Development of 3D Bioprinted *in Vitro* Distal Lung Cancer Models with Lung Fibrosis for Personalized Cancer Therapy

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Introduction

Lung cancer is the highest mortality rate worldwide, in order to increase the treatment efficiency of lung cancer, it is essential to understand the lung cancer microenvironment. Especially, lung cancer with fibrosis is known to have a poor prognosis. Pulmonary fibrosis mainly occurs in the distal region of the lung, and about 55% of lung cancers originate in the distal region. Therefore, there is a need for an *in vitro* distal lung cancer model that can recapitulate the microenvironment of lung cancer deriving from distal parts and investigate the progression and characteristics of lung cancer accompanied by fibrosis. Here, we developed distal lung tissue-derived decellularized extracellular matrix (LudECM) bioinks to mimic the specific microenvironment of the distal lung tissue and confirmed the thriving culture of patient-derived lung cancer organoids (LCOs). Our strategy for developing a distal lung cancer model proposes a tri-culture model of 3D bioprinting-based LCOs-iLFs-Endothelial cells (ECs).

Methods & Results

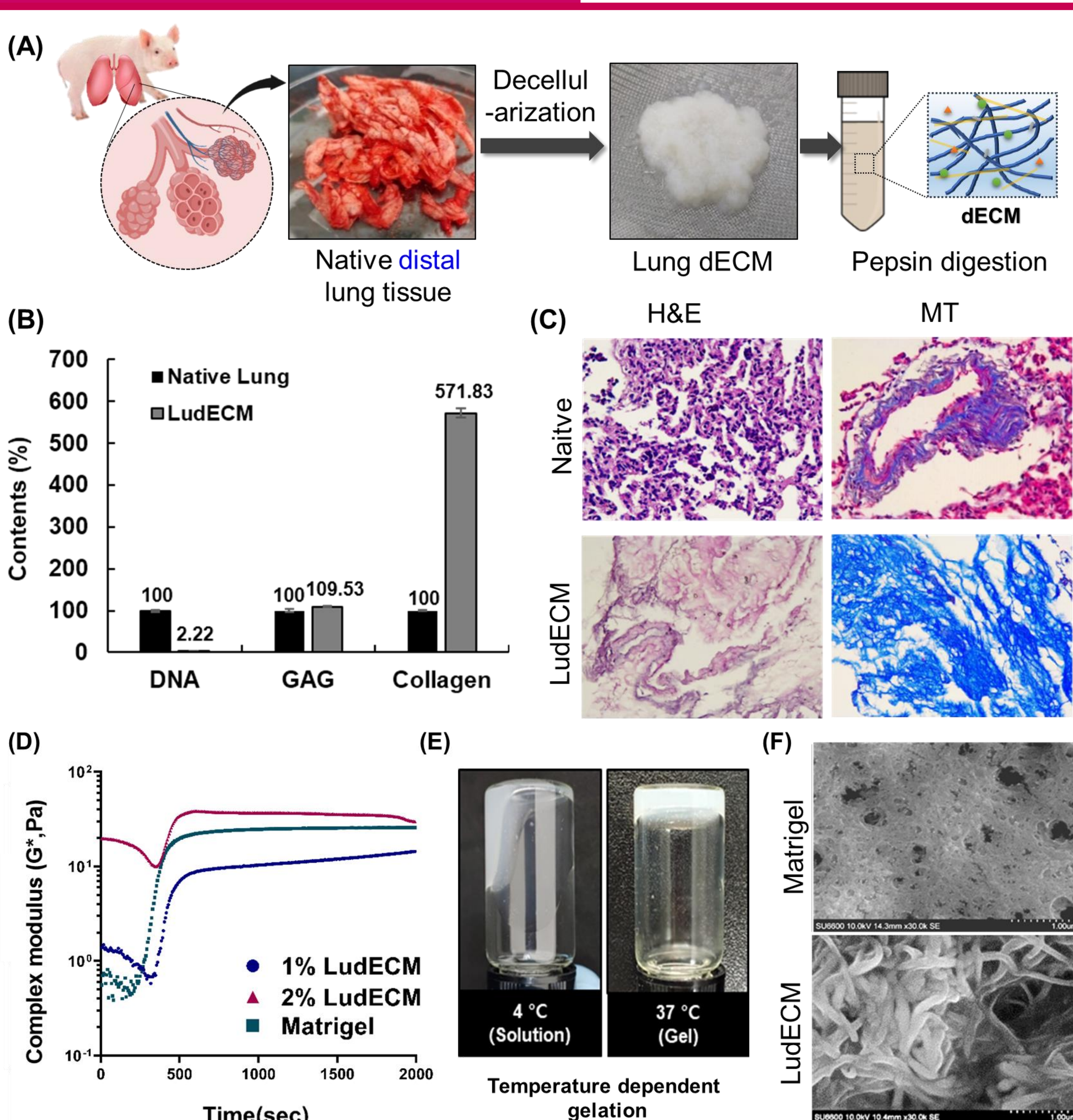

Figure. 1. Development of Distal Lung dECM Bioinks. (A) Establishment of the decellularization process of porcine distal lungs. (B)-(F) Characterization of distal lung dECM bioinks (LudECM); (B) Biochemical assay, (C) Histological analysis, (D) Rheological analysis, (E) Sol-gel transition, and (F) SEM image.

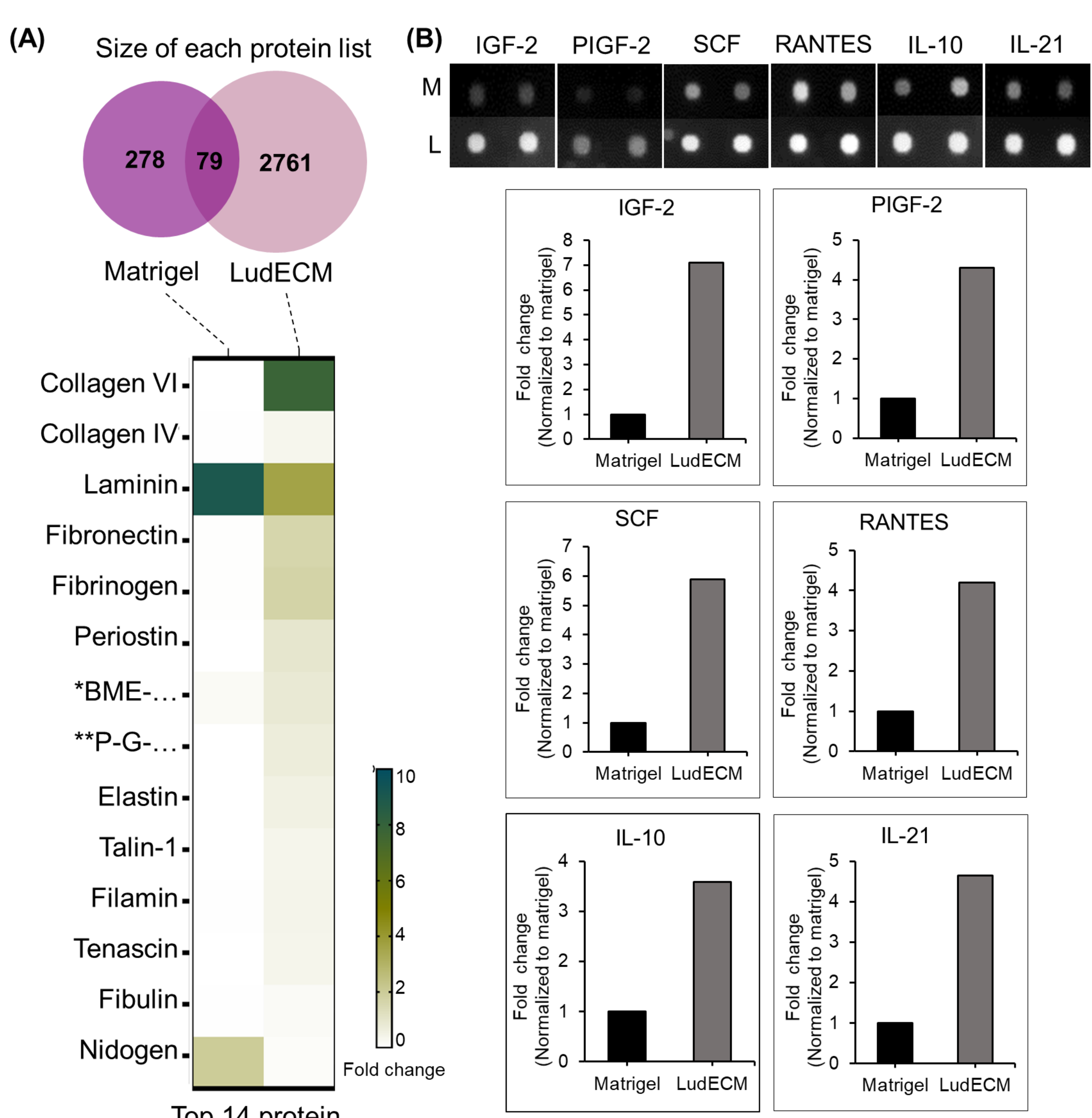

Figure. 2. Identification of protein contents(A) in LudECM using LC-MS/MS and residual cytokines(B) in LudECM.

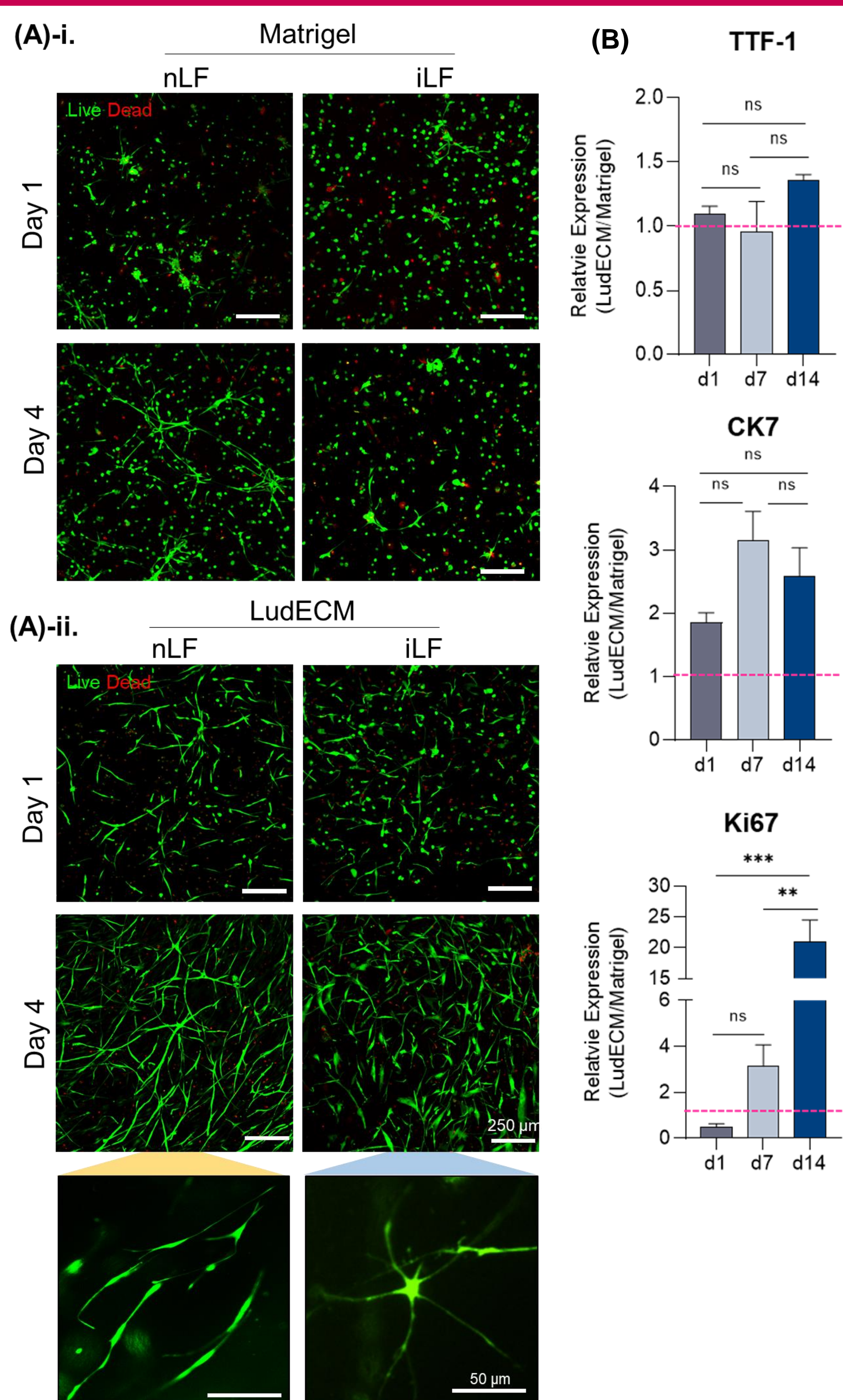

Figure. 3. Verification of the suitability of cell culture in LudECM (A) Viability test and morphology check of lung normal(A-i) and IPF-derived(A-ii) fibroblast in Matrigel and LudECM, respectively (scale bars, 250 μm or 50 μm) (B) Gene expression in LCOs cultured in Matrigel and the LudECM, respectively, normalized to each day of Matrigel expression(TTF-1 and CK7, lung cancer cell markers; Ki67, proliferation marker). Data represent mean ± S.D (n = 2; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant).

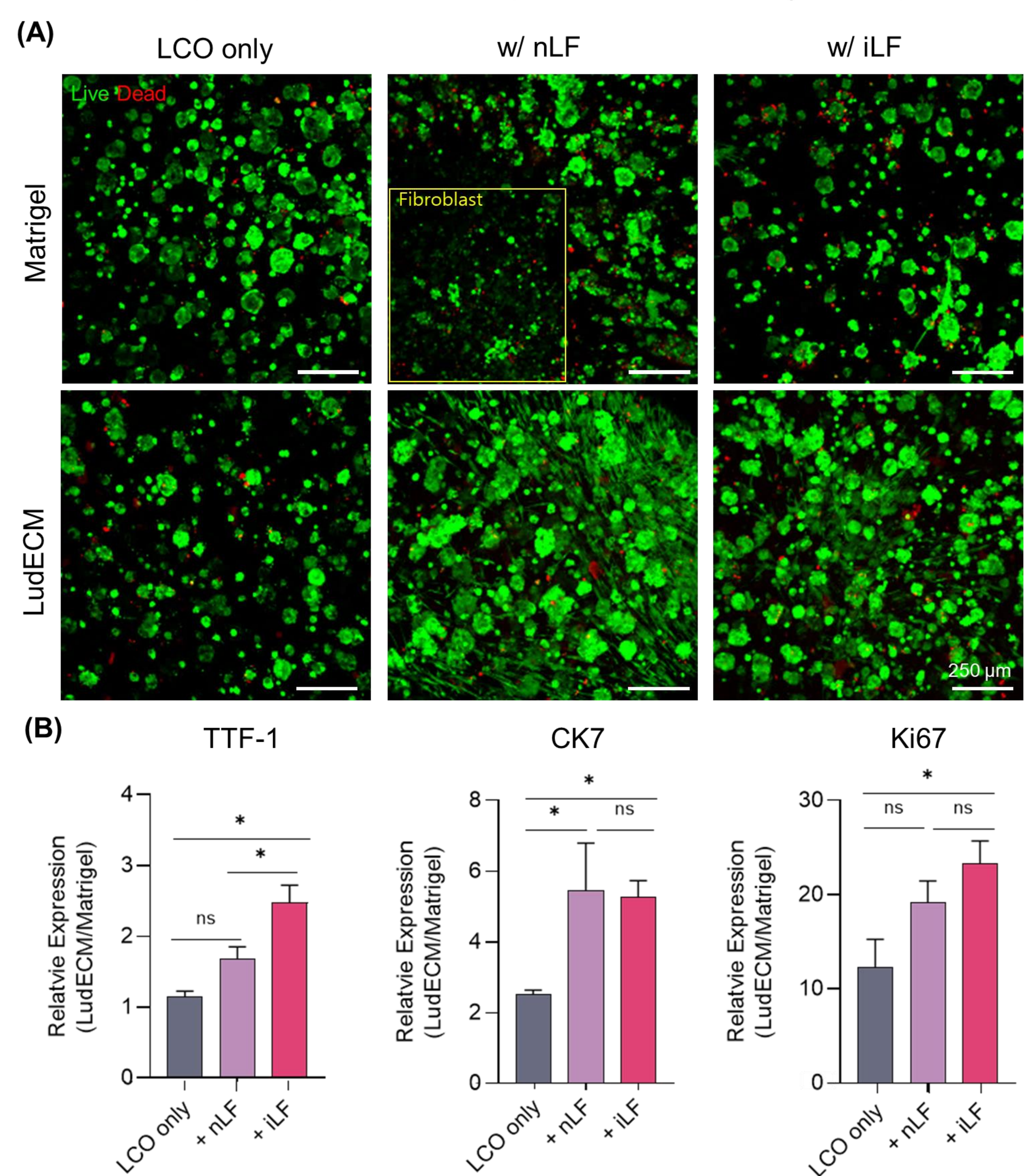

Figure. 4. Establishment of LCOs-lung normal and IPF-derived fibroblast co-culture condition in LudECM. (A) Live/dead assay was conducted at day 10, calcein-AM showed living cells (scale bars, 250 μm). (B) Gene expression of LCOs in monoculture or co-culture with 2 types of fibroblasts in Matrigel and LudECM respectively, normalized to Matrigel expression. Data represent mean ± S.D (n = 2; *, $p < 0.05$; ns, not significant).

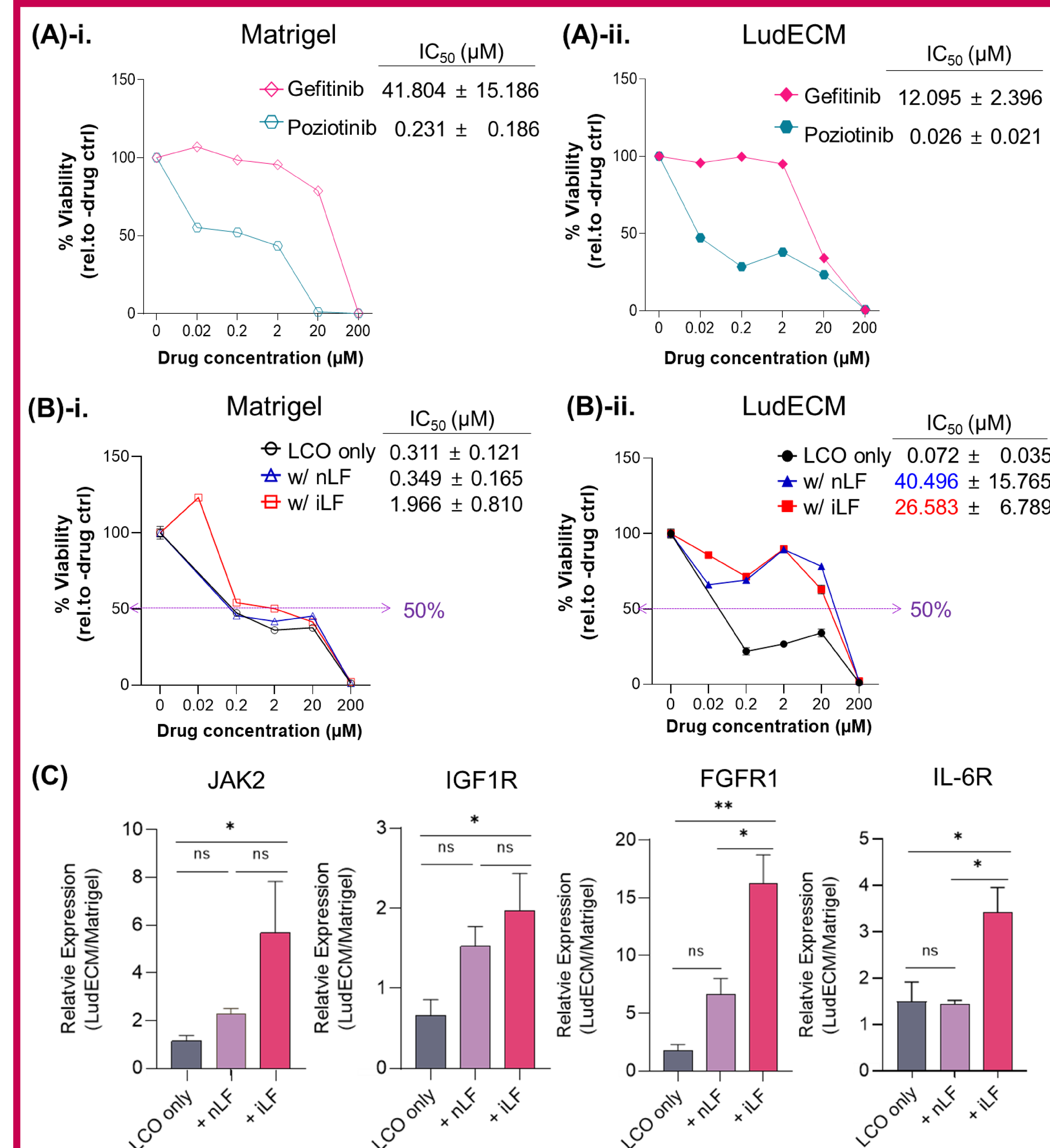

Figure. 5. Drug responsiveness in LCOs-Fibroblasts co-culture models. (A) Anticancer drug reactivity of monocultured LCOs and (B) co-cultured with 2 types of fibroblasts, 3 days of treatment with Gefitinib and Poziotinib, respectively. IC_{50} values are the average ± SD of each condition analyzed in triplicate. (C) Drug resistance-related gene expression levels of monocultured LCOs or co-cultured with two types of fibroblasts in Matrigel and LudECM. Data represent mean ± S.D (n = 2; *, $p < 0.05$; **, $p < 0.01$; ns, not significant).

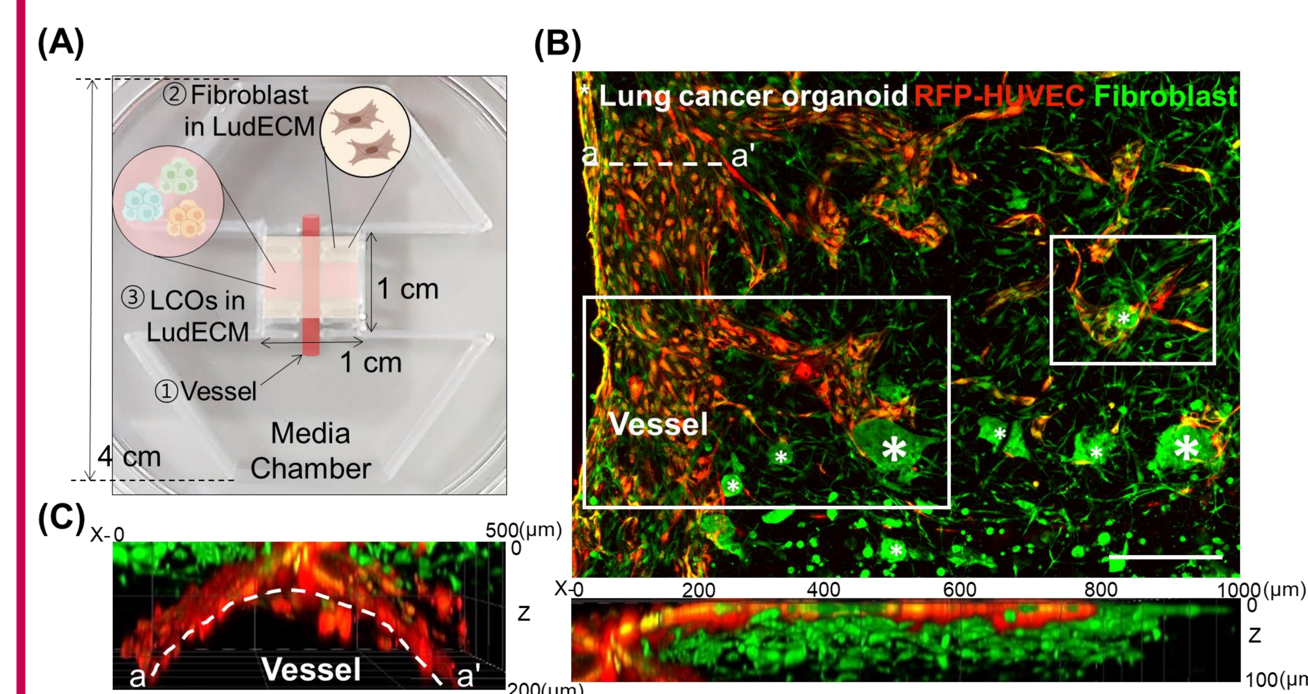

Figure. 6. 3D Bioprinted vascularized distal lung cancer models. (A) Actual printed image, (B) 3D bioprinted LCOs-iLFs-Ecs tri-culture model image, and (C) perfusable vessel cross-section image.

Conclusion

We developed distal lung tissue-derived decellularized extracellular matrix (LudECM) bioinks to mimic the specific microenvironment of the distal lung tissue and confirmed the thriving culture of patient-derived lung cancer organoids (LCOs). Interestingly, LCOs co-cultured with idiopathic pulmonary fibrosis-derived fibroblasts (iLFs) was confirmed to have increased resistance to sensitive anticancer drugs and enhanced gene expression related to drug resistance in LudECM. These results highlight the importance of cancer cell-matrix and cancer cell-stromal cell crosstalk for drug response. Furthermore, we successfully established a tri-culture lung cancer model of 3D bioprinting-based LCOs-iLFs-ECs. Our advanced printed cancer model could provide crucial information about the progression of lung cancer in a fibrous environment. We expect that this approach will be advantageous for modeling other solid cancers with fibrosis and ultimately beneficial for personalized medicine.

Acknowledgement

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