Chetprayoon et al.:

Multilayered Fibroblasts Constructed by Accelerated Cellular Self-Assembly and Applications for Regenerative Medicine

Supplementary Data

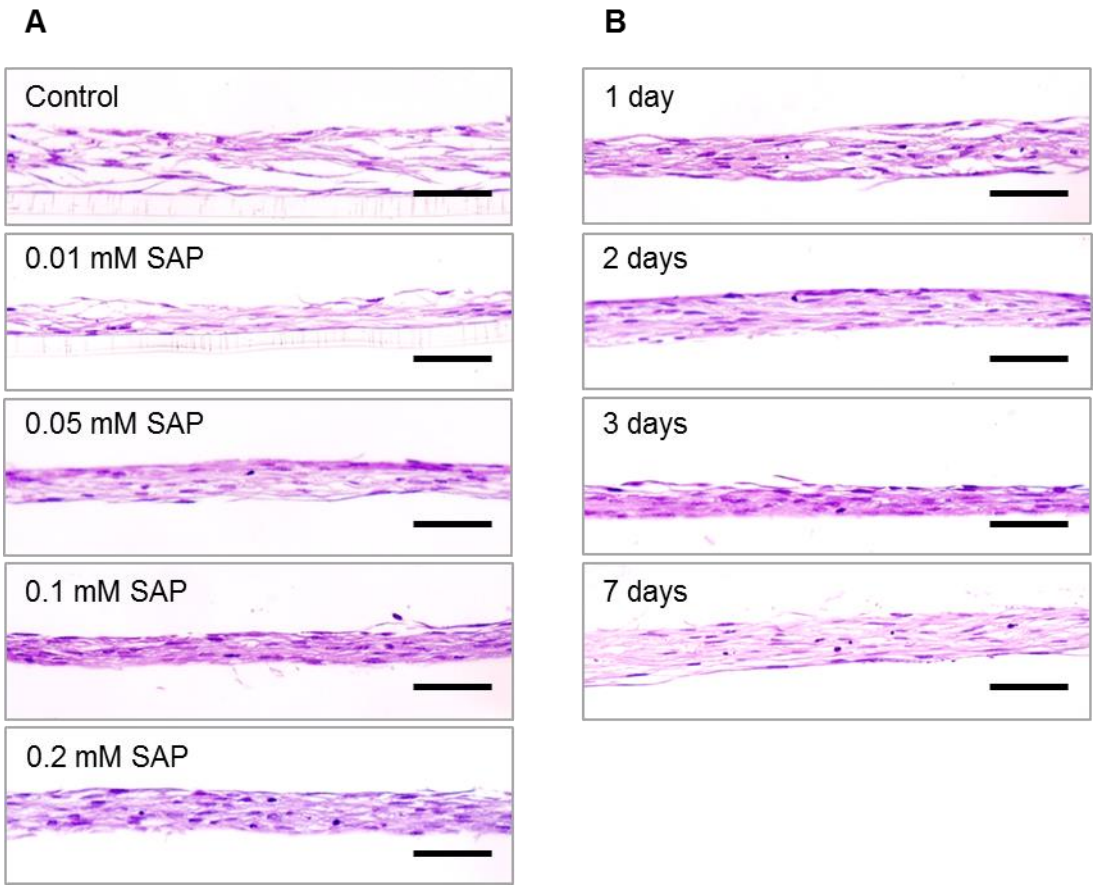

Fig. S1: Effects of culture conditions on the 3D-structure of 3D-DFs
Cells were seeded at a 5L cell density. (A) DFs seeded in complete medium with SAP at 0-0.2 mM for 3 days. (B) DFs cultured in medium containing 0.1 mM SAP for 1-7 days. Scale bars are 50 μm.

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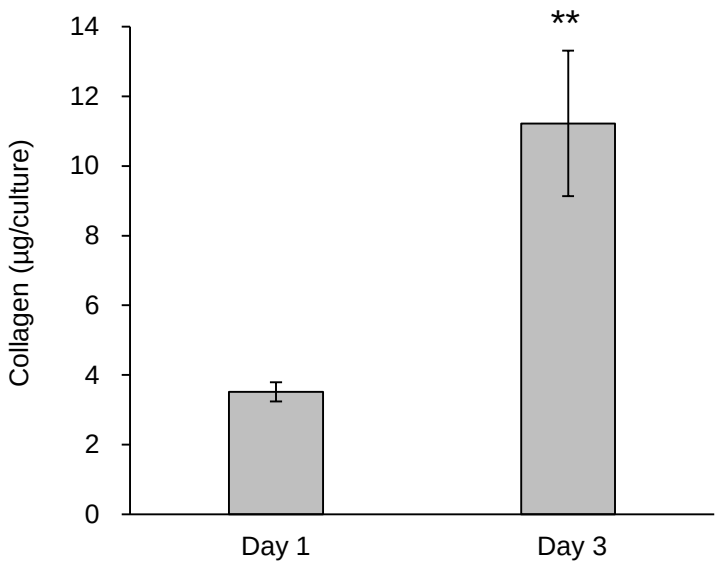

Fig. S2: Increasing amount of collagen in 3D-DFs (5L) during culture
Collagen content of 3D-DFs were cultured in medium containing 0.1 mM SAP for 1 day and 3 days as determined by hydroxyproline assay. **, $p < 0.01$ (*t*-test).

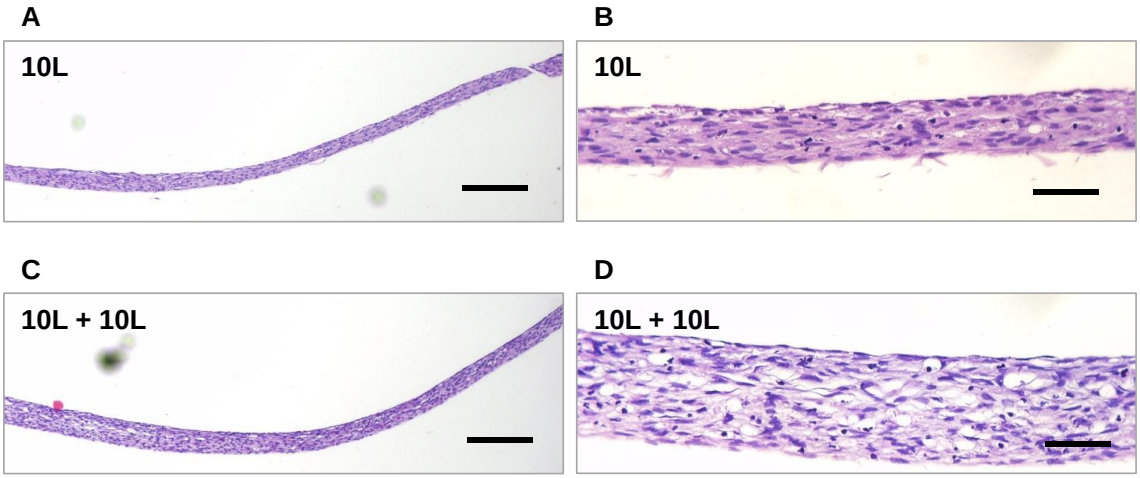

Fig. S3: Construction of 3D-DFs with a thickness greater than 10L by repeated cell seeding
H&E sections of (A, B) 10L-DFs constructed by seeding DFs at a density of 2 x 10^6 cells/cm^2 and culturing in medium containing 0.1 mM SAP for 5 days. (C, D) Representative 20L-DFs constructed by seeding 2 x 10^6 cells/cm^2 of DFs and culturing in medium containing 0.1 mM SAP. After 3 days of culture, DFs with a cell density of 2 x 10^6 cells/cm^2 were reseeded onto the construct and cultured for an additional 2 days (total of 5 days). Scale bars are 200 µm (A and C) and 50 µm (B and D).

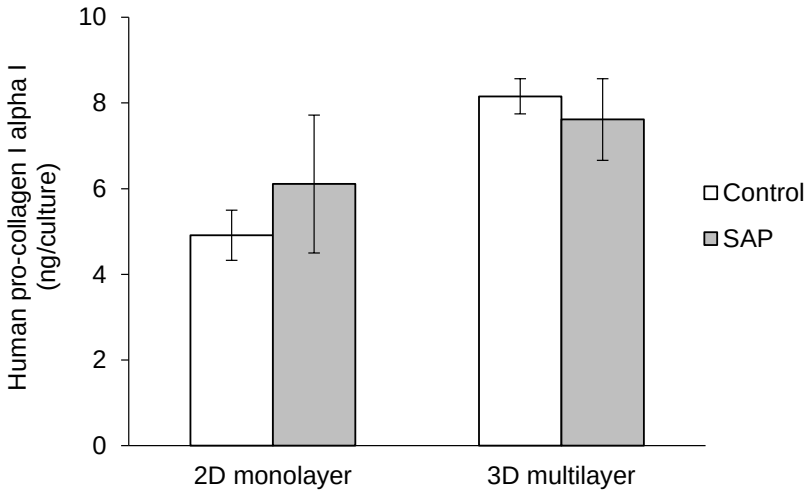

Fig. S4: Analysis of type I collagen in the culture medium
A 2D monolayer and 3D multilayer of DFs were constructed by seeding 3.35 x 10^5 cells to transwells (0.33 cm^2) and a 12-well plate (3.8 cm^2), respectively, and culturing either in the presence or absence of 0.1 mM SAP for 3 days. Culture medium was collected after 3 days and type I collagen was quantified by ELISA.

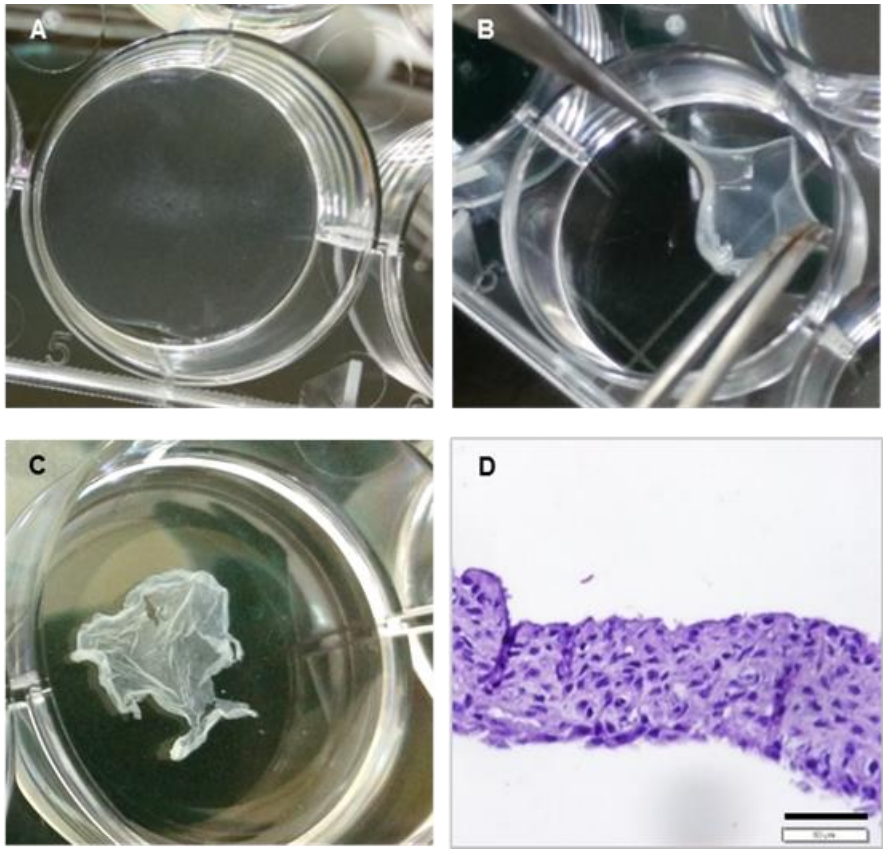

Fig. S5: Harvesting of the 3D-DF tissue sheet
The DFs were seeded onto a 6-well plate at a density of 8.5 x 10^5 cells/cm^2 (equivalent to 4.25L) and then cultured in medium containing 0.1 mM SAP for 3 days. (A) 3D-DF on the 6-well plate before harvesting. (B) Harvesting of the 3D-DF using forceps. (C) The contracted 3D-DF after harvesting. (D) Histological image of the contracted 3D-DF. Scale bar is 50 µm.

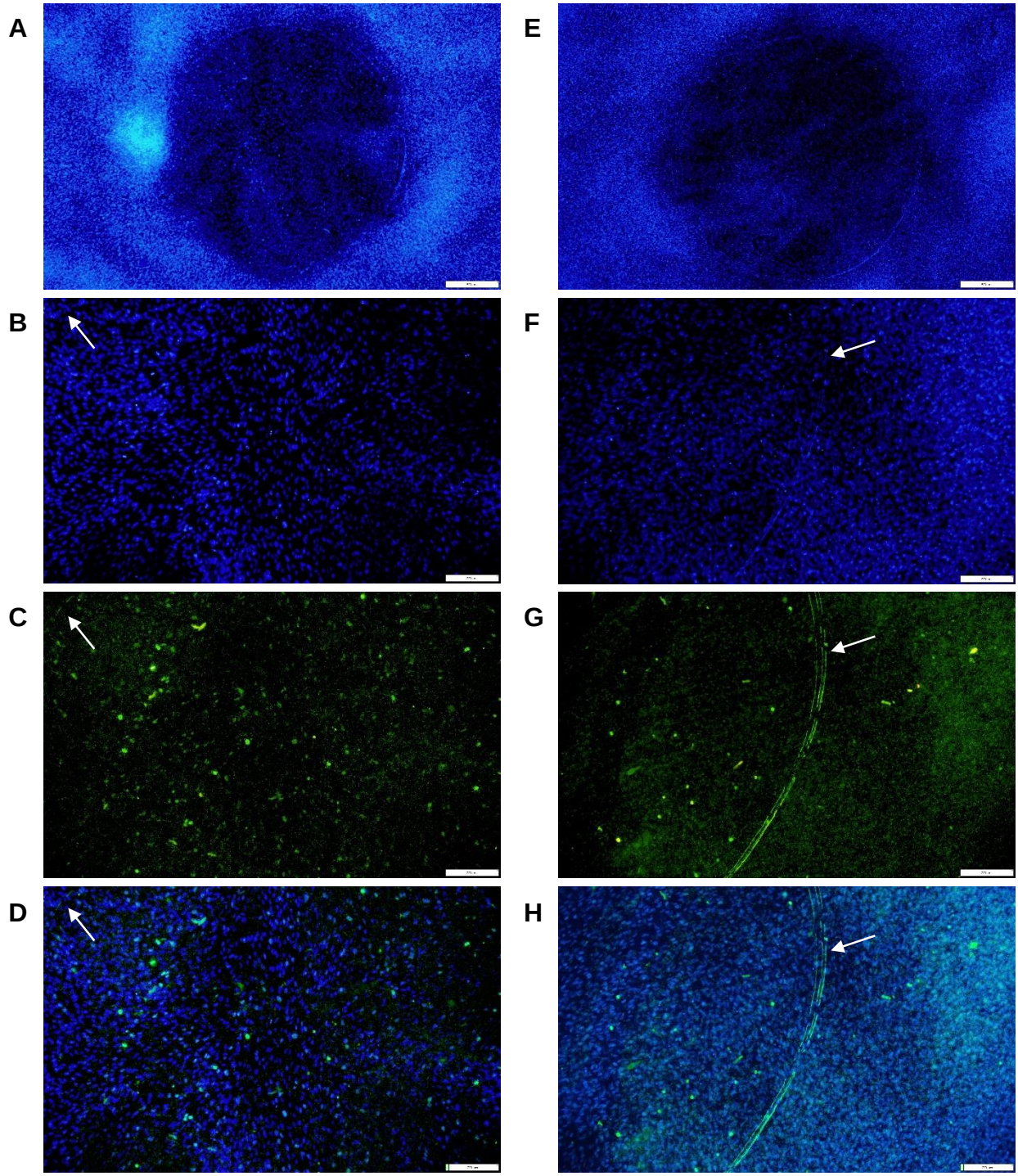

Fig. S6: Cell proliferation in the gap closure assays
5L-DFs were wounded with a 2 mm-diameter punch and the tissue in the punched area was removed by forceps. The punched tissues were cultured in complete culture medium without SAP and the medium was replaced with fresh medium three times a week. The wounded tissues were stained with Ki-67 (green), a proliferation marker, and nuclei were stained with DAPI (blue). Images shown are (A-D) 7 days after wounding and (E-H) 12 days after wounding. (A and E) Overall wounded area, showing only DAPI staining. (B-D and F-H) Images were taken close to the incision on the membrane (white arrow). Scale bars are 500 μm (A and E) and 200 μm (B-D and F-H).

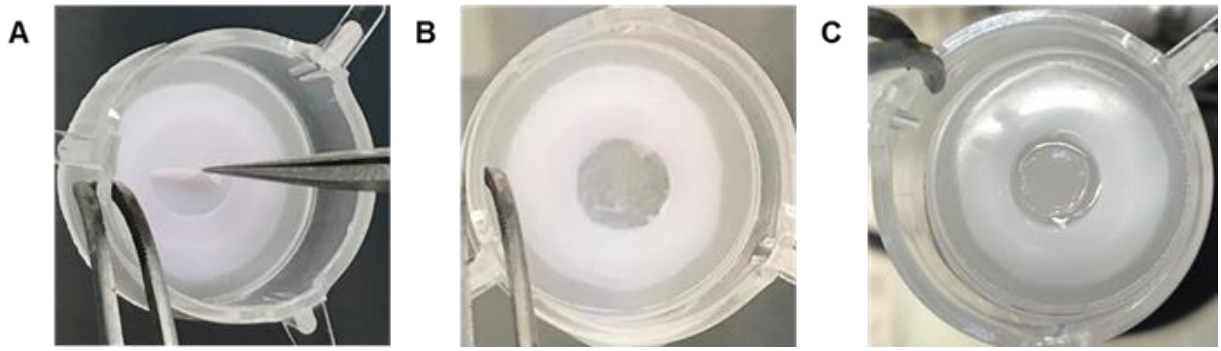

Fig. S7: Grafting of the 3D-DF on wounded FTMs
(A) The FTM after being punched; removal of the epidermis and the dermis. (B) The FTM after puncturing, where the dermis and the epidermis in the wounded area have been removed. Note that the membrane is ruffled due to removal of the collagen gel. (C) The wounded FTM after grafting with the 3D-DF.

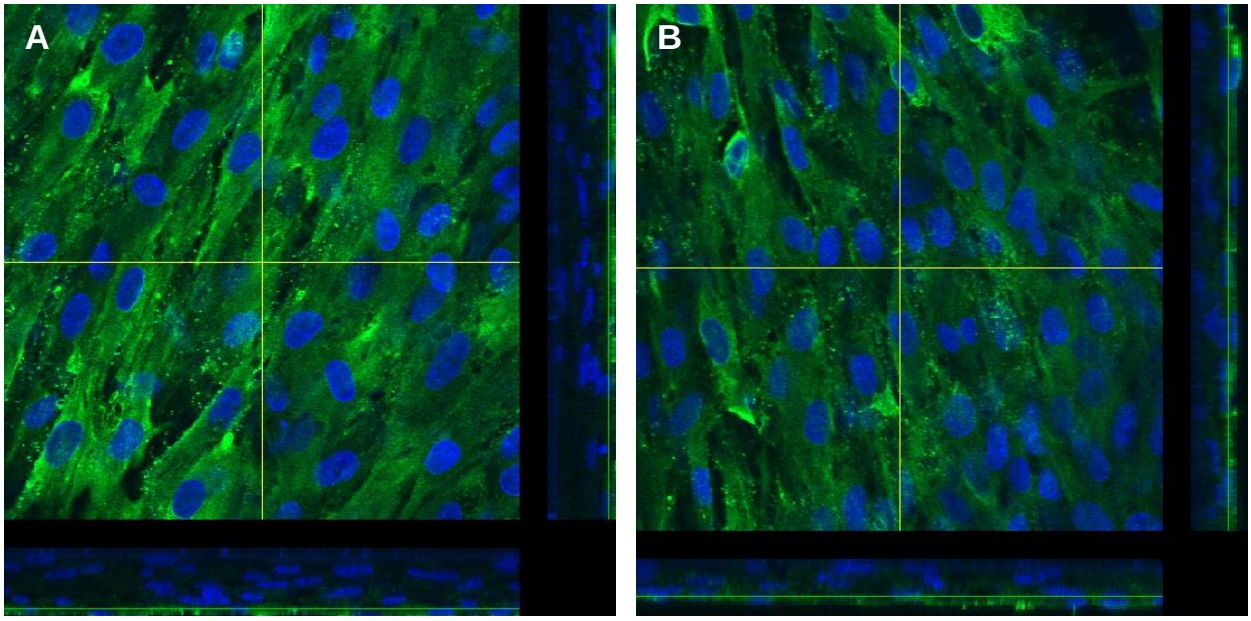

Fig. S8: Fibrillin-1 expression of 3D-DFs
5L-DFs were cultured in complete medium supplemented with 0.1 mM SAP. Anti-fibrillin-1 (green) staining of 5L-DFs after (A) 3 days and (B) 7 days. Nuclei are stained with DAPI (blue). Top layer of the tissue constructs is shown. Tissues were imaged with a confocal laser scanning microscope (FV10i, Olympus, Tokyo, Japan) in a Z-stack mode using same laser intensity. Note the reduction in fibrillin-1 expression on day 7. Scale bar is 50 µm.