Supplementary materials and methods

Optimization of medium volume

To analyze whether membrane size and medium volume supply have an effect based on the hypothesis that 3D culture usually requires more nutrition than conventional 2D culture HMGECs were cultured with proliferation medium and air-lift on differentially sized well inserts that were then placed in differently sized well plates, i.e. (A) 24-well inserts were placed in wells of 24-well plates (apical: 150 μL, basal: 800 μL), (B) 6-well inserts were placed in wells of 6-well plates (apical: 1 mL, basal: 3 mL) and (C) 24-well inserts were placed in wells of 6-well plates (apical: 150 μL, basal: 3 mL).
Supplementary Figure Legends

 Supplementary Figure S1. Microscopical view on 3D cultures of HMGECs in differently sized well plates after different cultivation periods.

HMGECs were cultivated on ThinCert™ membranes at the air-liquid interface with proliferation medium for 4 weeks. Influence of access to nutrition by insert size was tested on 24-well inserts in 24-well plates (A, D, G, J, M, left column), on 6-well inserts placed in 6-well plates (B, E, H, K, N, middle column) or on 24-well inserts placed in 6-well plates (C, F, I, L, O, right column). An increased volume of culture medium (24-well inserts in 6-well plates) improved the stratification outcome than other conditions. This was true for all time points analyzed, with the most intense stratification being reached after 1 week of culture (Figure F). Thereafter, this difference decreased continuously over time. From 3 weeks onwards, no further difference was detectable between the 6-well insert in 6-well group (Figures K, N) and the 24-well inserts in 6-well group (Figures L, O)

 Supplementary Figure 2. Lipid droplet accumulation during long-term 3D culture in Alvetex®.

HMGECs were cultured at the air-liquid interface, supplied with proliferation medium only for up to 4 weeks. Intracellular lipid droplets were stained by Sudan black, Sudan III and Oil red. Inevitable artifacts due to reagent binding within the scaffold were observed in all staining. Nevertheless, 3D culture promoted intracellular lipid accumulation. Scale bar: 50 µm (left, middle) and 10 µm (right, enlarged panel).
Supplementary Figures

Supplementary Figure 1

A
B
C

3 days

D
E
F

1 week

G
H
I

2 weeks

J
K
L

3 weeks

M
N
O

4 weeks
Supplementary Figure 2

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Supplementary Table S1
Primary and secondary antibodies used for immunohistochemistry.

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CK = cytokeratin