

## Pre-selection of fibroblasts subsets prompt prevascularization of tissue engineered skin analogues

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## Supplementary information

**Supplementary Table 1** – Fluorochrome-labeled antibodies used for cell sorting

Antibody	Brand	Ref	Host
CD324 PerCP-Cy5.5	BD Biosciences, USA	563573	Ms
CD45 PE-Cy7	BD Biosciences, USA	557748	Ms
CD31 APC-Cy7	BD Biosciences, USA	563653	Ms
CD90 FITC	eBiosciences, USA	11-0909-42	Dk
CD39 PE	BD Biosciences, USA	555464	Ms
CD26 PE-CF594	BD Biosciences, USA	565158	Ms
CD36 APC	BD Biosciences, USA	550956	Dk

Legend: Dk – donkey, Ms – mouse

**Supplementary Table 2** – Fluorochrome-labeled antibodies used for flow cytometry

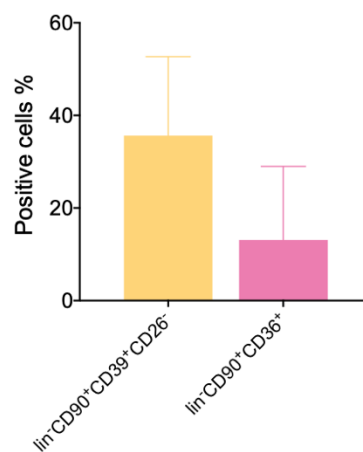
Antibody	Brand	Ref	Host
CD39 PE	BD Biosciences, USA	555464	Ms
CD26 PE-CF594	BD Biosciences, USA	565158	Ms
CD36 APC	BD Biosciences, USA	550956	Dk
PDNP PE	Biolegend, USA	337003	Rat
TGM2 FITC	Bioss, USA	bs-8589R-FITC	Rb

Legend: Dk – donkey, Ms – mouse, PDNP – podoplanin, Rb – rabbit, TGM2 – transglutaminase-2

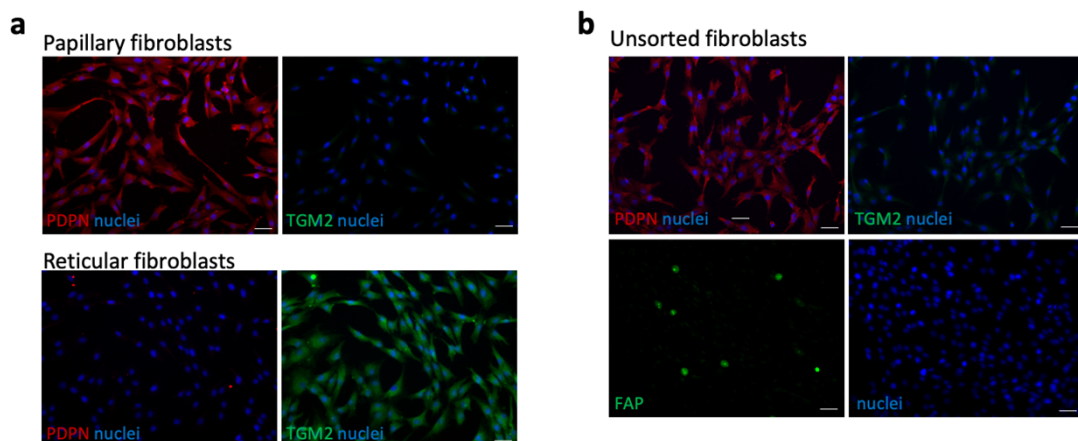
**Supplementary Table 3 – Immunolabelling antibodies**

	Antibody	Brand	Ref	Host	Dilution
Primary antibodies	Podoplanin	Abcam, UK	ab10288	Ms	1:250
	Transglutaminase-2	Abcam, UK	ab421	Rb	1:75
	FAP	Abcam, UK	ab53066	Rb	1:100
	CD31	Dako, Denmark	M0823	Ms	1:30
	Collagen type I	Abcam, UK	ab34710	Rb	1:100
	Fibronectin	Abcam, UK	ab2413	Rb	1:100
	Laminin	Abcam, UK	ab11575	Rb	1:30
	Keratin 14	Covance, USA	PRB-160P	Rb	1:800
Secondary antibodies	Alexa fluor 488 anti-rabbit	Invitrogen, USA	A-11008	Gt	1:500
	Alexa fluor 594 anti-mouse	Invitrogen, USA	A-11032	Gt	1:500
	Alexa fluor 594 anti-rabbit	Invitrogen, USA	A-11012	Gt	1:500

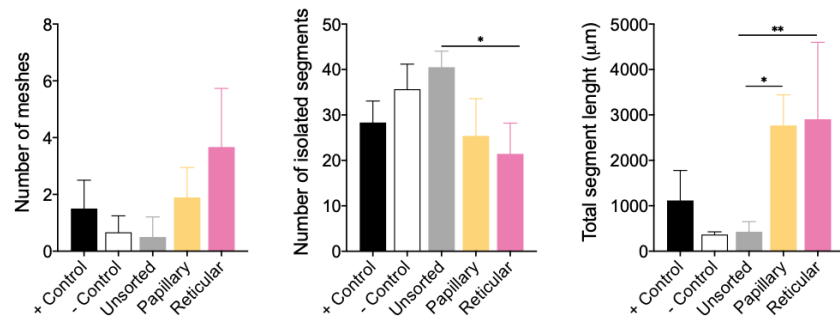
Legend: FAP – fibroblast activation protein, Gt – goat, Ms – mouse, Rb – rabbit



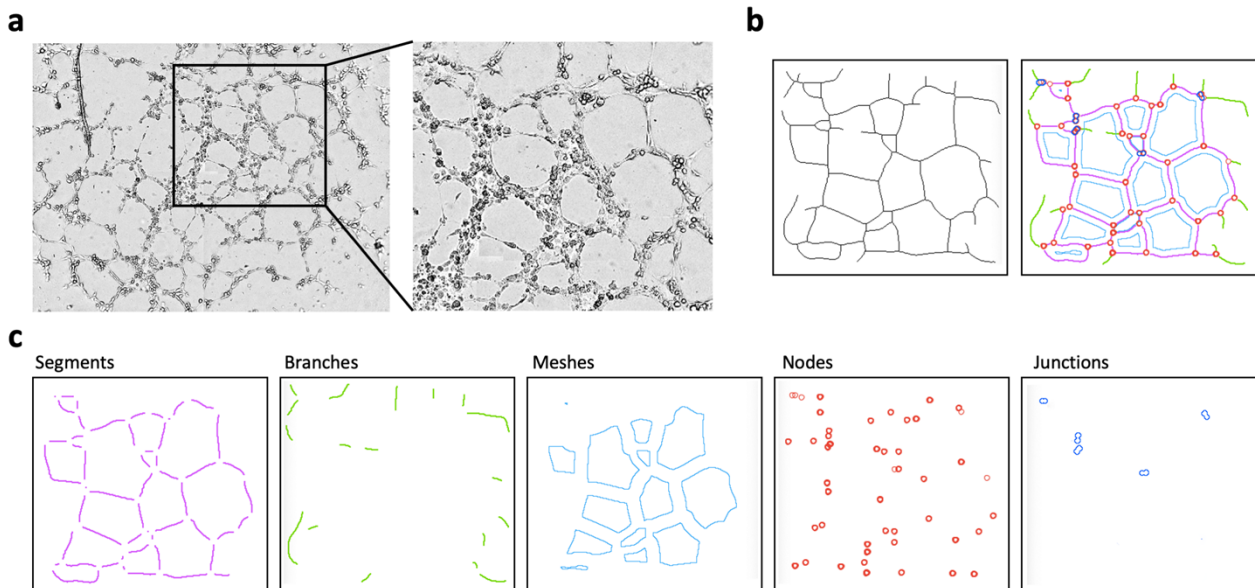
**Supplementary Figure 1 – Percentage of papillary and reticular fibroblasts present in the lin<sup>+</sup>CD90<sup>+</sup> fraction, N=6.**



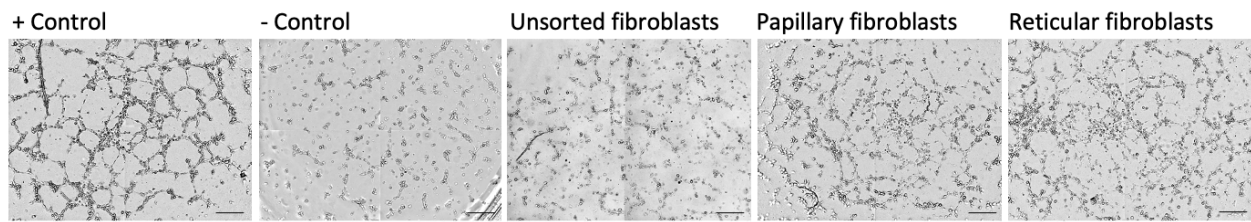
**Supplementary Figure 2 – Individual immunocytochemistry images of the expression of podoplanin (PDNP), transglutaminase-2 (TGM2) and fibroblast activated protein (FAP) by a papillary and reticular and b unsorted fibroblasts populations after isolation. Scale bar = 100  $\mu$ m. Nuclei were counterstained with DAPI.**



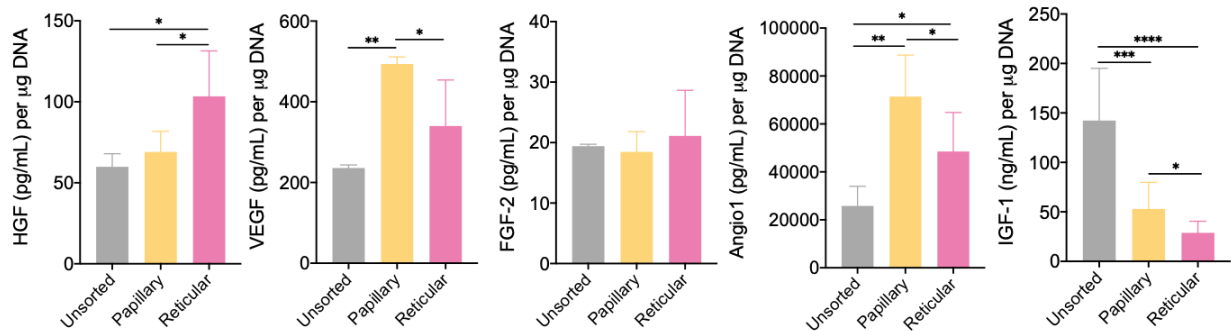
**Supplementary Figure 3** – Quantification of the number of meshes, isolated segments and total segment length of the tubular-like structures formed on Matrigel by hDMECs cultured with conditioned obtained from unsorted, papillary and reticular fibroblasts after 24 h of culture. Quantitative results are expressed as the mean  $\pm$  standard deviation where  $n = 3$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , one-way ANOVA with Tukey multiple comparison post-test.



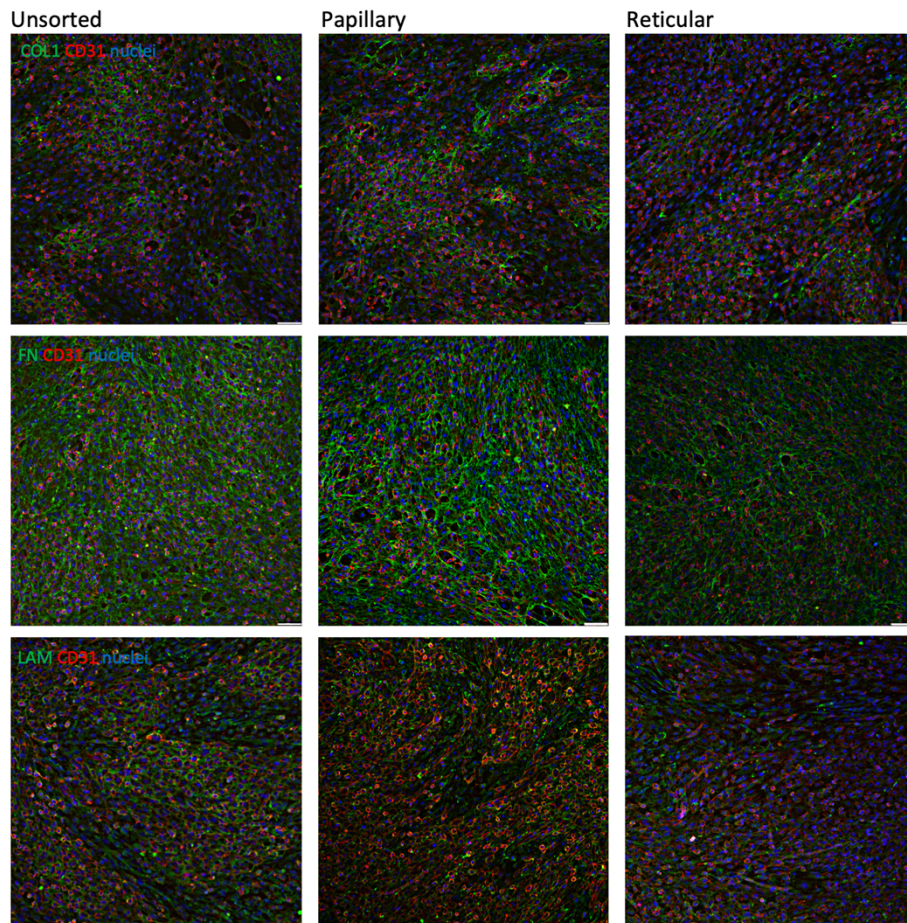
**Supplementary Figure 4** – Schematic representation of the measurements obtained from the Angiogenesis Analyzer in ImageJ. a Initial image used for the measurements. b Skeleton of the image. c Detection of segments (purple) – lines connected by two nodes, branches (green) – lines connected by a node, meshes (light blue) – closed areas surrounded by segments, nodes (red) – minimum structure allowing a bifurcation, and junctions (dark blue) – four or more connected segments.



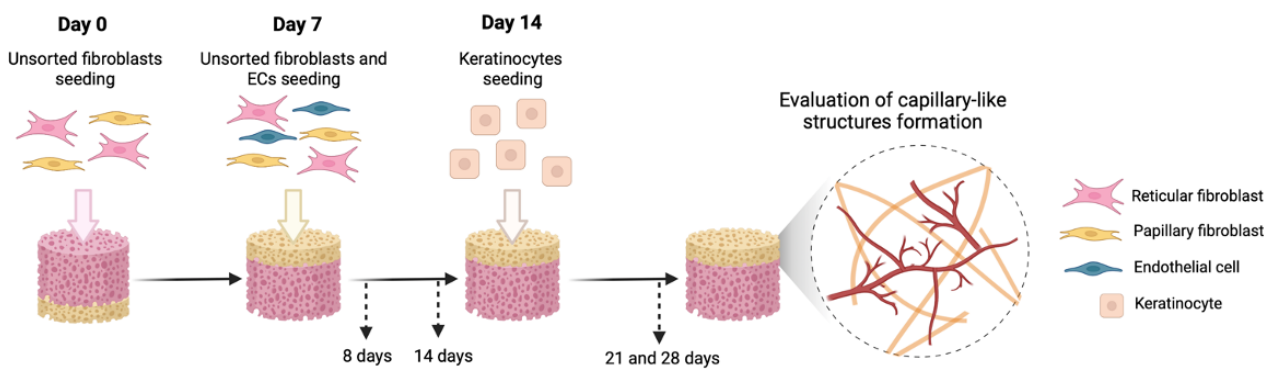
**Supplementary Figure 5** – Tubular-like structures formation after 8 h by hDMECs cultured on matrigel with conditioned media obtained from unsorted, papillary and reticular fibroblasts. Controls were set with VEGF-containing medium (+ Control) and with  $\alpha$ -MEM (- Control). Scale bar = 100  $\mu$ m.



**Supplementary Figure 6** – Amount of growth factors secreted by unsorted, papillary and reticular fibroblasts as quantified in the conditioned media normalized with their respective DNA amount. Quantitative results are expressed as the mean  $\pm$  standard deviation where  $n = 3$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , one-way ANOVA with Tukey multiple comparison post-test.

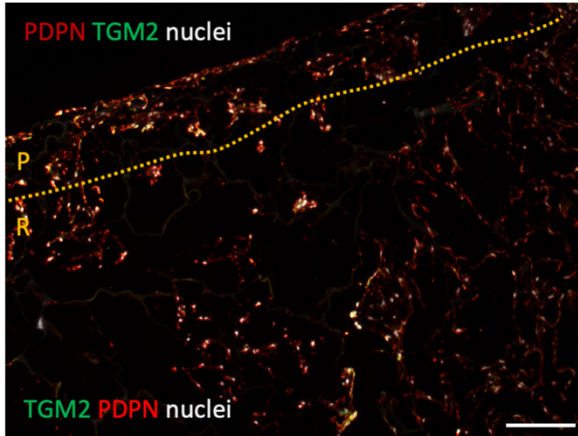


**Supplementary Figure 7** – Representative merged immunocytochemistry images of the expression of collagen type I (COL1), fibronectin (FN) and laminin (LAM) by the subpopulations of fibroblasts with the organization of CD31<sup>+</sup> hDMECs on the matrix of the different fibroblasts' subpopulations. Nuclei were counterstained with DAPI. Scale bar = 100  $\mu$ m.

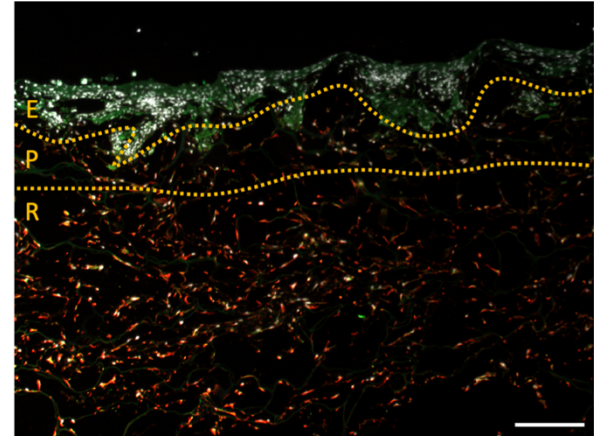


**Supplementary Figure 8** – Schematic representation of the unsorted fibroblasts, endothelial cells (ECs) and keratinocytes seeding strategy on the bilayer structure.

Day 8



Day 28



**Supplementary Figure 9** – Merged immunohistochemistry images of the expression of podoplanin (PDPN) and transglutaminase-2 (TGM2) in the bilayered structures with pre-selected fibroblasts at days 8 and 28. Nuclei were counterstained with DAPI. Scale bar = 200  $\mu$ m. E – epidermis, P – papillary-like dermis, R – reticular-like dermis.