A 3D Tissue Model-On-A-Chip for Studying the Effects of Human Senescent Fibroblasts on Blood Vessels

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SUPPLEMENTARY INFORMATION

Table of Contents

1. Supplementary Table

Table S1. List of TaqMan probes for RT-qPCR

2. Supplementary Figures

Figure S1. Traction force microscopy of young and senescent skin fibroblasts

Figure S2. Co-culture of microvessels with young and senescent skin fibroblasts

Figure S3. Quantification of collagen fibers in co-cultured microvessel models

Figure S4. Visualization of collagen fibers in microvessel co-cultured models

Figure S5. Immunofluorescence analysis of microvessels cultured with conditioned media

Figure S6. Microvessels cultured with conditioned media for six days

1. Supplementary Table

Gene	Assay ID	Fluorophore
hB2M	Human B2M (beta-2-microglobulin) Endogenous Control	VIC/MGB
hDLL4	Hs00184092_m1	FAM/MGB
hICAM-1	Hs00164932_m1	FAM/MGB
hIL6	Hs00174131_m1	FAM/MGB
hVCAM-1	Hs01003372_m1	FAM/MGB

Table S1. List of TaqMan probes for RT-qPCR

h: human

2. Supplementary Figures



Figure S1. Traction force microscopy of young and senescent skin fibroblasts. Analysis of traction stress caused by skin fibroblasts on a PAAm gel. (A) Quantification of the area showing a certain degree of traction stress per cell; areas were sorted and analyzed by traction stress's range of 100 Pa, except for values superior to 500 Pa; five and six cells were analyzed for young and senescent fibroblast, respectively; error bars: standard deviations. (B) Traction force's vectors map of a young and a senescent fibroblast.



Figure S2. Co-culture of microvessels with young and senescent skin fibroblasts. Maximum intensity projection views by epifluorescent microscopy of non-fixed microvessels visualized by transient staining with rhodamine-conjugated UEA1. This panel shows the most severe phenotypes, which were observed.



Figure S3. Quantification of collagen fibers in co-cultured microvessel models. (A) Schematics of the image processing methods used to quantify the collagen fiber density (upper panel) and cluster size (lower panel) from Masson's trichrome analysis (cf. M and M for details). (B) Comparison of collagen fibers among various conditions; C: monoculture, Y: co-cultured with young fibroblasts, S/-: co-cultured with senescent fibroblasts, and S/+R: co-cultured with senescent fibroblasts and treated with rapamycin. Collagen fibers density is expressed as a percentage of ROI; ROIs around eight cells were analyzed; error bars: standard deviations; *: p < 0.05 as compared with Y condition by Dunnett's test.



scales: 20 µm

Figure S4. Visualization of collagen fibers in microvessel co-cultured models. Collagen fibers from microvessels co-cultured with young or senescent fibroblasts were visualized by confocal reflection microscopy. White arrows indicate collagen fibers' directions. The right panel depicts MIP images of 20-µm-thick sections of collagen gel showing the collagen fibers in the immediate vicinity of fibroblast cells. The white triangles indicate cell bodies.



scale: 20 µm

Figure S5. Immunofluorescence analysis of microvessels cultured with conditioned media CLSM images (MIP) of microvessels cultured with normal medium (EGM2) or medium containing soluble factors secreted by young (EGM2^{Young}) or senescent fibroblasts (EGM2^{Sen.}). Adherens junctions and cytoskeletons were visualized by VE-cadherin (VE-cad, red) and actin (green), respectively. Nuclei were visualized with Hoechst 33343 (blue).



scales: 200 µm

Figure S6: Microvessels cultured with conditioned media for six days. Phase-contrast images of microvessels cultured with normal medium (EGM2) or medium containing soluble factors secreted by young (EGM2^{Young}) or senescent fibroblasts (EGM2^{Sen.}) for six days. The panel shows representative samples among four microvessels.