



# Rethinking Chronic Lung Disease Through Three-Dimensional Alveolar Models

Janaeya Z. Baher<sup>1</sup>, Amir Seyfoori<sup>1,2</sup>, Emmanuel T. Osei<sup>1,3</sup>

<sup>1</sup>Department of Biology, University of British Columbia, Kelowna, BC, Canada, BC

<sup>2</sup>Apricell Biotechnology, Victoria, BC, Canada

<sup>3</sup>Centre for Heart Lung Innovation, St. Paul's Hospital, Vancouver, BC, Canada



## Introduction

- Chronic lung diseases, including **chronic obstructive pulmonary disease (COPD)** and **pulmonary fibrosis**, remain leading causes of morbidity and mortality worldwide.
- Traditional 2D cell culture systems fail to **accurately replicate** the complex three-dimensional structure and cellular interactions of the human alveolus.
- Animal models, while widely used, are limited by species-specific **anatomical and physiological differences**, reducing translational relevance to human disease.
- To address these limitations, this study developed **three-dimensional multicellular alveolar spheroid models** to better mimic the native lung microenvironment.

### OBJECTIVES

- Establish a healthy multicellular alveolar spheroid model.
- Develop a fibrotic disease-state model.
- Compare structural and viability changes between conditions.
- Investigate fibrin hydrogel embedding as a model extension to promote preliminary vascular-like organization.

## Fibrotic Disease

- The model incorporated **A549 epithelial cells, MRC-5 fibroblasts, and EA.hy926 endothelial cells** to recapitulate key cellular components of the alveolar microenvironment.
- Fibrotic conditions were induced using **TGF-β1**, while exploratory vascularization studies were performed using **fibrinogen-thrombin hydrogel embedding**.
- TGF-β1 is a key **profibrotic cytokine** implicated in chronic lung disease and pulmonary fibrosis.
- Elevated TGF-β1 signaling promotes fibroblast activation, myofibroblast differentiation, extracellular matrix deposition, and **increased collagen production**, contributing to alveolar remodeling and tissue stiffening.

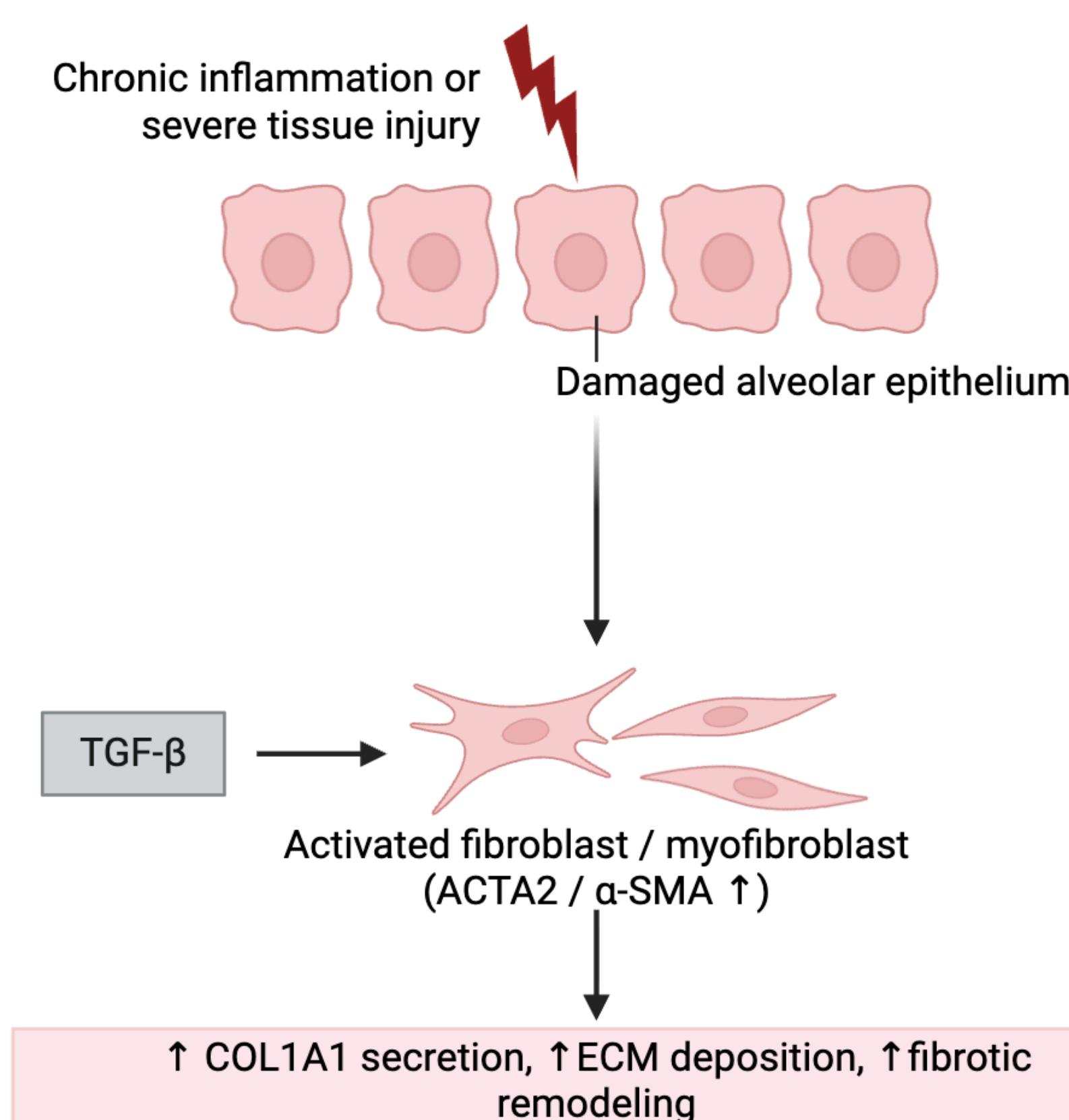


**Figure 1.** Schematic overview of TGF-β1-driven fibrotic remodeling in the alveolar model, illustrating epithelial injury, fibroblast activation, increased ACTA2/α-SMA expression, and enhanced COL1A1-mediated extracellular matrix deposition.

## Methods

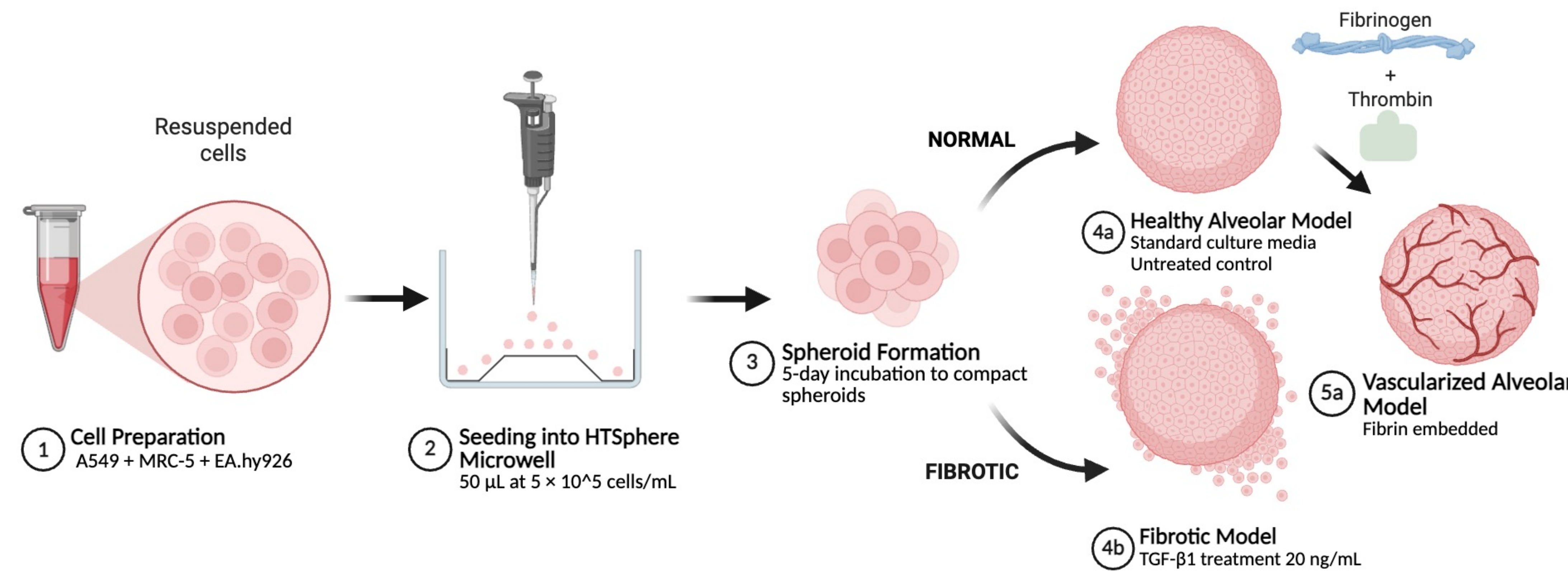


**Figure 2.** Experimental workflow for the establishment of healthy, fibrotic, and exploratory vascularized three-dimensional alveolar spheroid models. A549 epithelial cells, MRC-5 fibroblasts, and EA.hy926 endothelial cells were prepared and seeded into Apricell HTSpher microwell inserts at 50 μL of  $5 \times 10^5$  cells/mL, followed by 5 days of spheroid formation. Mature spheroids were then maintained as healthy controls, treated with TGF-β1 (20 ng/mL) to induce fibrotic remodeling or embedded within a fibrinogen–thrombin hydrogel to explore preliminary vascular-like outgrowth and model extension.

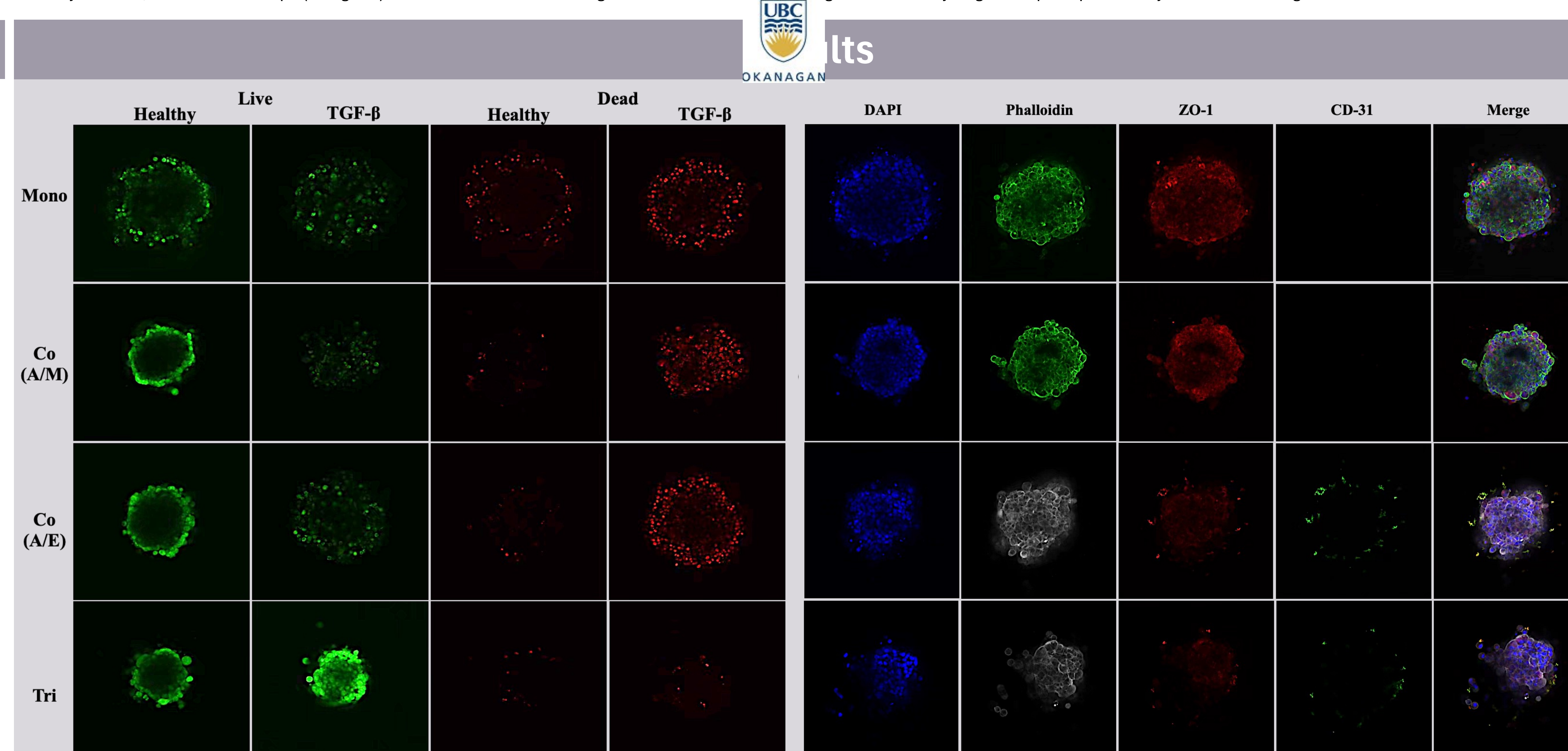


**Figure 3.** Representative live/dead characterization and quantitative assessment of healthy and fibrotic alveolar spheroid models. Live/dead fluorescence imaging compares mono-, co-, and tri-culture spheroids under healthy and TGF-β1-treated fibrotic conditions, with Calcein AM indicating viable cells (green) and Ethidium Homodimer indicating non-viable cells (red).

**Figure 4.** Representative immunofluorescence images of healthy alveolar spheroid models. Healthy mono-, co-, and tri-culture spheroids were stained with DAPI, phalloidin, ZO-1, and CD31 to assess nuclear distribution, cytoskeletal organization, epithelial junction integrity, and endothelial cell presence, respectively.

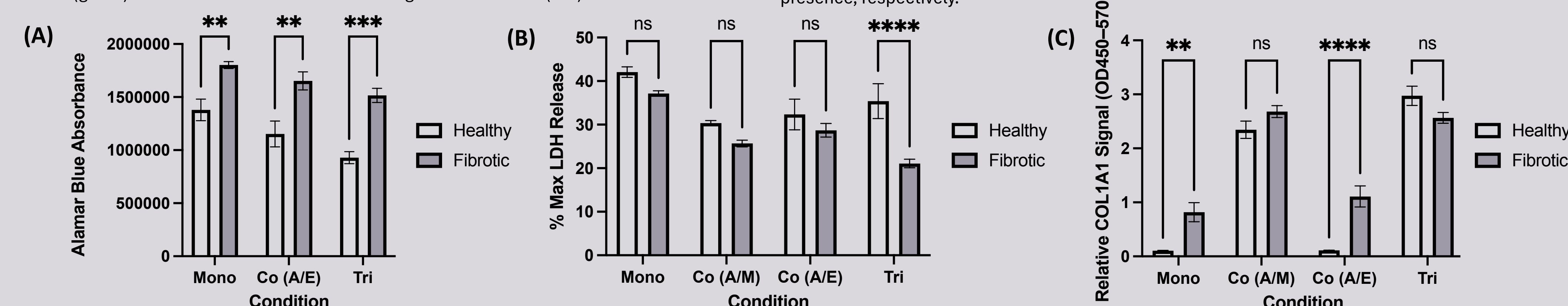


**Figure 5.** Quantitative assessment of healthy and fibrotic alveolar spheroid models. (A) Alamar Blue absorbance, representing metabolic activity across mono-, co-, and tri-culture spheroid conditions under healthy and TGF-β1-treated fibrotic states. (B) Percent maximum LDH release, representing relative cytotoxicity across culture conditions. (C) Relative COL1A1 signal (OD450–570), representing collagen output and fibrotic remodeling between healthy and fibrotic models. Data are presented as mean ± SEM, with statistical significance indicated where applicable.

## Model Extension

- Healthy alveolar spheroids were **embedded** in a fibrin hydrogel to assess **vascular-like organization** over 14 days.

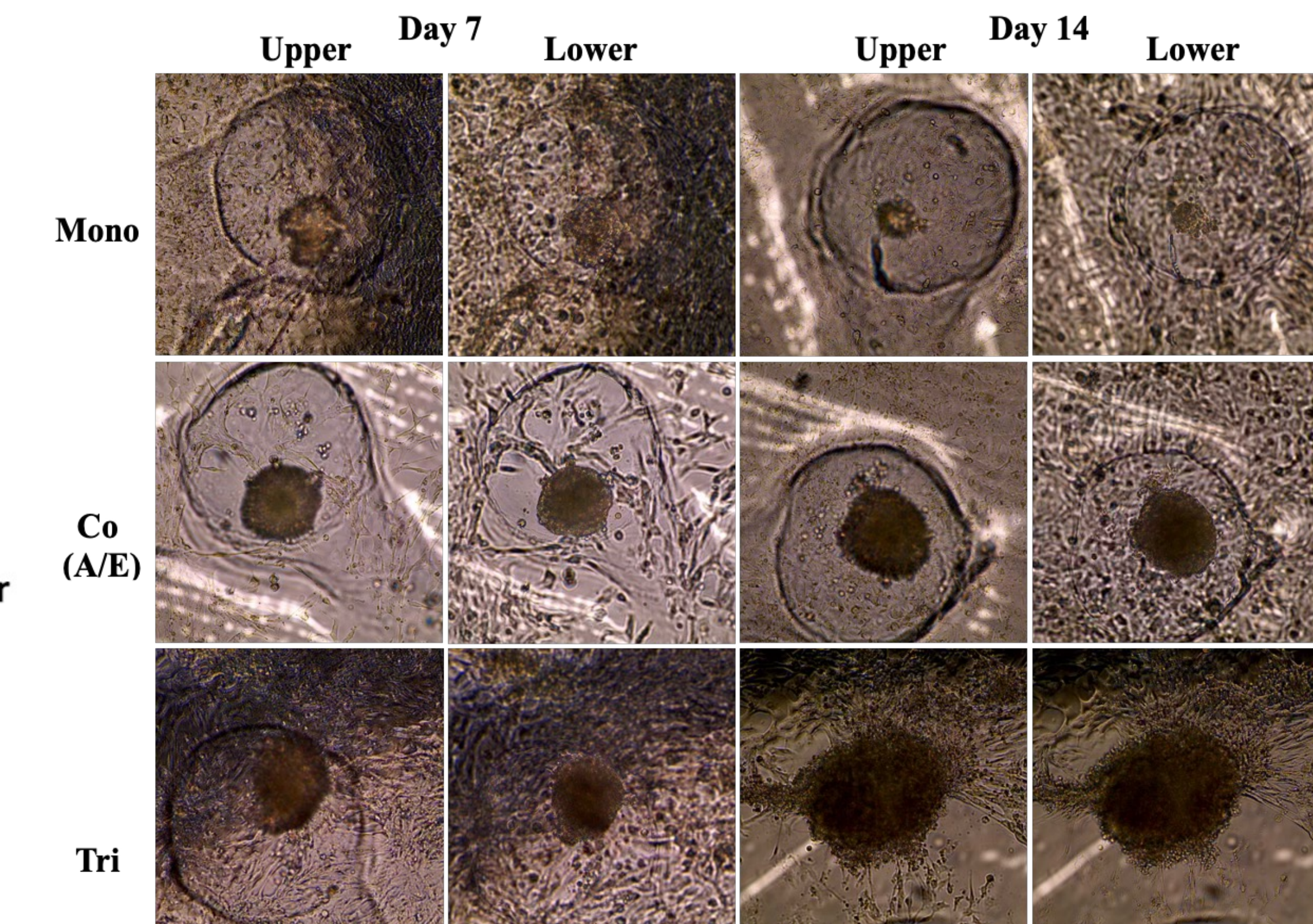


**Figure 6.** Representative brightfield images showing endothelial integration and vascular-like outgrowth in fibrin-embedded alveolar spheroid models over 14 days. Images from the upper and lower focal planes are shown at Days 7 and 14 to capture spatial organization.

## Concluding Remarks

- Collectively, these findings suggest that the inclusion of additional cell types, particularly within the **tri-culture model**, provides a **protective effect** against injury-induced damage, likely through enhanced cell–cell communication and microenvironmental support.
- Preliminary fibrin-based vascularization further supports the future development of a **more physiologically relevant** in vitro lung model for mechanistic and therapeutic studies.
- Current chronic lung disease research often emphasizes **airway damage**; however, pathological changes in the alveoli are **equally important**.

### Take-Home Message

*Three-dimensional alveolar spheroid models, specifically tri-culture models, successfully recapitulate healthy and fibrotic tissue states, highlighting the importance of studying alveolar remodeling alongside airway damage in chronic lung disease progression.*

- Future work will focus on **enhancing the physiological relevance** of this model through optimized vascularized fibrin extensions.
- Further validation of fibrotic and endothelial remodeling markers will support a **translational in vitro platform** for therapeutic screening of candidate antifibrotic treatments.

