Supplementary Figures



Supplementary Fig. 1. Rheology data for synthetic hydrogel composed of PEG at 6, 8, 10, and 12 PEG weight percentages. Sample size: 6% PEG = 7, 8% PEG = 7, 10% PEG = 8, 12% PEG = 6. Dotted lines above and below the regression represent 95% confidence intervals.



Supplementary Fig. 2. Images of Isotype controls for immunostaining in PEG hydrogels. (A) DAPI nuclear stain of lymphatic vessel from rat. (**B-D**) Staining for Mouse IgG2a, Mouse IgG1, and Rabbit poly IgG antibodies, respectively, of lymphatic vessel from rat in PEG hydrogel. Scale bar = 100 μm



Supplementary Fig. 3. Staining and isotype controls of intact rat LLV vessel. Merged DAPI stains with A) α SMA and B) VEGFR3 stains. Merged DAPI stains with the isotype controls (Iso Cntrl) for C) Mouse IgG2a and D) Rabbit poly IgG antibodies. Scale bar = 100 μ m



Supplementary Fig. 4. Density and distance of cells from embedded lymphatic vessel segment inversely proportional to collagen concentration. Representative images of collecting vessel segments sprouting at day 7 in (A) 1.00 mg/mL, (B) 1.88 mg/mL, (C) 3.00 mg/mL, and (D) 3.75 mg/mL collagen gels, respectively. Images are of intracellular fluorescent Calcein indicating live cells. Scale bar = $500 \mu m$. (E) Collagen concentration of 1.00 mg/mL had significantly increased sprout length compared to concentrations of 3.00 mg/mL or 3.75 mg/mL. (F) There was a significant decrease in the average number of sprouts for 3.75 mg/mL gels compared to 1.00 mg/mL. Data was analyzed by Kruskal-Wallis test followed by Dunn's multiple comparisons. (G,H) Linear regression shows the dependency of sprout length and sprout number on collagen concentration. Sample sizes (E-H): 1.00 mg/mL = 6, 1.88 mg/mL = 7, 3.00 mg/mL = 9, 3.75 mg/mL = 5. Dotted lines above and below the regression represent 95% confidence intervals and the p-values reflect the results of an F-test to determine the significance of a non-zero slope. * p<0.05



Supplementary Fig. 5. Representative images of daily growth of lymphatic segments through day 7. Lymphatic vessel segments were implanted into PEG-4MAL or collagen gels and cultured for 7 days with media changes on day 3 and day 6. Sprouting is visible between day 3 and 4 in both conditions. Live and dead staining (intracellular fluorescent Calcein-AM and Toto-3, respectively) on day 7 reveal high cellular viability in either condition on day 7. Scale bar = 100 μm.



Supplementary Fig. 6. Representative sprouting from various lymphatic tissues in variable media formulations cultured in collagen gels. (A) Mouse lymphatic thoracic duct sprouting network after 7 days of culture in 1.88 mg/mL collagen gels with media containing 10% FBS. (B) Mouse TD sprouting network after 7 days of culture in 1.88 mg/mL collagen gels with EBM-20% FBS. (C) Rat LLV sprouting network after 7 days of culture in 1.88 mg/mL collagen gels with media containing 20% FBS. Images are of intracellular fluorescent Calcein indicating live cells. Scale bar = 200 μm.

Α	-		_	-	_
	Day 1	Day 4	Day 7	Day 14	Day 14 (Live/Dead)
10% PEG – 2mM RGD		X	A A	(A)	
В	-			_	
	Davi 1	Davi 4	Day 7	Day 14	Dev. 20

	Day 1	Day 4	Day 7	Day 14	Day 20
10% PEG – 2mM RGD					
			X		A

Supplementary Fig. 7. Representative images of lymphatic sprouting networks in long term cultures through day 20. Select gels were cultured through day 20 to observe long term growth patterns. Media was changed every 3 days. Lymphatic sprouts continued growing through day 20, increasing in length and number, with no visible signs of degradation of the 10% PEG – 2mM RGD gels at day 20.



Supplementary Fig. 8: Impact of Chemotherapy Drugs on the Sprouting Phenotype in fully degradable 10% PEG – 2mM RGD Hydrogels: Representative images of collecting vessel segments labeled with Calcein-AM at day 10 cultured in A) ethanol (vehicle control), B) docetaxel, and C) carboplatin conditions. Images are of intracellular fluorescent Calcein indicating live cells. Scale bar = 500μ m. D) Lymphatic collecting vessels cultured with docetaxel (1mM concentration) at day 5 had significantly reduced max sprout length by day 10. E) Lymphatic collecting vessels cultured with docetaxel number of sprouts coming from the implanted vessel segment at day 10. There was no impact on sprout length or number from treatment with carboplatin. Data analyzed by Kruskal-Wallis test followed by Dunn's multiple comparisons. * p<0.05, ** p<0.01, *** p<0.001.