

Supporting Information

Rupture force of cell adhesion ligand tethers modulates biological activities of a cell-laden hydrogel

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Fabrication of RGD-DNA Tether and Their Rupture Force Calculation. The single stranded DNAs (Integrated DNA technologies, Inc.) were modified as follows: 5-/5Cy5/CAC AGC ACG GAG GCA CGA CAC/3ThiolMC3-D/-3 and 5-GG TCG TGC CTC CGT GCT GTG-3 with biotin label at various locations. Thiol-modified single stranded DNAs and RGDfK-NH₂ (Peptides International, Inc.) peptides were conjugated using hetero-bifunctional crosslinker. Sulfo-SMCC (Thermo Fisher Scientific Inc.) that has maleimide and NHS ester groups on two ends. RGDfK-DNA and biotin-DNA were annealed together in annealing buffer (150 mM NaCl, 10 mM Tris, pH 7.4).

Synthesis and characterization of Alginate-g-biotin. 200 mg alginate (Mw ~ 250,000 g/mol, LF 20/40, FMC Biopolymer) was dissolved in 20 ml (N-morpholino)ethanesulfonic acid (MES, Sigma-Aldrich) buffer at pH 6.5. To the alginate solution, 0.2 mmol biotin hydrazide (Vendor?) was added. Then, 0.1 mmol sulfonated N-hydroxysuccinimide (sulfo-NHS, Thermo Scientific) and 0.2 mmol 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Thermo Scientific) were added to the solution. The reaction was continued over 3 h. The resulting alginate-g-biotin was dialyzed against DI water for 3 days, sterilized via filtration, and lyophilized. The resulting polymer was kept dried in sterile condition until it was used. The conjugation of biotin to alginate was characterized using a ¹H NMR (Varian VXR 500).

Hydrogel Preparation and Functionalization with RGD-DNA Tether. Alginate-g-biotin was dissolved in 10 ml MES buffer to prepare 2 wt% polymer solution. Then, the (?) ml alginate-g-biotin solution was sequentially mixed with 0.5 mmol 1-hydroxybenzotriazole (HOBt), adipic acid dihydrazide (AAD), and 0.5 mmol EDC to activate cross-linking between AAD and uronic acids of alginate. The amount of AAD added to the solution was 0.05, 0.1, or 0.2

mmol. The solution was placed between two glass plates with a 2-mm spacer. The gel was punched in a form of 10-mm diameter disk. The resulting gel was incubated with 20 μ l avidin (0.2 mg/ml, Invitrogen) for 30 min. Then, excess avidin and AAD were removed by washing the gel with DI water. The RGD-DNA tether with controlled T_{tol} values was then added to the aqueous media immersed with the alginate-g-biotin gel that presents avidin on the surface. The gel and RGD-DNA tether were incubated for 30 min for the immobilization of the DNA-tether on the gel surface. The unbound DNA tethers were removed by washing the gel with DI water.

Characterization of Hydrogel. The elastic modulus of the hydrogel was measured using a mechanical testing system (MTS Insight). Before testing, the hydrogel was allowed to equilibrate with DI water for 24 h. Then, the elastic modulus was quantified by uniaxially compressing the gel at a rate of 1 mm per minute. The compressive elastic modulus was quantified from the slope of the stress (σ) versus first 10 % strain (ϵ) curve.

The immobilization of RGD-DNA tether on the gel surface was examined by using the RGD-DNA tether labeled with Cy5. After immobilization, fluorescent emission from the gel surface was examined using a fluorometer with excitation and emission wavelengths of 590 and 645 nm, respectively.

Cell Culture. Human bone marrow-derived MSCs (Lonza) with a passage number ranging from 5 to 7 were cultured in Dulbecco's modified Eagles medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 100 units/ml penicillin-streptomycin (Invitrogen).

Analysis of Cell Adhesion. 5×10^4 MSCs were plated onto 10-mm diameter hydrogel disks immobilized with RGD-DNA tether, and cultured in the FBS-supplemented DMEM for 24 h. Then, cells were fixed, permeabilized and sequentially incubated with Alexa fluor 488 phalloidin and 4',6-diamidino-2-phenylindole. Then, cell actins and nucleus were imaged using a laser scanning confocal microscope (LSM 710, Zeiss).

Neuronal Differentiation Assay of MSCs. 5×10^5 MSCs were seeded onto 10-mm diameter hydrogel disk and cultured in the FBS-supplemented DMEM for 5 days. Then, the media was replaced with neurogenic differentiation media (PromoCell) and cultured for 7 days. The cells were fixed with 3.7% (w/v) formaldehyde and stained with antibodies against microtubule-associated protein 2 (MAP2, Invitrogen). The positively stained MAP2 was visualized with Alexa Fluor 488-conjugated secondary antibodies (Invitrogen). The slides were counterstained with 4',6-diamidino-2-phenylindole (Invitrogen) to stain the nuclei of the cells. Then, cells were imaged using a laser scanning confocal microscope (LSM 710, Zeiss).

VEGF ELISA Analysis. MSCs were cultured on DNA tether-conjugated alginate gels without FBS for 24 h. The amount of VEGF secreted from cells into the media was then measured using an ELISA assay kit following the manufacturer's protocol. Briefly, a 96 well plate was coated with capture antibody overnight, washed, and blocked with bovine serum albumin for 1 h. After washing, the plates were incubated with MSC culture media and standards for 2 h followed by another washing step. The plates were then incubated with detection antibody for 2h followed by washing and further incubation with streptavidin-horseradish-peroxidase for

20 min. Following another washing step, the samples were incubated with a 1:1 mixture of hydrogen peroxide (color reagent A) and tetramethylbenzidine (color reagent B), called substrate solution. The colorimetric reaction was stopped with 2 N sulfuric acid. Finally, the absorbance of the plate at 570 nm was subtracted from the reading at 450 nm to determine the absorbance of the samples and standards. A standard curve was developed by measuring the absorbance of solutions with known VEGF concentrations and then used to back-calculate the concentrations of VEGF from the absorbance

In Vivo Angiogenesis Assay. The DNA tether-modified hydrogels were implanted on chick chorioallantoic membranes (CAM), and the stimulation of angiogenesis was examined at different time points. Briefly, fertilized eggs were incubated horizontally at 37 °C and 65% humidity for 7 days. Then, a window was made in the shell. Hydrogels were implanted onto the CAMs 37 °C for 7 days. Pictures of the CAM were taken at various time points using a Leica S6E stereomicroscope linked with a Leica D-Lux E Camera. The entire CAM experiment was performed under sterile conditions.

Statistical Analysis. Four samples were analyzed per condition. One-way analysis of variance (ANOVA) was used to determine the statistical significance of data. Tukey's post hoc tests were applied to all the pairwise differences between means. Data was considered significant for p values < 0.05 .

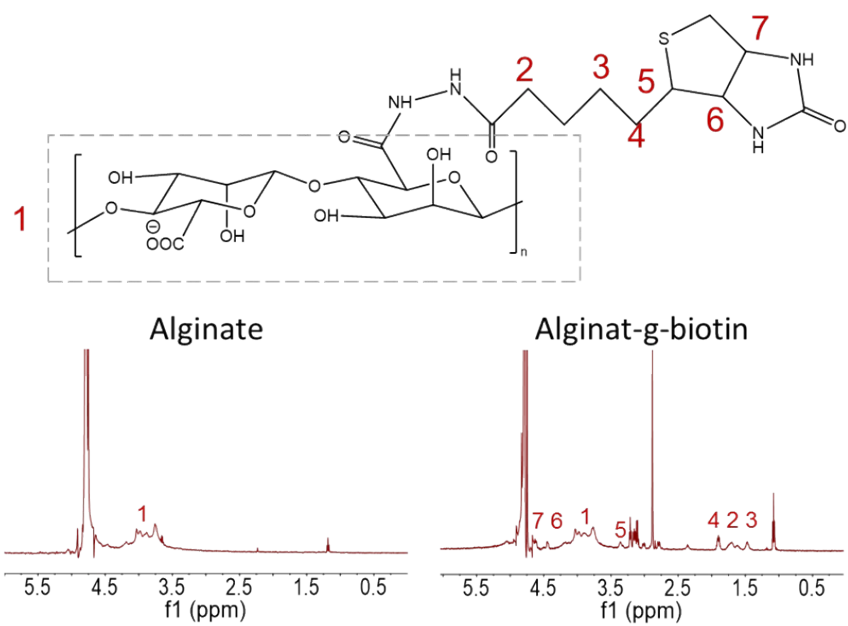


Figure S1. ¹H NMR spectrum that validates biotin coupled to the alginate.