[Supplemental Information]

Preparation of cell-interactive polymer: Synthetic oligopeptides with a sequence of $(Gly)_4$-Arg-Gly-Asp-Ser-Pro (Commonwelalth Biotechnology) were bound to sodium alginate molecules (LF20/40, Mw ~ 250,000 g/mol, FMC Biopolymer) using a previously described carbodiimide chemistry.[1] Briefly, N-hydroxysulfosuccinimide (sulfo-NHS, Pierce), 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC, Sigma), and RGD peptides were sequentially added to alginate solution prepared with 2-(*N*-morpholino)ethanesulfonic acid (MES, Sigma) buffer at pH 6.5. The degree of substitution, defined as the number of RGD peptides coupled to an alginate chain, was varied from 1 to 20 by altering the molar ratio between RGD peptides and uronic acids in alginate chains from 0.001:1 to 0.02:1. The molar ratio of uronic acid/sulfo-NHS/EDC was varied depending on the molar ratio of RGD peptides/uronic acid. The molar ratio of sulfo-NHS/EDC was kept constant at 1:2. After reaction for 24 hours, alginate molecules modified with RGD peptides were purified with dialysis against deionized water for four days (molecular weight cut-off, 3,500) and activated charcoal treatment followed by sterilization through a 0.22 μm filter. The degree of substitution was previously analyzed using ^{125}I-RGD peptides as tracer molecule.[1] The molecular size was analyzed with gel permeation chromatography (Viscotek) comprised of a laser refractometer, a differential viscometer, and a right angle laser light scattering detector. Following lyophilization, the samples were reconstituted with minimum essential medium α medium (α MEM, Invitrogen) at 2 % (w/w).

Preparation of hydrogels: Hydrogels to which cells adhere were prepared by mixing alginate solutions with $CaSO_4$ aqueous slurries (Sigma), which led to ionic cross-linking. The mixture was injected on a glass plate with a spacer of 2 mm and covered with a second glass plate. After 30 minutes, gel disks with a diameter of 10 mm were punched out. The overall density of RGD peptides (N_{RGD}) was varied by using alginate molecules with a different degree of substitution to form gels. Distance between islands of RGD peptides (d_{RGD}) was varied by mixing RGD peptide-modified alginate molecules and unmodified alginate at different volume ratios. In this approach, alginate molecules presenting different degrees of substitution were used to keep N_{RGD} constant as d_{RGD} was varied. Gel disks were incubated at 37 ^{o}C in α MEM for four days while exchanging the medium on a daily basis before using in cell experiments.

Preparation of pDNA condensates: The gWiz vector containing the gene encoding luciferase (Aldevron) and linear poly(ethyleneimine) (PEI, Fermentas) were first diluted in phosphate buffer saline (PBS, Invitrogen) at pH of 7.3. pDNA-PEI complexes were prepared by combining the PEI and pDNA solution, while vortexing the mixture. The charge ratio between PEI and pDNA (NH^{3+}:PO^{4-}) was kept constant at 7. In experiments monitoring gene transfer, pDNA labeled with rhodamine (Gelantis) was used.

Cell Culture: MC3T3-E1 preosteoblasts, a generous gift from Dr. Renny Franceschi (University of Michigan), were cultured in ascorbic α MEM (Invitrogen) supplemented with 10 % fetal bovine serum (FBS, Invitrogen) and 100 units/ml of penicillin-streptomycin (PS, Invitrogen). Cells with passage number between 14 and 20 were used in this study. For cell culture on gel disks, cells trypsinized from polystyrene cell culture flasks were seeded onto gel surfaces at a density of 2,500 cells cm^{-2}. For the 3D culture

experiment, cells were encapsulated in the gel matrix at a density of 1 million cells/ml by mixing cells with the alginate solution before mixing with calcium sulfate.

Immunostaining of intracellular actin filaments: Cells cultured on gel disks for 24 hours were first fixed with 4 % formaldehyde in PBS, and permeabilized and blocked with a mixture of 0.05 % Triton X-100 and 5 % bovine serum albumin (Sigma) in PBS. Fixed cells were then incubated with a rhodamine phalloidin (Invitrogen) solution for 15 minutes. Images of immunostained actin filaments were captured using a fluorescence microscope (Olympus).

Gene transfection studies: After 24 hours of cell culture on gel disks, cells were exposed to rhodamine-labeled pDNA condensates for 12 hours. Then, gel surfaces were washed with PBS to remove pDNA condensates not taken up by cells. The images of pDNA condensates transfected into cells were captured using a fluorescence microscope (Olympus). The efficiency of gene transfer was analyzed by quantifying fluorescence yield from pDNA condensates, which was quantified by counting the number of pixels that expressed rhodamine fluorescence, using NIH image processing software. The analysis was conducted with more than 20 cells at each condition.

Measurement of gene expression level: After 24 hours of cell culture on gel disks or in the gel matrix, cells were exposed to pDNA condensates for another 24 hours. Then, cells were collected by dissolving gels with 50 mM ethylene diamine tetraacetic acid (EDTA, Sigma) in PBS. Cells were lysed with cell lysis buffer (Promega) in ice. Following ultracentrifugation to remove cell debris, the supernatants were mixed with Dual-Glo™ luciferase assay reagents (Promega). The luminisecne from the mixture was recorded with a luminometer (Turner design). The readings were normalized to the protein

concentrations in the lysed cell samples, which were measured from the absorbance at wavelength of 595 nm after mixing samples with protein assay solution [Bio-rad]. The analysis was conducted with 4 samples at each condition.

3H thymidine analysis: Cell proliferation was analyzed by exposing cells to [^{3}H] thymidine and quantifying its incorporation into DNA. After 24 hours of cell culture on gel disks, cell culture medium was exchanged with fresh medium containing [^{3}H] labeled thymidine (Perkin Elmer). After 24 hours, cells were collected by dissolving gels in 50 mM EDTA-PBS solution. Cells were lysed with 12 M NaCl aqueous solution, and [^{3}H] thymidine incorporation was quantified using a scintillation counter (Pharmacia). The analysis was conducted with 4 samples at each condition.

Statistical analysis: Statistical significance was evaluated using an unpaired student *t*-test for two-tailed p-value determination using Microsoft Excel Software, and differences were considered to be statistically significant for $p < 0.05$.

References

1. Rowley, J.A.; Madlambayan, G.; Mooney D.J. *Biomaterials* **1999**, 20:45.