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Review article

Emerging themes and unifying concepts underlying cell behavior regulation by the pericellular space

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ABSTRACT

Cells reside in a complex three-dimensional (3D) microenvironment where physical, chemical, and architectural features of the pericellular space regulate important cellular functions like migration, differentiation, and morphogenesis. A major goal of tissue engineering is to identify which properties of the pericellular space orchestrate these emergent cell behaviors and how. In this review, we highlight recent studies at the interface of biomaterials and single cell biophysics that are lending deeper insight towards this goal. Advanced methods have enabled the decoupling of architectural and mechanical features of the microenvironment, revealing multiple mechanisms of adhesion and mechanosensing modulation by biomaterials. Such studies are revealing important roles for pericellular space degradability, hydration, and adhesion competition in cell shape, volume, and differentiation regulation.

Statement of significance

Cell fate and function are closely regulated by the local extracellular microenvironment. Advanced methods at the interface of single cell biophysics and biomaterials have shed new light on regulators of cell-pericellular space interactions by decoupling more features of the complex pericellular milieu than ever before. These findings lend deeper mechanistic insight into how biomaterials can be designed to fine-tune outcomes like differentiation, migration, and collective morphogenesis.

Contents

Abbreviations: ECM, extracellular matrix; PEG, polyethylene glycol; RGD, arginine-glycine-aspartic acid; 3D, three-dimensional; 2D, two-dimensional; EMT, epithelial-to-mesenchymal transition; MSC, mesenchymal stem cell; DAH, differential adhesion hypothesis; HA, hyaluronic acid; GAGs, glycosaminoglycans; M-TRAIL, matrix-labeling techniques for real-time and inferred location; TFM, traction force microscopy; MMC, macromolecular crowding; MMPs, matrix metalloproteinases; hMSC, human mesenchymal stem cells; GPCR, G-protein coupled receptor; SEM, scanning electron microscopy; AFM, atomic force microscopy; pMLC, phosphorylated myosin light chain; FAK, phosphorylated focal adhesion kinase; MeHA, methacrylated hyaluronic acid; PA, polyacrylamide; STORM, (stochastic optical reconstruction microscopy; eN&B, enhanced number and brightness; HUVECs, human umbilical vein epithelial cells; DexMA, dextran methacrylate.

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1. Introduction

The pericellular space is the region surrounding the cell by about 100 μm [1–4] and serves as a key source of chemical and mechanical stimuli [5] (Fig. 1a). Cells begin to actively and passively modify the pericellular space immediately after cell-seeding through processes including degradation [6–8] and/or extracellular matrix (ECM) deposition [9,10]. The initial stimuli provided by the pericellular space and its susceptibility to change both play important roles in directing cell behavior through the regulation of adhesion formation and stability [11], cytoskeletal polymerization and contractility [6,12], and changes in ion channel activity [13,14]. The collective action of these features determine the morphological landscape of cells [13]. Morphology, which includes cell shape, volume, and the organization of intracellular organelles, ultimately integrates mechanical and molecular cues into macroscopic scale outcomes such as migration, differentiation, and morphogenesis (Fig. 1, b and c). Thus, fundamental understanding of cellular behavior relies on knowledge of these dynamic cell-pericellular space interactions and their effect on morphology. Mechanistic understanding of these processes is particularly relevant for understanding diseases involving complex microenvironmental dysregulation, such as cancer, osteolysis, arthritis, and fibrotic diseases which include heart failure, pulmonary fibrosis, and cirrhosis [15]. Here, we review recent efforts to study these interactions and discuss the remaining challenges and promise of time dynamic studies and quantitative modeling.

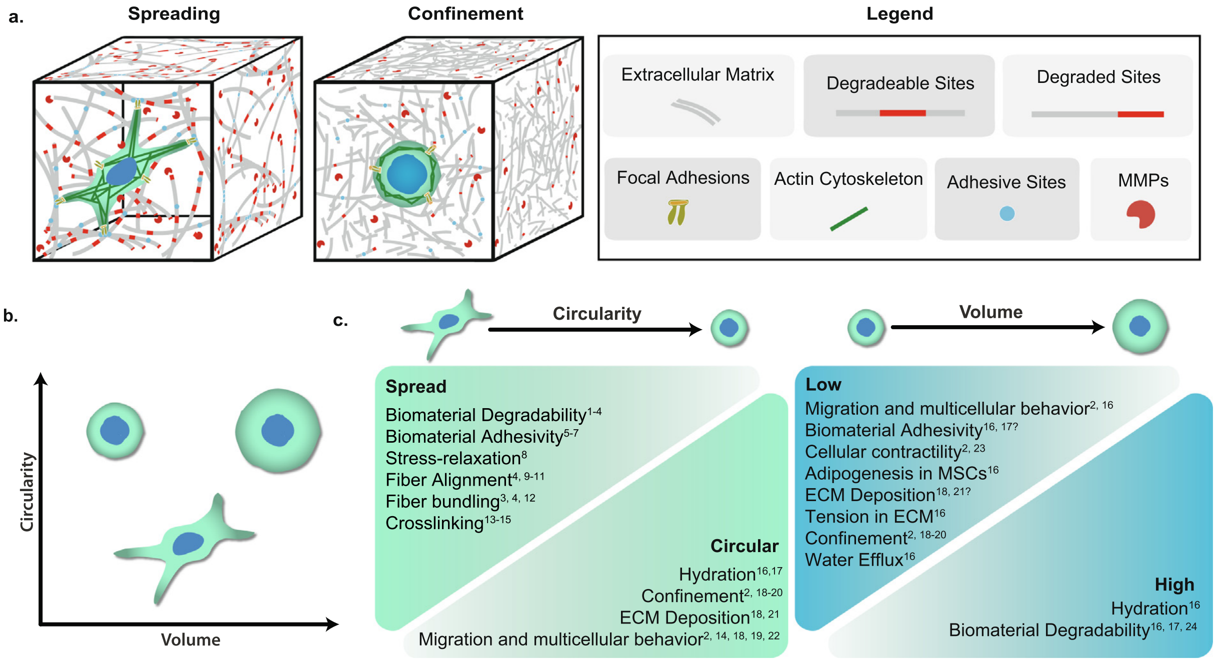

Fig. 1. The cell resides in a complex 3D pericellular space. a. The pericellular space regulates cellular response. The extracellular matrix (ECM) architecture depends on the fiber architecture and composition of the ECM [6,9,94]. Presentation, type and conformation of adhesive sites (blue) affects how the cell adheres and interacts with the matrix. Cellular focal adhesions (yellow) are the binding site of the cell to the matrix. Depending on the architecture of the matrix, a cell may be more spread and have more focal adhesions, or be more rounded and have fewer focal adhesions [6,158]. Degradable sites (red) similarly affect the ability of the cell to interact with the surrounding matrix, degrade ECM, and therefore, generate cleaved ends and pieces of ECM fibers which serve as signals regulating many cellular functions [5]. Cells can secrete MMPS to degrade the matrix. These features of the pericellular space regulate cytoskeletal components such as actin (dark green) as well as nuclear transcription [147]. All of these responses together inform cellular morphology including cell circularity and volume. b. A representation of the circularity volume space. A cell's morphology can be plotted as shown so that cells have a specified shape and circularity, which are the result of processes such as adhesion, degradation and protrusion. c. Morphology is defined by cellular response to the pericellular space. The shape of the cell represents the integration of intracellular and extracellular responses to the pericellular space. Cell shape can be defined using cell volume and cell shape metrics such as cell circularity. The key features of the shape are initially driven by the features of the pericellular space including adhesivity, degradability and architecture of the biomaterial. The cellular response to the biomaterial is includes short term effects such as regulation of contractility, transcription and ECM deposition, as well as long term effects such as migration, multicellularity, cancer progression and stem cell differentiation. The question marks represent papers that present potential links of the biomaterial feature to cellular response. List of references: 1. [44] 2. [6] 3. [7] 4. [8] 5. [57] 6. [58] 7. [59] 8. [55] 9. [40] 10. [42] 11. [34] 12. [147] 13. [43] 14.[44] 15. [45] 16. [13] 17. [14] 18. [9] 19. [75] 20. [62] 21. [10] 22. [73] 23. [12] 24. [111]

1.1. Challenges in decoupling the features of the pericellular space

The pericellular space is the region directly surrounding the cell, composed of ECM components, water, proteins, small and large signaling molecules, and other cells [5]. The profound influence of both the mechanical and chemical aspects of this milieu has been recognized for more than a century. However, its complexity makes it challenging to isolate individual features and determine their contribution to regulating cell behaviors. Synthetic biomaterials are well-suited to address some of these complexities as they can be engineered to mimic specific architectural features or mechanical properties. On the other hand, the use of native ECM polymers *in vitro* mimics native ligand presentation and may better capture the complexity of physiologic ECM. Using both native and synthetic polymers to mimic physiologic ECM is important to build an understanding of cellular response to the pericellular space. We discuss this further in the following section. To compare these studies in context of one another and the pericellular space of study, we utilize metrics of cell morphology, including cell shape and cell volume as key readouts of cell-matrix interactions (Section 1.2).

1.1.1. Synthetic and native biomaterials

Tissue engineering research in the last decade has seen a focus on recreating mechanical and chemical properties of native ECM from the bottom-up, using synthetic biomaterials such as polyethylene glycol (PEG) and alginate (Fig. 2a) [16–19]. Synthetic biomaterials provide both advantages and disadvantages. The major

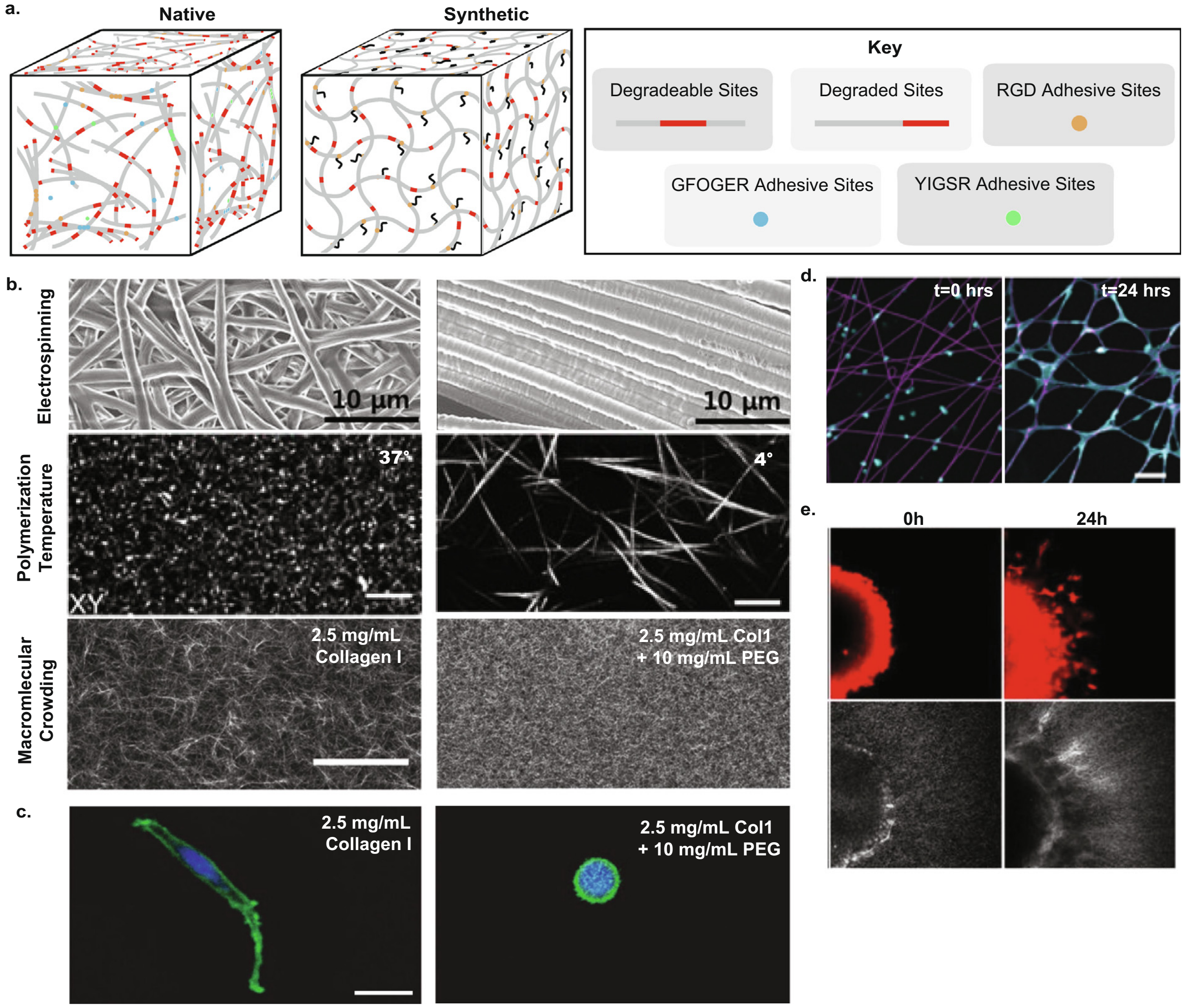

Fig. 2. In both native and synthetic ECM environments, the architecture can be manipulated to study features of cell structure and function. The cell also remodels the pericellular space by bundling and aligning fibers. a. Native vs. Synthetic ECM: Native ECM provides a variety of adhesive and degradable ligands which are presented to the cell in a specific architecture [159]. Synthetic biomaterials such as polyethylene glycol (PEG) and alginate [16–19] can be chemically modified to provide specific control over the adhesion and degradation sites available to cells, as well as mechanical and physical control of the cell's microenvironment. b. Methods to change architecture of native ECM materials: Representative images of electrospinning of a composite laminin core-polydioxanone/collagen shell fibers. These can be oriented in a random or aligned structure. Reproduced from Ref.[160] under the Creative Commons Attribution 4.0 International License. Decreasing polymerization temperature increases fiber length and pore size. Reproduced from Ref. [11] under the Creative Commons Attribution 4.0 International License. Small molecular weight crowders such as 8 kDa PEG decreases the fiber length and pore size in 2.5 mg/ml collagen gels. Reproduced from Ref. [6] with permission from The Royal Society of Chemistry. c. Degradability regulates confinement. In a confined collagen gel formed using a molecular crowding agent, encapsulated cells are not able to degrade the matrix and are more confined (circular) as opposed to a cell able to modify the matrix (spread) (phalloidin (actin, green) and DAPI (nuclei, blue). Scale bar is 25 μm.) Reproduced from Ref. [6] with permission from The Royal Society of Chemistry. d. Cells bundle fibers to remodel the pericellular space. Matrix reorganization occurs through recruitment of fibers by endothelial cells and lateral bundling of fibers between adhered cells and is accompanied by cell spreading and the formation of cell-cell contacts. Reproduced from Ref. [8] under the Creative Commons Attribution 4.0 International License. e. Cells align fibers in remodeling the pericellular space. Top row: tumor spheroids, dyed using CellTracker Red; second row: SHG images of collage fibers showing alignment over 4 hrs. Reproduced from Ref. [7] under the Creative Commons Attribution 4.0 International License.

advantages are that they can be chemically modified to provide exquisite control over the composition and presentation of adhesion and degradation sites available to cells. They also offer fine-tuned mechanical control of the cell's microenvironment (Fig. 2a) (see Table 1 for methods to tune synthetic matrices). In synthetic matrices, however, the conformation and presentation of the ligand may not sufficiently mimic native ligand presentation. Studies using such biomaterials have observed that ligands must not only be available, they must be presented properly for cells to "see" them, ie. to bind and activate the corresponding receptor [20–25]. For example, one recent study used alginate gels covalently modified with arginine-glycine-aspartic acid (RGD) peptides attached via varying length tethers and observed that increasing tether length decreased cell adhesion and cell spread area [25]. These results also echo lessons learned from the growth factor delivery field, where method of presentation and conformation of the growth factor affect ligand receptor activation [21,23]. Thus, the reduced chemical and topographical complexity that some synthetic ECMs provide also introduce new challenges of presentation. Biophysical and biochemical measurement of cell-biomaterial interactions, like adhesion, remain essential in confirming that materials are performing in the way that they are designed to. This has increasingly led to integrative research efforts spanning the fields of biomaterials and single cell biophysics.

1.1.2. Matrix architecture

The problem of ligand conformation and presentation within the scaffold has reinforced the idea that architectural features of the native pericellular scaffold are just as important as its composition. For example, type I collagen, one of the most abundant scaffolding proteins in the human body, has multiscale architecture that determines how adhesion and degradation sites are presented [26,27]. Architecture encompasses biopolymer size, alignment, crosslinking density, branching, and pore size (Table 1 for methods to vary architecture). Both microscale (~1 μm) and mesoscale (~100 μm) architectural features direct cell behavior through regulation of adhesion formation and topographic guidance, and when combined, mesoscale cues can enhance or compete against microscale cues [28–30]. Nanoscale topography can also regulate cell adhesion sensing [31].

Matrix architecture can be controlled across scales to some extent in both reconstituted native ECMs and synthetic ECMs by several established and newer methods (Table 1) (Fig. 2b). Pore size, crosslink density, structural organization, viscoelastic properties and stiffness can be manipulated to study cellular sensation of architecture [9,32,33]. For example, electrospinning can be used to generate nanofibrous scaffolds from either natural or synthetic biodegradable polymers with control over fiber size, pore size, and alignment [8,34]. Collagen fibers can be tuned to different lengths and thicknesses by changing polymerization temperature [11] (Fig. 2b) or introducing a crowding agent [6,10,35–39] or using a microchannel to align the fibers with flow [40]. However, specifying the architecture of a matrix usually has implications for its mechanics and degradability as well. Matrix architecture can regulate matrix degradability, modulating the degree to which encapsulated cells are confined (Fig. 2c) [6,9,41], or permitted to modify their local architecture by cutting, bundling, and aligning fibers [7,8,42]. Architecture can also influence local matrix stiffness, affecting the balance between cell adhesive and contractile forces [11]. Likewise, tuning the stiffness or tension in a scaffold is usually accomplished by changing architecture, such that the two cannot be decoupled. Much of the research literature has used the term "stiffness" broadly as an independent variable, when the experiments actually vary some chemical or architectural feature that contributes to stiffness (e.g. cross-links). Crosslinking often reduces degradability [43–45], so the phenotypic outcomes that are correlated to one feature may actually mechanistically result from another, related feature. Stiffness sensing by cells has been heavily explored over the last decade and was recently reviewed in detail [46–50]. Herein, we will discuss recently developed methods that now enable the decoupling of certain aspects of architecture from mechanics. These studies have shed new light on the underappreciated role of matrix degradability and hydration in dominating early cell-matrix interactions, as well as the complexity of biomaterial adhesive properties.

1.2. Cellular morphology as a readout of pericellular space interactions

A relatively new paradigm in the fields of biomechanics and three-dimensional (3D) biomaterials is that cell shape and volume serve as emergent properties, integrating multiple pericellular space cues to orchestrate cell outcomes (Fig. 1, b and c). Here, we use these two descriptors of cell morphology as a point of comparison across studies using different materials, since they are features that are easily measured from imaging data and used frequently [6,51,52]. Cell shape is often measured as circularity, which is a ratio of perimeter to area in a two-dimensional (2D) projection that effectively describes protrusion, cell spreading, and membrane ruffling in a single quantity for a given cross-section. It has recently been used to characterize and predict a wide variety of cell outcomes, form epithelial-to-mesenchymal transition (EMT) in cancer cells [53] to early stage lineage commitment in mesenchymal stem cell (MSC) differentiation [54]. Circularity has also been used to characterize the effect of stress-relaxation on cell shape [55]. While circularity captures a projection of the cell shape in 2D, cell shape can also be measured in 3D using metrics such as sphericity. Sphericity is the ratio of object volume and the surface area and captures the flatness of the cell in addition to the features of circularity. Sphericity has been used to develop a measure of cells shape to predict invasive capability of different cell lines [56]. Because of its inherent 3D nature, moving towards measures like sphericity will more reliably represent 3D cell-pericellular space interactions.

Another key metric of morphology, volume, can be measured from confocal z-stack imaging and has been linked to significant effects on cellular mechanics, osmotic balance, adhesion formation, and cytoskeletal polymerization. Cell volume responds to cortical tension [13], osmotic flux, intracellular signals, and environmental constraints [12,55]. Herein, we visualize cell morphology as regions in a circularity:volume space (Fig. 1b). Confinement and cellular spreading emerge as roughly opposite morphological conditions (Fig. 1a). We discuss recent studies that have identified emerging [2,3] features of the pericellular space responsible for modulating cell morphology, leading to phenotypic impacts (Fig. 1c). Cell type and genetic makeup play significant roles in these processes, so we include those details where appropriate but do not focus heavily on them in order to identify potential general design principles.

2. New insight into regulators of biomaterial adhesivity

If cells are able to adhere to a substrate, they will begin to spread and regulate their tensile state. This process is coupled with gene expression [57–59]. In the absence of adhesion, cells become more spherical and often stressed [60–63]. Thus, the adhesivity of the pericellular space is essential in the determination of cellular morphology and function. Despite incredible advancements in understanding the adhesion and spreading processes at the molecular and cellular levels, a surprising number of open questions remain [27,64–68]. Recent studies that couple synthetic and native

Table 1

Summary of select studies that analyze features of the pericellular space and their effects on cells.

Scaffold Material	Pericellular Space Feature	Study Results	Methods to modify ECM	Cell/tissue types	Ref
Native ECM Scaffolds					
Collagen	Fiber architecture, Pore size	Collagen matrices with small pores and short fibers trigger a conserved transcriptional response and subsequent motility switch in cancer cells which results in the formation of multicellular network structures. The transcriptional module associated with network formation is enriched for migration and vasculogenesis-associated genes that predict survival in patient data across nine tumor types.	Macromolecular crowding using PEG can be used to generate short fiber, small pore matrices with equivalent density to large pore, large fiber matrices. This method modifies the structure of the collagen fibers within the gels without changing the final collagen concentration, therefore decoupling ligand density and architecture	Fibrosarcoma cells (HT-1080), Mesenchymal human foreskin fibroblasts (HFF-1), Breast cancer cells (MDA-MB-231)	[9]
	Protein deposition, Confinement	Collagen type I gel promotes collagen type 4 deposition and brown adipogenesis.	3D encapsulation/confinement	Adipocytes	[10]
	Fiber architecture	Macromolecular crowding can decouple fiber architecture and matrix stiffness to study cancer cell morphology in confining environments.	Macromolecular crowding	Breast cancer cells MDA-MB-231	[6]
	Fiber architecture	Decreasing polymerization temperature increases fiber size and porosity and affects cell morphology, adhesion and motility.	Polymerization temperature	Human foreskin fibroblasts (HFF)	[11]
	Viscoelasticity	Collagen and fibrin gels stiffen with strain, but over time, the gel undergoes stress relaxation and the stiffening is reduced.	Measurements of stress relaxation were performed using an AR-G2 stress-controlled rheometer	N/A	[121]
	Degradability	Switching from single-cell to collective invasion modes is dependent on increasing collagen density and proteolytic collagen breakdown but independent of matrix stiffness.	Collagen density	Spheroids of MV3 melanoma and HT1080 fibrosarcoma cells	[73]
Matrigel	Stiffness	Endothelial cell network formation depends on thickness and stiffness of matrigel	Glutaraldehyde crosslinking	Endothelial cells	[8]
Synthetic and Hybrid ECM Scaffolds					
Poly ethylene glycol (PEG)	Adhesivity	Differentiation of iPSCs towards neural progenitor cell can be induced via integrin B1 binding motifs.	PEG-peptide-based hydrogels polymerized by photoinitiated step growth mechanisms facilitate adhesive ligand incorporation.	Induced pluripotent stem cells (iPSCs)	[16]
	Nano-architecture	Nanostructure of PEG hydrogels can be controlled by polymerization mechanism such that chain polymerized hydrogels are highly heterogeneous and step growth networks exhibit more uniform structures. These features affect cell proliferation and spreading; Step growth mechanisms promote cell spreading.	PEG polymerization via step-growth or chain growth mechanisms.	Human mesenchymal stem (hMSC)	[17]
Alginate	Ligand presentation	Controlling the spacer arm length of RGD ligand coupled to alginate hydrogels changes differentiation. Increasing spacer length promotes osteogenic and adipogenic differentiation of BMSCs but shows less enhancement of chondrogenic differentiation.	The length of spacer arms conjugated to alginate varied by varying the number of gly peptides before the adhesive ligand.	Mouse bone marrow stromal cells (BMSCs)	[18]
	Ligand presentation, Adhesivity	Peptide mimics of bone morphogenetic protein 2 (BMP-2) and adhesive ligands presented from both 2D surfaces and 3D alginate hydrogels, increase osteogenic activity in mMSCs.	BMP-2 peptides synthesized by solid phase Fmoc-peptide synthesis are covalently bound to alginate hydrogels via multiple strategies.	Mouse mesenchymal stem cells (mMSCs)	[21]
Alginate	Adhesivity, Stiffness, Viscoelasticity	Interpenetrating networks of alginate and reconstituted Basement Membrane (rBM) can simultaneously provide tunable mechanics and native adhesive ligands [19]. These matrices demonstrate that cells migrate through confining matrix if it exhibits sufficient mechanical plasticity [[51] .	The stiffness of interpenetrating networks formed by mixing rBM and alginate can be tuned by modulating the concentration of calcium used to cross-link the alginate while holding the concentration of rBM constant. This method can decouple the stiffness of the 3D microenvironment from the cell adhesion ligand concentration or pore size.	Breast cancer cell line MDA-MB-231	[19],[51]

(*continued on next page*)

Table 1 (*continued*)

Scaffold Material	Pericellular Space Feature	Study Results	Methods to modify ECM	Cell/tissue types	Ref
	Viscoelasticity	Gels with fastester strain relaxation enhance cell spreading, proliferation, and osteogenic differentiation of MSCs. In gels with a fast stress relaxation, MSCs form a mineralized, collagen-1-rich matrix similar to bone.	Two methods increase stress relaxation in 3D of alginate gels. Lowering the molecular weight of alginate polymers crosslinked by calcium decreases entanglement and crosslinking of the network. Another method to vary stress relaxation is to couple small PEG spacers to provide a steric spacing of crosslinking zones in the alginate. Using both methods together provides the fastest strain relaxation.	Mesenchymal stem cells (MSCs)	[104]
	Stress relaxation, Viscoelasticity	2D RGD-modified alginate hydrogel substrates with varying initial elastic moduli and rates of relaxation show that viscoelasticity and stress relaxation are key parameters affecting myoblast spreading and proliferation	Ionically crosslinking alginate with the divalent cation Ca2 + resulted in hydrogels exhibiting stress-relaxation whereas covalently crosslinking alginate with carbodiimide chemistry produced hydrogels with reduced stress-relaxation. Both types of gels have variable elastic moduli depending on crosslinking (2.8–49.5 kPa).	Mouse myoblast	[55]
Hyaluronic Acid (HA)	Degradability	hMSCs within HA hydrogels of equivalent elastic moduli that permit cell-mediated degradation exhibit high degrees of cell spreading and high tractions, and favour osteogenesis, while matrices that restrict cell-mediated degradation exhibit low cell traction/spreading and adipogenesis.	hMSCs encapsulated either into HA hydrogels using Michael addition reactions between MeMaHA maleimides and MMP degradable peptides.	Human mesenchymal stem (hMSC)	[75]
	Protein deposition	Local nascent protein deposition was investigated in 2 matrices of interest: proteolytically degradable, covalently crosslinked hyaluronic acid and dynamic viscoelastic hyaluronic acid hydrogels. Inhibiting nascent protein deposition in these hydrogels reduces mesenchymal stromal cell spreading and nuclear translocation of YAP/TAZ and results in a shift towards adipogenic differentiation.	HA degradability tuned by crosslinking via a thiol–ene reaction with MMP-degradable dithiol peptide crosslinkers. HA viscoelasticity can be tuned with a dynamic double-network HA hydrogel system based on covalent and supramolecular guest–host crosslinking.	Human mesenchymal stem (hMSC)	[103]
Dextran Methacrylate (DexMA)	Degradability	Matrix degradability is modified by inclusion of MMP degradable peptides in the DexMA gel. Matrix degradability switches 3D endothelial cell invasion between single-cell migration and the multicellular, strand-like invasion required for angiogenesis.	DexMA macromers are crosslinked through Michael-type addition with matrix metalloproteinase (MMP) labile dicysteine peptide sequences to study degradability effects on multicellular migration patterns.	Endothelial cells	[44]
	Fiber architecture	Macroporous scaffolds with tunable stiffness, fiber density and fiber modifiability demonstrates that cellular ability to bundle fibers improves endothelial cell network formation.	Electrospun DexMA fibers can be functionalized with adhesive peptides using Michael-like addition to study endothelial cell network formation.	Endothelial cells	[8]
Poly(glycerol sebacate) (PGS)	Mechanical properties and fiber architecture	PGS is a biodegradable elastomer that exhibits tunable mechanical properties and is a amenable to 3D bioprinting of complex scaffold geometries.	Photocurable thiol–ene click chemistry controls PGS crosslinking. These PGS networks exhibit tunable mechanical properties and degradation rates in the presence of different concentrations of crosslinker.	3 T3 fibroblast cells	[162]
	Porosity and Pore size	Higher porosity of PGS scaffolds promote cell infiltration, proliferation, and ECM production.	Porous elastomeric PGS scaffolds can be fabricated using salt leaching techniques. Plasma treating the scaffold decreases hydrophobicity and promotes cell infiltration, proliferation, and ECM production, with the greatest HA, sGAG, uronic acid, and collagen contents for chondrocytes.	Primary human chondrocytes	[163]

Poly(octanediol citrate) (POC)	Stiffness and protein deposition	Osteogenic differentiation of MSCs is improved with stiffer matrices as demonstrated by greater alkaline phosphatase activity and cellular mineralization.	Bioglass nanofiber composites are incorporated into electrospun POC matrices to increase overall modulus.	Mouse bone marrow mesenchymal stem cells (mBMSCs)	[164]
	Pore shape and permeability	To study the effects of permeability of a matrix on cartilage regeneration, POC matrices with different pore sizes and shapes were designed. Lower permeability with a spherical pore shape enhances matrix production and chondrogenic mRNA gene expressions *in vitro compared* to highly permeable scaffolds with a cubical pore shape.	POC scaffolds with specialized geometries are created by molding POC around a sacrificial mold of HA.	Primary porcine chondrocytes (pChon)	[165]
Polycaprolactone (PCL)	Fiber architecture	Electrospun scaffolds made of PCL mimic the morphological architecture of native ECM by recapitulating the micro- and nano-scale morphological features of fibers with diameter equal to 3.22 ± 0.42 µm and surface roughness of 17.84 ± 4.43 nm. These features promote proliferation of healthy human hepatocytes (HHH) over liver cancer cells (HEP-G2) in co-culture in the fibrous mesh.	Fibrous scaffolds were fabricated via electrospinning. Fiber morphology was adjusted using the voltage and time of deposition.	Human primary hepatocytes	[166]
	Fiber architecture, stiffness	Providing biomemtic ECM components, by coating electrospun ECM matrices in liver derived ECM, and providing stiff PCL structure improves hepatic cell adhesion and liver-like functions of hepatocytes .	The scaffold is constructed using liver-derived ECM, gelatin, and PCL by electrospinning.	Primary rat hepatocytes	[167]
	Porosity, fiber architecture	Deep cell migration occurs after implantation *in vivo* in PCL eletrospun scaffolds but does not occur in a gelatin gel, likely due to the large pores of the PCL scaffold.	PCL scaffolds generated by electrospinning.	Human fetal foreskin fibroblast cell line (HFFF2)	[168]
Polylactic acid (PLA)	Fiber architecture	Varying PLA fiber length and concentration as a reinforcement phase in a gelatin hydrogel matrix affects mechanical properties and cellular responses. With increasing fiber length and concentration, the ultimate tensile strength, modulus, and toughness increased. Cell viability is highest with the longest fibers (12.7 mm).	PLA fibers of various lengths and concentrations are embedded into a gelatin hydrogel using a wet-lay process. This process disperses fibers in a solvent, transfers them to a glass substrate, evaporates solvent and forms gelatin hydrogel around fibers.	Human mesenchymal stem cells (hMSCs)	[169]
	Degradability	PLA, a biodegradable and bioactive thermoplastic aliphatic polyester derived from renewable biomass, has a prolonged degradation profile. This allows for increased time for autologous cell colonization and extracellular matrix deposition to occur with the support of the PLA matrix. Electrospun arterial grafts from PLA show normal tissue properties after 12 months *in vivo*, including tissue ECM composition and matrix deposition.	PLA scaffolds spun into porous nanofiber conduits implanted as an arterial conduit in mice.	Artificial grafts implanted into mouse infra-renal artery	[170]
Poly(lactic-co-glycolic acid) (PLGA)	Matrix architecture, adhesivity, protein deposition	Macroporous substrates composed of PLGA and coated with artificial ECM molecules, including collagen type 1 and HA or sulphated HA derivatives, facilitate dermal colonization, decrease collagen type 1 mRNA expression, and increase MMP activity relative to PLGA scaffold alone.	Macroporous PLGA scaffolds are fabricated by lipid templating, where solid lipid microparticles are used as a porogen in PLGA polymer and extracted via n-hexane. The PLGA are coated in proteins via protein adsorption.	Human dermal fibroblasts (dFbs)	[171]
	Pore size, degradability	PLGA pore size was varied in to determine optimal pore size for cellular protein deposition and cell migration. PLGA constitutes a synthetic, degradable matrix suitable for implantation and *in vitro* models of intervertebral disc regeneration.	PLGA matrices with various pore sizes are generated by salt leaching techniques. These matrices were soaked in a small intestinal small mucosa solution, cross-linked and lyophilized to provide bioactivity.	Nucleus pulposus (NP) cells	[172]

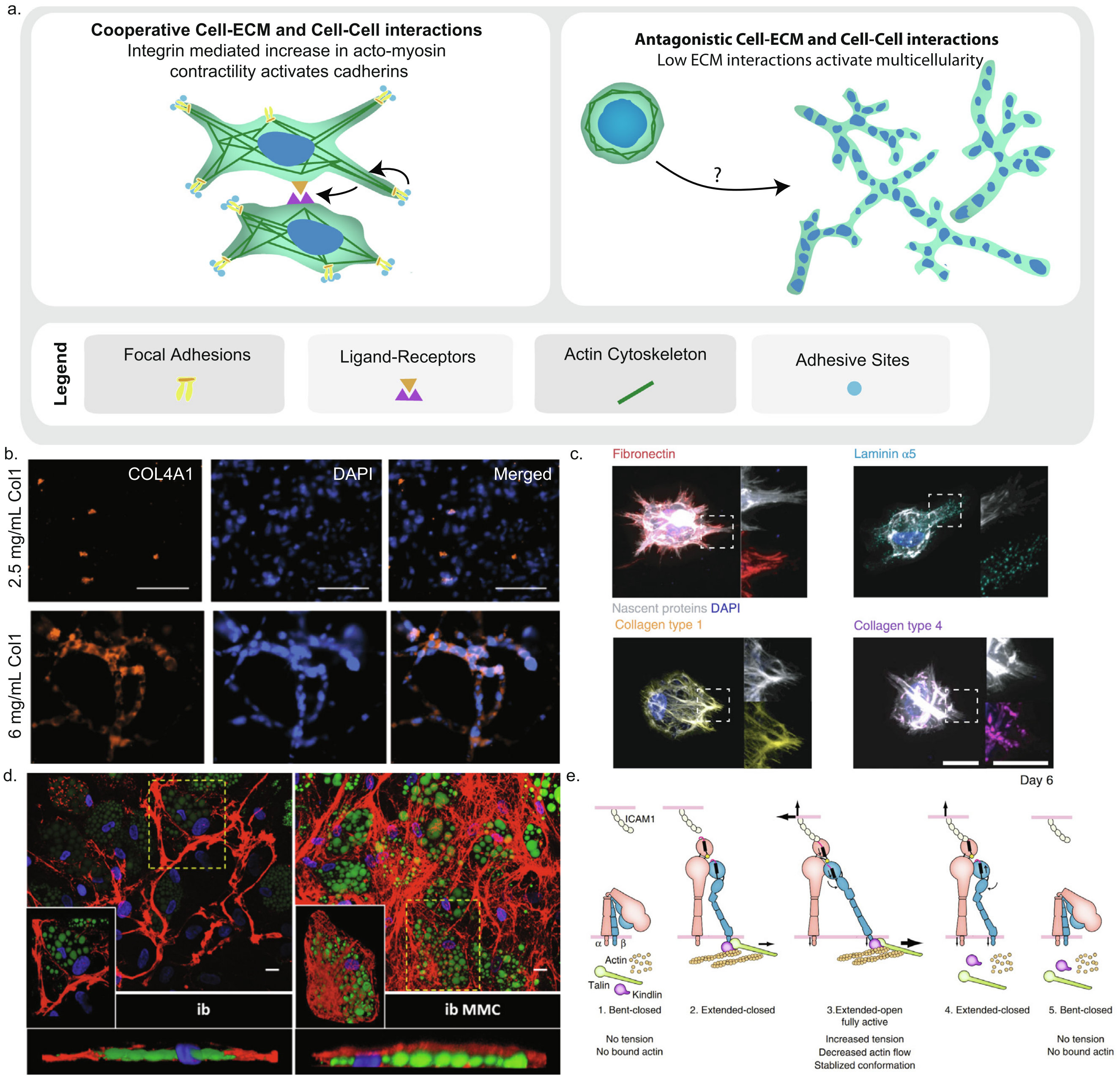

Fig. 3. New insights into regulators of biomaterial adhesivity. a. Cooperative vs Antagonistic mechanisms guide determination of cell-cell and cell-matrix adhesions. Cooperative adhesion: one mode of crosstalk between integrins and cadherins, is integrin-mediated increases in tension which can activate cadherin-based adhesions [69]. Antagonistic adhesion: In environments that promote cell-matrix adhesion, spreading, and increased intracellular tension, cells tend to remain as single cells even after cell division. Conversely, in confining environments cells tend to adhere to each other, forming multicellular structures [6,9,44,73,75]. b. In environments of low adhesion, cells deposit ECM. Collagen type 4 was deposited in confined environments. Immunofluorescence staining of a highly invasive breast cancer cell line, MDA-MB-231, cells for collagen type 4 after 7 days of culture in 6 vs. 2.5 mg ml^{-1}. Scale bar 100 µm. Reproduced from Ref. [9] under the Creative Commons Attribution 4.0 International License. c. Cell adhesion could be regulated by nascent protein deposition. Fluorescent images of nascent proteins including fibronectin, laminin α5 and collagen type 1 and type 4 of human mesenchymal stem cells seeded in degradable hyaluronic acid matrices for 6 days (scale bars, 20 µm). Reproduced with permission from Ref. [103] from Nature Materials d. Macromolecular crowding (MMC) activates ECM deposition. MMC in 2D culture media by addition of 18% Ficoll was able to induce significant increases in collagen type 4 deposition of MSCs undergoing adipogenesis, and the collagen type 4 (red) displays an intricate architecture. Z-projection images show nuclei (blue), collagen type 4 (red), and lipid droplets (green). Scale bar: 20 µm Reproduced from Ref. [10] under the Creative Commons Attribution 4.0 International License. e. Outside in vs. Inside out. A model for actin-dependent integrin activation. Reproduced from Ref. [161] under the Creative Commons Attribution 4.0 International License.

biomaterials to cell biophysics are revealing that multiple factors contribute to biomaterial adhesivity.

2.1. Cooperation versus competition in cell adhesion

When multiple cells share a pericellular space, two classes of adhesion molecules can be engaged simultaneously, facilitating cell-cell adhesion and cell-ECM adhesion. Controlling the balance between these two adhesion processes is essential to achieving functional outcomes such as differentiation and morphogenesis. Synergistic interactions between these two adhesion types is well established and has been reviewed in great detail [69,70] (Fig. 3a). In particular, crosstalk between integrins and cadherins is known to arise from integrin-mediated increases in tension, which can activate cadherin-based adhesions [69]. Elevations in the magnitude of integrin-mediated traction forces are accompanied by increases in myosin-dependent tension at cadherin-based adhesions [71]. Conversely, interfering with mechanotransduction at sites of cell–cell adhesion increases the degree of stiffening of cells in response to integrin ligands [72].

However, another mechanism of antagonistic interaction between cell-cell and cell-matrix adhesion is emerging (Fig. 3a). In environments that promote cell-matrix adhesion, spreading, and increased intracellular tension, cells tend to remain as single cells even after cell division [6,44,73,74]. Conversely, in confining environments where cells often exhibit reduced adhesion and traction with the ECM [75] and decreased cytoskeletal tension [6,11,76–80], cells tend to adhere to each other, forming multicellular structures [6,9,44,73–75]. In some cases, confinement has been shown to induce the expression of cell-cell adhesion genes, even prior to physical contact with neighboring cells [6,62]. Recent work by Siret et al. provides molecular insight into adhesion competition between cell-cell and cell-matrix interactions. Using 2D substrates to explore the impact of altered expression of cell–cell adhesion molecules (cadherins) and cell-extracellular matrix proteins (integrins) in melanoma cells, they found that in addition to localizing at sites of cell-ECM contact, a subset of $\alpha2\beta1$ integrin differentially localized with N-cadherin and E-cadherin. Interaction of $\alpha2\beta1$ integrin with type-I collagen disrupted E-cadherin localization at sites of cell-cell contact and promoted melanoma cell invasion and migration. On the other hand, blocking $\alpha2\beta1$ integrin interactions with type-I collagen promoted E-cadherin localization to cell-cell contacts and strong cell-cell adhesion [81]. These results reveal an interesting dichotomy, where on one hand, integrins are contributing to strong cell-cell adhesion and on the other hand, integrins are promoting cell-matrix adhesion and migration, depending on the type of adhesions available. These studies suggest that "tug-of-war" can exist between cell adhesion to the ECM and other cells [82].

This concept was originally explored by Foty and Steinberg in the context of cell-cell adhesion competition [83–85]. Their differential adhesion hypothesis (DAH) for cell-cell adhesion is based on a physical phenomena where different types of fluids have different surface tensions, which dictate the way they self-assemble into structures such as microspheres [86]. In this hypothesis, cells are modeled as fluids with specific surface tensions. Cellular populations with similar surface tensions therefore aggregate by moving away from cells with differing surface tensions. This concept was originally shown to explain cell sorting in development. In the developing embryo, cells with higher cortical tensions were shown to aggregate at the core of structures, while lower cortical tension pushed cells outward [83]. Similarly, it is known that cells can fine tune their adhesion to facilitate maintenance of a tensile state [87]. Extending this concept to cell-ECM adhesion suggests that a cell in a low-adhesive environment is like a water drop on a hydrophobic surface: if it encounters another water drop (cell), they will quickly aggregate and remain separated from the hydrophobic surface (low adhesion matrix). Conversely, a water drop (cell) interacting with a hydrophilic surface (high adhesion matrix) will increase its contact area and spread. Cells able to adhere to the surrounding ECM, will achieve equilibrium with the tension in the surrounding ECM by translating the tension of the ECM through cellular adhesions and the cytoskeleton and will be less likely to aggregate to other cells [58,59,87]. Interestingly, compressive stresses promote cell spreading and strengthen cell-ECM adhesion as well [88]. Collective cell behaviors are promoted in confining or low adhesive environments, reinforcing the analogy of cells to water droplets on hydrophobic substrates [[6,9,44,73–75]]. However, underlying molecular mechanisms continue to be elucidated. It appears that cells balance cell-ECM contact versus cell-cell contact through the regulation of binding affinities and relative tensions [89,90]. These results have important implications on the mechanisms of morphogenesis in both normal and pathologic physiologic processes.

Both cooperative and antagonistic mechanisms of cell-cell and cell-matrix adhesions are important in stem cell fate (Fig. 3a). The mechanical properties of the cell and ECM drive developmental patterning by modifying cell shape and structural integrity [91]. Goodwin et al. demonstrate a cooperative mechanism of cell-cell adhesion, by demonstrating that loss of cell–ECM adhesion (in drosophila integrin-null mutants) results in abnormal E-cadherin localization and stability, showing that cell-ECM adhesion regulates cell-cell adhesion [92]. One recent example of antagonistic cell-ECM and cell-cell interactions comes from a study on epithelial cell differentiation. In this study, Miroshnikova et al. find that cell division can lead to a cell crowding phenotype, and that cell crowding tends to increase intracellular tension and cell-cell adhesion via E-cadherin. Crowded cells tend to push away from the substrate, delaminating from the basal layer. Cells that are the most delaminated also display the greatest markers for epithelial differentiation [91], suggesting that the switch to high cell-cell adhesion plays a key role in epithelial differentiation and corresponds to increases in tension. Not only is the type of cell-cell adhesion and tension tightly regulated, but timing is critical. Barone et al. postulates that cells actually have a "memory", noting that the amount of cell-cell contacts over the course of a cell's lifetime effects its path of differentiation [93]. Cells with less cell-cell contacts over time have reduced nodal signaling and tend to differentiate into endoderm specification. Cells with greater E-cadherin-mediated cell-cell adhesion elicit increased nodal signaling which reinforces cell-cell contacts and tends to promote prechordal plate specifications. From these studies on adhesion in stem cells, it becomes clear that cell-cell and cell-ECM contact dynamics directly influence cell fate. It will be valuable to further investigate how biomaterials can be used regulate these cooperative and antagonistic adhesion modes in time-dynamic ways.

2.2. Cell-deposited ECM

Although substrates can be designed to present specific adhesive sites to cells, the ability of the cell to deposit ECM within biomaterial scaffolds complicates these systems. Proteins secreted by the cell or present in the culture medium adsorb and integrate with the biomaterial matrix, making it unclear which material the cell is adhering and therefore responding to. For example, our recent work shows that confinement of cells within a collagen type 1 matrix induces collagen type 4 upregulation and deposition relative to non-confining conditions in multiple cell types (Fig. 3b) [9]. Likewise, work by Loebel et al. emphasizes the importance of nascent protein deposition in cellular interactions with tissue-engineered matrices. In this study, deposition of fibronectin, laminin $\alpha5$, collagen type 4 and collagen type I by stem cells was observed within a variety of 3D engineered proteolytically degradable and dynamic viscoelastic hyaluronic acid (HA) hydrogels (Fig. 3c). Inhibiting nascent protein deposition in these gels decreased stem cell spreading and YAP/TAZ nuclear localization. This demonstrates that even in the presence of adhesive ligands, some cells depend primarily on nascent protein deposition for adhesion. Another study found that magnetically confining MSCs led to increased collagen deposition and secretion of other ECM such as glycosaminoglycans (GAGs), which supported chondrogenesis [94]. To resolve the relative contributions of the biomaterial scaffold and cell-deposited ECM to cell behavior, it will be necessary to conduct time-resolved functional experiments tracking cell-biomaterial interactions and matrix deposition simultaneously (see Table 1 for methods to study protein deposition). Tools such as matrix-labeling techniques for real-time and inferred location (M−TRAIL) as well as real-time biophysical analyses like 3D traction force microscopy (TFM) could be useful in such an endeavor [3,75,95,96].

Confinement of cells in 3D biomaterials may contribute to ECM deposition in a similar manner as macromolecular crowding (MMC) in 2D cell cultures. MMC techniques use high concentrations of macromolecules in the culture medium to reduce the volume of solvent available for other molecules in the solution [10,36–39,97]. This increases their effective concentrations and can promote nucleation and polymerization of ECM proteins [98,99]. MMC is used as a tool to promote ECM production in fibroblasts [99,100] and investigate cellular ECM deposition in stem cell differentiation and disease modeling [38]. For example, MMC in 2D culture media by addition of 18% Ficoll was able to induce significant increases in collagen type 4 deposition of MSCs undergoing adipogenesis, which had a fine meshwork architecture and induced many small focal adhesions (Fig. 3d). Interestingly, this led to brown fat formation instead of white fat [10]. For cells confined in 3D biomaterials, high concentrations of the biomaterial in the immediate pericellular space may similarly crowd cell-secreted molecules, promoting interactions that facilitate their precipitation out of solution and positive signaling feedback to the cell.

Alternatively, since 3D confinement has been associated with a low-adhesion and low-contractility phenotype (Fig. 3a), cell-deposited ECM could reflect a response to low-adhesion stress [6,74,101]. In suspension culture (no adhesion), cells can assemble a fibronectin matrix that promotes integrin-mediated cellular aggregation, compaction, and cohesion; inhibition of this fibronectin matrix assembly results in cell dispersal [102]. Likewise, in their study on protein deposition, Loebel et al. compared the intensity profile of focal adhesion proteins (paxillin) relative to the location of nascent protein secretion and found that they were aligned [103]. This result lends support to the conclusion that cells may not be adhering to the engineered matrix but to their own deposited ECM. Furthermore, the nascent protein deposition may be masking or supplementing cues from engineered materials.

Protein deposition is one key mechanism by which cells can not only interact with but also modify the pericellular space. Other matrix properties including degradability and viscoelasticity affect protein deposition. Increasing viscoelasticity for example, can affect protein deposition [104,105]. Loebel et al. observe that hydrogels that allowed either proteolytic degradation via matrix metalloproteinases (MMPs) or viscoelastic dynamic remodeling increased cell spreading. Blocking either nascent protein adhesion or remodelling of the matrix reduced spreading and downstream consequences on YAP/TAZ signalling and human mesenchymal stem cells (hMSC) differentiation [103]. The interplay of features affecting protein deposition in the pericellular space provides an example of how the cells ability to dynamically sense and modify the pericellular space over time contributes to downstream features and cell fate.

2.3. An update on outside-in versus inside-out regulation

To characterize the adhesivity of a biomaterial, it is also important to know how ready the cell's receptors are to ‘‘see” ECM binding sites (Fig. 3e). Cytoplasmic signals, e.g. GTPase mediated recruitment and binding of talin and kindlin to beta integrin cytoplasmic tail, are known to modulate increased affinity of integrin head domain for ECM binding sites [106]. However, it is unclear whether the effects of 3D culture, specifically in terms of confinement or cell shape restrictions, impact inside-out integrin activation signaling. Evidence from G-protein coupled receptor (GPCR) signaling experiments and modeling suggest that cell shape has dramatic impacts on cell signaling [107]. Physical confinement of the cell in a rounded shape has been associated with lower adhesion and cytoskeletal contractility [6,75]. However, contractility does not appear to be required for integrin activation and clustering in 3D fibrilar geometry [11].

By the same token, it is important to understand how binding sites are presented to the cell. Perhaps simplest example of this comes from studies using the collagen mimetic peptide RGD in a range of peptide lengths and structures (linear versus cyclic), where each promotes different strengths of adhesion [108,109]. Other studies have highlighted the importance of ECM spatial organization on the concept of adhesion competition, discussed above in Section 2.1, whereby cell-cell junctions are stabilized in subcellular locations void of ECM [110]. In native ECMs, architecture largely regulates presentation. Knowledge of binding site presentation in natural ECMs relies on models built from X-ray fiber diffraction, scanning electron microscopy (SEM), and atomic force microscopy (AFM) measurements. One model of the collagen type I fibril leaves high affinity recognition sequences for collagen-binding integrins buried and inaccessible, while another model proposes an alternative orientation with the opposite face exposed that would make these sites more accessible [27]. Since critical interaction domains may be buried within native ECM fibrils, how do biological processes that depend on these interactions occur? Is this an important feature to replicate in synthetic materials to direct cell behavior? One parameter that could control the cell's ability to access buried ligands is its ability to degrade and remodel the pericellular space. In the following section, we discuss new studies that highlight the importance of degradability on cell behavior [44,75]).

3. The underappreciated role of degradability

While the earliest events of cell spreading on a substrate have been historically characterized by passive adhesion and cell deformation [64–68], the degradability of the pericellular space is emerging as a predominant biomaterial property upstream of this process. New evidence comes in part from studies in 2D geometry using collagen-coated substrates, where MMP activity was found to be necessary for cell adhesion [111]. Inhibition of MMP activity led to loss of cell spreading and migration, suppression of traction forces, and cortical softening. These effects were associated with altered localization and expression of integrins and decreased phosphorylated focal adhesion kinase (FAK). Pre-conditioning the substrates with MMPs rescued cell spreading in the presence of MMP inhibitors. So, in this context, degradation must happen before a cell can sense other physical properties like substrate rigidity, at least through integrin-based mechanotransduction.

Recent studies using 3D native and synthetic materials also show that decreases in the degradability of a 3D scaffold often correlate with increases in cell circularity and decreases in phosphorylated myosin light chain (pMLC) levels and cell traction, a combination of contractility and adhesion (Fig. 4a) [6,44,75]. In natural ECM scaffolds, this effect may be the result of integrin binding sites being hidden prior to matrix degradation [27], or high internal strain within fibrils which protects from degradation [112–115]. In synthetic scaffolds, bundling and remodeling of the pericellular space through degradation could be important to enable aggregation of binding domains and adhesion maturation/stability. Alternatively, a lack of matrix remodeling capability seems to trap the cell in a rounded shape, impacting its intracellular tension [6,74]. This spatial confinement of cells after embedding in 3D scaffolds could affect inside-out adhesion regulation through cell shape and/or volume dependent mechanisms. For example, the inability to develop tension with the surrounding matrix while

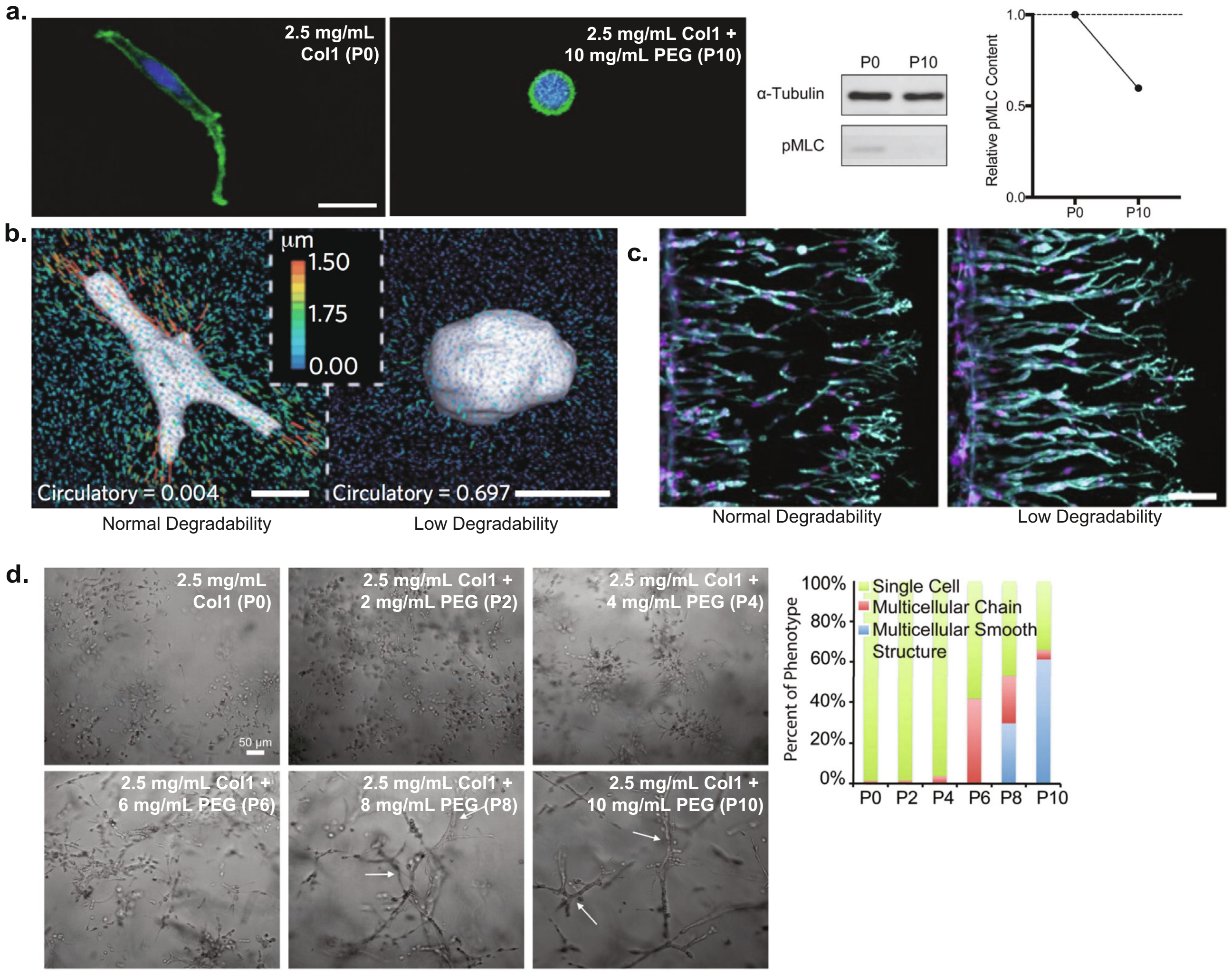

Fig. 4. Degradability is an important feature in regulating cell shape and multicellularity. a. Confinement increases circularity and decreases contractility. Confinement correlates with increases in cell circularity and decreases in phosphorylated myosin light chain (pMLC) levels and cell traction, a combination of contractility and adhesion (phalloidin (actin, green) and DAPI (nuclei, blue). Scale bar is 25 μm.) Reproduced from Ref. [6] with permission from The Royal Society of Chemistry. b. Degradability modulates adhesion, traction, and differentiation. Representative 3D traction force microscopy (TFM) images average drift-corrected bead displacements within 15 μm of the cell surface. Circularity is a measure of roundness of the cell ranging from 0 to 1 and in this case is calculated as a measure of the deviation of a point on the surface of the cell to the center of mass of the cell from TFM image. Reproduced from Ref. [75] with permission from The Nature Publishing Group. c. Degradability determined collective versus single cell phenotypes. Human umbilical vein epithelial cells (HUVECs) invading into dextran methacrylate (DexMA) gels crosslinked with degradable (right) and low degradable (left) peptide sequences. Reproduced from Ref. [44] under the Creative Commons Attribution 4.0 International License. d. Low degradability induces transition to multicellular phenotype. Representative brightfield micrographs of cells in crowded matrices with the concentration of crowder increasing from top left to bottom right after 1 week of culture. Increasing the amount of molecular crowder decreases degradability of the matrix [6]. Arrows indicate the multicellular smooth structure phenotype in confined conditions and graph shows frequency of phenotypes observed in each matrix construct. Reproduced from Ref. [6] with permission from The Royal Society of Chemistry.

simultaneously being confined could mimic cell-crowding. Interestingly, cell-crowding in development locally distorts cell shape and stress, reducing cortical tension and increasing cell-cell adhesion [91]. Perhaps cells in confining matrices follow a similar mechanism to upregulate cell-cell adhesion and collective behaviors.

3.1. Decoupling degradability from crosslinking and stiffness

Early approaches to control degradability in synthetic biomaterials involved modulating crosslinking [116,117]. However, in the context of 3D culture, changes in crosslinking density not only impact degradability but also matrix stiffness. To tease apart the relative contributions of degradability and stiffness, new materials have been developed by modifying the chemistry of crosslinking. For example, oxidation of an alginate backbone renders it susceptible to passive degradation by hydrolysis while crosslinking it through norbornene-tetrazine click-chemistry independently tunes its initial stiffness [118]. Likewise, hyaluronan-tyramine hydrogels can be cross-linked to achieve the same effective stiffness either by light exposure, which creates non-degradable linkages, or by horseradish peroxidase, which creates passively degradable linkages [119]. Modulating the susceptibility of the crosslinker sequence to MMP cleavage is another method in which the degradation rate of the gel can be tuned without altering stiffness. This can be achieved by replacing the standard sequence taken from the cleavage site of natural collagen with a similar sequence containing a single amino acid mismatch that lowers MMP binding affinity [44,120]. Photopolymerizable RGD-modified methacrylated hyaluronic acid (MeHA) hydrogels can also be used. In this case, hydrogel moduli are tuned by MeHA macromer concentration, degradability is enabled by the

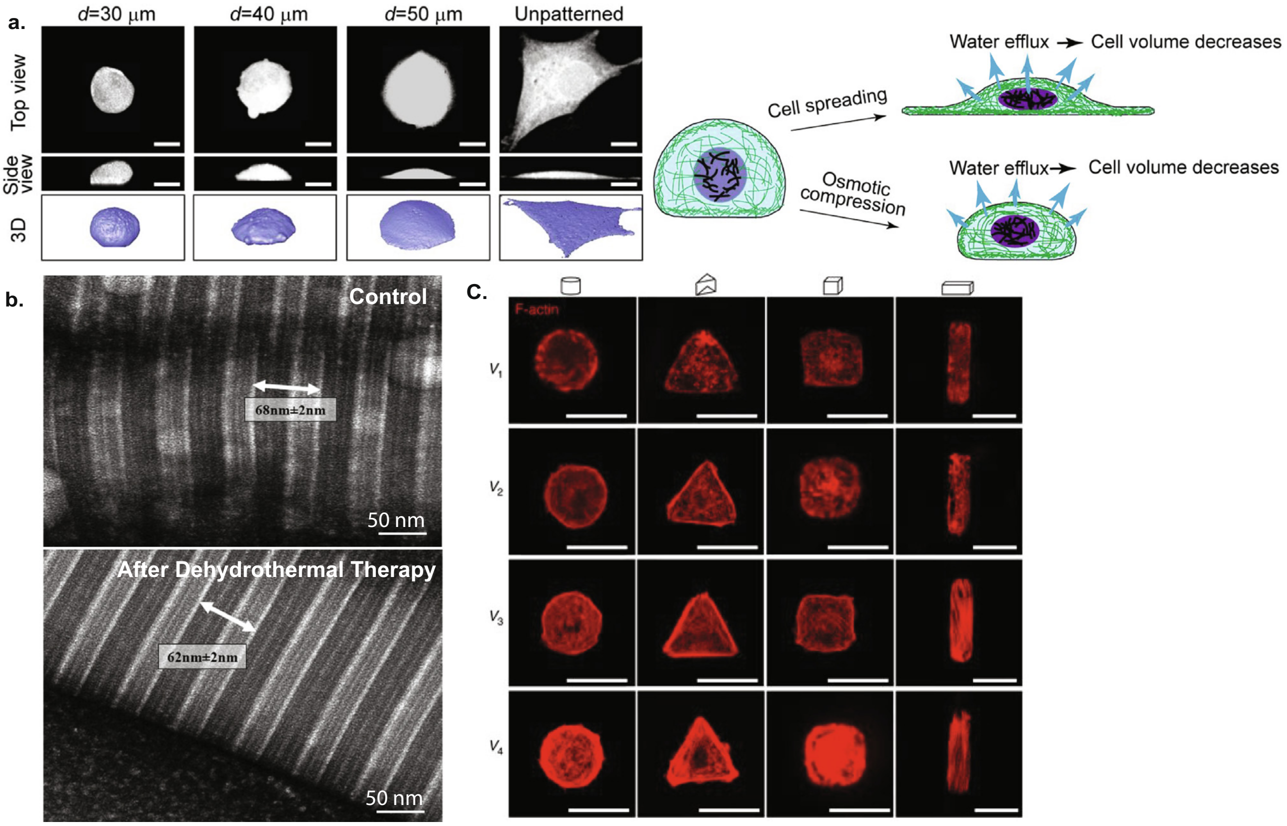

Fig. 5. Hydration regulates cell volume by affecting cellular contractility and ECM conformation. a. Cell volume increases as spread area decreases. Cell volume of cells grown on micropatterned collagen islands (d, diameter of micropatterned island) shows that cell volume increases as spread area decreases. 3D images of cells on micropatterned islands of different sizes on glass. Cells are labeled with cell tracker green (Scale bars, 20 μm.) (Right) Schematic illustration of cell volume decrease through water efflux, as cells spread out or are osmotically compressed. Reproduced from Ref. [13] with permission from PNAS. b. Dehydrating collagen fibers changes the conformation. Transmission electron microscopy (TEM) image of collagen fiber sample before and after dehydration treatments. The fundamental periodicity decreases from 68 to 62 nm. Reproduced from Ref. [126] with permission from American Chemical Society. c. Stress fiber formation and cell tension in a 3D microniches of various sizes and volumes. Representative images of vinculin staining for single human mesenchymal cells (hMSCs) cultured in 3D microniches with different volumes and geometries. Reproduced from Ref. [12] under the Creative Commons Attribution 4.0 International License.

introduction of proteolytically cleavable crosslinks, and degradability is independently tuned by introducing polymer chains that impede proteolytic degradation [75] (see Table 1 for methods to study degradability).

Studies using these various methods of tuning degradability independently of stiffness have revealed that synthetic hydrogel structural cues resulting from different crosslinking methods (covalent versus ionic) and dimensionality (2D versus 3D culture) modulate cell adhesion and differentiation distinctly [44,118–120]. In 3D geometries, degradability modulates adhesion, traction, and differentiation independently of stiffness and cell spreading (Fig. 4b) [44,74,75]. These methods have also revealed the importance of degradability in regulating collective cell behaviors. In low-degradable matrices, endothelial cells seeded as spheroids or in tubes molded from hydrogels invade the matrix and migrate collectively, forming long, strand-like networks required for angiogenesis (Fig. 4c) [44]. In high-degradable matrices, endothelial cells invade and migrate as single cells.

Similarly, degradability is associated with collective migration and morphogenesis of cancer cells in 3D collagen hydrogels, where cells are seeded as single cells and subsequently transition to collective behaviors (Fig. 4d) [6,9]. In these studies, slight modifications to collagen architecture through temporary macromolecular crowding are used to dramatically change degradation rates with little to no change in stiffness [6,9]. Cells in high-degradable gels (e.g. 2.5 mg/mL Col1 (P0) in Fig. 4d) tend to remain as single cells, but cells in low-degradable gels (e.g. 2.5 mg/mL Col1 + 10 mg/mL PEG (P10) in Fig. 4d) tend to form cell-cell adhesions and organize into multicellular structures. Interestingly, reduced degradability was associated with increased cell circularity and reduced pMLC, (Fig. 4a) prior to the upregulation of cell-cell adhesion and transition into collective behaviors. This is reminiscent of the cell-crowding effects observed during development, which were discussed above [91]. Together, these studies suggest that degradability is a key feature regulating cell behavior in covalently crosslinked native and synthetic 3D scaffolds.

The increase in adhesion and contractility that correspond to increased degradability may impact cell function in part by reducing cell volume. Although one may expect the increased area available to the cell through degradation would yield a higher cell volume, studies show that cell spreading is typically associated with a decrease in volume (Fig. 5a) [13,14]. Increased spreading increases chloride ion channel activity which ultimately results in water efflux and lower volume. Lower water content increases internal molecular crowding, stiffens the cell and nucleus, and leads to increased mechanical stability, which could explain differentiation responses such as osteogenesis in MSCs (Fig. 4b) [13,75].

It is important to mention that cells can also remodel the pericellular space via protease-independent mechanisms. The susceptibility of the ECM to remodeling by cell-generated forces, which are typically associated with blebbing and protrusive pushing, can be captured in the metric of viscoelasticity. Viscoelastic materials are malleable. Their mechanical properties are characterized by a dependence on the rate of strain, stress-strain hysteresis after loading, and often permanent deformation. Many native ECM biopolymers exhibit viscoelasticity [121], while synthetic hydrogels are more typically elastic [104]. Methods to vary viscoelasticity can be found in Table 1 [103]. Viscoelasticity tends to enhance the cell's ability to spread in the matrix [103,122]. Interestingly, viscoelasticity coupled with nascent protein deposition increases MSC

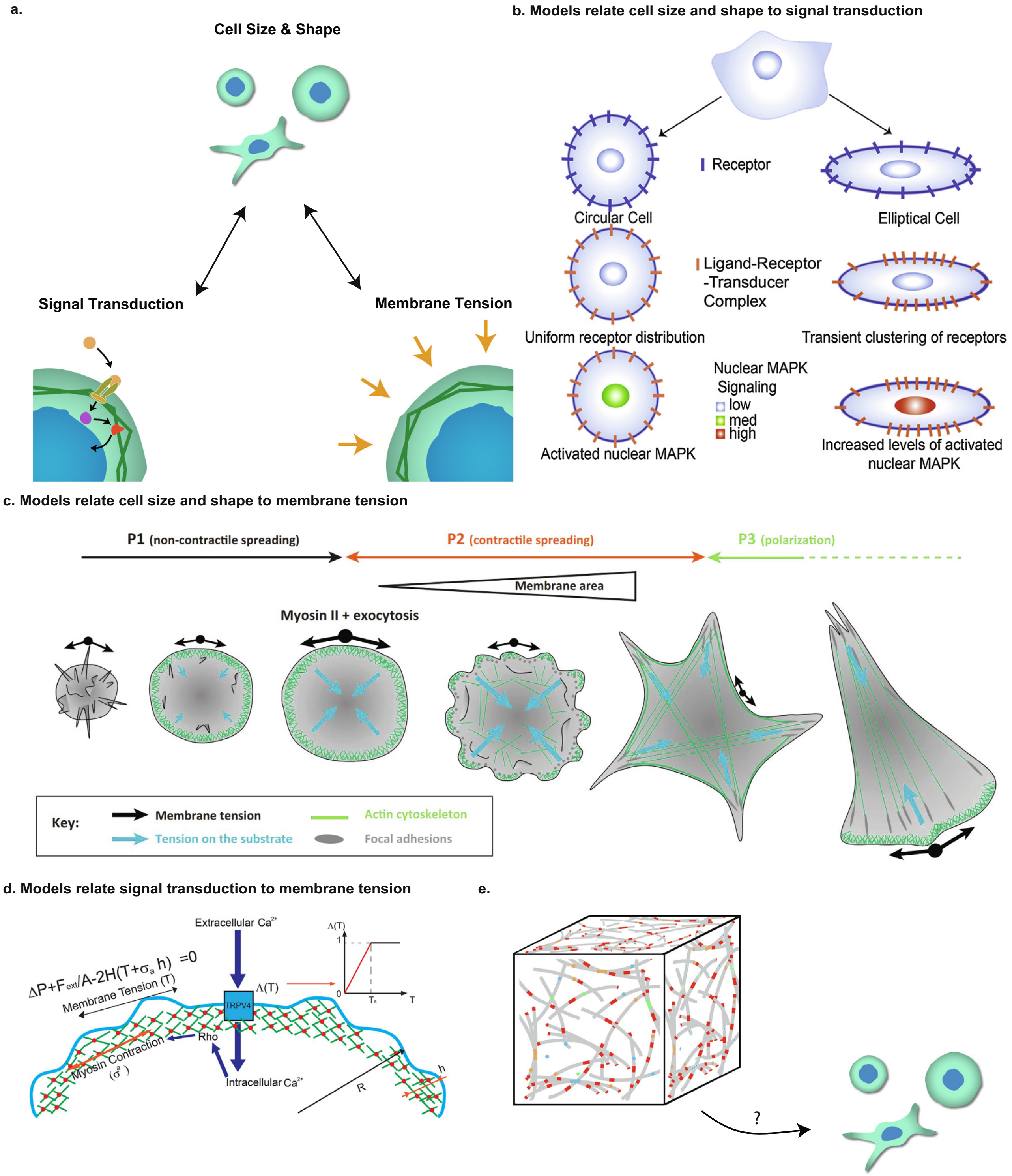

Fig. 6. Modeling links critical knowledge gaps between morphology, signal transduction and membrane tension. a. Modeling develops quantitative relationships between critical features of the cell. Current models relate cell size and shape to signal transduction (example shown in Fig. 6b.), cell size and shape and membrane tension (example shown in Fig. 6c.), and membrane tension and signal transduction (example shown in Fig. 6c.). b. Membrane shape informs signaling pathways including Src and MAPK. Reproduced from Ref. [107] with permission from Cell. c. Membrane tension regulates cell shape as in this model of cell spreading and polarization during the cell cycle. Reproduced from Ref. [142] with permission from Trends in Cell Biology. d) Membrane tension can inform cell signaling as in this model of tension activated ion receptor TRP4. Reproduced from Ref. [144] with permission from Journal of Cell Science. e) Computational models could address how features of the pericellular space affect cell shape by building on models of cell shape, membrane tension and signal transduction.

osteogenic differentiation and cell spreading. Material viscoelasticity has been shown to influence cell behavior, with faster stress relaxation or later onset of stress stiffening leading to enhanced ligand clustering/matrix bundling and MSC osteogenesis [104,123]. In these matrices with an initial elastic modulus of 17 kPa, MSCs form a mineralized, collagen type 1 enriched matrix similar to bone. These results again show an example of cellular interaction with the matrix correlating with protein deposition. Further time resolved studies are needed to mechanistically understand how cells sense and respond to the dynamic matrix remodeling that results from properties like degradability and viscoelasticity.

4. Hydration and cell volume regulation

Hydration of the pericellular space is another important feature that can directly impinge on cell volume and thereby cell fate. Cells transport water and ions across their membrane to maintain osmotic balance with the pericellular space. This transport can alter the concentration of intracellular material and the extent of molecular crowding in the cell, having numerous important consequences on cell mechanics by increasing stiffness [124], on protein folding and transport [125], and on regulation of chromatin condensation [125].

Several features of the pericellular space regulate hydration. For example, collagen fibers can hold differing amounts of water depending on their conformation (Fig. 5b) [126,127]. GAGs are another component of the ECM that control the hydration of the pericellular space by binding large amounts of water [128]. Joerges et al. found that when the GAG hyaluronan is added to cells in culture, its attraction to water influenced the cell membrane potential. This effect draws water out of the cell to maintain the osmotic pressure of the pericellular space. Thus, the cells exhibited a hyaluronan concentration-dependent volume change [128]. In addition, the structural elements of the ECM limit the space available and can exclude soluble macromolecules from the pericellular space [129]. Changes to ECM structure that affect hydration and therefore cell volume are especially important to understand in developing drug testing models, as the ECM itself may be crowding the drug and changing the apparent concentration to the cell [129].

Cell-ECM adhesion also plays an important role in hydration response to the pericellular space. As a cell adheres and spreads on a stiff 2D substrate, cytoskeletal tension increases, activating ion channels that lead to water efflux and volume reduction [13,14]. The cell also exhibits increased stiffness and decreased nuclear volume [13]. This relationship of volume regulation by varying rigidity is identical to that elicited by cell confinement on patterned substrates, suggesting the phenomena is fundamentally cell-shape dependent (Fig. 5a) [13]. That is, membrane curvature can directly link local cell shape to volume regulation through ion channel activity. Piezo proteins were identified in 2010 as important components of excitatory mechanosensitive ion channels [130]. Piezo1 relates membrane curvature to ion efflux and volume regulation, therefore integrating features of cell shape to volume in several cell types [131–133]. As discussed above, cell shape can be regulated by a number of biomaterial features in 3D scaffolds. It would be interesting to directly evaluate the effect of 3D biomaterial properties like confinement on the activity and expression of ion channels.

Utilizing an interesting technique to study the effects of cell shape and cell volume separately, a recent study used microcontact printing to create 3D microniches of varying volume and shape [12]. These 3D microniches are comprised of an array of polyacrylamide (PA) wells of different shapes and heights. The surface of the PA is modified and coated with fibronectin, then hMSCs are seeded, and a PA lid is applied to cover the microniche array. This system revealed that cellular features indicative of intracellular tension, such as actin stress fiber formation, myosin levels, focal adhesion formation, and nuclear YAP/TAZ localization, were maximized at intermediate cell volumes. These results were mostly independent of cell shape (slightly more prominent in angled or elongated geometries than for rounded or symmetric geometries), indicating the importance of distinguishing between the effects of cell shape and volume in the pericellular space (Fig. 5c) [12]. 3D microniches were also used to study whether cells with different volumes have different responses to matrix stiffness [134]. Interestingly, stress fibers and focal adhesions formed in soft, intermediate, and stiff (5, 12, and 23 kPa) matrices in the optimal volume, but stress fiber and focal adhesion formation was reduced in small or large cells, indicating that cell volume is a key feature regulating cellular mechanosensing.

5. Biophysical modeling of cell shape and volume regulation

In addition to the different experimental techniques described above, theoretical and computational modeling of cell shape and volume regulation in response to environmental cues from the pericellular space can enable the identification of general principles of cellular morphology in response to the physical features of the ECM (Fig. 6a) [135–138]. Recently, there have been several studies that have developed models that (1) relate cell size and shape to biochemical signal transduction (Fig. 6b) [107,138–141], (2) cell shape and membrane tension (Fig. 6c) [142,143], and (3) membrane tension and mechanosensation (Fig. 6d) [144]. These efforts span a range of cell types from hIPScs [145] to red blood cell [146]. From the wide variety of models and their application, it is becoming increasingly clear that close collaborations between experimentalists and the modeling community can enable deeper insights into time-dynamic biological processes comprising interactions between cells and their ECM.

Most mechanical models begin with a force balance on the cell membrane or the cell cortex and use viscoelastic models to represent the stress-strain response of cellular components. Contributions to the force balance arise from pressure within the cell, membrane/cortical tension, contractile forces due to myosin activity [147–149], and ion and water channel activity on the plasma membrane) [150–152]. Specifically, such models have identified that active regulation of cellular contractility through myosin coupled with osmotic regulation can control cell size [153]; that tension can couple the activity of RhoGTPases to modulate cell behavior ranging from contracted to relaxed states [154]; and that volume regulation of cell shape can result in altered nuclear morphology [155]. In some models that describe features of cell adhesion, including membrane tension or contact angle, cell volume is assumed to be constant or is ignored [156]. However, volume is a critical variable that changes in response to adhesion [13]. Importantly, none of these efforts have, to our knowledge, considered the interaction between the pericellular space and cellular morphology quantitatively. There is little doubt that models can complement experimental analyses and give rise to new hypotheses. The next frontier for the field is to develop mechanochemical models that are tightly constrained by experimental data from single cell interactions with the pericellular space. This will enable quantitative predictions to be made and tested. Additionally, there is a need for multiscale modeling tool development to effectively capture the interactions between cells and the ECM and between different cells. For example, consideration of the ECM architecture needs a representation that goes beyond the tortuosity and porosity of the matrix; a framework that captures ligand presentation, fiber orientation, and plasma membrane curvature locally is necessary.

These geometries can be informed by 3D reconstructions using volume electron microscopy or from Z-stacks obtained from confocal imaging [157] and simulations constrained by high-resolution imaging data from experiments (stochastic optical reconstruction microscopy (STORM), enhanced number and brightness (eN&B) analyses etc.). Simulations in these realistic representations can bridge the gap between phenomenological models and experimental measurements.

6. Conclusions and perspectives

A major opportunity for advancing tissue engineering lies in the adoption and advancement of 3D biophysical and biochemical analyses of cell-pericellular space interactions and their integration into predictive models. Investigating local cellular responses to biomaterials in a spatial and time-resolved manner will be critical to the development of quantitative models that reliably predict cellular outcomes. Our ability to design improved scaffolds for tissue regeneration and disease modeling will rely on the mechanistic insight gained through such studies.

Declaration of Competing Interest

The authors declare no competing interests.

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