Supplementary Information for

Decellularized Lung Extracellular Matrix Scaffold Promotes Human Embryonic Stem Cell Differentiation towards Alveolar Progenitors

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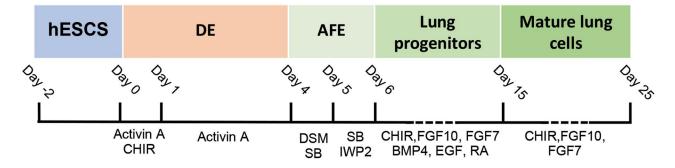


Fig.S1: The day-dependent addition of all the small molecules and growth factors used in the differentiation protocol. hESCs; Human embryonic stem cells, DE; Definitive endoderm, and AFE; Anterior foregut endoderm.

Received: 12/October/2022, Revised: 02/April/2023, Accepted: 16/April/2023 *Corresponding Addresses: P.O.Box: 1419963114, Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran P.O.Box: 16635-148, Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

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Royan Institute Cell Journal _(Yakhteh)

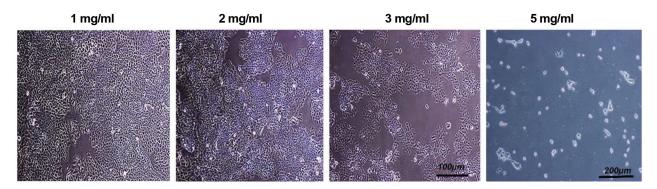


Fig.S2: Culture of A549 cell line on lung extracellular matrix (ECM)-derived hydrogel at concentrations of 1, 2, 3 and 5 mg/ml. Phase contrast images were taken after 6 days.

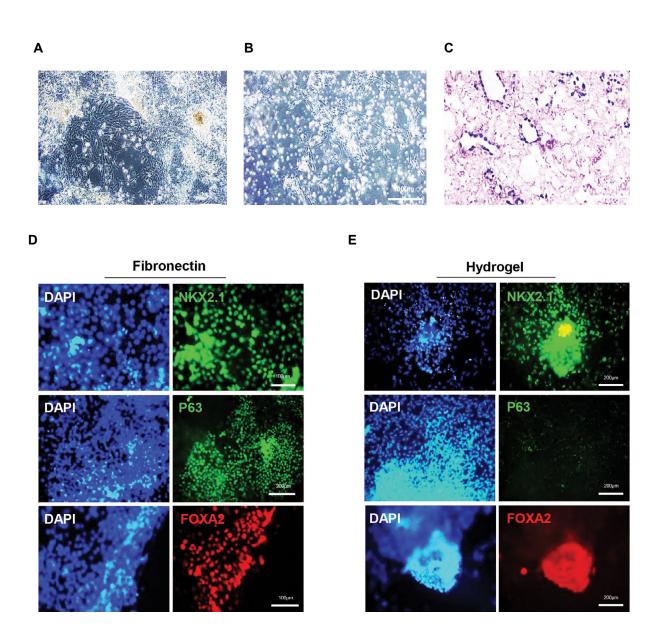


Fig.S3: Morphology and phenotypically characterization of human pluripotent stem cell (hPSC)-derived lung progenitor cells on fibronectin and hydrogel coating plates at day 15 of differentiation. Phase-contrast images of heterogeneous population of differentiated cells on **A**. Fibronectin and **B**. Hydrogel. **C**. H&E staining of scaffold after recellurization with endoderm cells at day 15 indicates that alveolar structures have been lined with epithelial cells. **D**. immunostaining of cells for NKX2-1, P63, and FOXA2 markers on fibronectin and **E**. On ECM lung hydrogel.

| TaqMan primers | Primer sequence (5'-3') |
|----------------|-----------------------------|
| GAPDH | F: GACAACAGCCTCAAGATCATCAG |
| | R: ATGGCATGGACTGTGGTCATGAG |
| NKX2 | F: GCATGAACATGAGCGGCAT |
| | R: CGACAGGTACTTCTGTTGCTTG |
| SOX2 | F: CCCAGCAGACTTCACATGT |
| | R: CCTCCCATTTCCCTCGTTTT |
| MUC5A | F: ATTGCTATTATGCCCTGTGTA |
| | R: TGGTGGACGGACAGTCACT |
| FOXJI | F: GCATAAGCGCAAACAGCCG |
| | R: TCGAAGATGGCCTCCCAGT |
| SOX9 | F: GAGGAAGTCGGTGAAGAACG |
| | R: GTTTTGGGGGGTGGTGGGT |
| SFTPC | F: CCTTCTTATCGTGGTGGTGGTGGT |
| | R: TCTCCGTGTGTTTCTGGCTCATGT |