REVIEW ARTICLE

Exploring *In Vitro* and *Ex Vivo* Models as Pharmacological Tool for Acute Lung Injury

Piyushkumar Sadhu, Nirmal Shah, Mamta Kumari

Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India

Abstract

Acute lung injury (ALI) remains a formidable clinical challenge, compounded by the scarcity of effective therapeutic options. Preclinical studies are crucial for evaluating the safety and efficacy of new drugs and formulations before clinical trials. Conventionally, animal models have been widely used for ALI research, but ethical concerns and differences from human physiology limit their effectiveness. This state-of-the-art review provides a comprehensive overview of *in vitro* and *ex vivo* models over traditional animal studies in ALI studies. While these models cannot entirely replace animal studies, they offer the potential to reduce the number of animal trials and improve drug efficacy and safety. Bridging the gap between *in vitro* studies and *in vivo* animal models, they address ethical concerns and provide more accurate data for human applications. Specifically, their application in ALI research highlights their potential in developing novel therapeutic approaches. However, challenges, such as maintaining tissue viability and replicating the complexity of human lungs still exist. Future advancements in bioengineering and personalized medicine promise to enhance these models' relevance, paving the way for more effective ALI therapies.

Key words: Acute lung injury, *ex vivo* models, *in vitro* models, inflammation, lung injury, preclinical study

INTRODUCTION

Over time, preclinical studies have primarily relied on animal models. However, an increase recognition of the constraints and ethical issues associated with the extensive use of animals in research redirected attention towards *in vitro* cell line studies and *ex vivo* models. While animal models have long essential in preclinical research, they pose significant challenges. Ethical concerns arise due to procedures causing pain or distress, and species differences often lead to inaccurate predictions of human responses, resulting in misleading data and failed clinical trials. In addition, animal studies are expensive and time-consuming, requiring extensive resources for breeding, housing, and care.[1,2] Figure 1 illustrates a comparative overview of preclinical models of acute lung injury (ALI) highlighting the balance between traditional and emerging approaches while addressing ethical concerns.

In vitro, models offer a promising alternative. Using human or animal-derived cells cultured in controlled environments, they eliminate the need for animal subjects, address ethical concerns, and provides more relevant data on human physiology.[3-5] Human cell lines, specifically, improve the accuracy of predicting treatment responses, potentially increasing clinical trial success rates. Moreover, *in vitro* studies are faster, less expensive, and allow precise control over experimental conditions, reducing variability.[6,7] Recent advancements in *in vitro* techniques have demonstrated their utility across various fields. In cancer research, they are used for high-throughput screening of anticancer drugs, while in toxicology, they evaluate the toxicity of chemicals and pharmaceuticals without animal testing.[8-10] In addition, genetically modified cell lines enable the study of specific genes in disease development and treatment response. As preclinical research evolves, integrating *in vitro* models alongside other advanced techniques will become increasingly important, providing

Address for correspondence:
Piyushkumar Sadhu, Department of Pharmacy,
Sumandeep Vidyapeeth Deemed to be University,
Piparia, Vadodara - 391 760, Gujarat, India.
Phone: +91-9033967019.
E-mail: piyush.sadhu@yahoo.in

Received: 10-12-2024
Revised: 23-02-2025
Accepted: 09-03-2025

ethical, cost-effective, and human-relevant data.[11,12] This review provides a comprehensive description of all such models, accompanied by case studies. Table 1 compares animal models with *in vitro* and *ex vivo* models.

ALI

ALI is a severe condition marked by the rapid onset of widespread inflammation and increased permeability in airspace of the lungs, leading to respiratory failure. ALI can result from infections, trauma, or inhalation of harmful substances. Despite the seriousness of the condition, effective treatments remain limited, necessitating the development of innovative therapies. One promising approach involves nanoformulations, where nanoparticles deliver drugs directly to the affected lung tissue, enhancing efficacy and reducing systemic side effects.[22]

To assess the potential of such nanoformulations, researchers often employ *in vitro* and *ex vivo* models before performing

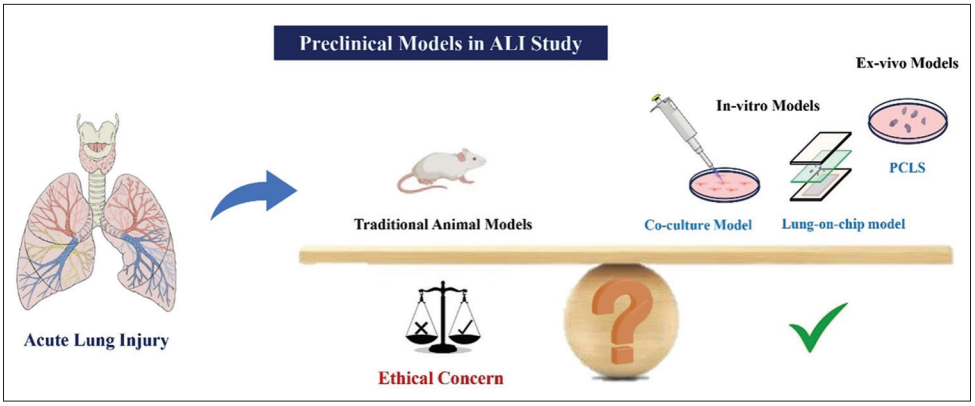

Figure 1: Illustration of different acute lung injury (ALI) preclinical models highlighting the balance between each type of models. The green checkmark suggests that *in vitro* and *ex vivo* models provide a viable and ethically favorable approach to studying ALI

Table 1: The key advantages of *in vitro* and *ex vivo* models over animal models

Parameter	*In vitro* models	*Ex vivo* models	Animal models	References
Complexity	Simplified, focusing on specific cell types or processes	Maintaining tissue architecture and cell diversity requires an intermediate level of complexity	Complexity is high, involving whole-organism interactions	[5,12,13]
Physiological relevance	The level is lower because of the absence of tissue architecture and systemic interactions	The preservation of tissue structure and cell interactions makes this method superior to *in vitro*	This is the highest level because it encompasses the entire organism's physiological context	[4,14]
Environmental control	The experimental conditions are highly controlled	Moderate control, maintaining near-physiological conditions	The complexity of whole-organism responses results in limited control	[5,15,16]
Ethical concerns	Minimal, as no animals are involved	The process is moderate, since we extract tissues or organs from organisms	High, involving the use of live animals and potential for pain and distress	[7,15]
Cost	The risk is minimal, utilizing fundamental laboratory tools and chemicals	Moderate, involving specialized equipment for maintaining tissue viability	The costs of animal care, breeding, and housing are high	[16,17]
Time efficiency	The level is high, enabling swift experiments and data gathering	It is moderate, requiring time to prepare and maintain tissue viability	The time required for animal breeding, handling, and longer experimental durations is low	[18,19]
Applications	High-throughput screening, mechanistic studies, genetic manipulation	Functional analysis, detailed tissue response studies, and closer approximation to *in vivo* conditions	There are comprehensive studies on drug efficacy, toxicity, pharmacokinetics, and whole-body responses	[20,21]
Examples	Cell line models, organ-on-a-chip etc.	PCLS, EVLP	Animal models like mice, rats, etc.	[18,20,21]

[†]PCLS: Precision-cut lung slices, EVLP: *Ex vivo* lung perfusion

animal studies. These models, obtained from human or animal lung cells or tissues, provide a controlled environment to study cellular responses to treatments.[23] Cell line models offer several advantages, including ethical benefits, cost-effectiveness, and relevance to human physiology. By allowing precise control of cellular mechanisms, they facilitate the evaluation of how well nanoformulations reduce inflammation and treat lung damage. Using these models helps in the efficient screening and optimization of treatments, accelerating the development of effective therapies for ALI.[24,25]

PATHOPHYSIOLOGY OF ALI

ALI involves a complex and rapidly progressive inflammation process that severely disrupts lung function. It can result from direct injury such as infection or aspiration, or with indirect injury like sepsis or trauma which damages the endothelial and epithelial cells of the alveolar-capillary barrier [Figure 2]. This damage increases permeability, allowing protein-rich fluid to enter the alveoli. This leads to the development of pulmonary edema and impaired gas exchange.[21,26] The injury induces a potent inflammatory response, attracting neutrophils to the lungs which release proinflammatory cytokines, chemokines, and reactive species exacerbate tissue damage. Furthermore, the inflammation leads to the reduction in surfactant produced by alveolar Type II cells, which causes alveolar collapse (atelectasis) and decrease lung compliance. As ALI progresses, fibroblasts proliferation and extracellular matrix deposition can lead to lung fibrosis, increasing the risk of long-term respiratory problems. Persistent inflammation and structural changes in the lungs intensify the initial tissue damage, severely impairing respiratory function and gas exchange.[27]

BIOMARKER OF ALI

Biomarkers are essential for identifying ALI, monitoring its progression, and evaluating the effectiveness of treatments. These biomarkers include cytokines, chemokines, cell adhesion molecules, and proteins, which reflect different aspects of the disease process. For example, interleukin-6 (IL-6) and tumor necrosis factor-alpha are key inflammatory cytokines elevated in the early stages of ALI, indicating a strong

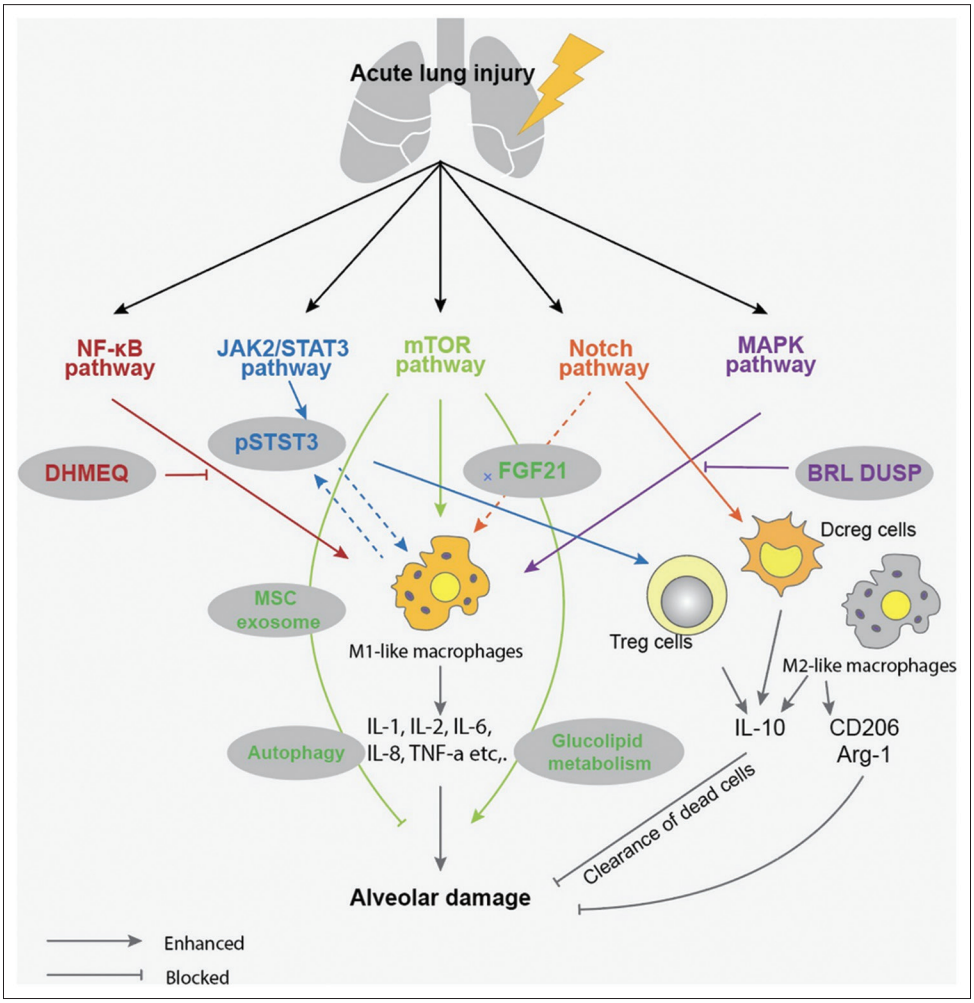

Figure 2: Illustration of the pathophysiology of acute lung injury due to sepsis, highlighting the release of different biomarkers. This figure is adapted from Sun *et al.* and is used under the Creative Commons Attribution 4.0 International License. No permission required for use[21]

inflammatory response. IL-8 acts as a chemokine, attracting neutrophils to the site of inflammation, further worsening the injury. Cell adhesion molecules like intercellular adhesion molecule-1 indicate endothelial activation and attachment of neutrophils to endothelial cells.[28]

Biomarkers reflecting endothelial and epithelial injury, such as angiopoietin-2 and receptors for advanced glycation end products, provide insights into the extent of cellular damage. Surfactant protein D and Clara cell protein 16 are specific to lung tissue and indicate damage to alveolar Type II cells and Clara cells, respectively.[29] In addition, matrix metalloproteinases (MMP-8 and MMP-9) are linked to tissue remodeling and neutrophil infiltration, representing the ongoing tissue degradation and repair processes.[30,31] The analysis of these biomarkers offers valuable information about the underlying pathophysiology of ALI, helps monitor disease progression, and guides evaluation of new treatments. Table 2 provides a detailed description of these biomarkers and their significance associated with ALI.

TYPES OF MODELS USED TO STUDY ALI

Different models are utilized to study ALI, each offering specific insights and assisting in treatment development. *In vivo,* animal models simulate ALI through methods like lipopolysaccharide (LPS) instillation and mechanical ventilation. However, these models have ethical limitations and are hindered by variations between different species. *Ex vivo* lung perfusion (EVLP) models use isolated lungs for controlled studies of lung function, while *in vitro* cell line models, using cultured lung cells, provide ethical and cost-effective options for drug screening. These models collectively enhance understanding of ALI and accelerate therapeutic development.[41,42] Figure 3 highlights the major properties of *in vitro* and *ex vivo* ALI models.

Each *in vitro* model offers distinct advantages. Single-cell line models provide simplicity and ease of manipulation, co-culture systems replicate cell-cell interactions, ALI models mimic respiratory tract conditions, 3D lung models recreate tissue architecture, and organ-on-a-chip models offer dynamic and physiologically relevant environments. Together, these *in vitro* systems form a comprehensive toolkit for advancing ALI research and accelerating the development of effective therapies. *Ex vivo* models provide substantial benefits compared to standard *in vivo* and *in vitro* methods by maintaining the physiological characteristics of lung tissue while allowing for controlled experimentation. These models are invaluable for enhancing the understanding of ALI pathophysiology and optimizing therapeutic interventions.

IN VITRO MODELS

In vitro models are essential for studying ALI since they provide a controlled environment to investigate cellular

Table 2: Potential biomarkers for acute lung injury

Category	Biomarkers	Significance	References
Pro-Inflammatory cytokines	IL-6, TNF-α, IL-2	These are indicators of early-stage inflammation and immune response activation	[32]
Chemokines	IL-8	Recruit neutrophils and other immune cells to the site of inflammation	[33]
Cell adhesion molecules	ICAM-1, VCAM-1	Endothelial activation and leukocyte adherence to the endothelium are reflected	[34]
Endothelial injury markers	Angiopoietin-2, E-selectin	These are indicators of endothelial cell damage and dysfunction	[35]
Epithelial injury markers	RAGE, SP-D, CC16	Damage to alveolar Type II cells and Clara cells is specific to lung tissue	[36]
Matrix metalloproteinases	MMP-8, MMP-9	Associated with neutrophil infiltration, tissue remodeling, and extracellular matrix degradation	[31]
Oxidative stress markers	8-isoprostane, malondialdehyde (MDA)	Reflect oxidative damage to cells and tissues	[37]
Coagulation markers	D-dimer, prothrombin fragments	Indicate the activation of coagulation pathways, often associated with inflammation and endothelial injury	[38]
Acute phase proteins	C-reactive protein, serum amyloid A	General markers of inflammation, produced by the liver in response to cytokines	[39]
Lung function markers	Lactate dehydrogenase, protein levels	Indicate cell damage and increased permeability of the alveolar-capillary barrier.	[40]

IL-6: Interlukin-6, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interlukin-1 β, IL-8, Interlukin-8, RAGE: Receptor for advanced glycation end products, SP-D: Surfactant protein D, CC16: Clara cell protein 16, MMP-8: Matrix metalloproteinases 8, MMP-9: Matrix metalloproteinases 9

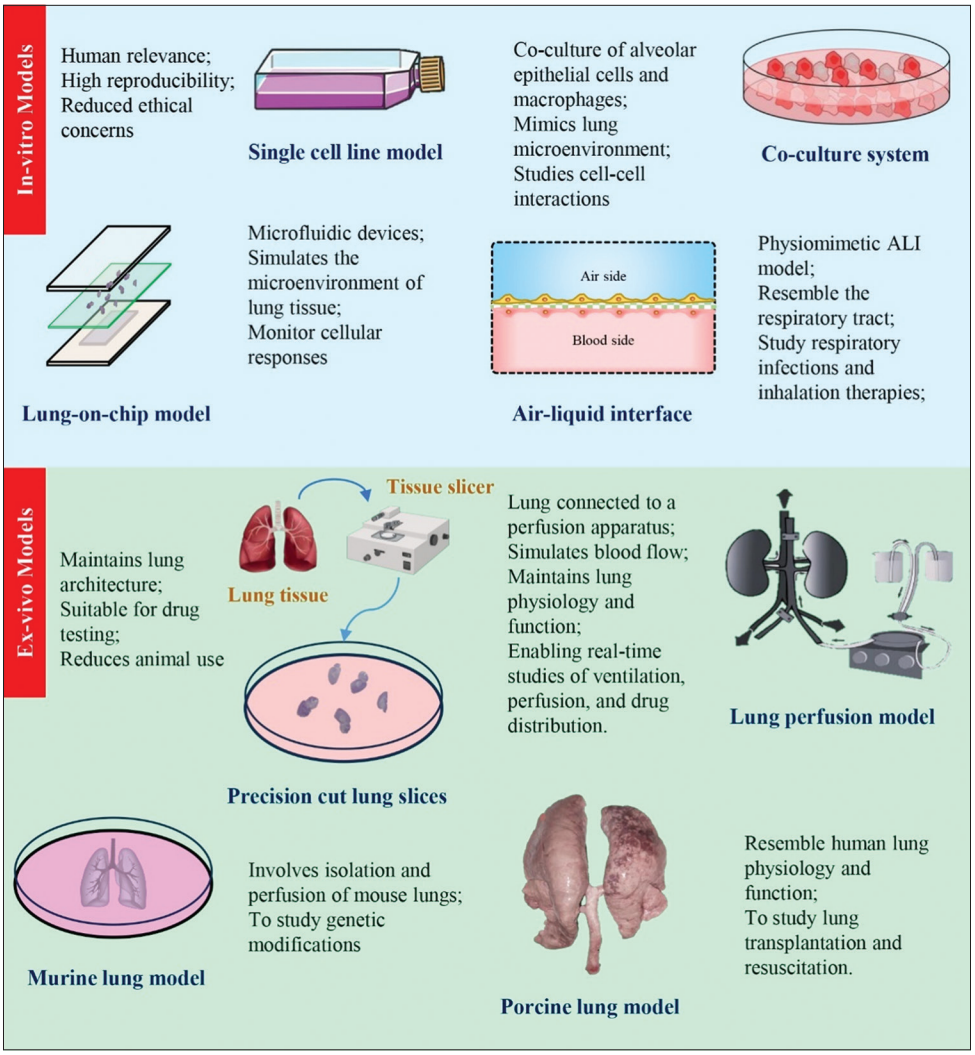

Figure 3: Illustration of different approaches of *in vitro* models and *ex vivo* models describing relevance of the models used for acute lung injury

mechanisms and evaluate therapeutic interventions. These models are essential for examining key aspects such as epithelial barrier function, mucociliary clearance, and cellular responses to pathogens and pollutants. The diverse types of *in vitro* models, including single cell lines, co-culture systems, air-liquid interface models, 3D lung models, and organ-on-a-chip models, provide distinct perspectives on the ALI pathophysiology and therapeutic development.[43]

Single-cell line models involve cultured lung cells, such as alveolar epithelial cells (e.g., A549, BEAS-2B) or endothelial cells, to enable researchers to study specific cellular responses to injury and treatment. Li *et al*. used A549 cells as a model to simulate the inflammatory response caused by LPS, a common inducer of ALI. The administration of anti-inflammatory drug formulation resulted in a notable decrease in proinflammatory cytokines, demonstrating the drug's potential in mitigating inflammation.[44-47]

Co-culture techniques, where multiple cell types are cultivated together, more closely resemble the complexity of the lung environment. These models offer valuable insights into cell-cell interactions during ALI. For example, Di Cristo and Sabella developed a co-culture model with alveolar epithelial cells and macrophages to study bacterial infection-induced inflammation. This approach helped identify critical inflammatory pathways and potential therapeutic targets.[48]

Air-liquid interface models are particularly effective for studying respiratory infections and inhalation therapies. By culturing epithelial cells at an air-liquid interface, these models closely simulate the conditions of the respiratory tract. A physiomimetic ALI model was used to investigate acute respiratory distress syndrome, enabling detailed studies on epithelial barrier function, mucociliary clearance, and treatment responses.[49,50] Another, three-dimensional (3D) lung models offer a more physiologically relevant environment by recreating lung tissue architecture. These models use scaffolds or hydrogels to support lung cell growth in a 3D structure, which better mimics the physical and mechanical conditions of lung tissue. Researchers have utilized 3D models to study the impact of mechanical stress

and inflammatory stimuli on lung tissue, providing more profound understanding of the development of ALI and potential therapeutic approaches.[51,52]

Organ-on-a-chip models are advanced microfluidic devices designed to replicate the specific conditions found in the lungs.[53,54] These models incorporate lung cells and microchannels that imitate blood flow and airways, providing dynamic circumstances for studying ALI. Dasgupta *et al*. employed a lung-on-a-chip model to explore the effects of cigarette smoke on lung tissue, enabling continuous observation of cellular reactions.[55] This technology has considerable promise for investigating intricate lung disorders and significant potential for studying complex lung diseases and testing novel treatments.

EX VIVO MODELS

Ex vivo models bridge the gap between *in vitro* studies and *in vivo* animal models, providing a more physiologically precise environment without the ethical and practical challenges of using live animals. Amongst different types, precision-cut lung slices (PCLS) *ex vivo* model indeed a sophisticated tool in respiratory study. They preserve lung's complex architecture and cellular diversity, allowing researchers to study the effects of various treatments in a controlled setting. To ensure the structural and functional integrity of lung tissue, PCLS necessitates the precise sectioning of the tissue. These slender sections are useful instruments for a multitude of scientific pursuits, especially those concerning respiratory disorders. PCLS enable researchers to analyze and understand the complex interactions and reactions occurring inside lung tissue, therefore enhancing our knowledge of pulmonary pathophysiology.[3] This is achieved by creating a controlled microenvironment. For example, Kim *et al*. used PCLS model derived from mice to simulate acid-induced lung injury. Their work demonstrated that PCLS is a useful tool for studying lung cell biology and screening potential therapeutic agents.[56,57] EVLP is a procedure where isolated lungs are perfused with a solution that is rich in nutrients, to sustain viability and functionality. This model allows for detailed analysis of lung function, cause of damage, and therapeutic interventions under settings that closely resemble the natural state. EVLP has been proven to be quite valuable in the examination of lung transplantation, ischemia-reperfusion injury, and the evaluation of novel therapeutic approaches. The technique has been effectively used in human lungs to evaluate novel therapeutics for ALI, demonstrating its potential for extending lung viability and translating laboratory findings into clinical applications, particularly in organ transplantation.[58,59] *Ex vivo* murine lung models allow the separation and perfusion of mouse lungs to study lung damage and healing. These models offer a controlled environment for examining genetic modifications, environmental exposures, and pharmacological interventions. An *ex vivo* murine lung model was used to evaluate the toxicity of ethyl acrylate, providing detailed insights into acute toxicity. Porcine lung models are employed to study ALI and other respiratory disorder in a system that closely mimics human lung anatomy and physiology. These models are particularly useful for assessing therapeutic approaches and studying lung transplantation and resuscitation.[60,61] Bhattacharya and Ramachandran used a porcine model of ALI induced by gastric aspiration to assess lung resuscitation strategies, demonstrating its efficacy as a platform for testing clinical therapeutics.[44]

Limitations and future perspectives

In vitro and *ex vivo* models are invaluable tools for studying ALI due to their ability to offer controlled environments to explore cellular mechanisms and potential therapeutic treatments. However, these models face several challenges that limit their potential. *In vitro* models, such as single-cell lines, tend to oversimplify complex biological systems, sometimes lacking the cellular diversity and interactions found in actual lung tissue. This can result in incomplete or misleading data when findings are translated to *in vivo* systems.[62] While co-culture and air-liquid interface models offer more realistic culture conditions by incorporating multiple cell types, they still struggle to fully replicate the lung environment, particularly in terms of immune cell interactions and systemic influences. *Ex vivo* models, such as PCLS and EVLP, maintain much of the lung's physiology and cellular interactions, providing a more physiologically relevant platform. However, they pose challenges related to maintaining tissue viability and function over extended periods. Precise control of variables like temperature, oxygen levels, and nutrient supply is required to keep lung tissues functional, which can be technically demanding. In addition, variability in human donor samples can introduce inconsistencies in experimental outcomes, further complicating the use of *ex vivo* models.[63,64]

Looking ahead, the future of *in vitro* and *ex vivo* models for ALI research holds significant promise. The development of advanced 3D cultures and organ-on-a-chip technologies will enhance the physiological relevance of these models by better replicating the lung's microenvironment, including cell-cell interactions and mechanical forces. These models, integrated with high-throughput screening technologies, will allow for more efficient drug testing and mechanistic studies. Advancements in tissue preservations and bioreactor technologies are anticipated to enhance the viability and performance of lung tissues in *ex vivo* models, resulting more reliable and precise data. In addition, the integration of *ex vivo* models with improved imaging and molecular tools would enable more detailed studies of tissue responses at the cellular and molecular levels. Personalized medicine presents another promising frontier for both *in vitro* and *ex vivo* models. Using tissues and cells derived from patients with specific genetic backgrounds or disease conditions, researchers can explore individual variability in disease

mechanisms and treatment responses.[65-67] This approach could lead to more personalized and effective therapeutic strategies for ALI. Collaboration between researchers, clinicians, and bioengineers will be crucial in overcoming current limitations and driving these advancements forward. As these models continue to evolve, they will provide deeper insights into ALI, ultimately facilitating the development of more effective treatments and improving patient outcomes.

CONCLUSION

In preclinical trials for ALI, *in vitro* and *ex vivo* models have become essential, in offering ethical and cost-effective aids to animal models. These models provide critical insights into cellular mechanisms and therapeutic responses, helping to bridge the gap between basic research and clinical applications. *In vitro* models, such as single cell line and co-culture systems, allow for detailed examinations of cellular processes and enable high-throughput screening of potential treatments. Meanwhile, *ex vivo* models such as PCLS and EVLP preserve lung structure and function, providing more physiologically relevant data. Although they possess significant worth, challenges persist. Ensuring the survival of tissue and precisely reproducing the intricate conditions of the lung continue to be significant hurdles. Future advancements, particularly in 3D culture systems, organ-on-a-chip technologies, and personalized medicine, are expected to improve model relevance and enable more precise and efficient drug and formulation testing. Through the process of refining these models and integrating emerging technologies, individual can gain more profound understandings of ALI, hence expediting the development of effective treatments and improving patient outcomes.

ACKNOWLEDGMENTS

Authors acknowledge the facilities provided by the Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, for the successful completion of review work.

REFERENCES

1. Mukherjee P, Roy S, Ghosh D, Nandi SK. Role of animal models in biomedical research: A review. Lab Anim Res 2022;38:18.
2. Robinson NB, Krieger K, Khan FM, Huffman W, Chang M, Naik A, *et al*. The current state of animal models in research: A review. Int J Surg 2019;72:9-13.
3. Bodenstein DF, Siebiger G, Zhao Y, Clasky AJ, Mukkala AN, Beroncal EL, *et al*. Bridging the gap between *in vitro* and *in vivo* models: A way forward to clinical translation of mitochondrial transplantation in acute disease states. Stem Cell Res Ther 2024;15:157.
4. Bahadoran Z, Mirmiran P, Kashfi K, Ghasemi A. Importance of systematic reviews and meta-analyses of animal studies: Challenges for animal-to-human translation. J Am Assoc Lab Anim Sci 2020;59:469-77.
5. Reddy N, Lynch B, Gujral J, Karnik K. Alternatives to animal testing in toxicity testing: Current status and future perspectives in food safety assessments. Food Chem Toxicol 2023;179:113944.
6. Hagiwara M, Koh I. Engineering approaches to control and design the *in vitro* environment towards the reconstruction of organs. Dev Growth Differ 2020;62:158-66.
7. Stresser DM, Kopec AK, Hewitt P, Hardwick RN, Van Vleet TR, Mahalingaiah PK, *et al*. Towards *in vitro* models for reducing or replacing the use of animals in drug testing. Nat Biomed Eng 2024;8:930-5.
8. Choi JR, Kozalak G, Di Bari I, Babar Q, Niknam Z, Rasmi Y, *et al*. *In vitro* human cancer models for biomedical applications. Cancers (Basel) 2022;14:2284.
9. Fortin MC, Szilagyi J. *In vitro* toxicology: Next generation models and methods to improve safety evaluation. In: Hock FJ, Pugsley MK, editors. Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays. Cham: Springer International Publishing; 2022. p. 1-29.
10. Madorran E, Stožer A, Bevc S, Maver U. *In vitro* toxicity model: Upgrades to bridge the gap between preclinical and clinical research. Bosn J Basic Med Sci 2020;20:157-68.
11. Gupta R, Rajpoot K, Tekade M, Sharma MC, Tekade RK. Methods and models for *in vitro* toxicity. In: Tekade RK, editor. Pharmacokinetics and Toxicokinetic Considerations. Vol. 2. Cambridge: Academic Press; 2022. p. 145-74.
12. Soufizadeh P, Mansouri V, Ahmadbeigi N. A review of animal models utilized in preclinical studies of approved gene therapy products: Trends and insights. Lab Anim Res 2024;40:17.
13. Kiani AK, Pheby D, Henehan G, Brown R, Sieving P, Sykora P, *et al*. Ethical considerations regarding animal experimentation. J Prev Med Hyg 2022;63 2 Suppl 3:e255-66.
14. Domínguez-Oliva A, Hernández-Ávalos I, Martínez-Burnes J, Olmos-Hernández A, Verduzco-Mendoza A, Mota-Rojas D. The importance of animal models in biomedical research: Current insights and applications. Animals (Basel) 2023;13:1223.
15. Owens RM. Advanced tissue engineering for *in vitro* drug safety testing. MRS Commun 2023;13:685-94.
16. Usui T, Macleod MR, McCann SK, Senior AM, Nakagawa S. Meta-analysis of variation suggests that embracing variability improves both replicability and generalizability in preclinical research. PLoS Biol 2021;19:e3001009.
17. Corleis B, Bastian M, Hoffmann D, Beer M, Dorhoi A. Animal models for COVID-19 and tuberculosis. Front

Immunol 2023;14:1223260.
18. Józsa L, Nemes D, Pető Á, Kósa D, Révész R, Bácskay I, *et al*. Recent options and techniques to assess improved bioavailability: *In vitro* and *ex vivo* methods. Pharmaceutics 2023;15:1146.
19. Sailer V, Von Amsberg G, Duensing S, Kirfel J, Lieb V, Metzger E, *et al*. Experimental *in vitro*, *ex vivo* and *in vivo* models in prostate cancer research. Nat Rev Urol 2023;20:158-78.
20. Mokrá D. Acute lung injury - from pathophysiology to treatment. Physiol Res 2020;69 Suppl 3:S353-66.
21. Sun B, Lei M, Zhang J, Kang H, Liu H, Zhou F. Acute lung injury caused by sepsis: How does it happen? Front Med (Lausanne) 2023;10:1289194.
22. Piyushkumar S, Mamta K, Rajput HS, Patel VP, Rathod F, Shah N, *et al*. Advances in nanoparticulate therapeutics for acute lung injury: Addressing unmet clinical needs through targeted therapy and controlled delivery of drug. Curr Nanomed 2025;15:142-56.
23. Narayan S. Experimental animal models to evaluate the therapeutic efficacy of nanoformulations against cancer. In: Chakraborti S, editor. Handbook of Oxidative Stress in Cancer: Therapeutic Aspects. Singapore: Springer Singapore; 2021. p. 1-21.
24. Deshmukh A, Patel JK, Pathak YV. Preclinical animal models for the experimental design of pharmacokinetic studies with nanoparticulate drug delivery systems. In: Patel JK, Pathak YV, editors. Pharmacokinetics and Pharmacodynamics of Nanoparticulate Drug Delivery Systems. Cham: Springer International Publishing; 2022. p. 79-100.
25. Vashishat A, Patel P, Das Gupta G, Das Kurmi B. Alternatives of animal models for biomedical research: A comprehensive review of modern approaches. Stem Cell Rev Rep 2024;20:881-99.
26. Yang T, Xiang CG, Wang XH, Li QQ, Lei SY, Zhang KR, *et al*. RIPK1 inhibitor ameliorates pulmonary injury by modulating the function of neutrophils and vascular endothelial cells. Cell Death Discov 2024;10:152.
27. Zhang J, Guo Y, Mak M, Tao Z. Translational medicine for acute lung injury. J Transl Med 2024;22:25.
28. Niri P, Saha A, Polopalli S, Kumar M, Das S, Chattopadhyay P. Role of biomarkers and molecular signaling pathways in acute lung injury. Fundam Clin Pharmacol 2024;38:640-57.
29. Ge R, Wang F, Peng Z. Advances in biomarkers for diagnosis and treatment of ARDS. Diagnostics (Basel) 2023;13:3296.
30. Atmowihardjo LN, Heijnen NF, Smit MR, Hagens LA, Filippini DF, Zimatore C, *et al*. Biomarkers of alveolar epithelial injury and endothelial dysfunction are associated with scores of pulmonary edema in invasively ventilated patients. Am J Physiol Lung Cell Mol Physiol 2023;324:L38-47.
31. Luchian I, Goriuc A, Sandu D, Covasa M. The role of matrix metalloproteinases (MMP-8, MMP-9, MMP-13) in periodontal and peri-implant pathological processes. Int J Mol Sci 2022;23:1806.
32. Ahmad A, Imran M, Ahsan H. Biomarkers as biomedical bioindicators: Approaches and techniques for the detection, analysis, and validation of novel biomarkers of diseases. Pharmaceutics 2023;15:1630.
33. Cambier S, Gouwy M, Proost P. The chemokines CXCL8 and CXCL12: Molecular and functional properties, role in disease and efforts towards pharmacological intervention. Cell Mol Immunol 2023;20:217-51.
34. Kong DH, Kim YK, Kim MR, Jang JH, Lee S. Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer. Int J Mol Sci 2018;19:1057.
35. Melegari G, Critelli RM, Lasagni S, Romagnoli D, Bertellini E, Villa E. Dynamic angiopoietin-2 serum level as endothelial damage marker and potential therapeutic target. Am J Pathol 2022;192:1336-7.
36. De Souza Xavier Costa N, Da Costa Sigrist G, Schalch AS, Belotti L, Dolhnikoff M, Da Silva LF. Lung tissue expression of epithelial injury markers is associated with acute lung injury severity but does not discriminate sepsis from ARDS. Respir Res 2024;25:129.
37. Menzel A, Samouda H, Dohet F, Loap S, Ellulu MS, Bohn T. Common and novel markers for measuring inflammation and oxidative stress *ex vivo* in research and clinical practice-which to use regarding disease outcomes? Antioxidants (Basel) 2021;10:414.
38. Chen AT, Wang CY, Zhu WL, Chen W. Coagulation disorders and thrombosis in COVID-19 patients and a possible mechanism involving endothelial cells: A review. Aging Dis 2022;13:144-56.
39. Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxid Med Cell Longev 2017;2017:6501046.
40. Newton DA, Lottes RG, Ryan RM, Spyropoulos DD, Baatz JE. Dysfunctional lactate metabolism in human alveolar type II cells from idiopathic pulmonary fibrosis lung explant tissue. Respir Res 2021;22:278.
41. Shi D, Mi G, Wang M, Webster TJ. *In vitro* and *ex vivo* systems at the forefront of infection modeling and drug discovery. Biomaterials 2019;198:228-49.
42. Watanabe T, Cypel M, Keshavjee S. *Ex vivo* lung perfusion. J Thorac Dis 2021;13:6602-17.
43. Barron SL, Saez J, Owens RM. *In vitro* models for studying respiratory host-pathogen interactions. Adv Biol (Weinh) 2021;5:e2000624.
44. Bhattacharya M, Ramachandran P. Immunology of human fibrosis. Nat Immunol 2023;24:1423-33.
45. Li J, Qin Y, Chen Y, Zhao P, Liu X, Dong H, *et al*. Mechanisms of the lipopolysaccharide-induced inflammatory response in alveolar epithelial cell/macrophage co-culture. Exp Ther Med 2020;20:76.
46. Lian J, Lin J, Zakaria N, Yahaya BH. Acute lung injury: Disease modelling and the therapeutic potential of stem cells. In: Turksen K, editor. Cell Biology and Translational Medicine. Stem Cells in Tissue Regeneration. Vol. 10. Cham: Springer International

Publishing; 2020. p. 149-66.
47. Zhang J, Liu Y. Epithelial stem cells and niches in lung alveolar regeneration and diseases. Chin Med J Pulm Crit Care Med 2024;2:17-26.
48. Di Cristo L, Sabella S. Cell cultures at the air-liquid interface and their application in cancer research. In: Movia D, Prina-Mello A, editors. Cancer Cell Culture: Methods and Protocols. New York: Springer US; 2023. p. 41-64.
49. Baldassi D, Gabold B, Merkel O. Air-liquid interface cultures of the healthy and diseased human respiratory tract: Promises, challenges and future directions. Adv Nanobiomed Res 2021;1:2000111.
50. Tran BM, Grimley SL, McAuley JL, Hachani A, Earnest L, Wong SL, *et al*. Air-liquid-interface differentiated human nose epithelium: A robust primary tissue culture model of SARS-CoV-2 infection. Int J Mol Sci 2022;23:835.
51. Nizamoglu M, Joglekar MM, Almeida CR, Larsson Callerfelt AK, Dupin I, Guenat OT, *et al*. Innovative three-dimensional models for understanding mechanisms underlying lung diseases: Powerful tools for translational research. Eur Respir Rev 2023;32:230042.
52. Sen C, Freund D, Gomperts BN. Three-dimensional models of the lung: Past, present and future: A mini review. Biochem Soc Trans 2022;50:1045-56.
53. Cao UM, Zhang Y, Chen J, Sayson D, Pillai S, Tran SD. Microfluidic organ-on-A-chip: A guide to biomaterial choice and fabrication. Int J Mol Sci 2023;24:3232.
54. Leung CM, De Haan P, Ronaldson-Bouchard K, Kim GA, Ko J, Rho HS, *et al*. A guide to the organ-on-a-chip. Nat Rev Method Primers 2022;2:33.
55. Dasgupta Q, Jiang A, Wen AM, Mannix RJ, Man Y, Hall S, *et al*. A human lung alveolus-on-a-chip model of acute radiation-induced lung injury. Nat Commun 2023;14:6506.
56. Kim SY, Mongey R, Griffiths M, Hind M, Dean CH. An *ex vivo* acid injury and repair (AIR) model using precision-cut lung slices to understand lung injury and repair. Curr Protoc Mouse Biol 2020;10:e85.
57. Viana F, O'Kane CM, Schroeder GN. Precision-cut lung slices: A powerful *ex vivo* model to investigate respiratory infectious diseases. Mol Microbiol 2022;117:578-88.
58. Ahmad K, Pluhacek JL, Brown AW. *Ex vivo* lung perfusion: A review of current and future application in lung transplantation. Pulm Ther 2022;8:149-65.
59. Yu J, Zhang N, Zhang Z, Li Y, Gao J, Chen C, *et al*. Diagnostic and therapeutic implications of *ex vivo* lung perfusion in lung transplantation: Potential benefits and inherent limitations. Transplantation 2023;107:105-16.
60. Shah DD, Raghani NR, Chorawala MR, Singh S, Prajapati BG. Harnessing three-dimensional (3D) cell culture models for pulmonary infections: State of the art and future directions. Naunyn Schmiedebergs Arch Pharmacol 2023;396:2861-80.
61. Xia JY, Zeng YF, Wu XJ, Xu F. Short-term *ex vivo* tissue culture models help study human lung infections A review. Medicine (Baltimore) 2023;102:e32589.
62. Lindstedt S, Wang Q, Niroomand A, Stenlo M, Hyllen S, Pierre L, *et al*. High resolution fluorescence imaging of the alveolar scaffold as a novel tool to assess lung injury. Sci Rep 2024;14:6662.
63. Abuwatfa WH, Pitt WG, Husseini GA. Scaffold-based 3D cell culture models in cancer research. J Biomed Sci 2024;31:7.
64. He RW, Braakhuis HM, Vandebriel RJ, Staal YC, Gremmer ER, Fokkens PH, *et al*. Optimization of an air-liquid interface *in vitro* cell co-culture model to estimate the hazard of aerosol exposures. J Aerosol Sci 2021;153:105703.
65. Anderson SR, Stagner EJ, Sivakumar H, Skardal A. Three-dimensional bioprinting of *in vitro* tumor organoid and organ-on-a-chip models. MRS Bull 2023;48:643-56.
66. Dichtl S, Posch W, Wilflingseder D. The breathtaking world of human respiratory *in vitro* models: Investigating lung diseases and infections in 3D models, organoids, and lung-on-chip. Eur J Immunol 2024;54:e2250356.
67. Ko J, Park D, Lee J, Jung S, Baek K, Sung KE, *et al*. Microfluidic high-throughput 3D cell culture. Nat Rev Bioeng 2024;2:453-69.

Source of Support: Nil. **Conflicts of Interest:** None declared.