Supporting information

Sustained delivery of anti-VEGFs from thermogel depots inhibits angiogenesis without the need for multiple injections

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Supplementary figures

Copolymer name	PEG:PPG ratio (wt%)	Feed ratio (wt%)			Actual ratio by ^{1}H NMR (wt%)		
		PEG	PPG	PCL	PEG	PPG	PCL
EPC 1:1	1.05	49.50	49.50	0.99	48.41	50.68	0.91
EPC 2:1	2.13	33.00	66.01	0.99	31.66	67.36	0.98
EPC 4:1	4.28	19.80	79.21	0.99	18.74	80.27	0.99
EPC 6:1	6.32	14.14	84.87	0.99	13.53	85.54	0.93

Table S1. List of ^{1}H NMR results for 4 different copolymers.

S/N	Sample ID	Concentration of polymer in thermogel					
		3wt%		7wt%		12wt%	
		Crossover temp (°C)	G' at 37°C (Pa)	Crossover temp (°C)	G' at 37°C (Pa)	Crossover temp (°C)	G' at 37°C (Pa)
1	EPC 1:1	No, Paste-like	0.04	10.75	379.46	7.86	2139.46
2	EPC 2:1	29.62	0.84	15.76	295.91	12.34	1612.39
3	EPC 4:1	No, Solution	0.022	35.24	30.08	27.18	359.36
4	EPC 6:1	No, Solution	0.009	No, Solution	0.19	No, Solution	18.27

Table S2. Rheological characterisation of various PEG:PPG ratios of thermogel by temperature ramp at different weight percent of polymer.

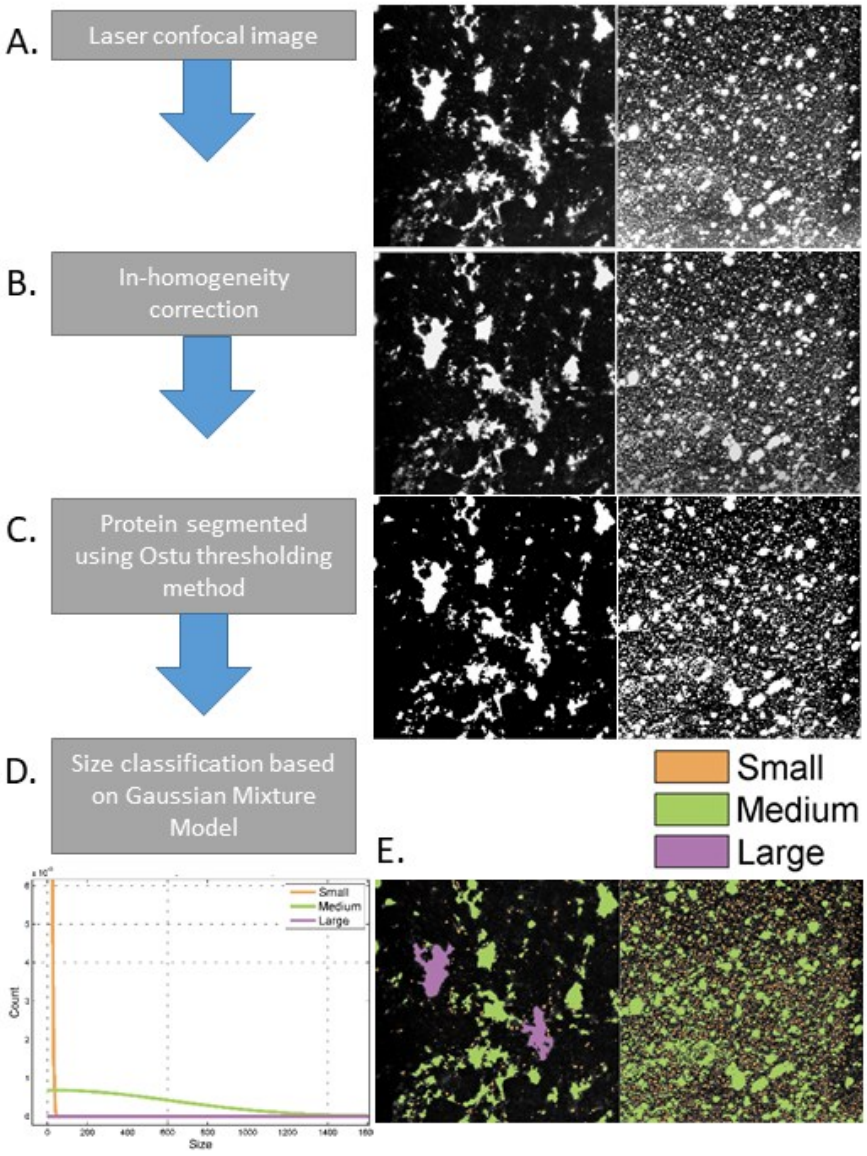

Figure S1. Confocal image processing and analysis pipeline. First, the green fluorescein channel in the images will be processed for inhomogeneity correction. The image after the inhomoneity correction (see Fig. 1B) will be used as input for image binarization. An optimized thresholding value was determined to binarize the image (see Fig. 1C) based on Otsu thresholding method. Hence, a segmented fluorescent protein domain in binary image format is obtained. The region properties such as area (in pixel) extracted from all images were collected. All sizes feature were used to construct the domain size classification model (see Fig. 1D) using expectation–maximization (EM) algorithm. An estimated mean and standard deviation for three clusters will be obtained based on EM method above. The three mode Gaussian mixture model (GMM) was generated based on the estimated mean and standard deviation. Subsequently, all sizes extracted will be used to classify the domain into "small", "medium", and "large" category (see Fig. 1E) based on the GMM accordingly. The average protein domain sizes in μm^2 were then calculated and quantified accordingly.

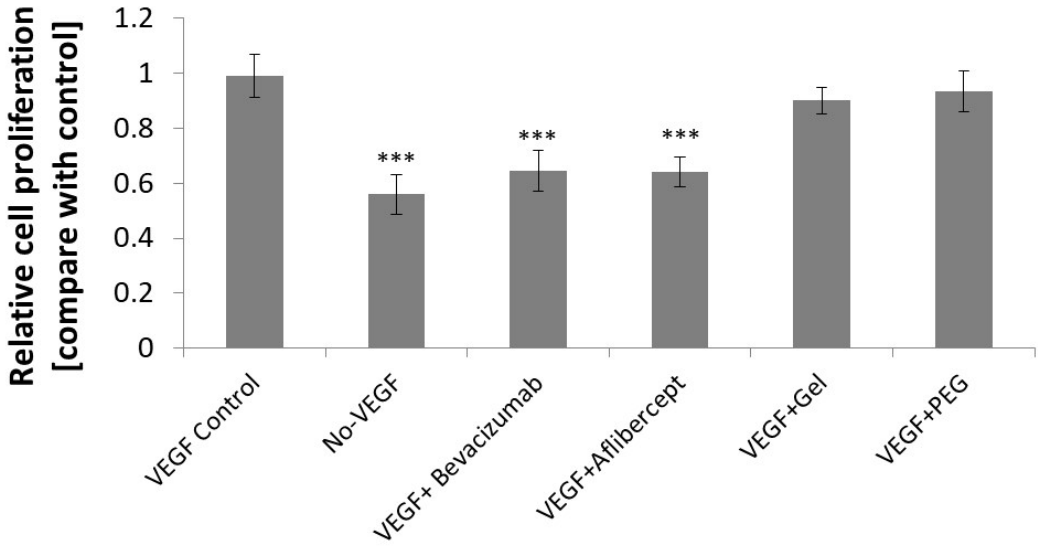

Figure S2. Anti-VEGF controls inhibit VEGF-induced HUVEC proliferation in vitro. HUVECs were treated with the VEGF only control, as well as anti-VEGF and thermogel separately. Cell proliferation was evaluated by WST-8 assay and compared with control (VEGF control) (n = 6). (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$)

Sample collecting time	5 days	10 days	20 days	40 days
Dissociated thermogel	0 ± 1.4	0 ± 1,5	1.4 ± 3.6	2.3 ± 1.8
Released Bevacizumab	0.3 ± 2.1	0 ± 2.9	1.6 ± 2.1	1.4 ± 1.4
Released Aflibercept	0.2 ± 1.4	0.8 ± 5.0	0 ± 4.6	3.2 ± 0.7

Table S3. Percentage of HUVEC cell death (Compared with none- treated control) after 3 days co-culture with In vitro release samples (LDH cell death assay)