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Getting the big picture of cell-matrix interactions: High-throughput biomaterial platforms and systems-level measurements

Ruoxing Lei [a,b], Sanjay Kumar [b,c,*]

[a] Department of Chemistry, University of California, Berkeley, CA 94720, United States
[b] Department of Bioengineering, University of California, Berkeley, CA 94720, United States
[c] Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, United States

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ABSTRACT

Living cells interact with the extracellular matrix (ECM) in a complex and reciprocal manner. Much has been learned over the past few decades about cell-ECM interactions from targeted studies in which a specific matrix parameter (e.g. stiffness, adhesivity) has been varied across a few discrete values, or in which the level or activity of a protein is controlled in an isolated fashion. As the field moves forward, there is growing interest in addressing cell-matrix interactions from a *systems* perspective, which has spurred a new generation of matrix platforms capable of interrogating multiple ECM inputs in a combinatorial and parallelized fashion. Efforts are also actively underway to integrate specialized, synthetic ECM platforms with global measures of cell behaviors, including at the transcriptomic, proteomic and epigenomic levels. Here we review recent advances in both areas. We describe how new combinatorial ECM technologies are revealing unexpected crosstalk and nonlinearity in the relationship between cell phenotype and matrix properties. Similarly, efforts to integrate "omics" measurements with synthetic ECM platforms are illuminating how ECM properties can control cell biology in surprising and functionally important ways. We expect that advances in both areas will deepen the field's understanding of cell-ECM interactions and offer valuable insight into the design of biomaterials for specific biomedical applications.

1. Introduction

Many cells within tissue are surrounded by a three-dimensional extracellular matrix (ECM), which is typically composed of proteins, polysaccharides, and proteoglycans. Historically, the ECM had been viewed as a passive scaffold that supports cell adhesion and tissue mechanics. However, it is now widely understood that the ECM profoundly regulates physiology and disease by providing a rich array of biophysical and biochemical cues that powerfully affect cell behavior [1–4]. The relationship between cells and the ECM is highly reciprocal, with cells deforming, digesting, and depositing matrix components. Cell-ECM interactions deeply regulate stem cell development [5], tumor progression [6,7], immune responses [8], and many other biological processes.

An important ongoing challenge in understanding and controlling cell-ECM interactions is the difficulty of presenting matrix cues to cells in a parallelized and multiplexed fashion, as is commonly done for soluble cues such as cytokines and small molecule effectors. The development of such platforms is as much a solid-state materials science problem as it is a cell biology problem, requiring combinatorial deployment of ECM-mimetic substrates of defined mechanics, adhesivity, and other properties. Moreover, while the synthetic ECM-mimetic materials used in culture platforms tend to be more scalable, tunable, and reproducible than their native counterparts, synthetic materials lack many of the structural and dynamic properties that regulate cell behavior in tissue [9]. Nonetheless, successful creation of combinatorial matrix platforms could both accelerate the field's understanding of cell-ECM crosstalk and serve as the basis for screening technologies.

In addition to permitting combinatorial presentation of ECM cues for discovery and screening, there is another level at which synthetic ECM fabrication and systems-level biology may be interfaced: The acquisition of high-content "omics" data from cells cultured on ECM scaffolds. Transcriptomic, proteomic, epigenomic, and other systems-level profiling in various ECM contexts all provide valuable high-level views of how matrix cues regulate biological processes such as metabolism, proliferation and apoptosis, both in normal and pathological systems. Systems-level measurements can often point to broad

* Corresponding author at: Department of Bioengineering, University of California, Berkeley, CA 94720, United States.
E-mail address: skumar@berkeley.edu (S. Kumar).

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mechanistic patterns that are difficult or impossible to discern from candidate-focused studies. Synthetic ECM-mimetic materials have unique value in conducting omics measurements because they are typically more bio-inert than naturally-derived ECM materials, which contain a large number of remnant cellular and ECM proteins [10]. However, obtaining these measurements in the context of 3D ECM scaffolds is still non-trivial given that large cell numbers are often needed for statistical power and that DNA/RNA or protein extraction is more difficult than on 2D culture dishes [11].

Despite these challenges, successful integration of synthetic matrix platforms and omics measurements is already yielding unprecedented mechanistic insights into cell-ECM interactions. In this review, we highlight recent progress in the development of high-throughput ECM platforms and in the application of systems biology to cells cultured in the context of synthetic biomaterials. We begin by discussing recently developed platforms that incorporate either single or combinatorial gradients of biophysical cues including matrix elasticity, ECM ligands and topography. We then focus on transcriptomic, proteomic and epigenomic level studies in mechanosensing events using biomaterial platforms.

2. High-throughput biomaterial platforms for probing cell-ECM interactions

Broadly speaking, we focus on two varieties of high-throughput ECM platforms in this review: First, we consider platforms in which one ECM biophysical property is systematically varied, often with the goal of decoupling the regulatory effects of this property from others. Another important objective of these platforms is to sample many more property values than would normally be possible with individually fabricated materials, so as to more quantitatively capture how the material property influences cell behavior (e.g. linear vs. nonlinear relationship). We will focus on platforms with varying stiffness and surface topography as both properties have been heavily studied using synthetic biomaterials. Second, we consider combinatorial platforms, where the aim is to deploy two or more biophysical cues in a single platform, with the goal of identifying synergies or other relationships between the parameters. Both types of platforms can provide valuable insight for understanding cell mechanobiology and guiding cell and tissue engineering.

For systems in which a single ECM parameter is systematically varied, ECM stiffness is a natural focal point given the importance of ECM viscoelastic properties in controlling cell growth and death [12–14], stem cell differentiation [15–17] and morphogenesis [18–20]. Stiffness gradients are ubiquitous in tissue physiology and disease, such as the transition between soft tissues to bone in joints and the interface between normal and tumor tissue [21,22]. Polymer hydrogels are a common scaffold for parallel deployment of a range of ECM stiffnesses within a single matrix material. Techniques such as photopatterning, layer-stacking, microfluidics and microprinting may be used to spatially control crosslinking level, thus generating stiffness gradients in the hydrogels. These fabrication approaches have been reviewed at length elsewhere [23,24].

Recent trends in developing stiffness gradient hydrogels include achieving precise control of stiffness distribution as a function of location and designing complex gradients intended to more closely mimic tissue structures. A double polymerization process was recently introduced to create spatially linear stiffness distributions [25]. The hydrogel was composed of two sequentially polymerized, ramp-shaped acrylamide/bisacrylamide components, where the slope of the stiffness gradient was tuned by altering the percentage of acrylamide or bisacrylamide. This platform captured nonlinear relationships between stiffness and the localization of stiffness-sensitive proteins such as YAP, MRTF-A and MRTF-B in human adipose-derived mesenchymal stem cells (MSCs). Another system featured a two-layered polyacrylamide (PA) gel hybrid, in which the corrugated, fiber-like topography of the underlying hydrogel created effective mechanical gradients at the surface of the flat, superficial layer [26]. In this system, cells migrated parallel to the axis of the underlying fibers, which recapitulated ECM fiber-mediated contact guidance seen in tissue. Electrospinning has also recently emerged as a valuable tool for the fabrication of parallelized ECM arrays, as exemplified by a recent study in which electrospun dextran vinyl sulfone (DexVS) fibers were arrayed into matrices with varying stiffness [27]. In contrast to non-fibrous culture substrates where soft matrices typically suppress cell spreading, fibroblasts in soft and deformable fibrous matrices exhibited increased spreading and focal adhesion formation. This observation is consistent with past studies indicating that nominally soft, fibrous ECMs can promote a stiff-ECM phenotype if cells are allowed to remodel and bundle the constituent fibers to create a locally stiff environment [28,29].

Surface topography determines how adhesion ligands align and are presented on the ECM, which significantly affects cell adhesion and tension [30]. Topographically patterned biomaterials are widely used in tissue engineering to direct cell differentiation, migration, and morphogenesis [31]. For example, surfaces microtextured with poly (lactose-co-glycolide) pillars of various heights and spacings were recently applied to control the differentiation of rat MSCs. The use of tall micropillars produced sharp, “piercing” nuclear deformations, which in turn correlated with greater osteogenic and less adipogenic differentiation [32]. Topographically patterned ECM fabricated from dip-coated catecholic polyglycerol-based substrates have revealed a biphasic relationship between MSC osteogenic efficiency and interfacial roughness, with the roughness of optimal osteogenesis also maximizing nuclear YAP localization [33]. There is great interest in incorporating topographically patterned substrates into standard multiwell culture plates to facilitate bioassays. In one such effort, various “trench-grid” topographies were deployed in a 96-well plate platform to screen effects on T cell growth and function. An optimal geometry was identified that maximized T cell interleukin 2 secretion, particularly when coupled with pharmacologic inhibition of myosin II [34].

There are many other examples of platforms for topographical control that employ polymer microfabrication techniques and thus have great potential for scale-up. We recently introduced a polydimethylsiloxane (PDMS) microchannel platform that allows contact-based AFM measurement of mechanical properties of cells migrating through confined spaces. Using this system, we showed that cells soften and rearrange their actomyosin networks as they squeeze through constructions [35]. In an important innovation relative to traditional, static ECM platforms, an optical embossing technique was recently exploited to create circular topographic patterns on azopolymer films while cells were migrating on these films. Cells showed evidence of adapting to newly introduced topographical features within two hours [36].

Combinatorial platforms incorporate more than one ECM properties orthogonally on a single device to investigate the coupling effects of multiple cues on directing cell behaviors. Stiffness and adhesive ligand density are two properties that have perhaps received the most attention in these applications, in part because they often synergistically impact cell adhesion, migration, and other phenotypes [37,38]. Conducting photochemistry in ECM hydrogels through gradient photomasks is a versatile and effective way of creating spatial gradients within materials [39]. Often, the polymer backbone is decorated with photoreactive moieties and then exposed to UV or visible light in the presence of a gradient photomask to create gradients within the hydrogel. We developed a 2D hydrogel platform based on methacrylated hyaluronic acid (HA) modified with a UV-sensitive moiety, 4,5-dimethoxy 2-nitrobenzyl-aminothiol (DMNBAT), allowing orthogonal photoreactions triggered by UV and visible light [40]. UV radiation through a printed photomask cleaved the DMNBAT groups to release free thiols for ECM protein conjugation, while transmitting visible light through an orthogonally rotated photomask induced crosslinking between methacrylate groups to form a stiffness gradient (Fig. 1A). We used this platform to show that ECM stiffness and fibronectin density interact to

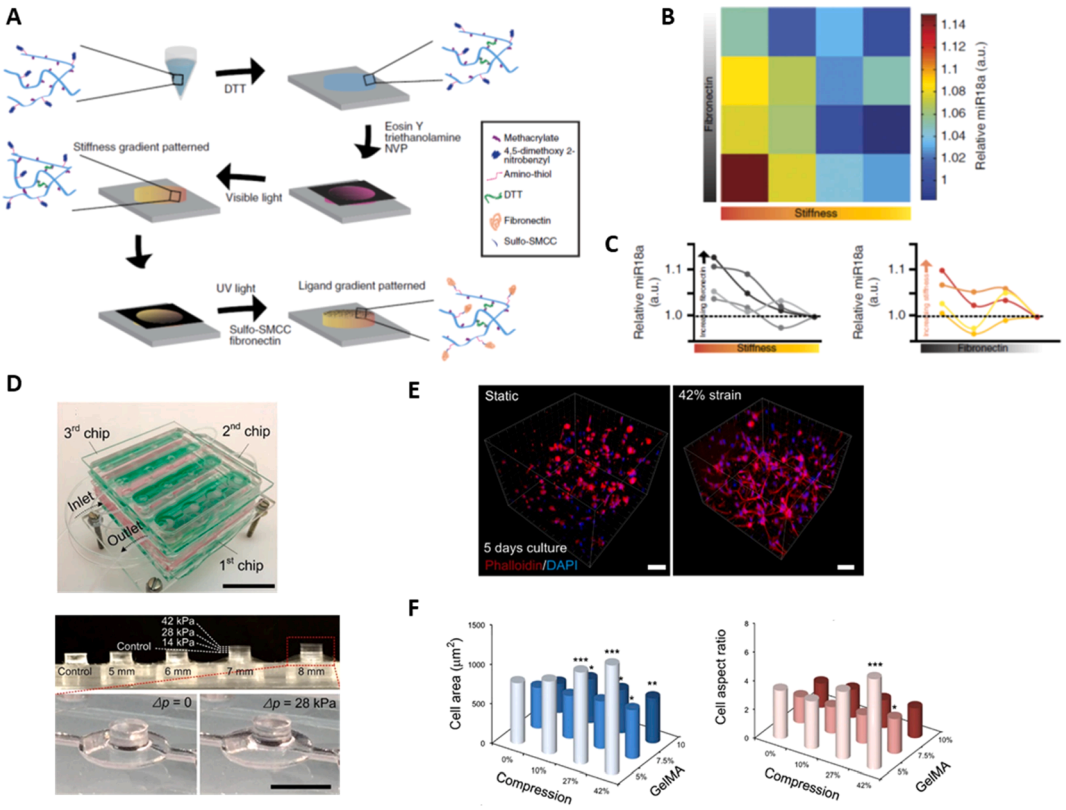

Fig. 1. Combinatorial hydrogel platforms for studying cell responses to stiffness and adhesivity (A–C) or to hydrogel composition and compression forces (D–F). (A) Dual gradient patterning of DMNBAT-HA-methacrylate hydrogel. (B) Heat map showing miR18a expression levels at 16 matrix stiffness-ligand combinations. (C) *Iso*-fibronectin curves (left) show that substrate stiffness regulates miR18a expression at all fibronectin densities tested. Similarly, *iso*-stiffness curves (right) show that fibronectin density only regulates miR18a expression at high stiffness. (D) Photographs of a platform in which cells are placed in interconnected bioreactors (top) and subjected to mechanical force thorugh displacement of pressure-controlled posts (bottom). Scale bars = 5 mm. (E) 3D reconstructed confocal photomicrographs of human MSCs in 5% GelMA hydrogel cultured under different strains. Scale bars = 100 μm. (F) 5% GelMA with 42% compression is optimal for cell spreading and elongation. (A–C) adapted with permission from [40]. (D–F) adapted with permission from [44]. Copyright (2018) American Chemical Society.

regulate expression of miR18a, an oncogenic microRNA in glioma cells (Fig. 1B, 1C). By varying stiffness and ligand density in parallel, we were able to uncover unexpected inter-parameter interactions. For example, stiffness influenced miR18a expression at all fibronectin densities examined, whereas fibronectin density modulated miR18a only on stiff substrates. Combinatorial hydrogels may use a single photosensitive moiety if excess unreacted sites from the first round can further react in the second round. This strategy has been used on hydrogels with norbornene groups as the only reactive "handles" in both rounds of photopatterning [41,42]. Matrix stiffness and adhesive properties can also be controlled without photopatterning; for example, plasma oxidation has been used to introduce stiffness and wettability gradients to surfaces, which indirectly modulate protein adsorption [43]. Using this platform, the authors revealed distinct nonlinear relationships between the engineered material properties and human MSC adhesion, spreading, nuclear size, and vinculin expression.

While most of the studies described above focus exclusively on modulating material properties, some approaches superimpose external dynamic mechanical loads on cells cultured on defined ECMs. In one notable example, coupling between biomaterial composition and dynamic mechanical compression in regulating human MSC osteogenesis was examined in 3D hydrogels [44]. Here, actuating posts were used to apply gradients of cyclic compressive strain, and GelMA concentration was used to vary ECM mechanics (Fig. 1D). The authors found that a low percentage (5%) of GelMA coupled with a high magnitude (42%) of dynamic compression provided the optimal combination for cell spreading and osteogenic differentiation (Fig. 1E, 1F), suggesting a coupling effect between hydrogel pore size and compressive strain. In another study, 3D miniaturized porous biomaterials were modified with 31 different protein formulations present in bone ECM, cell–cell junctions and enamel, and subsequently subjected to flow [45]. This approach captured unexpected interplay between protein composition and dynamic flow in regulating MSC adhesion and alkaline phosphatase production.

3. High-throughput systems biology analysis of cell mechanobiology on biomaterial platforms

Cell-ECM interactions can influence a wide variety of transcriptomic, proteomic, and epigenomic programs, which can be challenging or impossible to appreciate from candidate-based studies. Therefore, it is important to integrate engineered biomaterials with systems-level measurements as a first step in understanding how ECM controls and influences cellular responses [46–49]. We next highlight recent work on measuring how ECM biophysical properties influence transcriptomic programs, both at the level of mRNAs and non-coding RNAs. We also discuss more nascent but extremely promising efforts to extend these approaches to proteomic and epigenomic analysis.

To gain mechanistic insight into the interplay between bone morphogenetic protein 4 (BMP4) signaling and ECM mechanics in controlling the differentiation of glioblastoma (GBM) tumor initiating cells (TICs), we performed whole-genome RNA sequencing of GBM TICs on soft or stiff polyacrylamide hydrogels and in BMP4- or growth factor-supplemented medium [50]. Interestingly, whereas comparatively few transcripts were stiffness-sensitive in growth medium, many more transcripts were stiffness-sensitive in the presence of BMP4 (Fig. 2A).

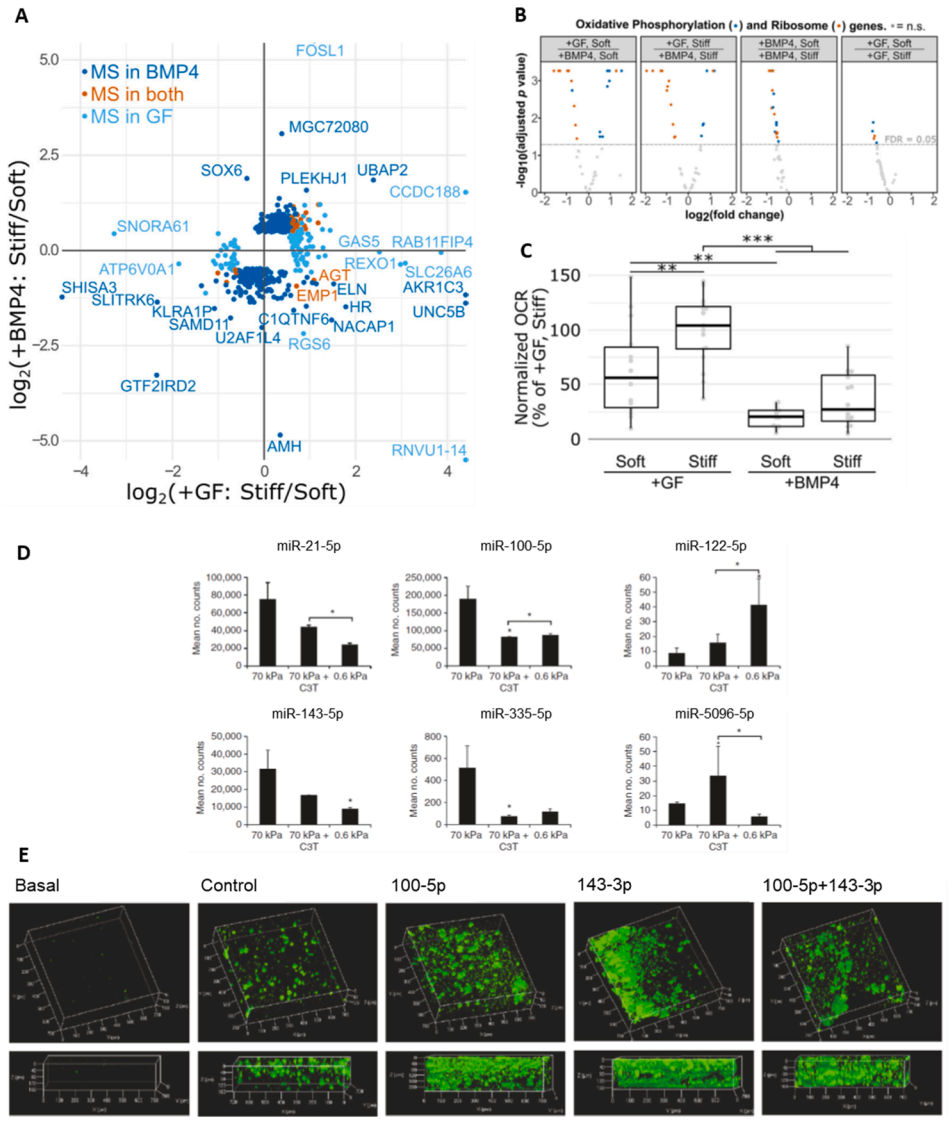

Fig. 2. Application of RNA-seq analysis on synthetic hydrogels to identify mechanosensitive genes and miRNAs. (A) Changes in mechanosensitive (MS) genes for BMP4-treated cells versus growth factor-treated cells. (B) Ribosome protein expression is upregulated by both BMP4 treatment and matrix stiffening. BMP4 upregulates a few oxidative phosphorylation (OXPHOS) genes but its net effect is to downregulate OXPHOS genes, while stiffness universally upregulates OXPHOS genes. (C) Both stiff ECM and BMP4 treatment significantly increase basal oxygen consumption rate (OCR). (D) Mean counts from miRNA sequencing of representative miRNAs whose expression is sensitive to ECM stiffness or RhoA inhibitor (C3T). (E) 3D projections of hydroxyapatite staining (green) showing a synergy in promoting MSC osteogenesis by miR-100-5p and miR-143-3p. (A–C) adapted with permission from [50]. (D–E) reproduced with permission from [54]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The notion that BMP4 sensitizes GBM TICs to stiffness cues confirmed earlier work in which we showed that TICs are largely stiffness-insensitive under self-renewal conditions [51]. Surprisingly, pathway analysis revealed oxidative phosphorylation as one of the few intrinsically stiffness-sensitive systems (Fig. 2B). We functionally confirmed this finding by showing that oxygen consumption varies with stiffness under both self-renewal conditions and in the presence of BMP4 (Fig. 2C). Transcriptomic analysis has also been applied to explore coupling effects between ECM parameters, in one example through RNA sequencing of stem cells on eight combinations of matrix stiffnesses, stress relaxation properties and adhesion ligand densities. In general, transcriptomic changes associated with one ECM property depended on the collective context provided by the other two properties [52]. Systems-level analysis can further reveal unexpected insights into therapeutic responses. For example, transcriptomic analysis of breast cancer cells on 2D/3D poly(ethylene glycol) (PEG) hydrogels and in uniform tumor spheroids enabled screens that uncovered synergies between MEK inhibition and the tyrosine kinase inhibitor sorafenib in reducing tumor burden in mouse xenograft models [53].

Many non-coding RNAs regulate gene expression at the transcriptional and post-transcriptional level, and there is much interest in understanding how non-coding RNAs are regulated by ECM properties. In human MSCs, transcriptomic studies indicate that ECM stiffness upregulates miR-100-5p and miR-143-3p (Fig. 2D). Subsequent functional studies implicated both miRNAs in promoting osteogenesis through mTOR pathway suppression (Fig. 2E) [54]. Such approaches have also proven valuable in studies of miRNA regulation of immunity and inflammation, including monocyte-to-macrophage differentiation, where a network of miRNAs was discovered to regulate macrophage behavior by influencing signaling through p53, integrins, and focal adhesions [55]. In still another study on stiffness-dependent differentiation of pluripotent stem cells, the long non-coding RNA (lncRNA) LINC00458 was found to increase on soft matrices, where it was necessary for endodermal lineage determination [56]. Finally, in vascular smooth muscle cells, matrix stiffening was found to enhance expression of the lncRNA MALAT1, which was then demonstrated to promote endothelial repair following vascular injury [57].

An emerging extension of proteomic studies that is especially relevant to cell and tissue engineering is the examination of the matrisome, i.e. the complement of ECM and ECM-related proteins, including those secreted or deposited in the process of ECM remodeling [58]. Proteomic analysis of the matrisome in 3D hydrogels has helped elucidate regulatory functions of the pericellular matrix. One such study utilized stable isotope labeling with amino acids in cell culture (SILAC) to distinguish newly secreted proteins of human MSCs in hyaluronic acid and PEG diacrylate-based hydrogels [59]. ECM proteins composed >40% of all

nascent proteins, with pericellular matrix deposition promoting adipogenesis on less cross-linked hydrogels. Label-free proteomic analysis has also been integrated with PEG hydrogel systems and has produced the observation that MSCs exhibit distinct collagen expression profiles under TGFβ1 or PDGF/EGF treatment. Here the analysis was facilitated by trypsin-degradable PEG hydrogels that allowed separate consideration of deposited ECM proteins and proteins from cell lysates [60]. Proteomic approaches are now being extended to study post-translational modifications on synthetic matrices [61,62]. For example, the nanoscale roughness of ZrO_x surfaces significantly alters the expression and phosphorylation profile of proteins that are involved in mechanotransduction and neuronal differentiation of neuro-like PC12 cells [61]. Surface nanostructures can also boost expression of anti-aging and anti-apoptotic proteins in human pancreatic β-cells, which are key to improving β-cell survival and differentiation [62].

Perhaps the newest frontier in these systems-level studies involves the dissection of epigenomic responses to ECM cues. The mechanical microenvironment is increasingly recognized to induce epigenetic modifications through nuclear deformations that may follow alterations in cell cytoskeletal deformation [63]. In recent years, synthetic biomaterials have been introduced to study epigenomic responses to ECM biophysical signals, as matrix stiffness, topography, and applied mechanical forces have all been reported to alter the epigenome [64,65]. Culturing human MSCs on TiO_2 nanotubes with diameters of 70 nm promoted osteogenic differentiation, which is associated with enhanced methylation of histone H3 at lysine 4 (H3K4) in the promoter regions of osteogenic genes Runx2 and osteocalcin [66]. In a separate study using PEG-based hydrogels that can soften upon photodegradation, MSC histone acetylation was found to increase, and chromatin condensation was found to decrease, on stiff microenvironments [67]. Notably, these changes depended strongly on the duration of cell exposure to stiff ECM, implying that the acetylation events may serve as the basis for mechanical memory.

4. Conclusions and future outlook

Our understanding of cell-ECM interactions is rapidly evolving thanks to the emergence of biomaterial models that are increasingly compatible with systems-level analysis. High-throughput biomaterial platforms and integration of biomaterials with systems-level “omics” measurements represent two key enabling tools. The continued advance of these approaches should greatly facilitate the development of technologies for cell and tissue engineering, disease modeling, and therapeutics.

One major challenge in the development of this new generation of ECM platforms is the need to balance experimental tractability against recapitulation of the complexities of tissue ECMs. Continued advances in polymer processing, 3D printing, and allied technologies should help make emerging ECM platforms more realistic, such as by incorporating fibrillar structures into hydrogels and introducing temporal control of matrix properties [68,69]. It will also be important to improve the compatibility of new ECM platforms with existing analytical assays and tools that were designed for standard culture plates and dishes, including plate readers [34] and advanced optical and force microscopies [35]. As this review has indicated, interest in conducting system biology studies on synthetic biomaterials is high and rising, fueled in part by the rich and unexpected regulatory relationships these studies have already revealed. However, current efforts in this space are still often limited to manipulations of one property at a time (e.g. stiffness) and, even then, on discrete numbers of conditions. Moving forward, it will be valuable to combine both approaches described in this review, i.e. systems-level analysis on high-throughput biomaterial platforms. To reach this goal, real challenges must be overcome in terms of workflow design and management of the massive data sets such efforts are likely to produce. It will also be important to ensure that the efficiency and sensitivity of the analytical techniques, including single-cell RNA-seq and proteomics, are maintained when deployed in biomaterial platforms. Statistical tools such as combinatorial design of experiments and post hoc correlation analysis may also help narrow the combinatorial space of material conditions to consider [49,70]. If these challenges can be overcome, the field will be poised for a completely new era in which cell-ECM interactions can be connected in rich and surprising ways to broader aspects of cell physiology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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*: *special interest*

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