Supplementary material (ESI) for Journal of Materials Chemistry

Supporting Information

Fabrication of Engineered M13 Bacteriophages into Liquid Crystalline Films and Fibers for Directional Growth and Encapsulation of Fibroblasts

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Inhibition Assay (NIH-3T3 Fibroblast Attachment to RGD-Phage Film)

NIH-3T3 fibroblast cells were seeded on RGD-phage drop-cast films at a density of ~1.8×10^3 cells/cm^2 in the presence of different concentrations of soluble RGD-phages (0-1 μg/mL), in the serum-free DMEM/F12 1:1 media. The cell morphology on the phage film was observed using the IX71 inverted research microscope (Olympus, Tokyo, Japan) at different time points (1.5, 3 and 5 h after co-seeding of cells and soluble RGD-phages) (Fig. S3). When we mixed higher concentrations of RGD-phage with cells, the adhesion of fibroblasts was retarded more strongly and fewer cells spread and flattened on the RGD-phage film.

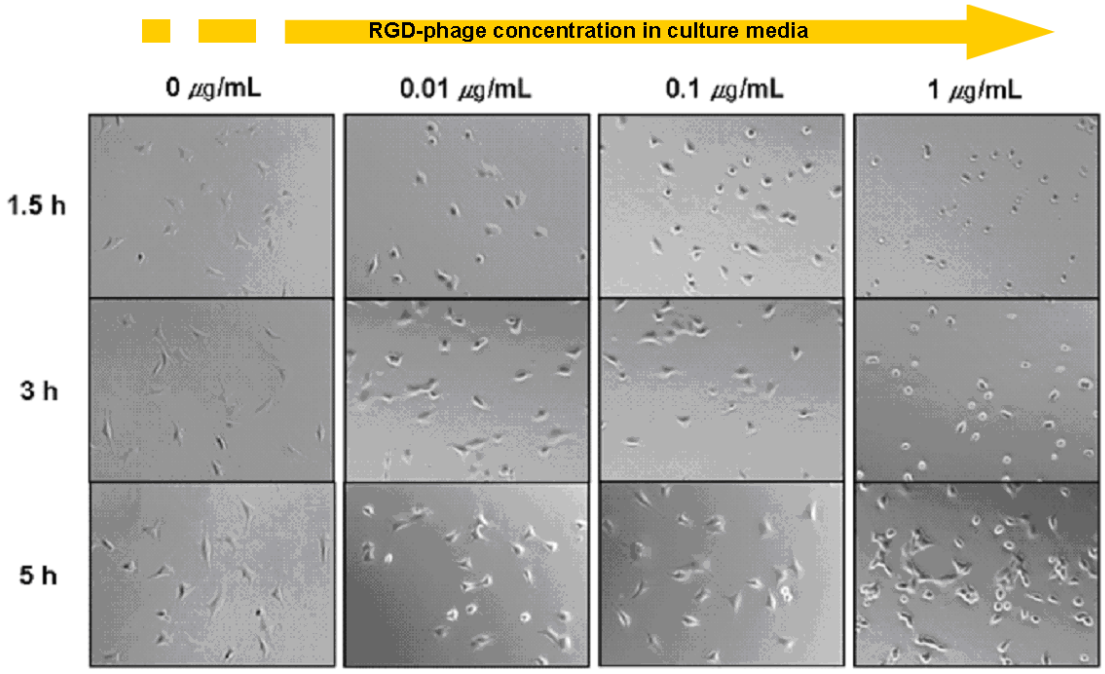

Figure S1. Cell morphology of NIH-3T3 fibroblasts cultured on the RGD-phage film (isotropic) in the presence of the soluble RGD-phages in the serum-free DMEM/F12 1:1 media. NIH-3T3 fibroblasts were seeded at densities of ~1.8 × 10^3 cells/cm^2 with different concentration of soluble RGD-phages as inhibitors. Scale bar indicates 100 μm.

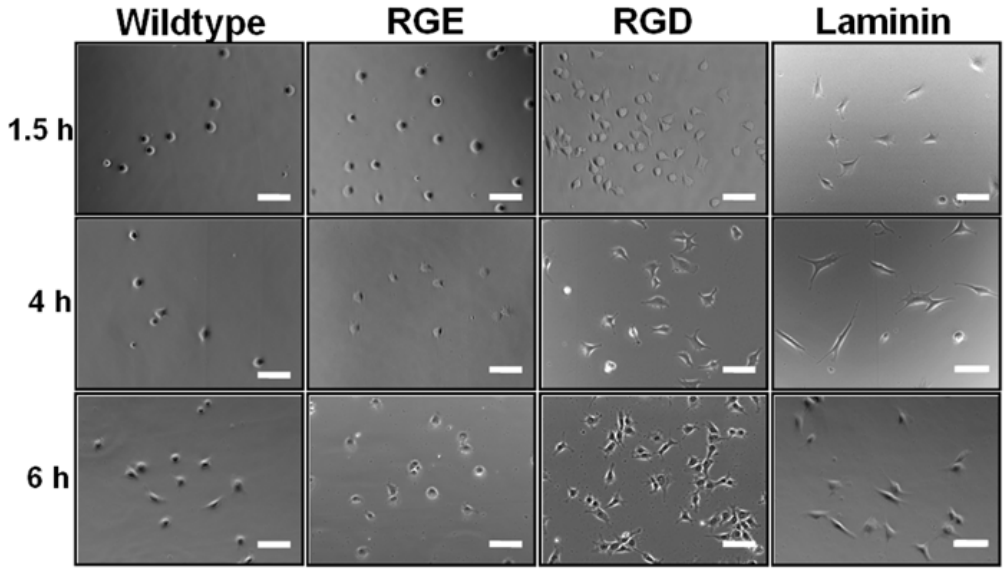

Figure S2. Cell morphology of NIH-3T3 fibroblasts cultured on phage films (isotropic) and laminin-coated substrate. Phase contrast microscope images of NIH-3T3 fibroblasts cultured for duration of 6 h on wildtype-, RGE-, RGD-phage film and laminin-coated substrates in serum-free culture media (DMEM/F12 1:1). NIH-3T3 fibroblasts were seeded at densities of ~2 × 10^3 cells/cm^2 on the phage film and the laminin-coated substrates. Scale bar indicates 100 μm.

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Atomic Force microscopy (AFM) Analysis of phage film: Topographic images of phage films were acquired in air using AFM (MFP-3D AFM, Asylum Research, Santa Barbara, CA) operating on tapping mode with silicon nitride cantilever (AC240TS, Olympus, Tokyo, Japan). Scan rate was 0.5 Hz. Root mean square (RMS) roughness of the phage film surface was measured using Igor Pro MFP-3D software (Asylum Research, Santa Barbara, CA). Height difference profiles were determined from line profiles traced perpendicularly through the features of interests on phage film.

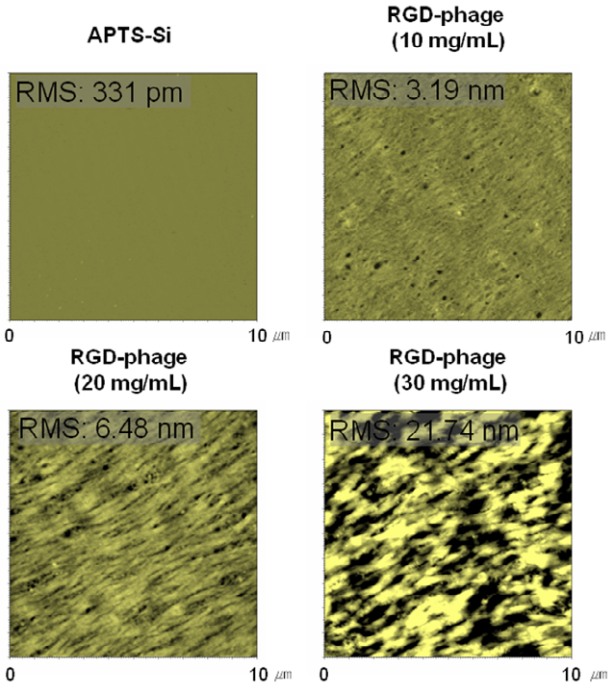

Figure S3. AFM images of the RGD-phage aligned films. 3-aminopropyl triethoxy silane (APTS)-treated substrate and anisotropic RGD-phage films topography prepared using 10 mg/mL, 20 mg/mL and 30 mg/mL of RGD-phage solution.

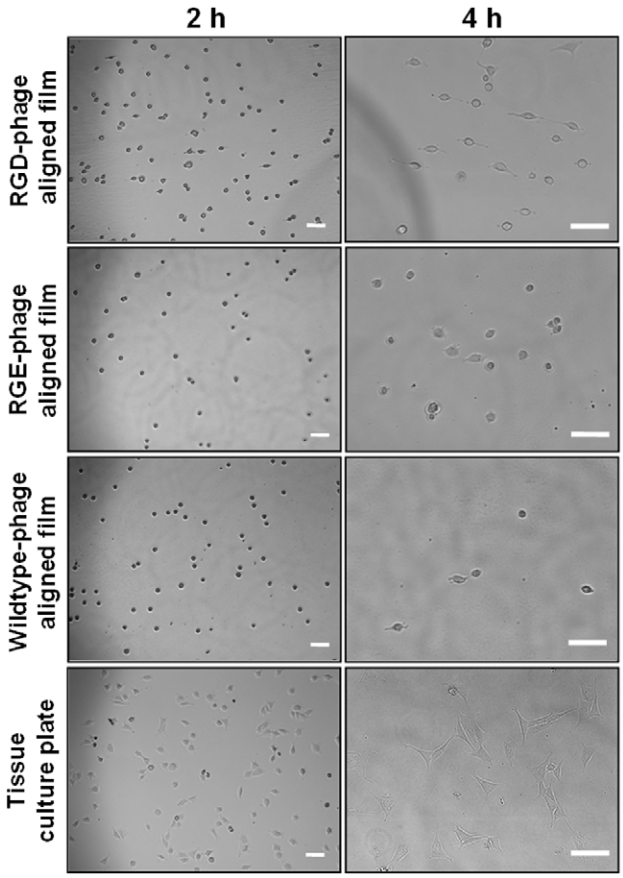

Figure S4. Optical microscope images of NIH-3T3 fibroblasts grown on aligned phage films (RGD-, RGE- and wildtype-) and the tissue culture plate for 2 and 4 h. NIH-3T3 fibroblasts were seeded with densities of ~3×10^4 cells/cm^2 on the phage films (prepared from 20 mg/mL of phage solution) and cultured in serum-free media (DMEM/F12 1:1) at 37 °C with 5% CO_2. Scale bars indicate 100 μm.

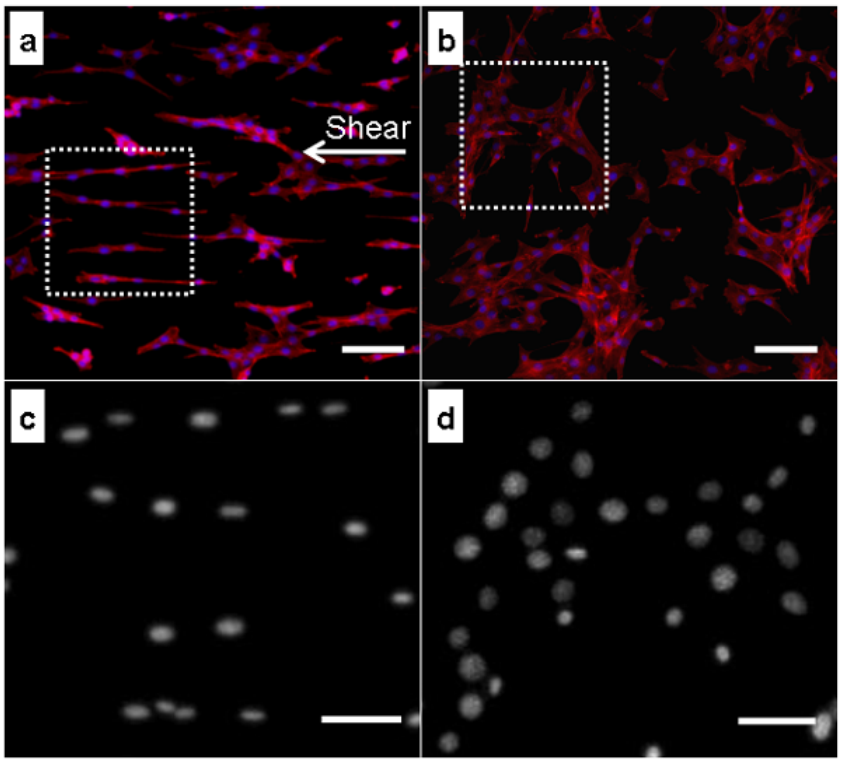

Figure S5. Directional guidance of the NIH-3T3 fibroblasts on RGD-phage films (a, b) Fluorescence images of NIH-3T3 fibroblasts, actin filaments stained with phalloidin (red), and nuclei counterstained with DAPI (blue) grown on aligned RGD-phage films with (a) anisotropic topography and (b) isotropic topography. NIH-3T3 fibroblasts were seeded with densities of ~3 × 10^4 cells/cm^2 on the phage films (prepared from 20 mg/mL of phage solution) and cultured for 24 h. (c, d) Differences in nucleus morphology, such as elongation and orientation are shown for the (c) aligned RGD-phage films and (d) isotropic RGD-phage films. Scale bar: (a, b) 100 μm, (c, d) 50 μm.

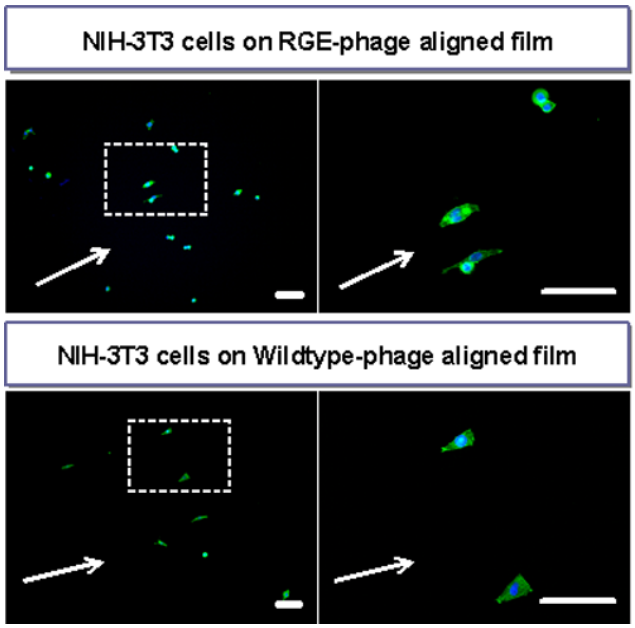

Figure S5. Directional growth of NIH-3T3 fibroblasts on control phage aligned films. Fluorescence images of fibroblasts grown on the RGE-phage aligned film and wildtype-phage aligned (after 12 h). Actin filaments stained with phalloidin (green), and nuclei counterstained with DAPI (blue). Scale bars indicate 100 μm.

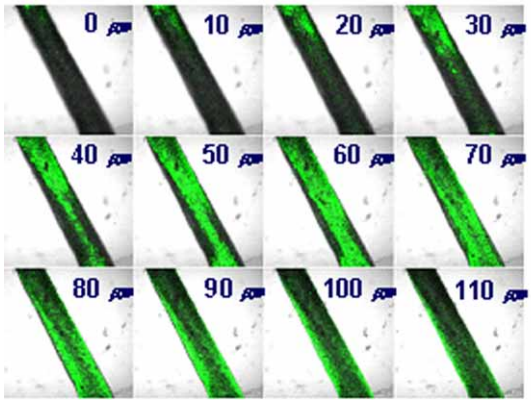

Figure S6. Confocal fluorescence z-section images of biotinlyated RGD-phage fiber treated with FITC-streptavidin (0.1 mg/mL in PBS) for 5 days.