Supporting information

Title

Synergistic effects of fibrin-enriched adipose decellularized extracellular matrix (AdECM) and microfluidic model on vascularization

Authors:

Yu-Yin Shih, [a] Chun-Wei Kao, [a] Yi-Rong Jhong, [a] Yi-An Chen [a] and Yi-Wen Chen [*a,b,c]

Affiliations:

a. Research & Development Center for *x*-Dimensional Extracellular Vesicles, China Medical University Hospital, Taichung, 404332, Taiwan

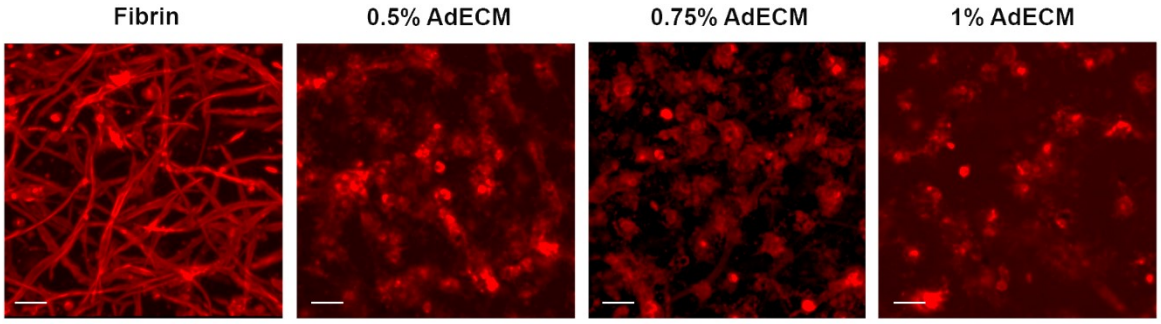
b. Department of Bioinformatics and Medical Engineering, Asia University, Taichung, 41354 Taiwan

c. Graduate Institute of Biomedical Sciences, China Medical University, Taichung, 406040 Taiwan

*Corresponding author: Yi-Wen Chen

E-mail address: evinchen@gmail.com

Fig. S1 Formation of microvasculature on different matrices. Fluorescence images of vasculature stained with F-actin after HUVECs and HDFs were cultured for 7 days. Self-assembled microvascular network on the matrices of fibrin and AdECM at concentrations of 0.5%, 0.75%, or 1%. Scale bar = 100 μm.



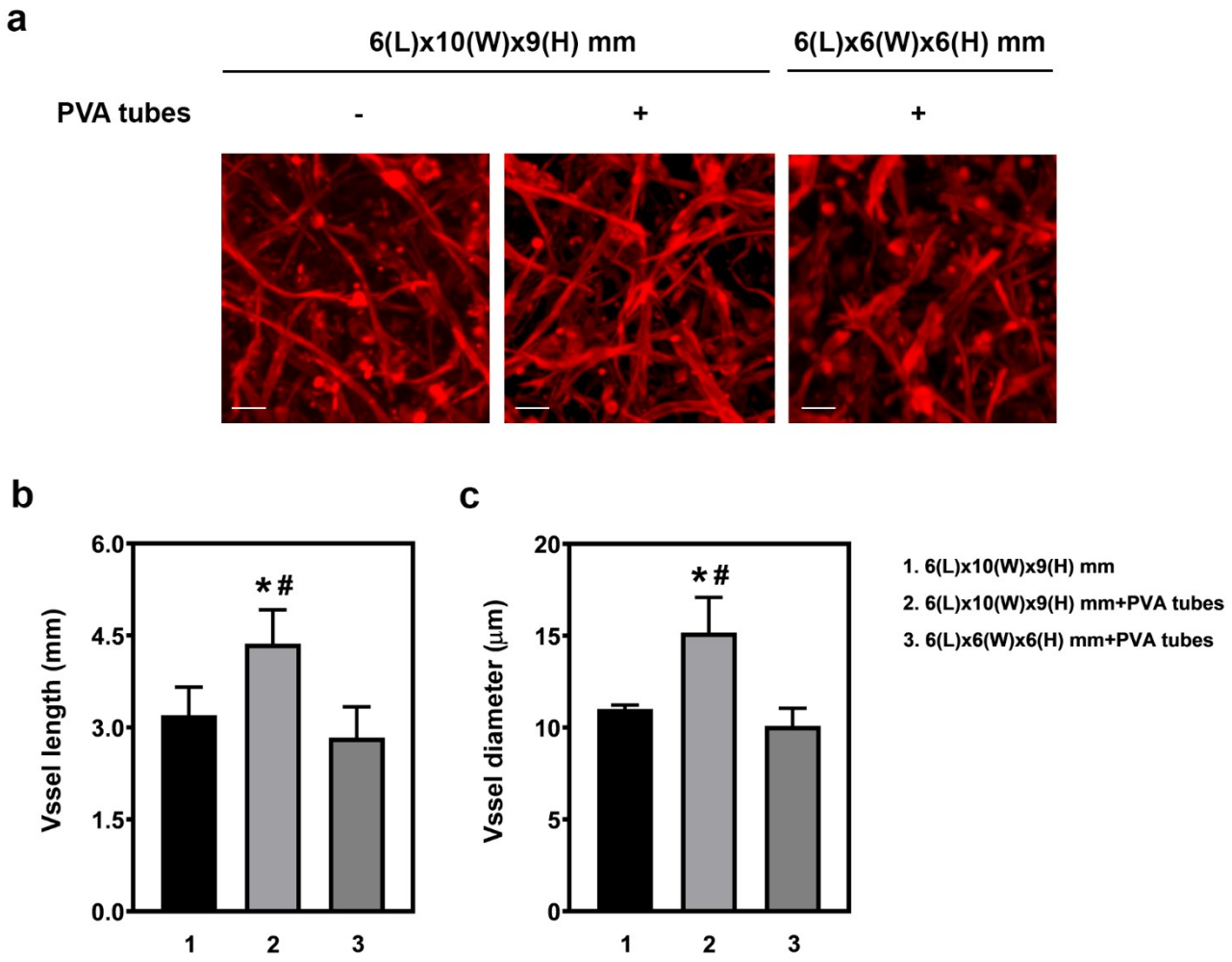


Fig. S2 Optimization of microfluidic device to improve the efficiency of media supply. Immunofluorescence staining of vasculature with anti-F-actin antibody: (a) After cultivating HUVECs and HDFs within a fibrin matrix containing 0.5% AdECM for 5 days in a microfluidic device placed on a rocker, a self-assembled microvascular network formed. The device dimensions were either 6(L) x 10(W) x 9(H) mm or 6(L) x 6(W) x 6(H) mm. Experiments were conducted with and without PVA tubes. Scale bar = 100 μm. (b, c) Vessel length and diameter were measured using ImageJ software and analyzed using Student's *t*-test from three independent experiments. *$P<0.05$, compared to vessels cultured in the device sized 6(L) x 10(W) x 9(H) mm without PVA tubes; $^{\#}P<0.05$, compared to a device sized 6(L) x 6(W) x 6(H) mm.

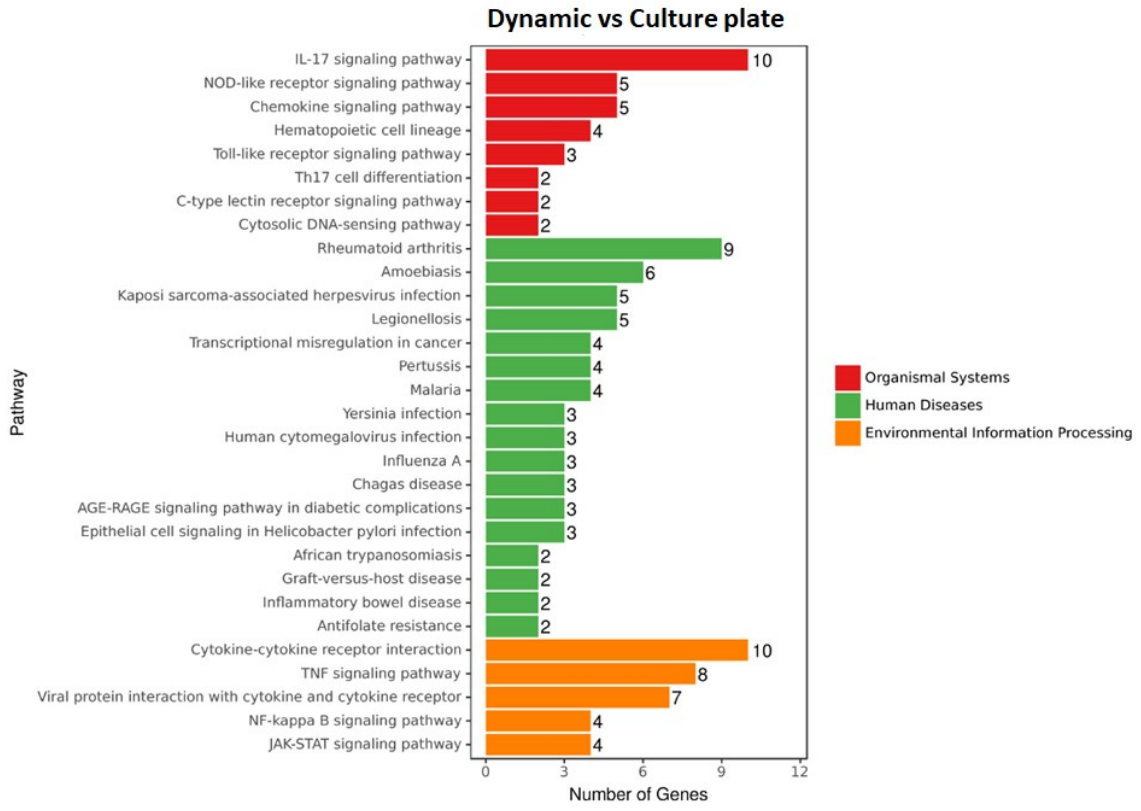


Fig. S3 Transcriptomic profile of vasculature formed within AdECM-containing gel in a culture plate or a microfluidic device (Dynamic) was analyzed by KEGG pathway.

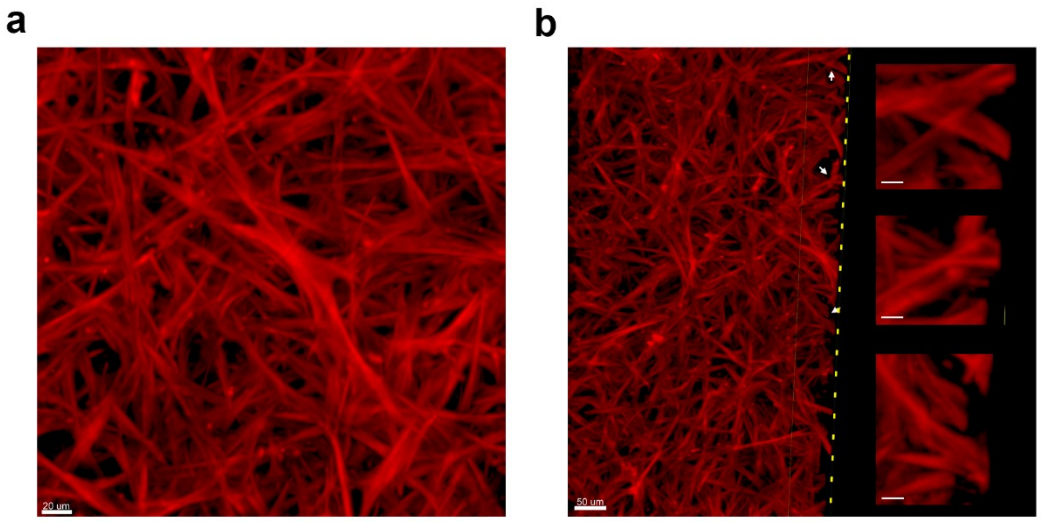


Fig. S4 Vasculature cultivated in a microfluidic system with an AdECM/Fibrin matrix displayed an intricate network and luminal structure. (a) HUVECs and HDFs were co-cultured in the AdECM/Fibrin matrix for 14 days, followed by immunofluorescence staining with an anti-F-actin antibody. Scale bar = 20 μm. (b) The 3D sectional view along the y-z plane of the vascular network. Scale bar = 50 μm. The yellow dashed line indicates the y-z cross-section area. The enlarged view shows the luminal structure of the vascular network as indicated by white arrows. Scale bar = 10 μm. 3D reconstructions of images and y-z cross-sections were conducted using Imaris software (Oxford).

Fig. S5 The movie displayed a 3D reconstructed image of vascular network.

Fig. S6 The movie showed the Y-Z cross-sectional images of the vasculature.

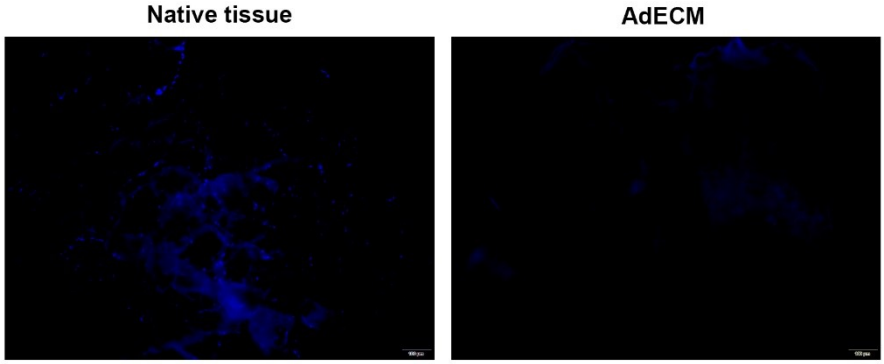


Fig. S7 DNA Content in Native Tissue and AdECM. Nuclei were stained with DAPI (blue) to visualize the presence of DNA. Scale bar = 100 μm.

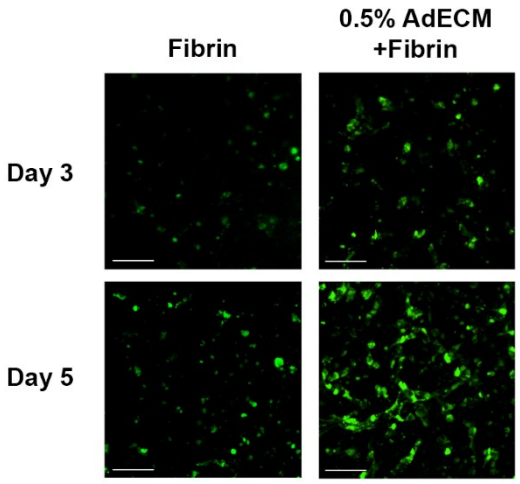


Fig. S8 VEGF content in fibrin and 0.5% AdECM/fibrin matrices. Expression of VEGF in matrix was detected by immunofluorescence staining with anti-VEGF antibody (green) after co-culturing of HUVECs and HDFs for 3 or 5 days. Scale bar = 100 μm.

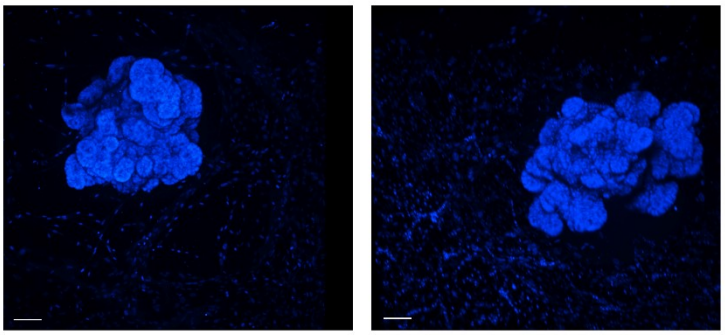


Fig. S9 The nuclei of vascularized colon tumoroids were visualized by DAPI staining. Scale bar = 100 μm.

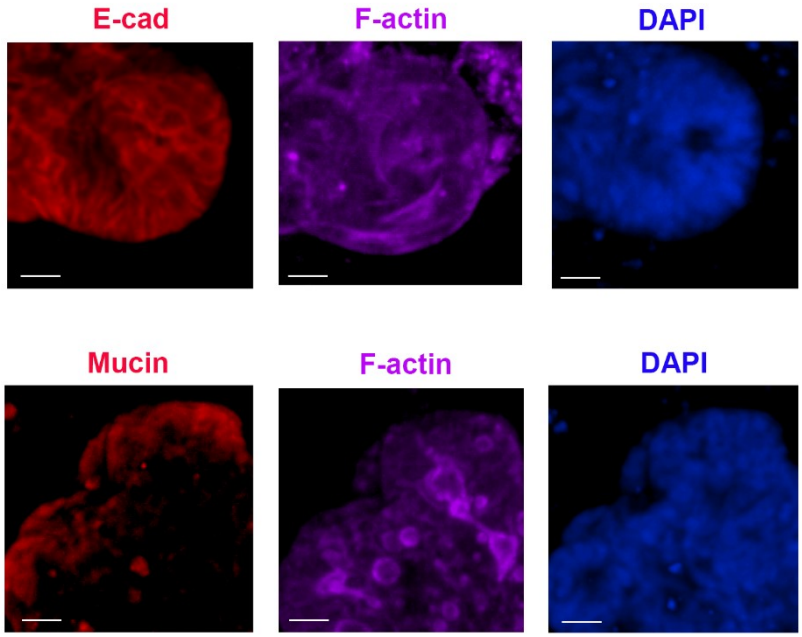


Fig. S10 Identification of human colon tumoroids. Immunofluorescence staining of human colon tumoroids with antibodies targeting for E-cadherin (E-Cad), a marker for enterocyte, and mucin, a marker for goblet cell. Scale bar = 25 μm.