Matrix Density Drives 3D Organotypic Lymphatic Vessel Activation in a Microfluidic Model of the Breast Tumor Microenvironment

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Electronic Supplementary Information

Tuble Striterpes for uniferent contagen ger densities und cultures contaitens							
	Lymphatic Vessels		Lymphatic Vessels				
	mono	cultures	res co-cultures				
Final collagen I density (mg/mL)	3	6	3	6			
Collagen gelª (µL)	80	80	80	80			
5x PBS (μL)	20	20	20	20			
0.5 M NaOH (μL)	3	3	3	3			
	Dilution	Dilutions to get to the final collagen I density:					
1 mg/mL Fibrinogen ^b (μL)	14.4	7.2	14.4	7.2			
30 μg/mL Fibronectin ^c	8.6	4.3	8.6	4.3			
250 cells/µL Cell suspension ^d			23.9	12			
Experimental media	172.6	36.4	148.7	24.4			
Total volume	298.6 µL	150.9 μL	298.6 µL	150.9 μL			

 Table S1. Recipes for different collagen gel densities and cultures conditions

^aStock concentration of 10.76 mg/mL ^bStock concentration of 20 mg/mL

Stock concentration of 1 mg/mL

^dInitial concentration 3,000 cells/µL

SI Materials and Methods

SI Results and Discussion

Supplemental video 1: Confocal 3D rendering of a lymphatic vessel.

Table S2. Primary and secondary antibodies used for immunofluorescent staining.

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Primary antibody	Reference code	Stock conc. (mg/mL)	Working conc. (μg/mL)	Dilution		
CD31	ab9498 (Abcam)	N/A	-	1:50		
PROX-1	PA5-11899 (Thermo Fisher Scientific)	0.5 mg/mL	N/A	1:25		
Primary antibody	Host species	Secondary antibody ^a	Working conc. (μg/mL)	Dilution		
CD31	mouse	Alexa Fluor 647 anti-mouse	10	1:200		
PROX-1	rabbit	Alexa Fluor 488 anti-rabbit	10	1:200		
Stool concentration of	F2 ma/mI Goat course					

^aStock concentration of 2 mg/mL. Goat source.



Figure S1: Representative image of diffusion assay in vessels cultured in LD and HD matrices at t=0 min. Arrows indicates localized leakage. Scale bar = 200 µm.