Electronic supplementary information (ESI)

Engineered phage nanofibers induce angiogenesis

So Young Yoo, Kshitiz Raj Shrestha, Su-Nam Jeong, Jeong-In Kang and Seung-Wuk Lee*

* Corresponding author at: BIO-IT Foundry Technology Institute, Pusan National University, Busan 46241 & Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 50612, Republic of Korea. E-mail: yoosy2@gmail.com; yoosy@pusan.ac.kr

Table S1. Primer sequences for pVIII and pIII engineering

Name	Oligonucleotide Primer Sequence*	Insert Peptide Sequence**
p8-fw RGD	5' ATATAT **CTGCAG** *NK (NNK)$_2$ CGTGGT GAT (NNK)$_2$* GATCCCGCAAAAGCGGCCTTTA ACTC CC 3'	A*XXXRGDXX*DP A*DSGRGDTE*DP ***
p8-fw SDKP	5' ATATAT **CTGCAG** *NK AGCGATAAACCG (NNK)$_2$* GATCCCGCAAAAGCGGCCTTTAACT CCCTGCAAGCC 3'	A*GSDKPYV*DP ***
p8-rev1376	5' CCTCTGCAGCGAAAGACAGCATCGG 3'	
p3-Fwd1626	5' AAACACT **CGGCCG** AAACTGTTGAAAGT TGTTTAGC 3'	
p3-rev RGD	5' TATATA **CGGCCG** A *TCCACCGCCGCAGC TATCGCCACGGCCGCACGC* CGAGTGAGAATAGAAAGGAACCACTAAA G GAATTGCG 3'	SHS*ACGRGDSCGGG*A

* For primer oligonucleotide sequences the restriction sites are shown in **bold**, and the insert is *underlined and italic*

** For the resulting peptide sequence the insert is *underlined and italic*

*** Constructed from partial library approach,[1] selected sequence indicated

Table S2. Phage cloning PCR conditions

PCR Ingredients	pVIII PCR Conditions	pIII PCR Conditions
~25ng dsDNA template*		
2.5µL 10µM forward primer		
2.5µL 10µM reverse primer		
1µL dNTP (10mM mix of A, T, G & C bases)	98C 1min / 98 °C 15 sec	98C 1min / 98 °C 15 sec
1µL DMSO	25x < 58 °C** 20 sec	25x < 61 °C 20 sec
10 µL 5X HF Phusion Polymerase Buffer	\ 72 °C 3min 30sec	\ 72 °C 3min 30sec
	72C 4 min	72C 4 min
balance with sterile H_20 to 50µL	4C ∞	4C ∞
1 µL Phusion Polymerase Enzyme		

* ~1 µL, use any template that has a PstI and a BamHI site for the pVIII M13 engineering; have the EagI and the Acc65I sites for pIII M13 engineering

** Primer annealing temperature = Primer Tm (lower of the two primers) – 2

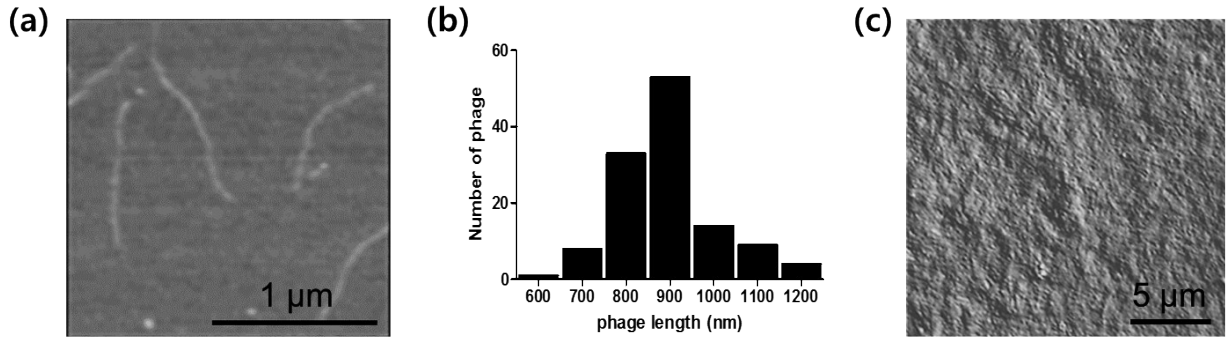

Fig. S1. AFM images of engineered phage nanofiber and its drop-cast film surface. (a) AFM image showed that the engineered phage have nanofibrous structure. 250 ng/ml phage solution was used. (b) Distribution of individual phage length shows that phage have ≈880nm in length. (c) The resulting phage drop-cast could form nanofibrous phage film.

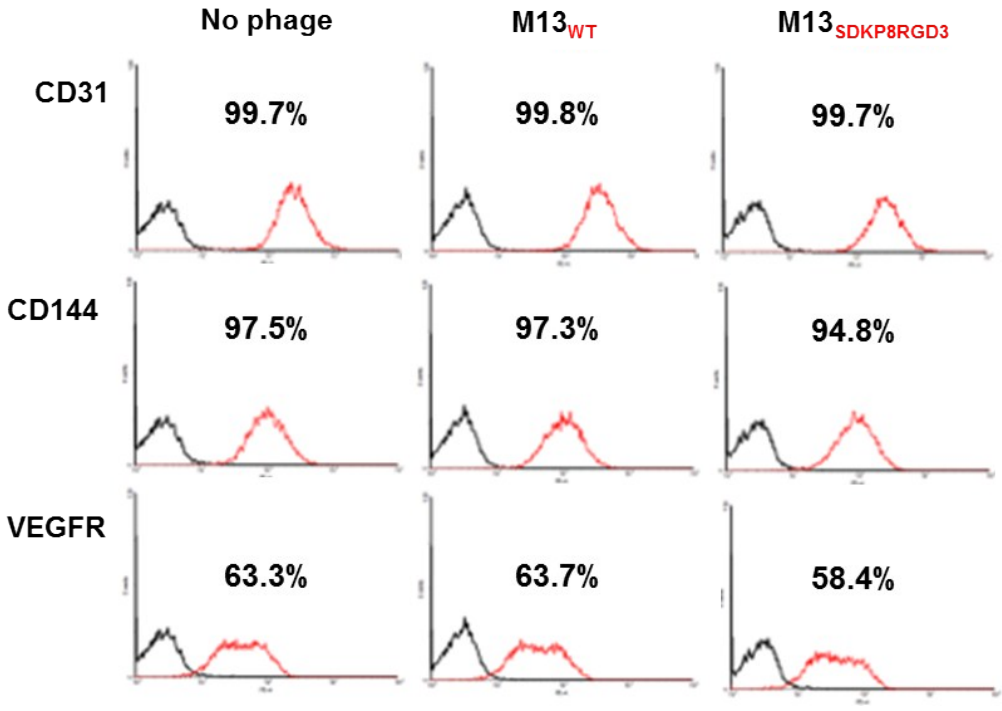

Fig. S2. The surface markers of endothelial cells were detected by flow cytometry analysis for CD31, CD144 and VEGF2. Histograms represent the cell number (y-axis) versus the fluorescent intensity (x-axis, log scale). EPCs were used. Flow cytometry analysis gating was performed using cells stained with isotype-matched IgG as a negative control. Black lines indicate the negative control cells and red lines indicate cells stained with each corresponding antibody.

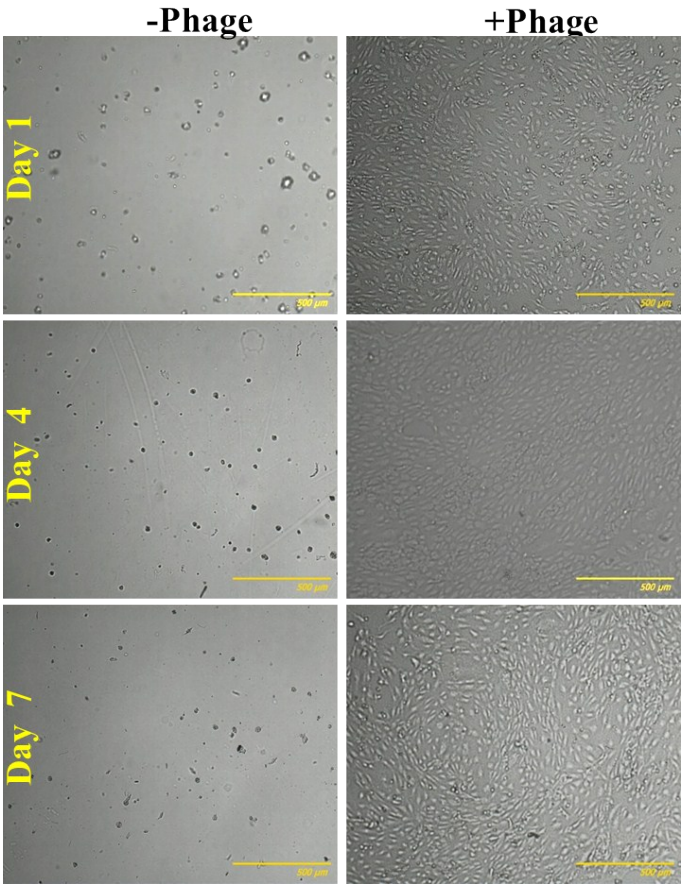

Fig. S3. HUVECs cultured on PA hydrogel substrate with or without phage. Phages were resuspended in PA hydrogel substrate at a final concentration of 1 mg/ml (+Phage). PA hydrogel consisted of 4% acrylamide and 0.04% bisacrylamide.

References

1. A. Merzlyak, S. Indrakanti and S. W. Lee, *Nano Lett*, 2009, **9**, 846-852.