Supplementary Information

Material	Example Cell Culture Studies and Experimental Outcomes	Specific Recipes and Properties of Hydrogel Used
Natural Materials		
Collagen	Mouse fibroblasts were shown to invade into collagen hydrogels in an MT1-MMP-dependent manner[1]	2.2 mg ml^{-1} rat or mice tail type I collagen hydrogel; Hydrogels were formed at 37°C for 45 min in bottom chamber of Transwell setup while cells were seeded in upper chamber (pore size 3 μm)[1]
	MSCs cultured in collagen hydrogels require dynamic loading for maintaining tenogenic markers and suggest a role for Wnt in tenogenesis similar to chondrogenesis[2]	2.4 mg ml^{-1} type I collagen hydrogel in PBS at pH 7.4 (Vitrogen, Cohesion Inc); MSCs encapsulated at 2 x 10^6 cells ml^{-1}; Hydrogels formed via self-assembly for 1-2 h at 37°C[2]
	3T3 fibroblasts cultured on soft (G ~ 100-400 Pa) collagen hydrogels show robust spreading and focal adhesion formation without YAP nuclear localization, perhaps highlighting the importance of fibrous architecture and substrate viscoelasticity in cell mechanotransduction studies[3]	2 and 4 mg ml^{-1} rat tail type I collagen hydrogel in PBS at pH 7.3 (BD Biosciences); Hydrogels formed via self-assembly in 35 mm glass bottom dishes for 90 min at 37°C; Cells seeded on top of hydrogels; Hydrogel shear moduli were 104 and 391 Pa for 2 and 4 mg ml^{-1} formulation respectively[3]
	HT-1080 fibrosarcoma and MDA-MB-231 carcinoma cells cultured in 3D collagen matrices showed protease-independent mechanisms of migration/invasion by transitioning from a mesenchymal to amoeboid morphology[4]	1.67 mg ml^{-1} dermal bovine type I collagen hydrogels (Vitrogen, Cohesion Inc)[4]
Fibrin	Embryonic chick tendon fibroblasts were seeded on fibrin Hydrogels anchored into well plates to form contraction-mediated collagenous microtissues[5]	5.7 mg ml^{-1} fibrinogen (Sigma), 35.7 U ml^{-1} thrombin (Calbiochem), and 14.3 μg ml^{-1} aprotinin (Roche) were combined in cell culture media; Gelation occurred at

		37°C for 1 h; Embryonic chick tendon fibroblasts were cultured on top of hydrogels[5]
	Increased pore elongation in fibrin constructs resulted in improved myofibroblast formation and alignment, leading to increased total contractile force[6]	4 mg ml^{-1} bovine fibrinogen (Sigma), 0.8 U ml^{-1} thrombin (Sigma), and 10% v/v Matrigel in PDMS mold; 10 million rat neonatal rat skeletal myoblasts per ml were encapsulated; Gelation was for 45-60 min at 37°C[6]
Alginate	Mouse MSCs were shown to sense 3D substrate stiffness with osteogenesis preferentially occurring at higher (11-30 kPa) stiffnesses; this finding was not dependent on cell shape but instead on traction-mediated nanoscale re-organization of adhesion ligands[7]	1-5 wt% alginate (FMC Biopolymer) with 6.25-50 mM calcium content yielded hydrogels with mechanics ranging from 2.5-110 kPa; Hydrogels were covalently modified with either 189 or 754 μM RGD; Mouse MSCs (D1) were encapsulated at 2 x 10^7 cells ml^{-1} [7]
	3T3 fibroblasts showed increased spreading and YAP nuclear localization when cultured on stress relaxing hydrogels mimicking tissue viscoelasticity compared to stiffness-matched elastic substrates[8]	1 wt% guluronic acid-rich high molecular weight (280 kDa) alginate (FMC Biopolymer) was used with RGD peptide conjugated via carbodiimide chemistry at either 150, 750, or 1500 μM; Hydrogels were either ionically crosslinked with calcium sulfate or covalently crosslinked using carbodiimide chemistry; Cells were seeded at a density of 1 x 10^4 cells cm^{-2} and cultured for 20 h[8]
Synthetic Materials		
Polyacrylamide (PA)	Rat kidney epithelial cells and 3T3 fibroblasts were cultured on collagen-coated polyacrylamide	10% acrylamide and bis-acrylamide ranging from 0.03 to 0.26%; Increased bis-

	substrates of varying stiffness; Cells on softer substrates showed reduced spreading, increased motility, and irregularly shaped focal adhesions compared to cells on stiffer substrates[9]	acrylamide corresponds to increased elastic modulus; Hydrogels were coated with type I collagen (0.2 mg ml^{-1}) using sulfo-SANPAH chemistry[9]
	Lineage commitment of naïve human MSCs was shown to be heavily influenced by substrate stiffness alone, with MSCs displaying neurogenic, myogenic, and osteogenic phenotypes on soft (0.1-1 kPa), stiff (8-17 kPa), and rigid (25-40 kPa) polyacrylamide substrates respectively[10]	5 or 10% acrylamide and bis-acrylamide ranging from 0.03-0.3%; 5% acrylamide hydrogel elastic modulus ranged from ~ 1 kPa (0.03% bis-acrylamide) to ~ 8 kPa (0.3%); Hydrogels were coated with type I collagen (0.25-1 μg cm^{-2}) using sulfo-SANPAH chemistry[10]
	The stiffness of 2D cell culture substrates was shown to regulate human ASC and MSC fate independent of hydrogel pore size and protein tethering[11]	A range of acrylamide/bis-acrylamide percentages were used depending on hydrogel elastic modulus: 4 kPa (4/0.40, 6/0.06, 10/0.02), 13 kPa (6/0.45, 10/0.10, 20/0.03), and 30 kPa (8/0.55, 10/0.30, 20/0.18); Higher acrylamide/bis-acrylamide ratios for a given stiffness resulted in larger pore size; Human ASCs or MSCs were seeded at 1000 cells cm^{-2}; Hydrogels were coated with type I collagen (0.02-1 mg ml^{-1}) using sulfo-SANPAH chemistry[11]
	Human MSCs were cultured on hydrogels with constant compressive modulus but varying loss modulus; MSCs on substrates with more viscoelastic character (higher loss modulus) had greater spread area, less mature focal adhesions, and were capable of more efficient differentiation down various lineages[12]	Hydrogels with constant shear modulus (G' ~ 4.5 kPa) but varying loss modulus (G'') were synthesized by changing acrylamide/bis-acrylamide percentages; 15/0.0125 (G" ~ 130 Pa), 12/0.0358 (G" ~ 10 Pa), and 8/1 (G" ~ 1 Pa); Human MSCs were seeded at 4000 cells cm^{-2}; Hydrogels were coated with type I collagen (50 μg ml^{-1}) using sulfo-

		SANPAH chemistry[12]
Polyethylene glycol (PEG)	PEG hydrogels modified with RGD were used to photoencapsulate osteoblasts; Osteoblasts showed better survival at lower (10%) PEG concentrations and improved attachment, spreading, and mineralization with RGD incorporation[13]	10-30% PEG-diacrylate modified with 0.5 or 5 mM acrylated RGD; Rat calvarial osteoblasts were photoencapsulated at 50 x 10^6 cells ml^{-1} with UV light at ~ 4 mW cm^{-2} for 5 min[13]
	PEG hydrogels incorporating crosslinkers susceptible to MMP degradation were developed; Human fibroblasts invaded the hydrogel in an MMP-dependent manner[14]	10% 4 arm-PEG-tetravinyl sulfone (20 kDa) was reacted with cysteine-containing peptides containing RGD or MMP-sensitive sequences; Crosslinking occurs via Michael addition at pH 8 within a few minutes; Fibrin-protected human foreskin fibroblasts were embedded within hydrogels to assess invasion[14]
	PEG-based photodegradable hydrogels were developed to enable spatiotemporal control of hydrogel features to explore cell phenomena including migration and differentiation[15]	PEG-*bis*-amine is reacted with nitrobenzyl ether-derived moiety to create photolabile groups; The photolabile crosslinker is reacted with PEG acrylate using APS/TEMED redox to form hydrogels; RGD is incorportated to permit cell attachment; Human MSCs were encapsulated at 2 x 10^7 cells ml^{-1} [15]
	Maleimide-functionalized PEG hydrogels are shown to hydrogel more rapidly (1-5 min) compared to other modified PEG hydrogels formed by Michael addition (acrylate, vinyl sulfone); PEG-maleimide hydrogels can be	10% 4-arm-PEG-maleimide (20 kDa) was reacted with cysteine (thiol)-containing RGD and MMP-sensitive sequences; Crosslinking occurs rapidly via Michael addition at pH 7.4; Murine

	formed at lower concentrations and facilitate improved cell survival and spreading[16]	C2C12 myoblasts were encapsulated at 3 x 10^6 cells ml^{-1} [16]
Hybrid materials		
Hyaluronic acid (HA)	Methacrylated HA planar hydrogels are described that can be stiffened in the presence of cells from ~ 3 kPa to ~ 30 kPa via sequential crosslinking; Human MSCs spread rapidly and exert greater traction forces in response to stiffening; In mixed osteogenic/adipogenic culture late stiffening time points favor adipogenesis while earlier stiffening preferentially promotes osteogenesis[17]	3 wt% methacrylated HA (100% modification) crosslinked via Michael addition at pH 10 for 1 h with DTT (~ 18% methacrylate consumption) to yield hydrogel with E ~ 3 kPa; Hydrogels incorporated 0.5-2 mM RGD peptide; Hydrogels could be stiffened via photocrosslinking of remaining network methacrylates in presence of photointiator (0.05 wt% I2959) and light (UV light 365 nm 2 min at 10 mW cm^{-2}) to E ~ 30 kPa[17]
	Degradation-mediated traction force generation directs human MSC differentiation in covalently crosslinked HA hydrogels; Restrictive hydrogels promote adipogenesis regardless of hydrogel modulus while hydrogels permissive to cell-mediated degradation promote traction force generation and osteogenesis[18]	1.5-2.5 wt% HA hydrogels modified with methacrylates (or both methacrylates and maleimides) were fabricated; Methacrylated HA was formed by UV crosslinking (0.05 wt% I2959, 10 mW cm^{-2}); Methacrylate/Maleimide HA hydrogels were formed by an initial addition reaction with MMP-sensitive peptides consuming the maleimides followed by photocrosslinking of hydrogel methacrylates at same conditions as above; Human MSCs were encapsulated at 15 million cells ml^{-1} (MeHA) or 1 million cells ml^{-1} (MeMaHA)[18]
Polypeptides	A self-assembled peptide hydrogel was developed for	0.5 wt% KLD12 peptide (AcN-KLDLKLDLKLDL-CNH_2) in

	cartilage tissue engineering; chondrocytes were encapsulated for 4 weeks *in vitro* and showed phenotypic maintenance, deposition of cartilage-like ECM, and increasing stiffness over culture time[19]	295 mM sucrose; Bovine chondrocytes were encapsulated at 15 x 10^6 cells ml^{-1}; Hydrogel was self-assembled in PBS bath for 25 min[19]
	3D peptide hydrogels encapsulating mouse ESCs or iPSCs supported improved generation of dopaminergic neurons compared to 2D substrates or 3D Matrigel cultures[20]	Mouse ESCs or iPSCs were mixed with RADA16 peptide (PuraMatrix, BD Biosciences) to form hydrogels; Cells were encapsulated at 5 x 10^7 cells ml^{-1} [20]
	VEGF-mimetic peptide amphiphile was designed to self-assemble into nanofibrils that displayed the VEGF-mimetic sequence on their surface; This peptide promoted phosphorylation of VEGF receptors and proangiogenic responses[21]	VEGF-mimetic presenting peptide C_{16}-$V_2A_2K_3$-GKLTWQELYQLKYKGI-NH_2 where the underlined sequence is the VEGF mimetic was synthesized by standard solid phase Fmoc chemistry[21]

Supplementary Table 1. Specific examples of cell studies performed with hydrogels with details of hydrogel formation included.

Supplementary References

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