Supplementary Material (ESI) for Soft Matter

Electronic supplementary information (ESI)

Facile Growth Factor Immobilization Platform Based on Engineered Phage Matrices

*So Young Yoo, Anna Merzlyak, and Seung-Wuk Lee**

* Corresponding author at: Department of Bioengineering, University of California, Berkeley, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley Nanoscience and Nanoengineering Institute, Berkeley, California 94720 (U.S.A.). E-mail: leesw@berkeley.edu

Genetic engineering and purification of M13 bacteriophage: To present peptide motifs on every copy of the M13 major (pVIII) and minor (pIII) coat proteins, an inverse PCR cloning method was adapted.[1, 2] Phages presenting HPQ or RGD motifs at the N-terminus of every copy of pVIII were constructed using inverse PCR methods as described in [3] (see Table S1 for primer sequences, Table S2 for PCR conditions). For pIII peptide display, a slightly altered approach was used. The peptide motifs were inserted immediately at the N-terminus of pIII. The M13KE phage vector from New England Biolabs has an engineered EagI restriction enzyme site at the N-terminus of the mature pIII protein. The pIII reverse primer was designed to include the EagI restriction site, the insert sequence, and a segment complimentary to the gIII 5'-3' strand. The pIII forward primer was designed to make the vector linear and was fully complimentary to the engineered gIII 3'-5' region, including an EagI restriction site (see Table I for primer sequences, Table S2 for PCR conditions). To incorporate the gene sequences, polymerase chain reaction (PCR) was performed using Phusion™ High-Fidelity DNA Polymerase, the two primers, and an M13KE vector, either as supplied or previously pVIII engineered (See Table S2). The obtained product was purified on an agarose gel, eluted with spin column purification, digested with Eag I enzyme (New England Biolabs, Ipswich, MA), and re-circularized overnight at 16°C with T4 DNA Ligase (New England Biolabs).[4] The ligated DNA vector was then transformed into XL10-

Gold® Ultracompetent bacteria cells (Stratagene, La Jolla, CA), and the amplified plasmid was verified via DNA sequencing at the UC Berkeley DNA Sequencing Facility (Berkeley, CA).

Neural Progenitor Cell Culture: Neural stem cells originally isolated from the hippocampi of adult female Fischer 344 rats were the kind gift of Prof. David Schaffer (University of California, Berkeley). Cells with passage number 35-44 were seeded onto various surfaces and grown as previously described[5] in serum free DMEM/Hams F-12 medium supplemented with N-2 (Invitrogen,, Carlsbad, CA) and 20 ng/mL bFGF (Peprotech, Rocky Hill, NJ) (Invitrogen, Carlsbad, CA) at 37°C, 5% CO_2 and 95% humidity.

Streptavidin conjugation of FGFb or NGF: The Lightning-Link^{TM} conjugation kit (Innova Biosciences, Cambridge, UK) was used to conjugate FGFb or NGF with streptavidin. The amine group of FGFb or NGF was activated by adding LL-modifier reagent, and incubation with the Lightning-Link mixture resulted in coupling of the FGFb or NGF to streptavidin.

Phage drop cast film: Phage-coated surfaces were initially coated with polyornithine to allow for better adhesion of the negatively charged phage. Phage solutions in PBS (~10^{12} pfu/mL) were then drop-cast on the surface and allowed to dry overnight at room temperature.

WST1 Proliferation Assay The WST-1 assay (Cell Proliferation Reagent WST-1; Roche Applied Science, Basel, Switzerland) was performed as per the manufacturer's instructions. It is a colorimetric assay for the quantification of cell proliferation and cell viability, based on measuring the metabolic activity using the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. 10 µl of reagent was added to a 0.1 ml volume of growth medium. The activities were quantified by measuring absorbance (450– 690 nm) on an ELISA reader (Safire, Tecan Group Ltd., Männedorf, Switzerland).

Immunostaining and Fluorescence Microscopy: Cells were fixed in 3.7% formaldehyde solution for 15 min and then blocked with a solution of 0.3% Triton X-100 and 5% normal goat serum in 1X PBS for 30 min. To stain the cell cultures, primary antibodies for identifying cell markers and for M13 bacteriophage were incubated with the cells overnight at 4°C. The primary antibodies used in this investigation were mouse anti-β-tubulin III antibody (1:400, Sigma Aldrich, St. Louis, MO), mouse anti-nestin antibody (1:1000, BD Biosciences, San Jose, CA), and rabbit anti-fd antibody (1:500, Sigma Aldrich, St. Louis, MO). Secondary goat Alexa flourochrome-conjugated antibodies (Molecular Probes, Eugene, OR) were used at a dilution of 1:250 to visualize the markers and incubated with the cells for 2hrs at room temperature. A 300nm DAPI (Molecular Probes, Eugene, OR) solution in PBS was used as a nuclear counterstain for all samples. The fluorescence images were collected using an IX71 Fluorescence Microscope (Olympus, Tokyo, Japan). Effects of the growth factors immobilized on the HPQ modified phages on cellular distribution (cell to cell distance), cell density (cell numbers per mm^2 spot area), neurite growth measurement, and cell numbers in each aggregated cell island were analyzed using NIH ImageJ (NIH, http://rsb.info.nih.gov/ij/).

Table S1. Primer sequences for pVIII and pIII engineering

Name	Oligonucleotide Primer Sequence*	Insert Peptide Sequence**
p8-RGD	5' ATATAT **CTGCAG** *NK (NNK)$_2$ CGTGGT GAT (NNK)$_2$* GATCCCGCAAAAGCGGCCTTTA ACTC CC 3'	A*XXXRGDXX*DP A*DSGRGDTE*DP ***
p8-HPQ	5' TATAT **CTGCAG** *AATTTAGCCATCCGCAGA ATACC* GATCCCGCAAAAGCGGCCTTTAACT CCCTGCAAGCC 3'	A*EFSHPQNT*DP
p8-rev1376	5' CCTCTGCAGCGAAAGACAGCATCGG 3'	
p3-HPQ	5' TATATA **CGGCCG** A *TCCACCGCCGCACG GCGGGCCCTGCGGATGGCACGC* CGAGTGAGAATAGAAAGGAACCACTAA AG GAATTGCG 3'	SHS*ACHPQGPPCGGG*A
p3-Fwd1626	5' AAACACT **CGGCCG** AAACTGTTGAAAGT TGTTTAGC 3'	

* 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,[3] 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
balance with sterile H_20 to 50µL	72C 4 min	72C 4 min
1 µL Phusion Polymerase Enzyme	4C ∞	4C ∞

* ~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

References

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