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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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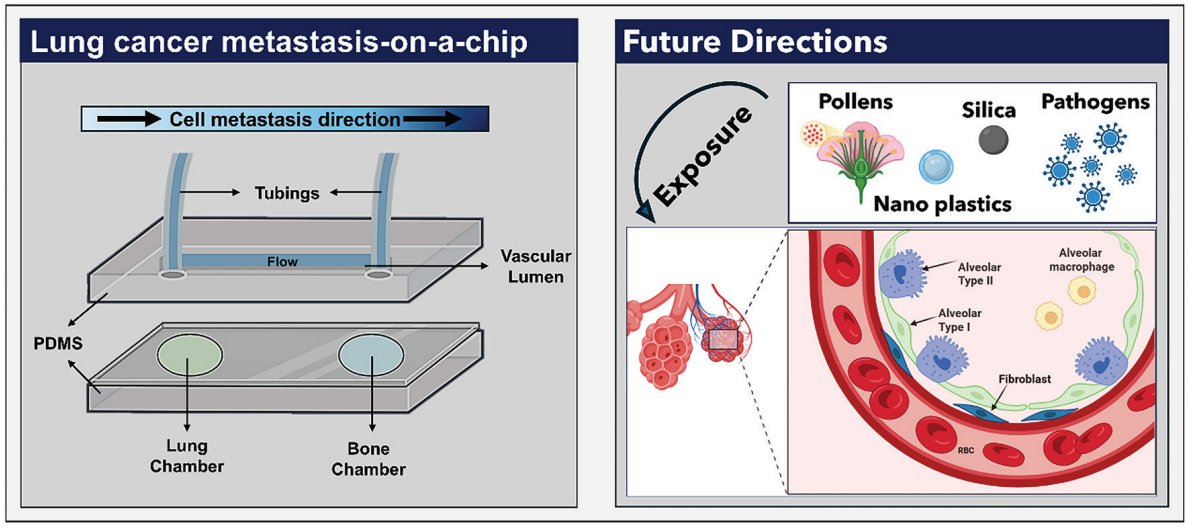


**Figure 1.** Lung cancer metastasis-on-a-chip design and demonstration as a potential tool to study the effects of exposome exposure on lung cancer progression.

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