements represent a source of immunogenic recurrent neo-antigens in patients with lung cancer. *Sci Immunol.* 2023;8:eabm6359.
doi: 10.1126/sciimmunol.abm6359

148. Vokes NI, Galan Cobo A, Fernandez-Chas M, et al. ATM mutations associate with distinct co-mutational patterns and therapeutic vulnerabilities in NSCLC. *Clin Cancer Res.* 2023;29:4958-4972.
doi: 10.1158/1078-0432.CCR-23-1122

149. Bartfeld S, Clevers H. Stem cell-derived organoids and their application for medical research and patient treatment. *J Mol Med (Berl).* 2017;95:729-738.
doi: 10.1007/s00109-017-1531-7