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ABOUT

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Thoracic Research and Practice is a bimonthly journal that is published in English in January, March, May, July, September, and November.

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Thoracic Research and Practice covers a wide range of topics related to adult and pediatric pulmonary diseases, as well as thoracic imaging, environmental and occupational disorders, intensive care, sleep disorders and thoracic surgery, including diagnostic methods, treatment techniques, and prevention strategies. The journal is interested in publishing original research that addresses important clinical questions and advances the understanding and treatment of these conditions. This may include studies on the effectiveness of different treatments, new diagnostic tools or techniques, and novel approaches to preventing or managing pulmonary diseases.

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Volume 26 November Supplement 1-2025

Thoracic Research & Practice

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CONTENTS

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Bioengineered Humanoid-on-Chip Platforms: Tools for Evaluating the Effects of Environmental Exposure on Human Physiological Barriers

Pelin Saglam-Metiner[1-3], Ozlem Goksel[1,4], Tuncay Goksel[1,4], Omer H. Yilmaz[1,5], Esra Erdal[3], Ozlem Yesil-Celiktas[1,2,6]

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INTRODUCTION: Environmental pollutants; bioaerosols, chemicals and micro/nano-plastics (MNPs) can disrupt the mechano-biological processes of the human body, impairing structural, cellular, and molecular functions. Such disruptions may ultimately contribute to the onset of toxicity-associated pharmacokinetic disorders.[1] Traditional *in vivo* and 2D *in vitro* models often fall short in accurately mimicking the complexity of human physiological responses to such environmental exposures. In response, recent advances in microfluidic organ-on-a-chip (OoC) platforms, organoid models, and induced pluripotent stem cell (iPSC) technologies have enabled the development of biomimetic, animal-free, and human-relevant *in vitro* models. These systems increasingly replicate the molecular, structural, and functional characteristics of both healthy[2] and diseased human tissues, providing powerful tools for toxicological, pharmacological, and pathophysiological research.[3,4] To further enhance the predictive power of these models, multi-organ integration strategies are gaining attention particularly those emulating organ axis that are functionally and biochemically interconnected.[5] Among these, the brain-lung-liver-intestine axis represents a critical multi-organ network that mediates systemic responses to external insults. For example, inhaled airborne pollutants can initiate inflammatory signaling in the lung, which may be translocated via the bloodstream to the liver and brain.[6,7] Similarly, orally ingested MNPs or pharmaceuticals undergo metabolism in the intestine and liver, potentially generating bioactive metabolites that affect the central nervous system (CNS).[5,8] Such complex inter-organ interactions are essential to understanding the full scope of environmental toxicity and human pathophysiology, yet are poorly captured in isolated organ models.[5] Bioengineered humanoid-on-chip systems that incorporate the brain-lung-liver-intestine axis within a single fluidically linked microphysiological platform offer a novel solution. These integrated systems can simulate real-time organ-organ crosstalk under dynamic flow conditions, mimicking human circulatory, absorptive, and barrier functions with high fidelity.[5,6,8] For instance, the intestine-on-chip component can model nutrient or xenobiotic absorption, followed by first-pass metabolism in the liver module,[8] systemic immune or endocrine responses via the lung module,[6] and downstream neuroinflammatory

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effects observed in the brain-on-chip compartment.[5,7] Such systems also allow for the controlled exposure of specific organs to pollutants or therapeutics, enabling precise mechanistic dissection of toxicity pathways across the entire axis.[9] By combining tissue-specific cellular architectures, mechanical cues (e.g., peristalsis, breathing motions), and physiologically relevant flow dynamics, brain-lung-liver-intestine axis OoC models represent a transformative step toward predictive environmental health assessment.[5,10] These platforms not only reduce the need for animal testing but also offer scalable, human-relevant alternatives for screening environmental toxins, investigating multi-organ disease mechanisms, and evaluating therapeutic safety and efficacy.[5,6,7,8,9,10] In an era of rising environmental health concerns, such integrative platforms are poised to play a pivotal role in translational toxicology and precision medicine.[9]

CASE REPORT: Herein, we highlighted our iPSCs or patient-derived brain, lung, liver and intestinal organoid models, iPSCs-differentiated alveolar epithelial cell (AEC) and brain microvascular endothelial cell (BMVEC) barriers, and their OoC models to evaluate the effect of kinds of exposomes. We successfully characterized SPC+ AECs and CD31+ BMVECs with highly transepithelial electrical resistance values as a gold standart, IBA1+ microglia and CD31+ endothelial cells enriched, cortical plate structured advanced matured functional brain organoids, MUC1+ lung-like organoids, EPCAM+/ALB+ liver organoids and human crypt-derived intestinal organoids (Figure 1). Subsequently, we developed integratable organ-specific OoC models utilizing a layer-by-layer fabrication approach to enable co-culture systems of cells and organoids.

CONCLUSION: Our advanced bioengineered models demonstrated that environmental exposures significantly compromised barrier integrity, leading to increased translocation across the tissue construct, reduced cell-organoid viability, and dysregulated expression of inflammatory cytokines and immune cell activity. The resilience of human physiological barriers can be effectively modelled using humanized bioengineering platforms that emulate the dynamic mechanical and biochemical forces present *in vivo*. The integration of organoids, epithelial-endothelial barriers, and OoC technologies that mimics different kinds of organs, holds significant promise as robust and physiologically relevant systems for studying exposure-related responses.

Keywords: Environmental exposure, iPSCs-derived models, organoids, organ-on-chips

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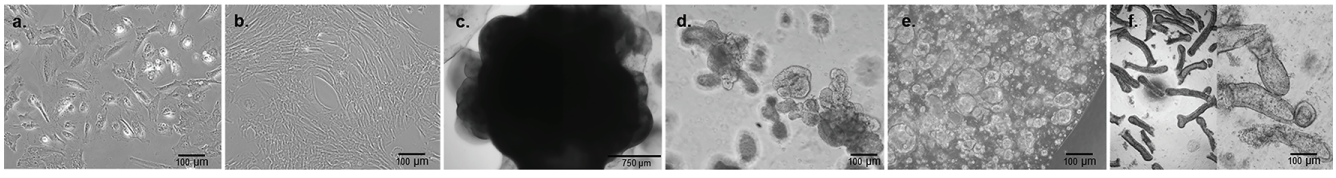

Figure 1. The brighfield images of (a) AECs, (b) BMVECs, (c) brain organoid, (d) lung organoid, (e) liver organoid and (f) intestinal organoid models
AECs: Alveolar epithelial cell, BMVEC: Brain microvascular endothelial cell

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A NAMs-Based Microphysiological System for Metastasis and Mechanobiology Studies

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INTRODUCTION: Lung and breast cancers are among the most common types of cancer worldwide, with approximately 2.5 million new cases occurring each year.[1] Lung cancer often remains asymptomatic until advanced stages, leading to delayed diagnosis and high mortality. Bone metastasis is a frequent complication of lung cancer; after breast and prostate cancers, lung cancer accounts for 30–40 % of bonemetastatic case.[2] As in other cancers, the tumor microenvironment (TME) plays a crucial role in the pathogenesis of lung cancer. Fibroblasts, endothelial cells, and immune cells, as well as the extracellular matrix (ECM), are the primary TME components.[3] The lung ECM, which consists largely of collagen, hardens under the influence of other proteins in the cancerous state, contributing to tumor prognosis.[4] Epithelial-mesenchymal transition (EMT) is one of the most important phenomena in the progression and metastatic process of lung cancer.[5] During EMT, epithelial cells lose their apical polarity, detach from the basement membrane, and transition to a mesenchymal phenotype. This initiates a cascade characterized by increased cellular invasiveness, called metastasis. EMT is triggered by signaling pathways such as TGF-β, HIF-1α, and Notch, and is generally characterized by a loss of E-cadherin and an increase in vimentin and N-cadherin.[5] This process enhances the ability to metastasize, particularly to distant organs such as bone. The bone microenvironment, with its rich calcium content, serves as an important signal transmitter for cancer cells. Osteoblasts, osteoclasts, bone matrix proteins, and local immune cells play a decisive role in the localization, dormancy, and reactivation of metastatic cells.[6] Lung-derived metastases may gain a proliferative advantage due to the high calcium concentration and growth factors in bone. Conventional 2D culture and animal models insufficiently reproduce the complex tumor–bone interactions, limiting translational progress.[7,8] Organ-on-a-chip models developed to evaluate therapeutic strategies mimic cell-cell and cell-matrix interactions at the physiological level through microfluidic systems.[9] These systems are compatible with New Approach Methodologies strategies, which include organoids, *in silico* studies, and omics technology, and offer platforms with high translational value from both ethical and scientific perspectives.[10] In this context, we developed a lung cancer metastasis-on-a-chip platform as a physiologically relevant *in vitro* alternative (Figure 1).

MATERIAL AND METHODS: Lung cancer metastasis-on-a-chip platform consists of two PDMS layers, where two tissue chambers represent the lung and bone compartments, and a single microchannel serve as the vascular lumen. The dual-chamber microfluidic system integrates lung cancer epithelial and stromal cells in one compartment and human osteoblasts encapsulated in gelatin methacrylate hydrogel in the other, enabling dynamic study of EMT and

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cancer cell migration. Time-lapse fluorescence microscopy was employed to visualize real-time cellular dynamics and migration events. ELISA was used to quantify secreted EMT-related proteins, while quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to assess gene expression changes associated with metastatic progression.

RESULTS AND CONCLUSION: Microscopic observations demonstrated that lung cancer cells successfully metastasize to bone stroma and disrupt the bone microenvironment, like the *in vivo* effect. ELISA and qRT-PCR analyses also demonstrated an increase in associated mesenchymal cytokines and genes. Furthermore, an increase in epithelial genes related to proliferation was observed in the lung chamber. ELISA analysis also revealed an increase in epithelial genes. All analyses show that lung cancer cells successfully metastasized to the bone chamber, highlighting the physiological relevance of our platform in simulating *in vivo* metastatic behavior. It's interesting to note that the lung chamber's parallel upregulation of markers of epithelial proliferation points to a spatially separate but biologically related dynamic where primary tumor dissemination and secondary site colonization take place simultaneously.[11] The lung cancer metastasis-on-a-chip platform can be adapted to study how a broad range of exposome factors, such as environmental pollutants, dietary components, and lifestyle-related exposures, affect cancer progression and metastasis in besides modeling tumor–bone interactions.[12] his system, similar to our previous airway epithelial barrier-on-a-chip work, could bring exposome research into oncology, providing a versatile bridge between cancer biology, toxicology, and regulatory science (Figure 1).[13] This helps to remove animal testing worldwide and advances precision medicine and cancer prevention.

KEYWORDS: Epithelial-mesenchymal transition, lung cancer, cell migration, exposome, NAMs, mechanobiology

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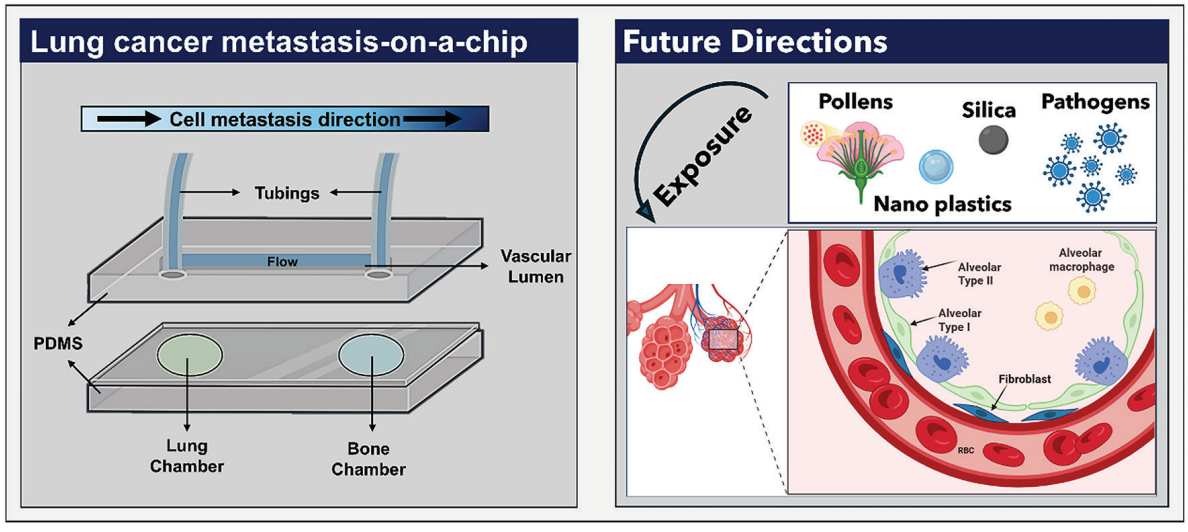

Figure 1. Lung cancer metastasis-on-a-chip design and demonstration as a potential tool to study the effects of exposome exposure on lung cancer progression.

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Occupational and Environmental Exposures: Trends in Publications, Diseases, and Experimental Models

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INTRODUCTION: Environmental exposures such as silica dust, microplastics, and general dust have significant health impacts. We performed a PubMed-based analysis (2000-2025) of research trends on these exposures, associated diseases, and experimental models. Pulmonary fibrosis was the most studied disease, while animal models and conventional *in vitro* systems dominated experimental approaches. Organ-on-chip platforms remain scarcely used. The results highlight growing research interest and the need for advanced human-relevant models to study exposure effects on respiratory and systemic health. Diverse environmental agents (particulates, gases, volatile organic compounds, biological agents, and nanomaterials) disseminate through the atmosphere, water, and soil, resulting in human exposure via numerous pathways. The result of exposure is influenced by the physicochemical characteristics of the substance (dimensions, morphology, surface chemistry), the exposure dosage, the duration of exposure, and the exposure pathway.[1] Especially, fine-ultrafine particles in the atmosphere can penetrate through the upper and lower respiratory pathways due to their aerodynamic features; PM 2.5[2] or smaller particles can directly interact with epithelial cells at the alveolar level. The airway epithelium serves as the main defense mechanism against inhaled substances. Also orchestrate the homeostasis via physical and immunological defense mechanisms. To form the epithelial barrier, epithelial cells are connected to each other by cell-cell junctions (tight, gap, and adherents).[3] Due to the inhalation of 6-12 liters of air per minute, this tissue is subjected to a significant quantity of deleterious substances and pathogenic organisms.[4] Because of the lack of early diagnosis methods and the gaps that need to be addressed, particularly in studies conducted with animal models, the number of studies observing the effects of exosomes on the lungs and other organs is increasing. For example, pneumoconiosis, one of the world's most common occupational diseases, or silicosis, considered an occupational lung disease, is characterized by the inhalation of crystalline silica dust, inflammation, and progressive fibrosis.[5] On the other hand, physiological and anatomical mimicry models of the respiratory system have been proven to be essential for drug development and elucidating the pathophysiological mechanisms of diseases triggered by epigenetics and hereditary genetic factors. Lung epithelial models can be organized as monoculture/multicultural cells with supporting scaffolds such as trans-wells and bio-gels. Notably, as a novel study niche, organ-on-a-chips provide controllable physiological conditions that recapitulate tissue specific features.

MATERIAL AND METHODS: We performed a PubMed search (2000-2025) to quantify publications on occupational exposures (silica, coal dust, microplastics, dust), related diseases [asthma, chronic obstructive pulmonary disease (COPD), lung cancer, pulmonary fibrosis, cardiovascular disease], and experimental models (transwell, organ-on-a-chip, *in vitro*, *ex vivo*, animal). Counts were retrieved using the PubMed API, either annually or as total sums, with

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optional epithelial-focused filtering. Data were compiled and visualized in Python using trend plots, bar charts, and heatmaps of exposure-disease and exposure-model relationships.

RESULTS: Annual publication trends for silica dust, microplastic, dust, and coal dust showed an overall increase from 2000 to 2025, with silica dust and dust exhibiting steady growth and microplastic-related publications rising sharply after 2016. Total publications were highest for silica dust and dust, while coal dust and microplastic were less studied. Exposure-disease analysis revealed pulmonary fibrosis as the primary outcome associated with silica dust (619 publications) and dust (373), whereas asthma and COPD were less frequently studied; microplastic and coal dust publications were comparatively few. Experimental model usage indicated a clear predominance of animal models and *in vitro* studies across all exposures, with transwell systems used sporadically and organ-on-chip platforms largely unexplored (Figure 1).

CONCLUSION: The observed trends reflect increasing research focus on occupational and environmental exposures, particularly for silica dust and microplastics, with pulmonary fibrosis as the most frequently studied disease outcome. The predominant use on animal and conventional *in vitro* models underscores a gap in physiologically relevant human-based experimental systems. Organ-on-chip technology, despite its potential to recapitulate human epithelial barriers and tissue-specific responses, remains minimally utilized, highlighting a clear opportunity for future mechanistic studies. These findings emphasize the need for innovative experimental models to better understand exposure-related pathophysiology and to bridge the translational gap between *in vitro* and *in vivo* studies.

KEYWORDS: Environmental exposures, silica dust, microplastics, pulmonary fibrosis, organ-on-chip, *in vitro* models, bibliometric analysis

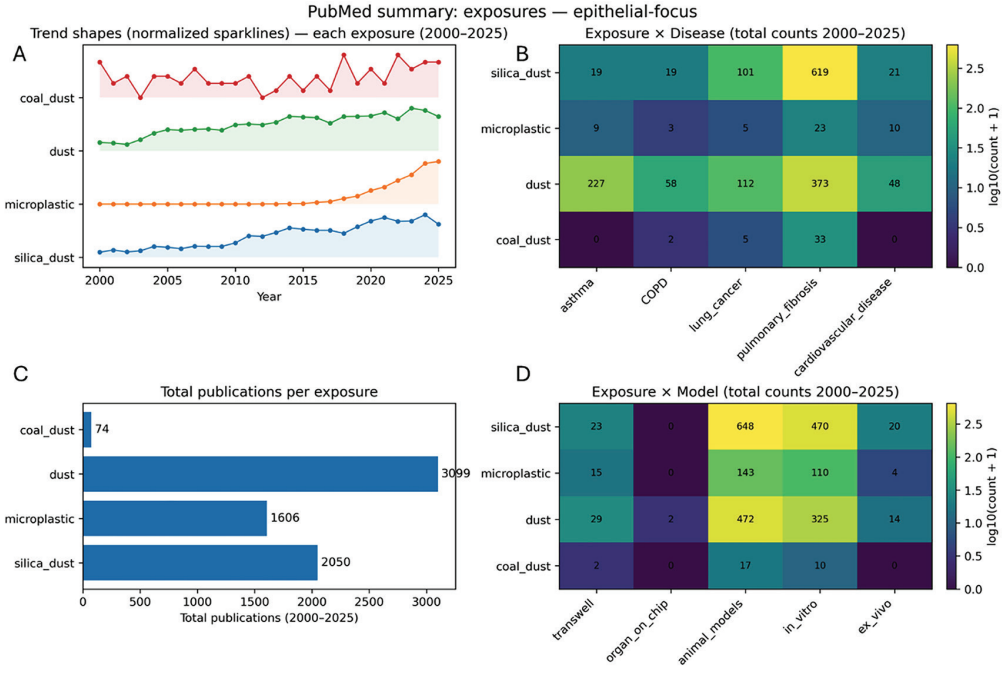

Figure 1. PubMed publication trends and experimental model usage for selected environmental exposures (2000-2025). A) Normalized annual publication trends for silica dust, microplastic, dust, and coal dust. B) Total publications per exposure across 2000-2025. C) Heatmap of total publications for each exposure-disease pair. D) Heatmap of total publications for each exposure-model combination

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Hydrogel Formulations to Investigate Lung Cancer Mechanism

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INTRODUCTION: Lung cancer remains a leading cause of cancer-related deaths worldwide, largely due to late diagnosis and the complexity of the tumor microenvironment (TME).[1] A key factor in lung cancer is the extracellular matrix (ECM), a 3D network composed of structural proteins, glycoproteins, proteoglycans, and growth factors that together regulate cell adhesion, proliferation, and signaling. ECM architecture and its changes are closely related to cancer mechanisms.[2] Thus, physiological models that recapitulate ECM composition and mechanics are essential. 2D cultures fail to replicate the organization and biochemical and mechanical signals of the TME, whereas microfluidic platforms offer dynamic, 3D cell culture systems hydrogel-integrated.[3] A broad range of biomaterials (synthetic, semi-synthetic, natural) is used to recapitulate the dynamics of ECM. Natural hydrogels such as collagen, gelatin methacrylate (GelMA), Matrigel, alginate, fibrin, and decellularized ECM (dECM) are widely used due to their inherent bioactivity and ability to support cell adhesion and proliferation.[4] Synthetic hydrogels, such as polyethylene glycol (PEG) and polyacrylamide, provide tunable stiffness and control over matrix composition, while semi-synthetic hybrids (e.g., PEG-GelMA, GelMA-dECM) combine biological cues with structural stability (Figure 1).[5]

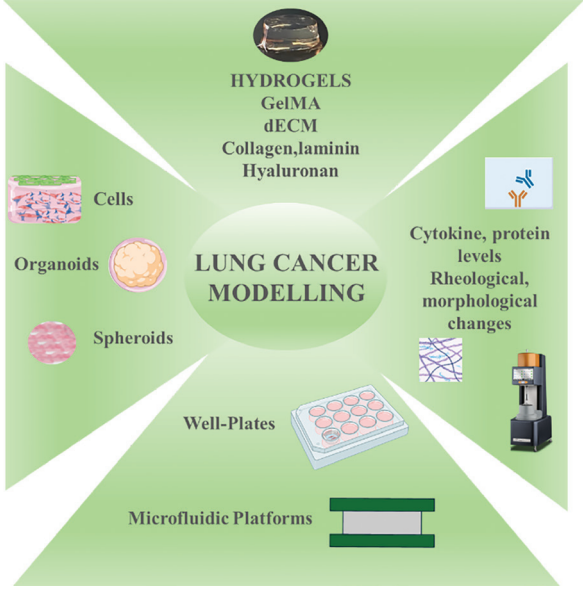

Figure 1. Schematic representation of hydrogel utilizattion in lung cancer modelling

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MATERIAL AND METHODS: Cancer cell lines A549 (adenocarcinoma), H1299 (non-small cell lung cancer), and H460 (large cell carcinoma) are frequently used in cancer modelling. Co-culture systems integrate fibroblasts, endothelial cells, and immune cells (e.g., macrophages) to simulate the TME and study cell-matrix-cell interactions. Patient-derived organoids preserve tumor heterogeneity, genetic mutations, and drug response profiles, representing a personalized *in vitro* cancer model. Tumor spheroids embedded in hydrogels recapitulate diffusion gradients and are used to evaluate drug penetration and metastasis. Further, they can be integrated into microfluidic platforms or well plates. As a next step, it is important to investigate ECM rheology, determine mechanical structure and cytokine expression levels, and further validate cell-matrix interactions.[6,7]

RESULTS: These models have shown that tumor cells embedded in hydrogels exhibit enhanced invasive behavior and increased expression of matrix metalloproteinases (enzymes responsible for ECM degradation and remodeling). From a biomechanical perspective, rheological analyses revealed that cancer-associated hydrogels typically exhibit higher storage modulus than healthy matrices, reflecting a stiffer microenvironment and increased collagen levels.[8]

CONCLUSION: Collectively, recent findings underscore the central role of the ECM and hydrogel-based systems in modeling lung cancer progression. The development of tissue-specific, mechanically tunable, and microfluidic-integrated hydrogels has transformed *in vitro* modeling from static 2D monolayers to dynamic, physiologically relevant 3D systems. Increased stiffness is now recognized as a key regulator of cancer cell fate, governing proliferation, EMT, and metastasis through mechanotransduction pathways such as YAP/TAZ and integrin–FAK signaling.[9] Despite significant progress, variability in dECM composition and crosslinking chemistry still challenges reproducibility and bioactivity. Moreover, current hydrogel-based models often lack immune cell components and vascular complexity, limiting their ability to fully emulate the native TME. Future efforts should integrate dECM-based hydrogels with organoid and microfluidic systems, supported by multi-omics profiling, to achieve patient-specific and physiologically relevant lung cancer models.

KEYWORDS: Organ-on-chip, biomaterial, hydrogel, extracellular matrix, tissue engineering, lung tumor-stroma interface

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The Development of Laboratory-made NTD Sensor Systems for the Analysis of Volatile Organic Compounds (VOCs) in Synthetic Breath Samples

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INTRODUCTION: In recent years, the analysis of volatile organic compounds (VOCs) has emerged as a highly attractive sample preparation method due to its ease of application to a wide range of samples. These techniques, which significantly increase the sensitivity of the analysis by reducing the interference effect, especially in biological samples containing many components, have the possibility of being applied in many ways.[1] These systems, which are widely used for the analysis of a wide range of compounds from pesticides to biomarkers, have many advantages over systems obtained by preparing the adsorbent polymer in fiber form. NTD sensor systems, which are prepared to be more protected by the presence of the polymer on the inner surface of the needle, have a longer service life.[2] For this purpose, NTD systems can be prepared using many different types of polymers.In addition, the surface area is significantly increased by coating it onto a cylindrical outer surface. This significantly increases the sensitivity of the method. Breath analysis has become quite popular over the years.[3] These analyses offer advantages such as ease of use and rapid diagnosis, particularly in crucial areas such as early-stage disease diagnosis, and can be performed without causing any pain to the patient due to their non-invasive nature.[4] Additionally, breath samples, which contain less interfering matrix than other biological samples, allow for highly sensitive analyses. Furthermore, due to the very low concentrations of VOCs in breath, more sensitive analytical techniques are needed for these analyses. In this context, the study investigated the applicability of laboratory-developed NTD sensor systems to synthetic breath samples. For this purpose, lab-made NTD sensors prepared with polyaniline polymer in laboratory environment were used for the determination of benzene, toluene, ethylbenzene and and o-, p-xylene (BTEX) compounds, which are important for environmental exposure, in synthetic breath medium.

MATERIAL AND METHODS: The NTD systems used in the study were made using four different conductive polymers. Surface and physical characterization of these polymers prepared by electropolymerization were investigated using scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis. Following surface characterization, the responses of NTDs prepared with each polymer to BTEX solutions were compared and the polymer with the best response was selected. Subsequently, the sampling technique (active and passive), adsorption temperature, adsorption time and desorption time were optimised. Finally, the analytical properties of the method developed for BTEX analysis in synthetic breath samples were determined using the optimum parameters obtained from the optimisation studies.

RESULTS: SEM images of the prepared NTDs showed that the polymers obtained were on porous surfaces, indicating

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that the lab-made NTDs had suitable properties for adsorption. FTIR spectra obtained with the attenuated total reflectance (ATR) probe confirmed the functional groups in the existing structures and supported the chemical properties of the structures by confirming that the targeted polymers were obtained. FTIR spectra obtained with the ATR probe confirmed the functional groups in the existing structures and supported the chemical properties of the structures by confirming that the targeted polymers were obtained. When the effects of four different polymers (polyaniline, polypyrrole, polythiophene and polyfuran) prepared in the study were examined on the responses of BTEX, it was seen that polyaniline gave the most effective response. When it comes to optimizing the experimental operating conditions, firstly the effects of active and passive sampling on BTEX responses were investigated (Figure 1). The results showed that passive sampling had better effect on analyte signals than active sampling. Optimizing the adsorption temperature revealed that an adsorption temperature of 45 °C provided the optimal response. Lower adsorption temperatures reduce analyte interaction with the polymer, while higher temperatures facilitate desorption from the surface, reducing adsorption efficiency. Therefore, 45 °C was determined to be the ideal value for adsorption. On the other hand, adsorption time is also a crucial parameter for establishing the necessary analyte-polymer balance. A 20-minute adsorption process is sufficient for this purpose. Longer adsorption processes do not result in a positive response due to surface saturation, but they do experience a loss of efficiency due to the loss of analytes from the surface due to environmental conditions. The final parameter optimized was the desorption time, which corresponds to the time spent by NTDs in the gas chromatography injection block. The optimum condition for this value was determined to be 4 min.

CONCLUSION: BTEX compounds are important in both environmental and biological samples. The PANI-based NTDs prepared in this study were successfully applied in these analyses in synthetic breath media. Data obtained through optimization of experimental conditions demonstrated that these analyses can be successfully applied in synthetic breath media at concentrations ranging from 89 to 395 µg/m^3.

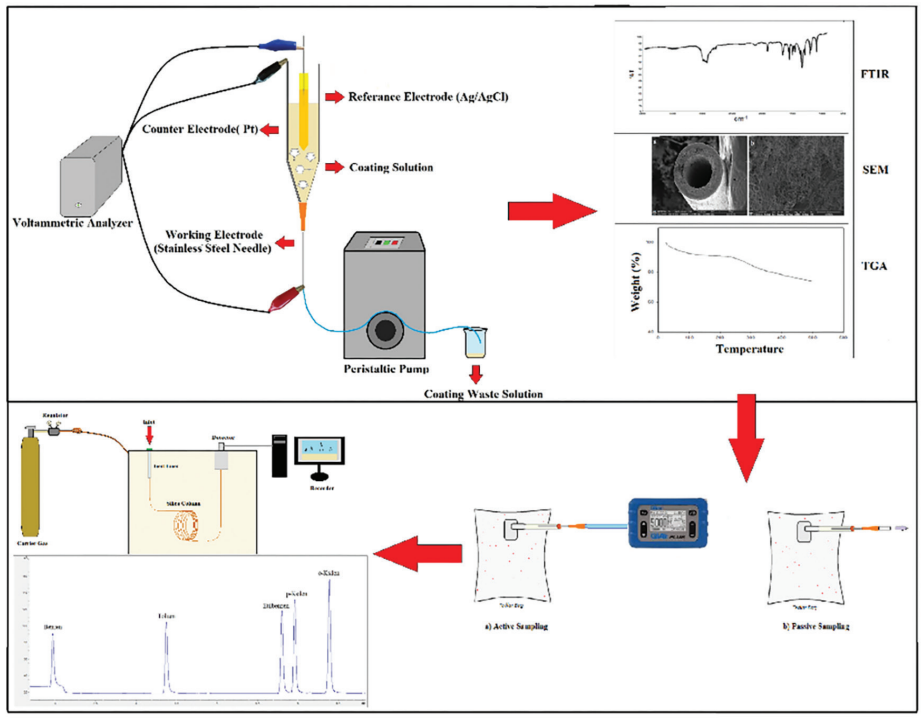

Figure 1. Experimental procedure of the study

KEYWORDS: Needle trap devices, breath analysis, gas chromatography, sample preparation

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Determination of Untargeted Metabolomic Profile in Breath Condensate Using LC-QTOF/MS and Investigation of Potential Lung Cancer-Specific Biomarkers

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INTRODUCTION: Lung cancer remains the leading cause of cancer-related mortality worldwide, primarily due to late diagnosis and the lack of sensitive, non-invasive biomarkers for early detection. Conventional diagnostic methods such as imaging and biopsy are either invasive or incapable of identifying disease at its earliest stages. Therefore, there is a growing demand for analytical strategies that enable non-invasive, rapid, and reliable diagnosis of lung cancer.[1] In recent years, exhaled breath condensate (EBC) has emerged as an attractive biological matrix in clinical metabolomics. EBC collection is completely non-invasive, painless, and repeatable, allowing real-time reflection of the metabolic status of the respiratory tract. It contains a variety of low-molecular-weight compounds, including volatile and non-volatile metabolites derived from airway and alveolar surfaces. Metabolomic profiling of EBC, particularly through high-resolution mass spectrometry, offers valuable insight into the biochemical alterations associated with lung cancer pathogenesis.[2] Metabolomics, as a systems biology approach, enables comprehensive analysis of metabolites that serve as the final products of cellular processes. This field provides a functional readout of the physiological state and disease progression, bridging the gap between genotype and phenotype. The untargeted metabolomics approach—unlike targeted methods that focus on predefined molecules—captures the widest possible range of metabolites, making it ideal for exploratory biomarker discovery.[3] The present study aimed to investigate potential lung cancer-specific biomarkers through untargeted metabolomic profiling of EBC samples using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). Breath samples were collected from lung cancer patients and healthy controls, and data were analyzed using multivariate statistical approaches to identify significant metabolic differences between the two groups.

MATERIAL AND METHODS: EBC samples were collected in the morning after overnight fasting. Participants inhaled filtered air through their noses and exhaled through their mouths into a thermostat-controlled condenser system maintained at +4 °C for 15 minutes. Saliva contamination was prevented using a U-shaped glass trap. The collected condensate (approximately 2-2.5 mL) was transferred into Teflon tubes and stored at -80 °C until analysis. Metabolites were separated by LC-QTOF/MS, a high-resolution analytical platform suitable for untargeted metabolomics due to its excellent mass accuracy and sensitivity. Raw data were processed using Signpost MS software for peak alignment, normalization, and statistical comparison. Principal component analysis (PCA) and hierarchical clustering were

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applied to visualize group separation and identify metabolite patterns associated with lung cancer. The workflow of the method is shown in Figure 1.

RESULTS: The PCA score plot revealed a clear separation between lung cancer patients (Group A) and healthy controls (Group B) along the PC2 axis, accounting for 8.5% of total variance, while PC1 explained 21.7%. The partial overlap between groups indicated some biological heterogeneity within the cancer cohort, which is expected due to tumor stage and individual metabolic variability. Nevertheless, the distinct clustering pattern confirmed that EBC contains disease-specific metabolic information. A heatmap visualization demonstrated differential metabolite expression profiles between the two groups. Notably, several metabolites exhibited increased intensity in the cancer group, suggesting potential involvement in tumor metabolism. Subsequent statistical evaluation (P <0.05) identified six significant metabolites, among which L-alanine and creatine were highlighted as potential endogenous biomarker candidates. Both metabolites play essential roles in cellular energy metabolism and biosynthetic pathways. Elevated creatine levels in EBC may reflect altered energy demands and mitochondrial dysfunction characteristic of cancerous cells. Meanwhile, L-alanine accumulation is consistent with enhanced glycolytic flux ("Warburg effect"), a hallmark of cancer metabolism.[3] Additional compounds such as N-(2,4-dichlorophenyl) hydrazinecarboxamide and 4-dimethylaminobenzophenone were also detected, possibly representing exogenous exposures or metabolic byproducts of oxidative stress.These findings collectively indicate that EBC metabolomics provides a rich biochemical snapshot of lung pathophysiology. The untargeted LC-QTOF/MS approach offers both qualitative and quantitative insights into metabolite alterations, thereby establishing EBC as a promising diagnostic medium for early lung cancer screening.

CONCLUSION: The results of this study demonstrated that untargeted metabolomic profiling of EBC can successfully distinguish lung cancer patients from healthy individuals. Identified biomarkers such as L-alanine and creatine show potential for early disease detection and could serve as the foundation for developing non-invasive screening tools. Future studies should validate these findings in larger cohorts and explore longitudinal sampling to assess biomarker stability and diagnostic accuracy. The integration of high-resolution mass spectrometry with non-invasive sampling techniques represents a powerful direction in personalized oncology. Such approaches may complement existing diagnostic modalities and contribute to earlier detection, better prognosis, and improved patient outcomes.

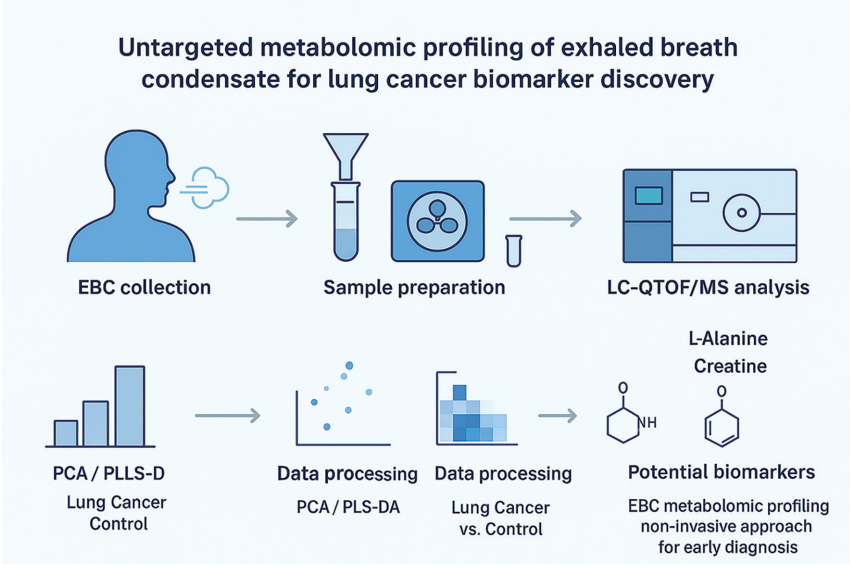

Figure 1: Graphical Abstract

KEYWORDS: Biomarker, quadrupole time-of-flight liquid chromatography/mass spectrometry, metabolomics, exhaled breath condensate, principal component analysis

ACKNOWLEDGMENTS: This study was supported by The Scientific and Technological Research Council of Türkiye (TUBITAK, Grant No. 122Z749) and the Presidency of Strategy and Budget of the Republic of Türkiye (2019K12-149080).

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A Thin Film Micro-Extraction Based Salivary Metabolomics and Chemometric Strategy for Rapid Lung Cancer Diagnosis

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INTRODUCTION: Lung cancer (LC) remains one of the leading causes of cancer-related mortality worldwide, largely due to the lack of reliable biomarkers for early detection.[1] Despite advances in diagnostic imaging and targeted therapies, the five-year survival rate remains low because most cases are diagnosed at advanced stages. Consequently, the development of sensitive, non-invasive, and cost-effective diagnostic approaches is a major clinical priority. Metabolomics, the comprehensive profiling of small-molecule metabolites, has emerged as a powerful tool for uncovering cancer-associated metabolic alterations, providing insights into tumor biology and facilitating the discovery of novel biomarkers for accurate diagnosis and disease monitoring. Among biological matrices, saliva is a promising diagnostic biofluid because it can be collected non-invasively, is simple to obtain, and reflects systemic and local metabolic changes. Recent studies have demonstrated its potential for detecting various cancers, including lung cancer, highlighting its value for biomarker-based early diagnosis.[2,3] In this study, a novel thin-film microextraction (TFME) technique integrated with liquid chromatography-tandem mass spectrometry (LC-MS/MS) is introduced for the rapid, selective, and reproducible extraction of salivary metabolites. The developed TFME approach offers high throughput, reduced solvent consumption, and enhanced analytical performance, enabling the identification and quantification of key metabolic biomarkers associated with lung cancer. The objective of this workflow is to advance saliva-based metabolomics toward clinical translation, offering a promising avenue for the early and non-invasive diagnosis of lung cancer.

MATERIAL AND METHODS

Synthesis of SiO_2 Nanoparticles and TFME blade Preparation: SiO_2 nanoparticles were synthesized using the Stöber method, followed by post-coating with tetraethyl orthosilicate, centrifugation, washing with ethanol, and drying. The nanoparticles were incorporated into a polyacrylonitrile (PAN) matrix and coated onto steel TFME blades via a controlled dip-coating process to ensure uniform film thickness.

Participants and Sample Collection: Saliva samples were collected from 40 histopathologically confirmed lung

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cancer patients and 38 healthy volunteers following an overnight fast and an oral rinse. Ethical approval and informed consent were obtained (Ege University Ethics Committee, protocol: 15-11.1/46). Saliva samples were centrifuged, diluted (1:2), and stored at -80 °C until analysis.

TFME Sampling and Analysis: A 96-well plate system equipped with PAN/SiO_2-coated TFME blades was used for metabolite extraction (Figure 1). Blades were immersed in diluted saliva samples and rotated at 850 rpm for 150 minutes to allow analyte adsorption, followed by desorption of analytes in 0.1% formic acid for 30 minutes. Desorbed solutions were spiked with 0.5 µg/mL ornidazole as an internal standard prior to LC-MS/MS analysis.

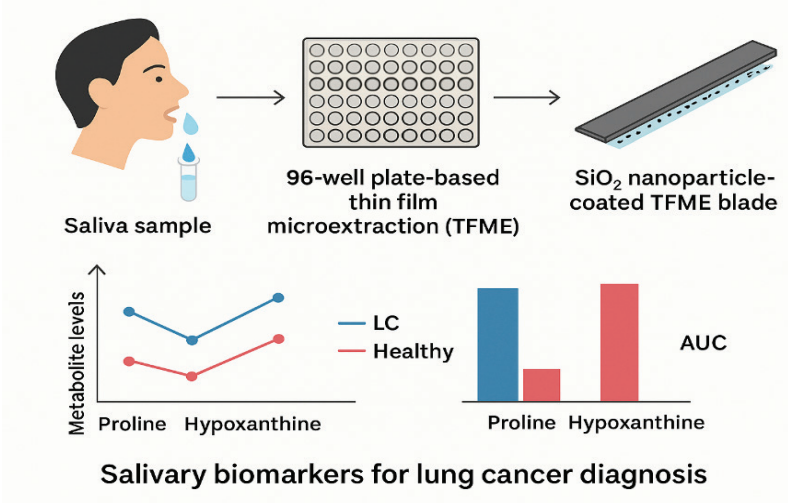

Figure 1. Graphical abstract

RESULTS: The TFME method was optimized to detect 18 metabolites in pre-treatment saliva samples from lung cancer patients. Chromatographic evaluation demonstrated that the Inertsil 100 column, employing isocratic elution with ornidazole as the internal standard, provided optimal separation efficiency and reproducibility. Extraction parameters, including desorption solution type and pH, were optimized; desorption solution type 2 at pH 8-9 yielding the highest metabolite recovery. Analytical validation indicated robust linearity (R^2: 0.9841-0.9975), sensitivity (limit of detection: 0.014-0.97 µg/mL; limit of quantification: 0.046-3.20 µg/mL), precision (%relative standard deviation <20%), and accuracy (85-125% for most metabolites). Pathway analysis revealed significant alterations in the metabolism of phenylalanine, purine, tyrosine, histidine, and methionine. The Heatmap visualization showed increased levels of proline, hypoxanthine, phenylalanine, and tyrosine in lung cancer patients. receiver operating characteristic curve analysis highlighted these metabolites as potential biomarkers, with proline exhibiting the highest diagnostic performance [area under the curve (AUC): 0.946], followed by hypoxanthine (AUC: 0.933) and phenylalanine (AUC: 0.905)

CONCLUSION: The findings of this study demonstrate that the TFME approach is a reliable and efficient platform for metabolomic profiling in lung cancer. Using pre-treatment saliva samples, the method achieved a sensitivity exceeding 90% for detecting newly diagnosed histopathologically confirmed patients. Among the metabolites analyzed, proline, hypoxanthine, and phenylalanine showed strong diagnostic potential, consistent with the pathway analyses implicating purine and phenylalanine metabolism. These results underscore the potential of salivary metabolomics as a non-invasive screening alternative in the absence of validated early lung cancer biomarkers. Additionally, TFME's high-throughput capacity, cost-effectiveness, and environmental sustainability

support its feasibility for routine clinical application.

KEYWORDS: Biomarker, metabolomics, thin-film microextraction, LC-MS/MS, saliva

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From Scarcity to Survival: Adaptive Strategies of A549 Lung Cancer Cells Under Serum Starvation

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INTRODUCTION: Serum plays a critical role in regulating fundamental biological processes, and variations in serum levels can directly influence tumor cell metabolic adaptations, stress responses, and traits associated with tumor progression. The lung adenocarcinoma cell line A549 serves as a widely used *in vitro* model for studying these mechanisms. This study aims to investigate how short- and long-term exposure to different serum concentrations affects A549 cell morphology, proliferation, migration, cell cycle dynamics, gene expression profiles, three-dimensional spheroid formation capacity, and molecular features related to stemness, metastatic potential, and drug resistance. Serum availability is a key determinant of tumor cell behavior, influencing proliferation, differentiation, and survival, while nutrient deprivation triggers adaptive mechanisms that enable cancer cells to endure stress conditions.[1,2]

MATERIAL AND METHODS: A549 cells were cultured under different serum concentrations [1%, 10%, and 20% fetal bovine serum (FBS)], with cells maintained in 10% FBS serving as the control group. To evaluate the effects of serum availability on cellular behavior, various assays were performed, including Giemsa staining for morphology, wound healing assay for migration, flow cytometric analysis of CD44 and CD133 for stemness, XTT assay with flavopiridol for drug resistance, colony formation assay for clonogenic capacity, three-dimensional spheroid formation assay, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) for gene expression profiling.

RESULTS: Significant differences in proliferation and morphology were observed in A549 cells depending on serum concentration. Cell cycle analyses showed that exposure to different serum concentrations caused significant changes in phase distribution. RT-qPCR results revealed significant expression differences in specific genes associated with cellular stress response and proliferation. These findings contribute to elucidating adaptive response mechanisms of tumor cells to nutritional conditions and provide an important approach for the development of *in vitro* tumor biology models.

CONCLUSION: Serum deprivation triggers complex adaptive responses in A549 cells, influencing morphology, migration, stemness, drug sensitivity, and gene expression. Low-serum conditions promoted stress-adaptive and survival phenotypes relevant for modeling tumor microenvironments. These findings suggest that serum availability is a crucial determinant of cancer cell plasticity and behavior. Moreover, long-term adaptation to serum limitation

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may mimic the nutrient-restricted conditions of solid tumors, providing a valuable *in vitro* model for studying metabolic flexibility and therapy resistance mechanisms in lung cancer cells.

KEYWORDS: Lung cancer, serum starvation, gene expression, cell migration

ACKNOWLEDGEMENTS: This study was supported by Ege University Scientific Research Projects Coordination Unit with General Research Project-32942 project number.

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Effects of *Staphylococcus aureus* on Lung Cancer Cells

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INTRODUCTION: Lung cancer continues to be a leading cause of morbidity and mortality.[1,2] In addition to genetic, epigenetic, and stromal microenvironmental factors, the host microbiota, an integral part of the human body, significantly contributes to cancer development and progression.[3] *Staphylococcus aureus* (*S. aureus*), a Gram-positive bacterium, is known to cause DNA damage through its toxins and may promote carcinogenesis and metastasis in various cancer types, including lung cancer.[4,5] The aim of our study was to establish a *S. aureus* infection model and investigate its molecular and cellular effects on lung cancer and normal bronchial epithelial cells.

MATERIAL AND METHODS: To establish an intracellular infection model, A549 lung cancer and BEAS-2B bronchial epithelial cell lines were infected with *S. aureus* at infection ratios of 1:25, 1:50, and 1:100 for 2 hours and treated with gentamicin for 2-4 hours to eliminate extracellular bacteria. Intracellular infection was visualized by Giemsa staining. Intracellular *S. aureus* load was determined by CFU analysis, and the infection index was calculated. The effect of *S. aureus* infection on cell cycle and cell death was analyzed using flow cytometry. Wound closure assay was performed to assess metastatic potential. Expression levels of key genes involved in cancer mechanisms were examined using reverse transcription quantitative polymerase chain reaction (RT-qPCR).

RESULTS: Intracellular *S. aureus* colonies were demonstrated in A549 and BEAS-2B cell lines at ratios of 1:25, 1:50, and 1:100 (Figure 1A, B). BEAS-2B cells were observed to be more infected than A549 cells (Figure 1C). According to the migration assay results, A549 cells infected with *S. aureus* showed a significant increase in migration capacity compared to the control group. This finding suggests that *S. aureus* infection increases cell migration in A549 cells. Cell cycle analysis revealed that infected A549 cells accumulated, particularly in the G2 phase, 24 h after infection. These results suggest that *S. aureus* infection differentially affects these two cell lines. Furthermore, RT-qPCR analysis revealed changes in the expression levels of genes involved in important cellular processes.

CONCLUSION: This study demonstrates that *S. aureus* infection induces cell-type-specific responses in lung epithelial cells. BEAS-2B cells were more susceptible to colonization, while A549 cells exhibited increased migration and G2/M phase arrest, along with changes in gene expression associated with key cellular pathways. These findings suggest that *S. aureus* may contribute to pathological processes such as chronic infection or tumor progression at the cellular level.

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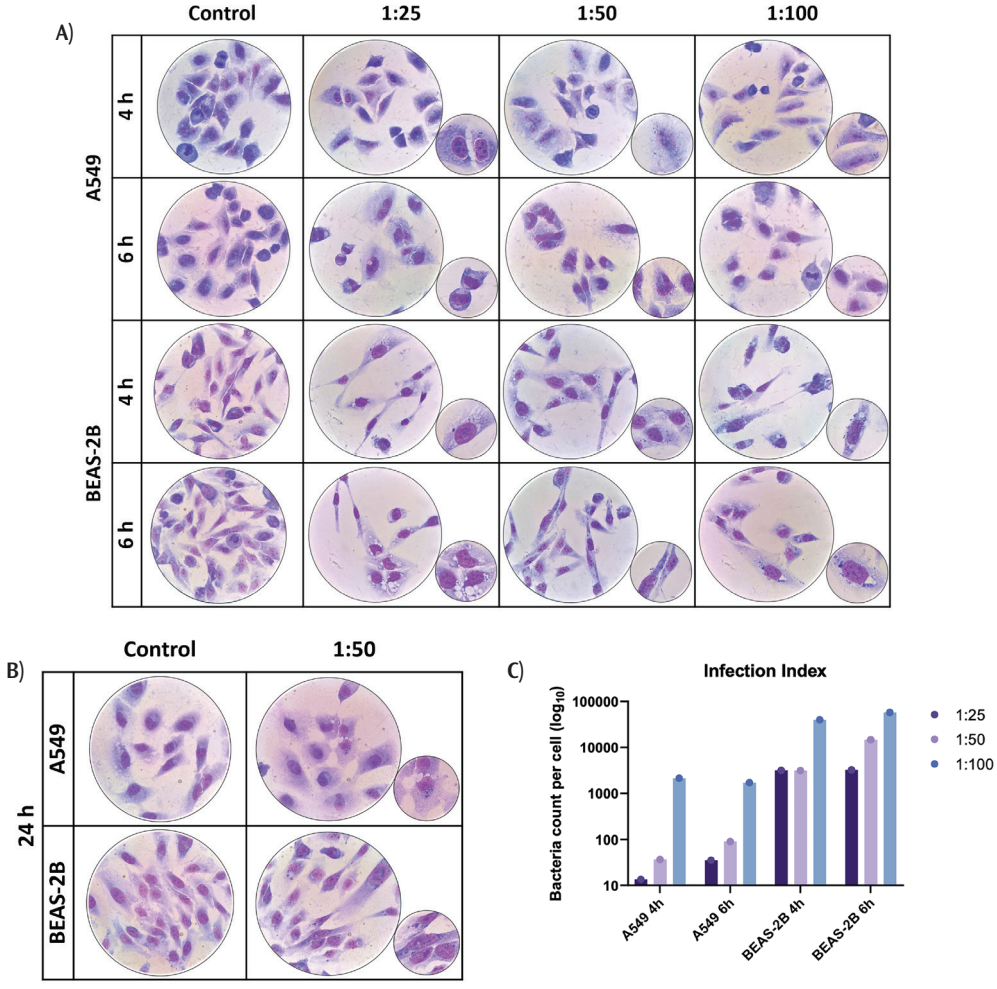

Figure 1. Infection of A549 and BEAS-2B cells with *S. aureus*. A) Demonstration of infection of A549 and BEAS-2B cells with *S. aureus* at MOIs of 1:25, 1:50, and 1:100 for 4 and 6 hours using Giemsa staining at 100x focus. B) Demonstration of infection of A549 and BEAS-2B cells with *S. aureus* at MOIs of 1:50 for 24 hours using Giemsa staining. C) Infection index graphs of A549 and BEAS-2B cells after *S. aureus* infection at 1:25, 1:50 and 1:100 ratios at 4 and 6 hours

KEYWORDS: Cell culture, lung cancer, infection, *Staphylococcus aureus*

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Expression Profile of circRNA in Lung Cancer Cells Infected with *Staphylococcus aureus*

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INTRODUCTION: Lung cancer is one of the main causes of cancer -related deaths on a global scale and ranks first with approximately 1.8 million deaths according to 2022 data.[1] Tumor formation is shaped not only by genetic and epigenetic changes, but also by microorganism-hycre interactions. The cancer load attributed to infections was reported in 2018 as approximately 2.2 million new cases.[2] This shows that chronic bacterial infections can support inflammatory signaling and tumor progression. *Staphylococcus aureus*, one of these pathogens, is known for adhesion, invasion and immune response modulation to the host cell. It can also affect cell behavior by disrupting the epithelial barrier through toxins.[3] In recent years, it has been suggested that these effects on lung epithelium can re -program processes such as proliferation, apoptosis and migration in cancer cells. On the other hand, Circular RNAs (circRNA) show high stability due to the closed ringing of 5′ and 3′ ends and have functions such as miRNA/protein binding or regulation of gene expression. circRNA played a role in the basic processes of cancer biology such as proliferation, metastasis and drug resistance; It is also reported that it can be used as biomarker and therapeutic target in lung cancer.[4,5] This study aims to examine the circRNA expression changes after infection of A549 (lung adenocarcinoma) and Beas-2b (normal bronchial epithelial) cells with *S. aureus*.

MATERIAL AND METHODS: In order to create an intracellular infection model, A549 and Beas-2B cell lines are infected with *S. aureus* 6538p for 2 hours of 1:50 infection (MOI). It was then treated with gentamicin for 2-24 hours to destroy non-cell bacteria. Intracellular infection was displayed using Giemsa painting method and intracellular *S. aureus* load was measured by CFU analysis, infection index was calculated (infection index = dilution factor × 10). In the 4th and 24th hours of 1:50 infected cells, trizol was insulated with RNA and the concentrations were measured and CDNA was synthesized. circRNA levels were analyzed with reverse transcription quantitative polymerase chain reaction (RT-qPCR). The circular RNA candidates used in the study were determined through CircBank database. The potential target genes and expression levels of these circRNAs were examined in detail using CircAtlas 3.0 database.

RESULTS: In the A549 and Beas-2b cells infected with 1:50 *S. aureus*, the presence of intracellular bacteria was evaluated microscopic with Giemsa dyeing performed at the 4th and 24th hours of infection. According to the infection index analysis at 4 hours of infection, Beas-2B cells were found to be higher than A549 cells. This suggests that normal bronchial epithelial cells may be more sensitive to bacterial internalization. As a result of the RT-qPCR analysis performed in cells after infection, a significant decrease in the expression levels of hsa_circ_0000003,

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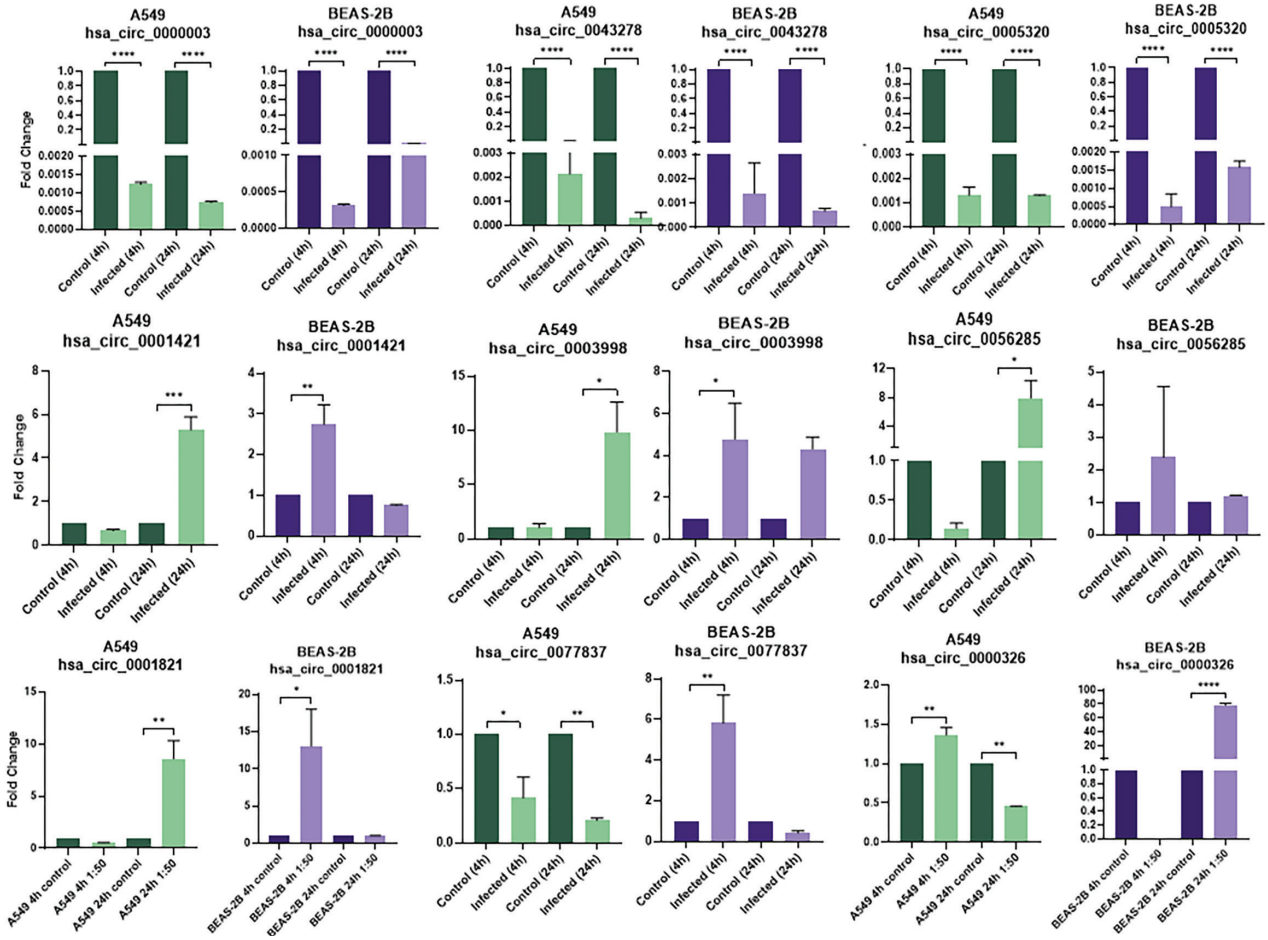

Figure 1. RT-qPCR floor charts after 4 and 24 hours of infection of A549 and Beas-2B cells at a rate of 1:50 ratio
RT-qPCR: Reverse transcription quantitative polymerase chain reaction

hsa_circ_0043278, hsa_circ_0005320, hsa_circ_0001421, hsa_circ_0003998, hsa_circ_0056285, hsa_circ_0001821, hsa_circ_0077837 and hsa_circ_0000326 circular RNAs were observed in both A549 and Beas-2B cells ($P \leq 0.0001$) (Figure 1). These findings show that *S. aureus* infection may suppress the expression of the related circRNA and that these molecules may play a potentially role in the response of infection.

CONCLUSION: In this study, *S. aureus* infection A549 lung cancer and BEAS-2B normal bronchial epithelial cells were successfully achieved and the effects of infection on the circRNA expression profile were evaluated. The data obtained show that infection changes the expression of hsa_circ_0000003, hsa_circ_0043278, hsa_circ_0005320, hsa_circ_0001421, hsa_circ_0003998, hsa_circ_0056285, hsa_circ_0001821, hsa_circ_0077837 and hsa_circ_0000326 CIRCRNA in host cells, and that these molecules can undertake potential regulatory roles in the infection response. These different expressions in the infection process of circRNA can both contribute to a better understanding of disease mechanisms and can be considered as biomarker or therapeutic target in the future.

KEYWORDS: Lung cancer, *Staphylococcus aureus*, circRNA, infection, biomarker

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Large-Scale Production and Therapeutic Evaluation of Exosomes for Cancer Treatment

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INTRODUCTION: Lung cancer remains one of the most prevalent and deadly malignancies worldwide, representing a major global health challenge. According to the latest global cancer statistics, lung cancer accounts for approximately 11.6% of all new cancer diagnoses and 19.8% of cancer-related deaths, making it the leading cause of cancer mortality.[1] Current therapeutic strategies, including surgery, chemotherapy, radiotherapy, and targeted therapies, vary depending on the histological type and stage of the tumor. While these approaches have improved survival in select patient populations, their overall effectiveness remains unsatisfactory due to systemic toxicity, drug resistance, and tumor recurrence. Consequently, there is an urgent need to develop safer, more effective, and targeted therapeutic strategies capable of overcoming these limitations.[2]

Recent advances in immunotherapy and nanotechnology have transformed the landscape of cancer treatment, enabling precise modulation of tumor immunity and site-specific delivery of therapeutic agents. Among the various nanocarrier systems, such as liposomes, polymeric nanoparticles, developed to date, exosomes have attracted attention as a promising next-generation therapeutic tool for cancer diagnosis, treatment, and prognosis.[3] Exosomes are naturally derived, cell-secreted nanovesicles with intrinsic biological functions. Exosomes are lipid bilayer vesicles with diameters typically ranging between 30 and 150 nm, secreted by most eukaryotic and prokaryotic cells under both physiological and pathological conditions. They are formed through the endosomal pathway and encapsulate a rich cargo of proteins, lipids, nucleic acids, and metabolites reflective of their cell of origin. Due to their endogenous origin, exosomes exhibit exceptional biocompatibility, low immunogenicity, and the ability to cross biological barriers such as the blood–brain barrier, features that confer them a distinct advantage over synthetic nanoparticles. Furthermore, their inherent role in intercellular communication allows them to mediate the transfer of bioactive molecules between cells, influencing diverse biological processes including immune regulation, angiogenesis, and metastasis.[4,5] These properties position exosomes as promising candidates for both diagnostic and therapeutic applications in cancer.

In recent years, the use of exosomes as liquid biopsy biomarkers for early detection of cancer has gained attention. Because exosomes can be readily isolated from non-invasive sources such as plasma, serum, or bronchoalveolar lavage fluid, they provide valuable molecular insights into tumor progression and response to therapy.[6] Beyond diagnostics, exosomes also offer unique advantages as therapeutic delivery vehicles. Their natural targeting capabilities, long circulation time, and ability to encapsulate and protect therapeutic molecules such as small RNAs, proteins, or antigens make them ideal candidates for precision drug delivery. Specifically, in oncology, exosome-based delivery systems can enhance drug accumulation within tumor tissues, minimize systemic toxicity, and improve overall therapeutic efficacy compared with conventional chemotherapeutics. Despite these advantages,

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one of the major challenges impeding the clinical translation of exosome-based therapeutics is the difficulty of large-scale production and purification. Exosomes are typically secreted at low concentrations, and traditional isolation methods such as ultracentrifugation, precipitation, or size-exclusion chromatography are often labor-intensive, time-consuming, and yield-limited. Therefore, optimizing scalable, reproducible and cost-effective bioprocesses for the large-scale production and purification of exosomes is a critical prerequisite for their preclinical and clinical application.[4,5] Addressing these limitations will be a crucial step toward realizing the full potential of exosome-based nanomedicine as a next-generation therapeutic strategy in the treatment of lung cancer and other malignancies.

MATERIAL AND METHODS: In the present study, THP-1 cells, a well-established human pro-monocytic cell line, were selected for exosome production due to their immune-regulatory potential and capacity to secrete vesicles rich in functional proteins and cytokines. To ensure the isolation of exosomes exclusively secreted by THP-1 cells, the culture system was adapted to serum-free conditions, eliminating contamination from animal-derived exosomes commonly present in fetal bovine serum.[7] Subsequently, cells were produced in a stirred-tank bioreactor, and a cross-flow ultrafiltration system was optimized for isolation, creating a bioprocess system for large-scale production of exosomes. The isolated exosomes were characterized for size distribution, morphology, homogeneity, concentration, protein content, and surface markers. Finally, after loading the cargo molecule into exosomes, their therapeutic efficacy was further evaluated in a three-dimensional carcinoma spheroid model (Figure 1).

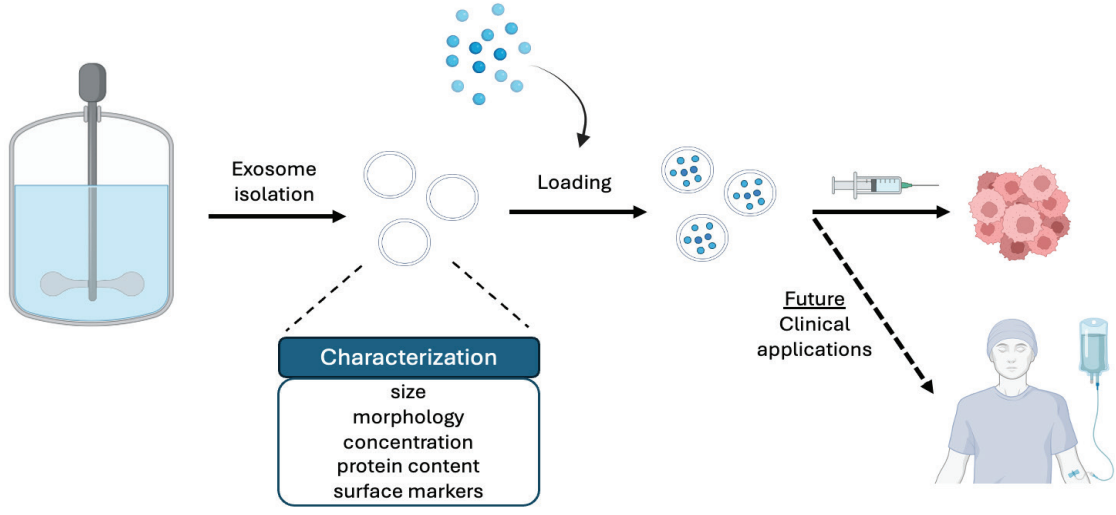

Figure 1. Schematic representation of the large-scale production, isolation, and therapeutic evaluation of THP-1-derived exosomes

RESULTS: THP-1 cells successfully adapted to serum-free culture conditions and produced exosomes efficiently in a stirred-tank bioreactor. The optimized ultrafiltration system achieved high recovery rates and excellent exosome purity, as validated by nanoparticle tracking analysis, scanning transmission electron microscopy, and immunoblotting for characteristic exosomal markers. The improved bioprocess significantly increased exosome yield, thereby overcoming one of the major bottlenecks in their clinical scalability. Functionally, the application of loaded THP-1-derived exosomes to carcinoma spheroids led to a notable reduction in spheroid size and cell viability, demonstrating their potential tumor-suppressive and antigen-delivery capabilities. These findings highlight the immunostimulatory potential of immune cell-derived exosomes, which may act through pathways involving antigen presentation and modulation of immune signaling cascades. The scalability of this bioprocess,

combined with the therapeutic efficacy of THP-1-derived exosomes, emphasize their promise as next-generation immunotherapeutic platforms for cancer treatment.

CONCLUSION: This study comprises a progress about a scalable bioprocess platform for the efficient production, purification, and functional validation of THP-1-derived exosomes. The optimized stirred-tank bioreactor and cross-flow ultrafiltration system significantly improved exosome yield and purity, enabling the quantities required for preclinical applications. Functionally, the resulting exosomes demonstrated potent antitumor effects in 3D tumor models, supporting their potential use as immunomodulatory nanotherapeutics in oncology. Future research should focus on optimizing cargo loading strategies, *in vivo* biodistribution analysis, and optimization of targeting strategies for specific tumor types, including lung cancer. Ultimately, integrating scalable manufacturing with precise therapeutic design will accelerate the clinical translation of immune cell-derived exosomes as safe and effective platforms in cancer nanomedicine.

KEYWORDS: Exosomes, cancer, large-scale production, THP-1 cells, nanotechnology in oncology

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Nanoparticle-Based Therapeutic Strategies in Respiratory Diseases: Current Approaches and Future Perspectives

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INTRODUCTION: Respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), lung cancer, tuberculosis, and acute respiratory distress syndrome (ARDS) remain major global health challenges, causing significant morbidity and mortality worldwide.[1,2] Despite the availability of pharmacological treatments such as bronchodilators, corticosteroids, antibiotics, and chemotherapeutics, most conventional drug administration routes (oral or intravenous) are often associated with critical limitations. These systemic delivery methods lead to the widespread distribution of drugs throughout the body rather than targeted accumulation at the site of infection or inflammation. This lack of specificity reduces the drug concentration at the affected region while increasing off-target toxicity in healthy tissues.[3] Furthermore, many orally administered drugs suffer degradation in the gastrointestinal tract or undergo first-pass metabolism in the liver, which significantly decreases the amount of active drug reaching the systemic circulation and ultimately the target lung tissues.[4] Many therapeutic drugs also have short half-lives, necessitating frequent dosing to maintain therapeutic levels, reducing patient compliance in chronic or long-term treatments.[5] Therefore, there is an urgent need for advanced drug delivery systems capable of improving pulmonary targeting, enhancing therapeutic efficacy, and minimizing systemic side effects.

Nanotechnology offers a promising and innovative alternative platform for addressing these challenges. Nanoparticles, ranging from 1-100 nm and employed as drug delivery systems, display unique physical, chemical, and biological properties compared to their macro-scale counterparts. As a transformative field in biomedical science, nanotechnology enables the design of novel nanoformulations to overcome the major limitations of conventional treatments. Nano-based drug delivery systems can enhance solubility, extend drug half-life, and achieve localized accumulation in the lungs by penetrating mucosal barriers.[6] Different nanoparticle types, such as polymeric, lipid-based, and metallic nanoparticles, have been developed to optimize therapeutic efficiency and minimize side effects.[7] Considering liposomes, polymeric nanoparticles, solid lipid nanoparticles, and inorganic nanoparticles as representative platforms for pulmonary applications a comparison of conventional and nanotechnology-based drug delivery systems is important (Figure 1).

Recent studies highlight the great potential of nanoparticle-based systems for respiratory diseases For example, inhalable reactive oxygen species (ROS)-responsive nanoparticles were designed to release anti-inflammatory drugs only in regions with high oxidative stress, reducing inflammation and tissue damage in COPD and ARDS models.[8] Similarly, glutathione (GSH)-triggered nanoparticles utilize redox-sensitive linkers to release antibiotics selectively in infection sites, improving bacterial clearance in pulmonary infections.[9] Nanoliposomal formulations of salbutamol sulfate provide controlled bronchodilator release and prolonged lung retention in asthma therapy.[10] Biodegradable poly(lactic acid) (PLA) nanoparticles enhance the stability and controlled release of anti-inflammatory agents,

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offering improved safety profiles.[11] Meanwhile, mannose-conjugated chitosan nanoparticles effectively target alveolar macrophages for the treatment of tuberculosis, improving drug accumulation and antimicrobial efficacy.[12] Collectively, these examples underscore that nanotechnology-based systems hold great promise for overcoming the intrinsic barriers of conventional therapies by achieving site-specific, sustained, and safer drug delivery in respiratory diseases.

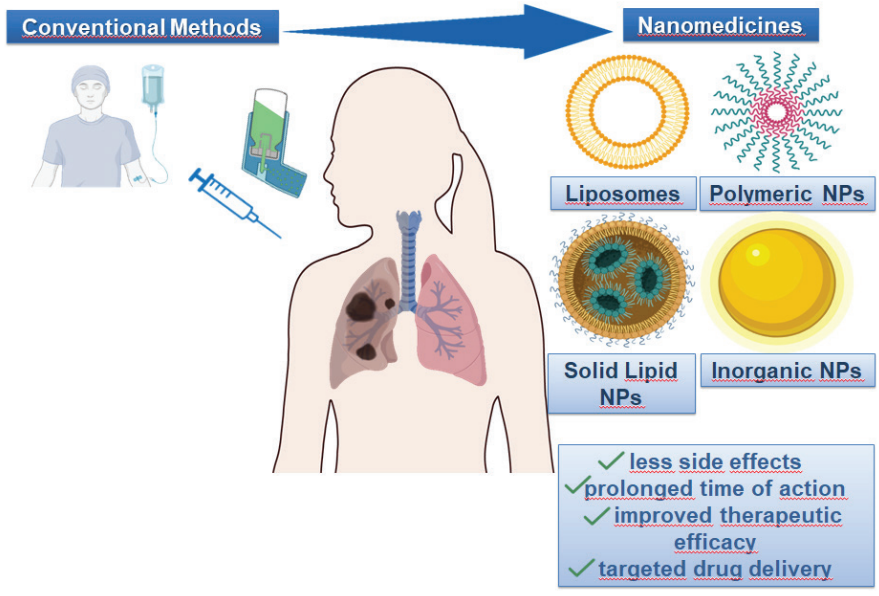

Figure 1. Schematic representation of conventional and nanotechnology-based drug delivery systems

CONCLUSION: Nanoparticle-based drug delivery systems provide a significant advancement in the treatment of respiratory diseases by overcoming the fundamental limitations of conventional therapies. Through their small size, tunable physicochemical properties, and ability to target specific lung regions, nanoparticles ensure improved bioavailability, controlled release, and reduced systemic toxicity. Studies on various nanocarriers —such as ROS-responsive and GSH-triggered nanoparticles, nanoliposomal salbutamol sulfate, PLA nanoparticles, and mannose-conjugated chitosan nanoparticles— have demonstrated promising outcomes in enhancing drug retention, reducing inflammation, and improving therapeutic efficacy in respiratory disorders. Despite these achievements, challenges such as mucus barrier penetration, long-term pulmonary toxicity, and large-scale reproducibility still remain. However, ongoing interdisciplinary research combining materials science, pharmacology, and pulmonary biology continues to improve the design, safety, and performance of nanoparticle systems. Collectively, these advancements indicate that nanotechnology can transform the current therapeutic landscape of respiratory medicine, making treatments more effective, safer, and more patient-centered.

Future Perspectives

The future of nanoparticle-based pulmonary therapies lies in the development of next-generation smart and personalized nanomedicines. **Stimuli-responsive nanoparticles** capable of detecting disease-specific microenvironments, such as pH shifts, oxidative stress, or enzymatic activity, will enable localized and on-demand drug release, minimizing off-target effects. **Personalized nanomedicine** approaches will allow the design of patient-specific formulations that combine multiple therapeutic agents, offering synergistic efficacy for complex respiratory

disorders. **Hybrid nanoplatforms** that integrate metallic nanoparticles (e.g., silver, gold) with natural bioactive compounds such as phycocyanin are expected to exhibit both therapeutic and diagnostic potential, enhancing the scope of precision medicine. Furthermore, advancements in inhalation device technology, aerosol engineering, and biocompatible excipient development will further support the translation of nanoparticle-based formulations from laboratory research to clinical application. As large-scale production, regulatory harmonization, and long-term safety validation advance, nanoparticle-based systems are expected to become a cornerstone of future respiratory therapies, offering precision, safety, and efficacy beyond the limitations of current treatments.

KEYWORDS: Nano-delivery systems, respiratory diseases, nanoparticles

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MSC-Exosomes as Novel Therapeutics in Asthma and Allergic Airway Inflammation

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INTRODUCTION: Allergic respiratory diseases, including asthma and allergic rhinitis, are chronic inflammatory diseases that affect millions of people worldwide and constitute a significant health problem.[1] These diseases are characterized by airway obstruction, mucus overproduction, eosinophilic infiltration, and airway hyperresponsiveness, which together lead to impaired respiratory function and reduced quality of life. Despite significant advances in pharmacotherapy, current treatments such as corticosteroids and bronchodilators mainly target symptom control rather than underlying immune dysregulation, often resulting in relapse or steroid resistance.[2] Consequently, there is a growing need for novel, effective, and safe therapeutic strategies that address the root causes of airway inflammation rather than providing temporary relief.

In recent years, extracellular vesicles, particularly exosomes (Exos), have emerged as key mediators of intercellular communication and as promising candidates for next-generation biologics. Exos are lipid bilayer nanosized vesicles (30-150 nm) secreted by almost all cell types, containing proteins, lipids, mRNAs, and microRNAs that reflect the physiological state of their parent cells.[3] Their natural stability, biocompatibility, and ability to traverse biological barriers with minimal immunogenicity confer several advantages over synthetic nanocarriers. Due to their intrinsic targeting capacity and role in cell-cell signaling, Exos are increasingly explored for use in drug delivery, immune modulation, and regenerative medicine.[4] Among various exosome sources, mesenchymal stem cell (MSC)-derived Exos have emerged as highly attractive candidates for cell-free therapies. MSCs are well-known for their immunoregulatory and regenerative capabilities, and their secreted Exos retain most of these biological functions. Importantly, MSC-Exos can interact with immune cells and modulate inflammatory signaling pathways, suggesting their potential as next-generation biotherapeutics in allergic airway diseases.[5]

The immunopathogenesis of allergic airway diseases is mainly driven by the imbalance between Th1 and Th2 immune responses, with Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13 playing central roles in eosinophilic inflammation and immunoglobulin E (IgE) production.[6] MSC-Exos have been shown to mitigate these pathological processes through multiple mechanisms: attenuating Th2-dominated responses, downregulating pro-inflammatory cytokines, and promoting the expansion of regulatory T-cells (Tregs), which are crucial for maintaining immune tolerance.[7] Furthermore, MSC-Exos promotes the polarization of M2 macrophages, a phenotype associated with tissue repair and resolution of inflammation. At the molecular level, Exos derived from MSCs deliver bioactive microRNAs that modulate critical signaling cascades, including NF-κB, STAT6, and MAPK pathways, key regulators of inflammation and immune activation.[8] For instance, microRNAs such as miR-146a-5p, miR-126-3p, and miR-1470 carried by MSC-Exos have been implicated in suppressing inflammatory mediators and restoring immune balance in airway tissues.[6] Through these mechanisms, MSC-Exos effectively attenuate airway hyperreactivity and remodeling,

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leading to functional improvement in preclinical asthma models. MSC-Exos exert their therapeutic effects through multiple mechanisms, including modulation of immune cell activity, suppression of Th2 cytokines, and promotion of Treg and M2 macrophage responses that collectively alleviate airway inflammation (Figure 1).

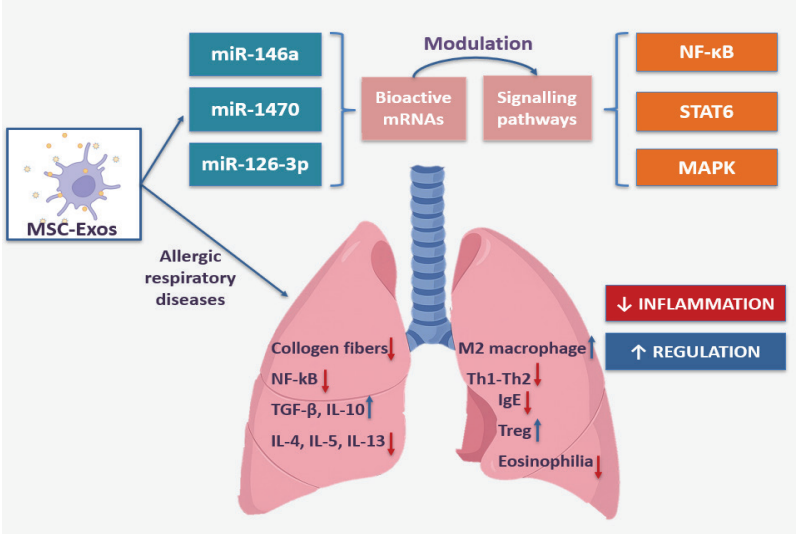

Figure 1. Schematic representation of the therapeutic mechanisms of MSC-derived exosomes in allergic airway inflammation

CONCLUSION: Preclinical studies using both systemic and intranasal administration of MSC-Exos have demonstrated significant therapeutic benefits. In murine models of allergic airway inflammation, treatment with MSC-Exos resulted in reduced eosinophilic infiltration, decreased serum IgE levels, and suppression of mucus hypersecretion. These effects were accompanied by enhanced secretion of IL-10 and TGF-β, two key anti-inflammatory cytokines that contribute to an immunosuppressive microenvironment.[9] Collectively, these findings indicate that MSC-Exos can recapitulate many of the beneficial immunomodulatory effects of MSC therapy while avoiding several of the risks associated with live cell transplantation, such as immune rejection or tumorigenicity.

A major advantage of MSC-Exos therapy is its cell-free and safer nature, avoiding risks associated with stem cell transplantation. Due to their nanoscale size, lipid bilayer structure, and endogenous cargo, MSC-Exos can efficiently deliver regulatory molecules to target tissues, outperforming many synthetic nanocarrier systems. Nevertheless, translating these promising preclinical findings into clinical practice requires overcoming several challenges, including standardized isolation and characterization protocols, scalable GMP-compliant production, dose optimization, and rigorous long-term safety evaluation.

Future Perspectives

MSC-Exos represent an innovative and safe therapeutic platform for allergic respiratory diseases such as asthma and rhinitis. By combining the regenerative and immunomodulatory properties of MSCs with the advantages of a cell-free system, they effectively regulate Th2 cytokines and restore immune balance. Future research should prioritize the scalable production and bioengineering optimization of MSC-Exos to enhance their stability, targeting efficiency, and bioactivity. Advances in exosome surface modification, such as ligand conjugation or genetic engineering of parent MSCs, may further improve selective delivery to inflamed airway tissues. Additionally, large-scale clinical trials are necessary to confirm therapeutic efficacy, establish optimal administration routes (e.g., intranasal vs.

systemic), and ensure long-term safety. Beyond allergic airway diseases, the versatility of MSC-Exos may extend to other inflammatory and fibrotic lung conditions, including chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. As understanding of exosome biology deepens and bioengineering techniques advance, MSC-Exos are poised to become a cornerstone of next-generation immunomodulatory and regenerative therapies, bridging molecular biology, nanotechnology, and clinical immunology for transformative outcomes in respiratory medicine.

KEYWORDS: Mesenchymal stem cell, exosomes, allergic diseases, airway inflammation, immunomodulatory therapy

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