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

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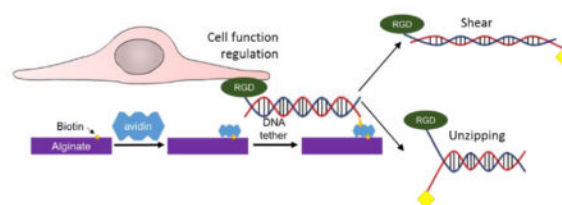
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Abstract

Recent efforts to design a synthetic extracellular matrix for cell culture, engineering, and therapies greatly contributed to addressing biological roles of types and spatial organization of cell adhesion ligands. It is often suggested that ligand-matrix bond strength is another path to regulate cell adhesion and activities; however tools are lacking. To this end, this study demonstrates that a hydrogel coupled by integrin-binding deoxyribonucleic acid (DNA) tethers with pre-defined rupture forces can modulate cell adhesion, differentiation, and secretion activities due to the changes in the number and, likely, force of cells adhered to a gel. The rupture force of DNA tethers was tuned by altering spatial arrangement of matrix-binding biotin groups. The DNA tethers were immobilized on a hydrogel of alginate grafted with biotin using avidin. Mesenchymal stem cells showed enhanced adhesion, neural differentiation, and paracrine secretion when cultured on the gel coupled with DNA tethers with higher rupture forces. Such innovative cell-matrix interface engineering would be broadly useful to a series of materials used for fundamental and applied studies on biological cells.

Graphical Abstract



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Integrin-binding peptides and proteins have been extensively employed to regulate adhesion of biological cells to materials of interests used as a synthetic extracellular matrix (ECM).¹ These cell adhesion ligands are chemically conjugated to or physically associated with molecular building blocks of the synthetic ECM. Previous studies have demonstrated that cell adhesion and functionality could be controlled with the type, density, and spatial organization of cell adhesion ligands.²⁻⁴ For instance, the affinity and density of the cell adhesion ligands on a matrix can regulate differentiation levels of various precursor, progenitor, and stem cells.⁵ The distance between the ligands at the nanometer scale can also change the morphology, focal adhesion formation, and differentiation of cells.⁶ Additionally, the ligand density can direct cells adhered to a soft matrix to behave like those adhered to a rigid material.^{4, 7}

Furthermore, linkage of the cell adhesion ligands to a matrix may mediate phenotypic activities of cells. For example, the absence of chemical linkages between cell adhesion ligands and a matrix significantly reduces the cell adhesion force and, subsequently, the extent of cell spreading and frequency of cell division.⁸ Therefore, it has been suggested that tailoring strength of the cell-matrix linkage may further mediate cellular activities in a refined manner.⁹ Recently, it was demonstrated that deoxyribonucleic acid (DNA) with pre-defined rupture forces can be used as a tension gauge at the molecular level by inserting it between cell adhesion peptides and a matrix. Then, cells can rupture or retain DNA tethers, termed tension gauge tethers (TGTs) depending on the magnitude of the force that cells exert.¹⁰

We therefore hypothesized that the strength of a molecular linker that connects a cell adhesion ligand to a material would play important roles in regulating cellular function. To examine this hypothesis, DNA tether coupled with biotin and cell adhesion peptide containing Arg-Gly-Asp (RGD) sequence was used as a model linker that tunes the strength of RGD peptide-matrix bonds. The biotin group was connected to different sites in the DNA tether in order to regulate the rupture force. The resulting DNA tether was linked to hydrogels of alginate conjugated with biotins via specific biotin-avidin bonds. Bone marrow-derived mesenchymal stem cells (MSCs) were cultured on the hydrogels. Then, the role of DNA tether was evaluated by analyzing adhesion, neural differentiation, and proangiogenic factor secretion of MSCs. Taken together, the results of this study would greatly serve to advance the ability to regulate cellular activities by engineering cell-matrix interface.

As previously reported, integrin-binding DNA tethers were prepared by annealing two single-stranded DNA (ssDNA) chains (Fig. 1a).¹⁰ One ssDNA chain was modified with thiol group at 3' end and Cy5 at 5' end, while the complementary DNA strand is modified with biotin at different sites. The thiolated strand of DNA tether was further modified to present RGD peptides via Michael addition reaction between amine ends (or lysine residue) of the peptides and the thiol group. The resulting RGD-ssDNA was annealed with the complementary ssDNA conjugated with biotin at different locations by incubating two molecules in the annealing buffer. A previous study demonstrated that the tension tolerance value (T_{tol}) of the TGT in the unzipping mode, where biotin is attached to the complementary strand opposite to the RGD peptide, is about 12 pN regardless of the length

of DNA tether.¹¹ On the other hand, T_{tol} of TGTs in shear mode or intermediate mode, where the other end or internal locations of the complementary strand are modified with biotin, varies depending on the site of biotin labeling.¹² In this study, we used DNA tethers with T_{tol} values of 12, 43, and 54 pN, which were estimated by the equation

$F=2f_c \left[X^{-1} \tanh \left(X \frac{L}{2} \right) + 1 \right]$, where $2f_c$ is the rupture force for one single bond, L is the number of DNA base pairs, $X^{-1} = \sqrt{Q/2R}$ where Q is the spring constant of the backbone, and R is the spring constant of the bond between base pairs. Empirical data measured using magnetic tweezers showed that $f_c p = 3.9$ pN and $X^{-1} = 6.8$.¹²

Separately, alginate molecules, a molecular building block of a hydrogel, were chemically coupled with biotin groups using N-hydroxy-succinimide/ethyl-dimethyl-aminopropyl-carbodiimide (NHS/EDC) chemistry (Fig. 1b). The molar ratio between biotin and alginate units was kept constant at 20%. The conjugation of biotin to alginate was confirmed with peaks in the ¹H nuclear magnetic resonance (NMR) spectra. (Fig. S1).

The DNA tether coupled with RGD peptides, termed RGD-DNA tether, was immobilized to alginate conjugated with biotin, termed alginate-g-biotin. The hydrogel was formed by cross-linking alginate-g-biotin molecules with adipic acid dihydrazide (AAD) in aqueous media using NHS/EDC chemistry. By varying the molar ratio between the AAD cross-linker and uronic acids of the alginate-g-biotin from 0.05 to 0.2, the elastic modulus of alginate-g-biotin gel was tuned from 21 to 82 kPa (Fig. 2a). The conjugation of biotin minimally affected gel elastic modulus compared to unconjugated alginate gels (Fig. 2a).

The resulting gel was incubated with avidin, rinsed, and further incubated with DNA tethers (Fig. 2b). In the presence of avidin, alginate-g-biotin gel incubated with the Cy5-labeled DNA tether presented fluorescence emission upon excitation (Fig. 2c). The fluorescence emission intensity was independent of an elastic modulus of the gel, but the fluorescence emission was significantly lower on the gel incubated with Cy5-labeled DNA tether without avidin, although certain amount of DNA tether was non-specifically bound to the alginate-g-biotin hydrogel. (Fig. 2c).

We studied the effects of T_{tol} on cellular adhesion by plating MSCs on the gel coated with RGD-DNA tether. Previous studies demonstrated that alginate gel without RGD peptides does not support cellular adhesion.¹³ More cells adhered to the 44 kPa hydrogel modified with the RGD-DNA tether with higher T_{tol} values (Fig. 3a). Extent of cell spreading also increased with increasing T_{tol} from 12 to 54 pN (Fig. 3b).^{14, 15}

The hydrogel-TGT system was also used to examine whether the RGD-DNA tether can regulate neural differentiation level of MSCs at a given RGD density and elastic modulus. MSCs adhered to the hydrogel-TGT system were incubated in a media formulated to activate neural differentiation (Fig. 3c). Differentiated neural cells were marked by antibody to microtubule-associated protein 2 (MAP2). Interestingly, the ratio of MAP2-positive cells increased with increasing T_{tol} value in 44 kPa hydrogel-TGT system (Fig. 3c). On gels functionalized by the RGD-DNA tether with T_{tol} of 54 and 43 pN, 95% and 88% of the cells

expressed MAP2, respectively (Fig. 3c). On the other hand, only 27% of the cells were MAP2 positive on hydrogel-TGT system with T_{tol} value of 12 pN (Fig. 3c).

We next tested if T_{tol} values can modulate cellular activities to secrete growth factors (Fig. 3d). Vascular endothelial growth factor (VEGF) is known to stimulate vascular sprouting from existing blood vessels, in a process known as angiogenesis.¹⁶ Thus, VEGF enhances outcomes of wound healing and tissue regeneration.¹⁷ Interestingly, the 82 kPa hydrogel-TGT system with T_{tol} value of 54 pN released 1.6-fold higher amount of VEGF than that with T_{tol} value of 12 pN (Fig. 3d). Additionally, the dependency of VEGF secretion level on T_{tol} value was mediated by an elastic modulus of the gel (Fig. 3d). In particular, the gel with an elastic modulus of 21 kPa abolished effects of T_{tol} values on cellular VEGF secretion level while increasing T_{tol} enhanced the secretion of VEGF in 44 and 82 kPa gels.

The MSC-adhered gel was further implanted on chicken chorioallantoic membrane in order to assess the capability of the RGD-DNA tether to promote vascularization *in vivo*. The 82 kPa gel immobilized with the RGD-DNA tether with T_{tol} of 54 pN led to a significant increase of vascular density at the site of implantation as compared with the gel coupled with the RGD-DNA tether with T_{tol} of 12 pN (Fig. 3e).

Taken together, this study demonstrates that the ability of cell-laden hydrogels to form neural networks and release proangiogenic factor can be modulated by coupling integrin-binding RGD-DNA tether with predefined T_{tol} values to the gel. We propose that such dependency of cell-laden gel function on T_{tol} values is mainly attributed to the number of cells adhered to the gel as experimentally confirmed. As expected, cells plated on a gel functionalized by the RGD-DNA with a lower T_{tol} value, in particular 12 pN, would readily separate ssDNA and fail to adhere to the gel. Conversely, the RGD-DNA with higher T_{tol} values may resist cell-induced separation, because the ultimate strength is higher than cell-exerting forces. Furthermore, the stronger RGD-DNA tether may be advantageous in activating cellular signaling involved in differentiation and secretion activities, because a small increase of T_{tol} from 43 to 54 pN elevated cellular differentiation and secretion levels despite the small difference of the number of cells adhered to the gel. These results implicate that RGD-DNA tether may partially modulate cellular traction forces by controlling cell spreading and further cell signaling pathways, independent of gel mechanics and cell adhesion ligand type and density. To the best of our knowledge, this is the first work to demonstrate a capability to regulate cell differentiation and growth factor secretion with ligand-matrix bond strength.

More interestingly, effects of tether strength on cellular secretion activities are mediated by gel stiffness. This result implicates combinatorial effects of matrix stiffness and interfacial mechanics on cellular signaling. This perspective will be more thoroughly studied in near future studies via both theoretical modeling and experimental measurements.

In conclusion, a hydrogel engineered with RGD-DNA tethers of pre-defined strength allows us to regulate adhesion and phenotypic activities of biological cells by modulating both number and, likely, traction force of cells adhered to the gel matrix. We propose that the results of this study would be advantageous in assembling cell-laden gel implants that

should remain structurally stable at the site of implantation while providing cells an environment appropriate to stimulate cellular activities desirable for therapies. This method would also allow us to orthogonally regulate cellular activities with bulk gel properties and cell-gel interface. Overall, the results of this study to engineer cell-hydrogel interface mechanics present a new perspective of material design used for culture and therapies of a wide array of stem and progenitor cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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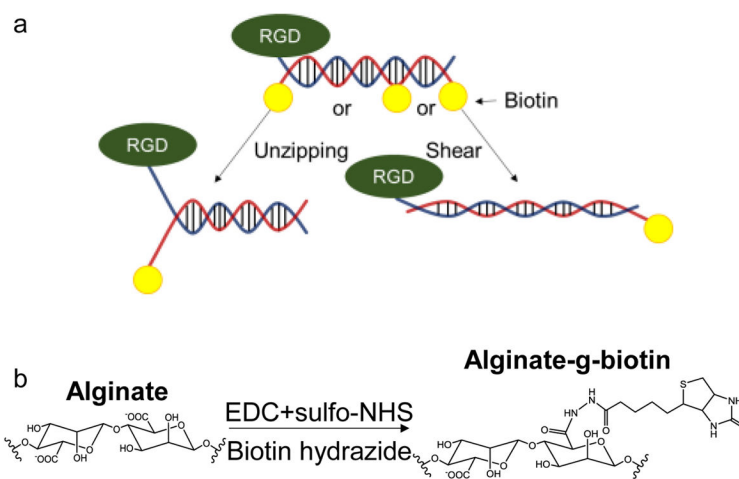


Figure 1. Synthesis and characterization of RGD-DNA tether and alginate-g-biotin
 a. Scheme of different force geometry of DNA tethers. b. Reaction scheme to synthesize alginate-g-biotin.

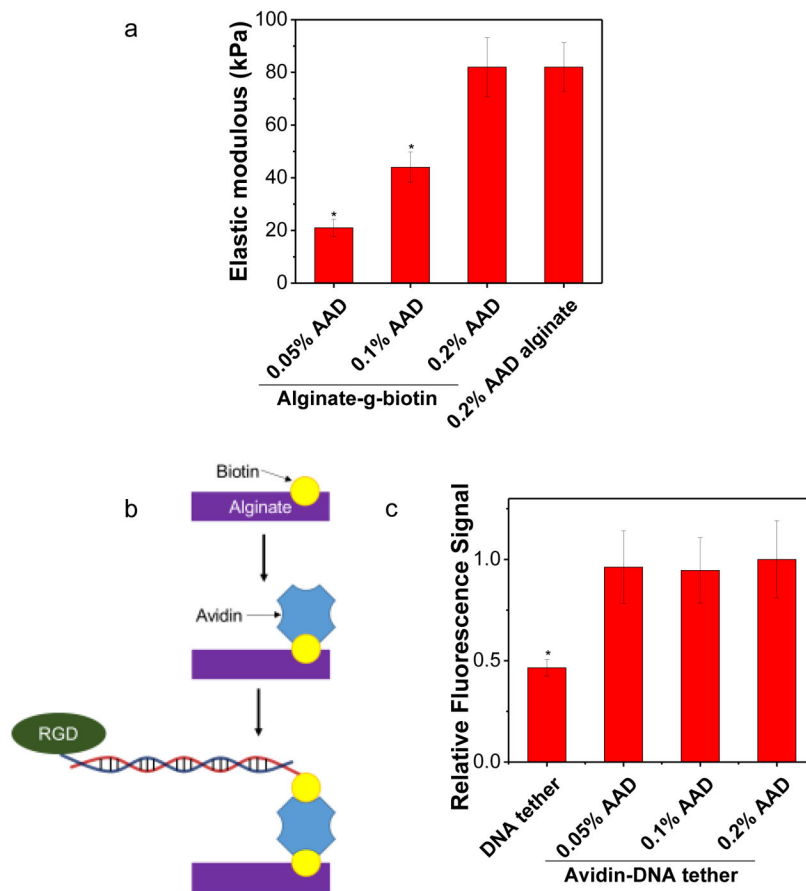


Figure 2. Surface conjugation of RGD-DNA tethers to the alginate-g-biotin hydrogel
 a, Elastic modulus of alginate-g-biotin hydrogels controlled with different molar ratio between the AAD cross-linker and alginate-g-biotin. b, Schematics of the process to immobilize RGD-DNA tether to the alginate-g-biotin gel. c, Fluorescence from the DNA tether coupled to the gel due to biotin-avidin bonds, independent of the molar ratio between the AAD cross-linker and alginate-g-biotin. “DNA tether” represents a condition at which RGD-DNA tether was incubated with the gel in the absence of avidin. The error bars represent the standard deviation of three measurements. * indicates statistical significance of the difference compared to any other group (* $p < 0.05$).

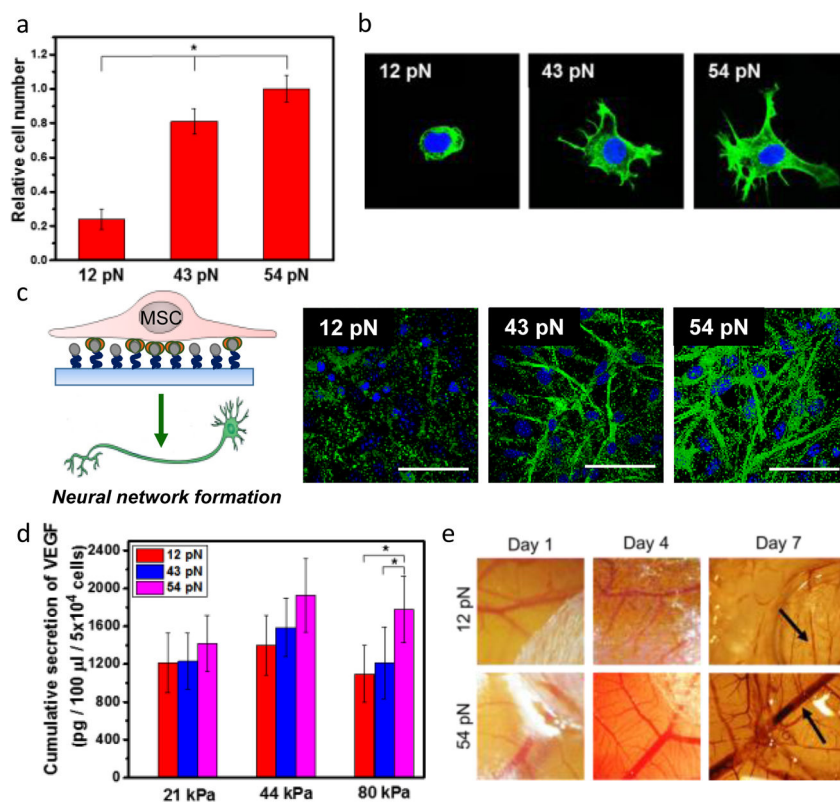


Figure 3. Analysis of the role of T_{tol} of RGD-DNA tether on adhesion and phenotypic activities of MSCs

a, Quantification of the number of MSCs adhered to a hydrogel modified with RGD-DNA tether with controlled T_{tol} . b, Fluorescence image of F-actin (green) of MSCs. c, Analysis of neural differentiation of MSCs. Differentiated neural cells were positively stained for MAP-2 (green). d, The total amount of VEGF secreted by MSCs cultured on alginate-g-biotin hydrogels with different elastic moduli modified with RGD-DNA tether with controlled T_{tol} . e, Microvascular networks of the CAM implanted with the MSC-hydrogel constructs. Arrows indicate blood vessels. The error bars represent the standard deviation of three measurements. * indicates statistical significance of the difference (* $p < 0.05$).