ADVANCED MATERIALS

Supporting Information

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Independent Control of Topography for 3D Patterning of the ECM Microenvironment

*Jiyun Kim, Jack Rory Staunton, and Kandice Tanner**

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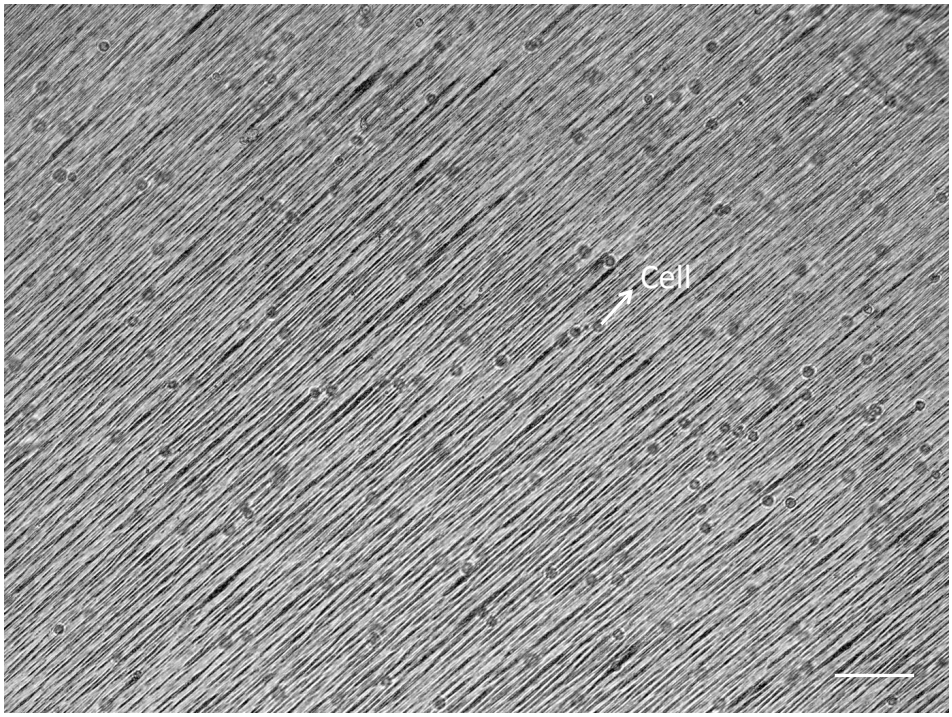

Figure S1. NIH 3T3 fibroblasts seeded in anisotropic matrix directly following matrix solidification. 0.2 mg/ml of superparamagnetic particles coated with fibronectins in Matrigel were mixed with cells, and an external magnetic field was applied to this mixture at 4°C for 5 min to cause the particles to assemble. Matrigel was solidified at 35°C for 20 min to confine both magnetic chains and cells in Matrigel. Then serum-free media was applied to this matrix to observe long-term cell behavior. Scale bars, 50 μm.

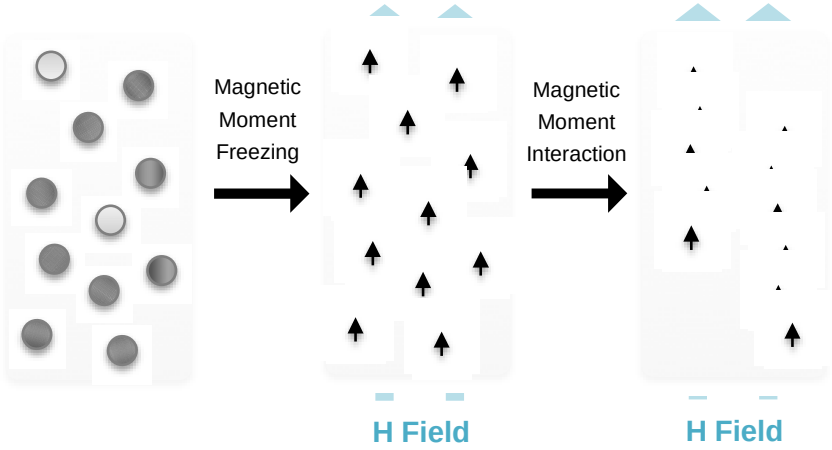

Figure S2. Schematic that describes the principle of magnetic field-directed assembly of superparamagnetic particles. Magnetic particles used as topographic building blocks are superparamagnetic materials whose magnetization randomly flips direction under the influence of temperature. Their magnetic susceptibility is much higher than that of paramagnetic materials. In the absence of an external magnetic field, these particles are randomly dispersed in a liquid, as they have no net magnetic moments at room temperature. When an external magnetic field is applied, the interaction energy between the external magnetic field and the intrinsic magnetic moment of a particle overcomes the thermal fluctuation energy, thus fixing the magnetic moments parallel to the direction of the applied magnetic field line. Then the particles are arranged into chain-like aggregates, minimizing the interaction energy of all magnetic moments. In other words, the assembled particles form nanostructures akin to "nano-fibers" along the magnetic field lines. Specifically, under the external magnetic field, the attractive magnetic force due to the magnetic dipoles of particles is balanced with the rheological resistance due to the fluid, and the particles in the fiber-like nanostructure dynamically build or maintain their arrangement, establishing equilibrium among the many forces involved in the assembling process. This process is termed magnetic field-directed self-assembly. Particles frozen into the nano-fibers are released when we remove the external magnetic field. Therefore, to maintain the arrangement without the aid of the external magnetic field, we must solidify the liquid matrix.

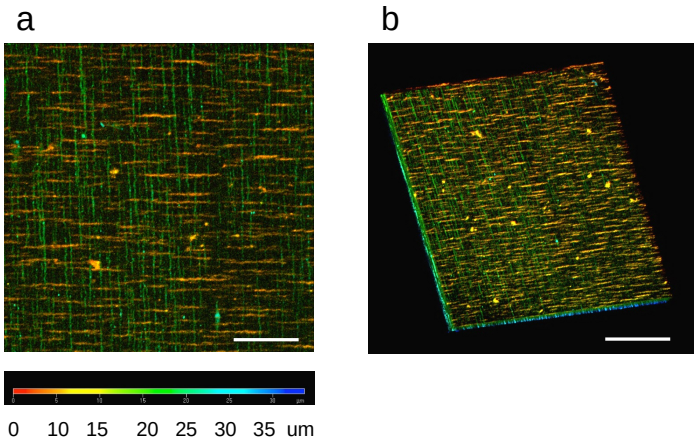

Figure S3. 3D images of stacked matrices with different topographies. (a-b) A hybrid structure that is stacked in layers is fabricated by sequential matrix polymerization. The topographies, composed of different types of ECM protein (fibronectin and laminin), are perpendicular to each other. Color gradation represents the z-length from the bottom. One limitation of this technology is different topographies cannot occur in the same space. Scale bars, 15 μm.

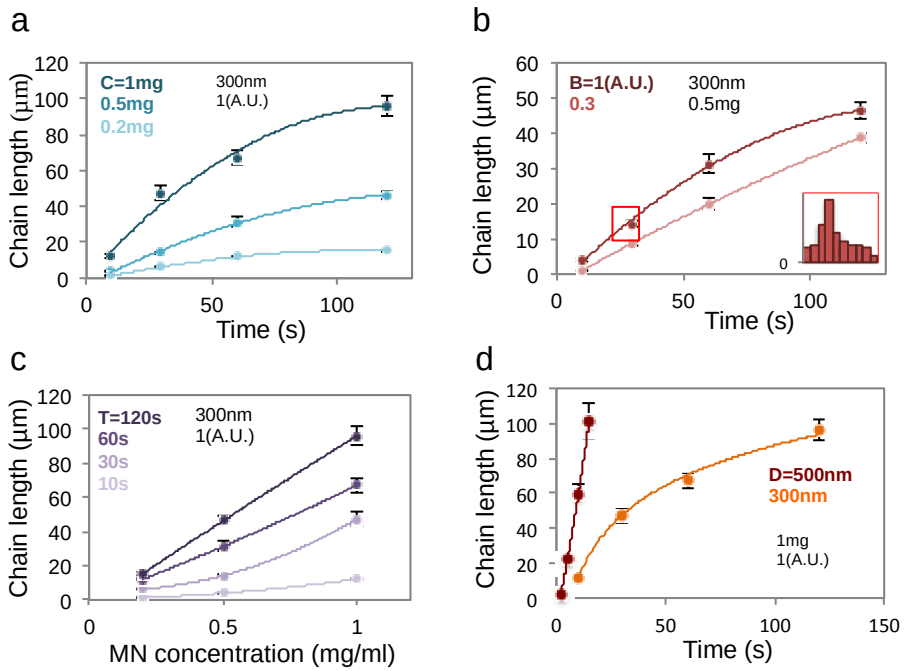

Figure S4. Self-assembling characteristics of superparamagnetic particles. The dimensions of nano-chains fabricated by magnetic field-directed self-assembly can be manipulated by various factors. Incremental change of self-assembling profile according to (a) the magnetic particle concentration (b) the magnetic field intensity determined by the distance from the magnet (c) magnetic field application time (Diameter of magnetic particles: 300 nm) (d) the diameters of magnetic particles.

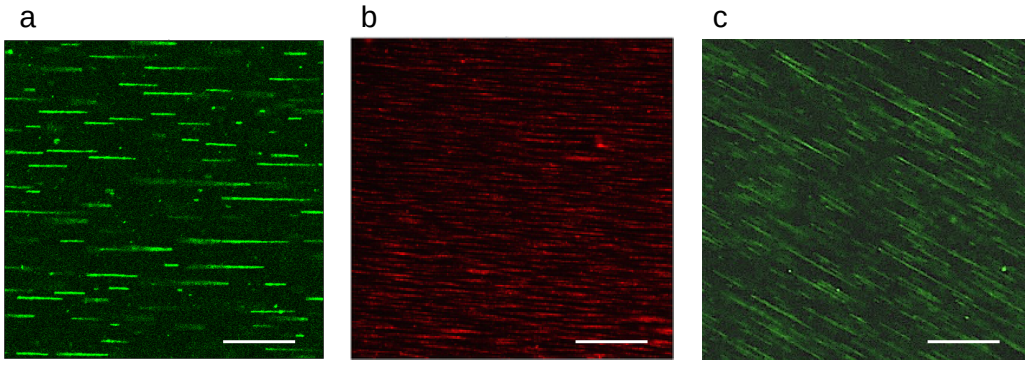

Figure S5. Introduction of fibril architecture to various matrices using magnetic field-directed self-assembly. (a) Culture media. (b) Matrigel. (c) Hyaluronic acid. Green and red colors represent fluorescent fibronectins and laminins that are chemically cross-linked on the carboxylated superparamagnetic particles, respectively. The self-assembling profiles differ according to the types of matrix that have distinct rheological characteristics. Scale bars, 100 μm.

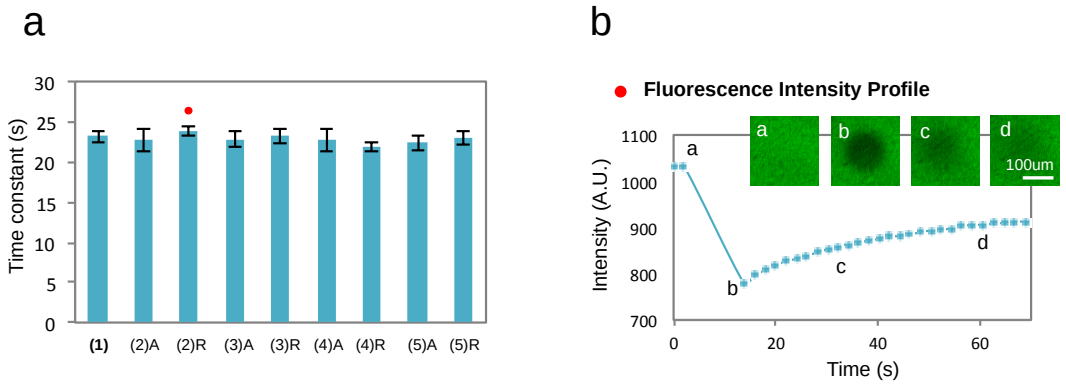

Figure S6. Comparison of biomolecule diffusional characteristics of engineered matrices using fluorescence recovery after photobleaching (FRAP). (a) We measured the diffusional time constant of Fluorescein isothiocyanate-dextrans (wt. 150000, Sigma Aldrich, FD150S). (1) 100% Matrigel. 200 nm of superparamagnetic particles coated with (2) Fibronectin and (3) Laminin. 300 nm of superparamagnetic particles coated with (4) Fibronectin, and (5) Laminin. 'R' represents isotropic matrix and 'A' represents anisotropic matrix. All samples contain 0.2 mg/ml of particles in Matrigel. Each bar represents an average of 10 cases obtained from 2 samples and each error bar represents the standard error. Clearly, the rate of diffusion of the proteins is not significantly affected by the incorporation of particles in the matrix.

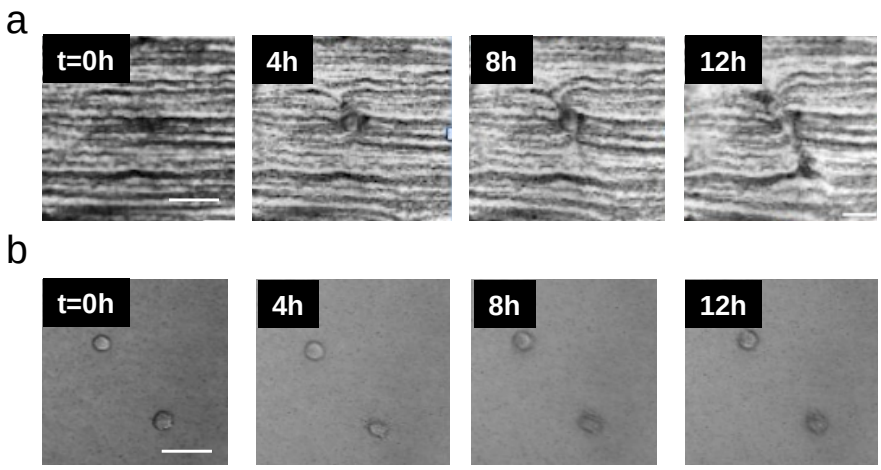

Figure S7. Time-lapse images of NIH 3T3 fibroblast growth in an engineered matrix. (a) Fibroblasts in anisotropic, and (b) in isotropic matrices. The particles are initially coated with fibronectin and 0.2 mg/ml of these particles are added to Matrigel with fibroblasts. In the anisotropic matrix, a single cell elongates its body and extends the dendrites, deforming the fibril architecture composed of nano-chains. On the other hand, the cells in an isotropic matrix explore the architecture of the matrix by stretching their filopodia in all directions, but do not elongate during 12 hours. Scale bars, 30 μm.

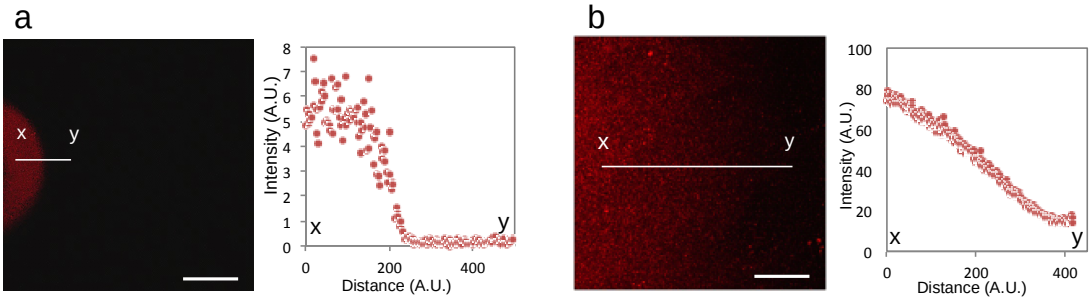

Figure S8. Distribution of laminin-coated magnetic particles achieved by diffusion in Matrigel. (a) Localization of particles. Scale bar, 100 μm. (b) Gradated distribution of particles. Scale bar, 50 μm. Matrigel is a relatively viscous material and its gelation depends on temperature and time; at lower temperatures from 2-8 degrees, Matrigel remains in its liquid state, and at higher temperatures, the gelation process is accelerated. When we drop a liquid droplet containing a high concentration of particles into the matrix, the particles slowly diffuse into the adjacent area, creating a gradated distribution (the particles move by diffusion, down to the concentration gradient, into the matrix). To obtain a localized pattern of particles, as shown in panel (a), we increased the temperature immediately after the particle droplet was added to the Matrigel. We also could obtain a gradated distribution of particles by allowing time for the particles to diffuse into the matrix before we increased the temperature for gelation. In other words, the matrix type determines the diffusion characteristics and the solidification time determines the gradation pattern at the final state. Unlike other polymer-crosslinking-based ligand binding methods, this method does depend on increasing the concentration of ligand-binding sites to increase the material stiffness.

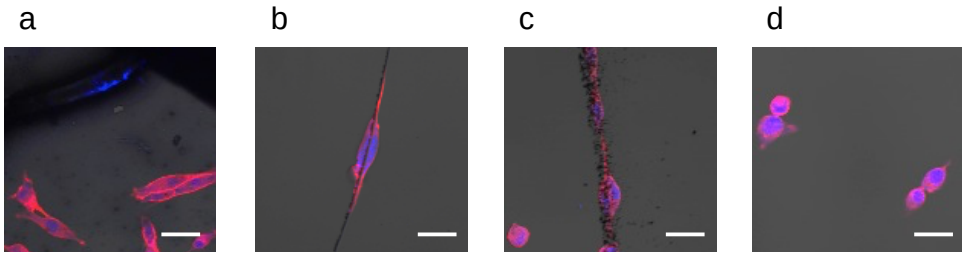

Figure S9. Morphology of NIH 3T3 fibroblasts in 2D where fibronectin-coated particles are physically immobilized. (a) Fibroblasts on particle-coated substrate. (b) Fibroblasts grown on a fiber composed of particles. (c) Fibroblasts grown on narrow line composed of particles. (d) Fibroblasts grown on glass substrate. To physically immobilize the particles on the glass substrate, liquid containing particles coated with fibronectin was dropped onto an 8-well glass plate and evaporated at room temperature overnight. By drying, some particles are aggregated, forming a planar particle sheet and some particles are dried into the shape of fibers due to the coffee ring effect. We spread the cells (10K) on both particle-immobilized substrate and pure glass substrate, and observed their behavior for 6 hours. Clearly, the fibroblasts grew better on the protein-coated particles. Scale bars, 15 μm.