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Designer Biomaterials for Mechanobiology

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Abstract

Biomaterials engineered with specific bioactive ligands, tunable mechanical properties, and complex architectural features have emerged as powerful tools to probe how cells sense and respond to the physical properties of their material surroundings, and ultimately provide designer approaches to control cell function.

Introduction

A variety of physical forces are ever present throughout the human body, ranging from the pumping of blood by the heart and flow-induced shear stress in blood vessels to tensional and compressive stresses from skeletal muscle contraction, tendon ligament stretching, joint loading, and the vibrations of vocal folds phonation. While such forces are vital for the physical movements that enable us to breath, move or digest, mechanical forces are also critical regulators of biochemical signaling, cell behavior, and tissue function. At the tissue level, physical forces regulate dorsal closure¹, epithelial morphogenesis², and skeletal development during embryogenesis³, extracellular matrix (ECM) remodeling during tissue homeostasis⁴, vascular inflammation and sprouting^{5–7} and repair of injured tissue during wound healing^{8,9}. At the cellular scale, cell-generated contractile forces play a fundamental role in assembling the cytoskeleton and organizing of the cellular architecture, which affects activation of biochemical signaling pathways and downstream gene transcription, ultimately controlling cell adhesion, migration, proliferation, differentiation, and apoptosis^{10–12}. Given the profound impact of mechanical forces on most, if not all, cellular functions, understanding how physical forces are converted into biochemical signals, a process called mechanotransduction, is imperative in order to comprehend embryonic development, regeneration and disease^{13–16}.

The first class of materials employed to study the impact of matrix mechanics on cellular function were hydrogels based on isolated natural ECM components such as collagen I, fibrin or basement membrane constituents (e.g., Matrigel). These materials bear structural

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resemblance and cell adhesive properties comparable to those in *in vivo* microenvironments^{17,18}, wherein cells are embedded in a three-dimensional fiber-rich network composed of proteins, glycoproteins and proteoglycans⁴. However, in the reconstituted native ECMs, the non-linear stress-strain mechanics, the fibrous structural features and the compositionally constrained biological ligands are typically intertwined, which makes it difficult to identify the contribution to cell behavior of each individual material property, such as the binding affinity for cells, mechanical stiffness, porosity, fibrous organization, and viscoelasticity (Fig. 1a). These challenges in manipulating natural ECM properties initially hampered researchers' ability to isolate which aspects of the ECM impacted cell signaling and behavior; however, the limitations ultimately inspired the development of novel synthetic biomaterials with tunable material properties to parse out how cells sense, probe and integrate physical forces.

In this Commentary, we will discuss various approaches to control specific properties of synthetic analogs of ECMs, and how these materials have revealed previously unappreciated mechanisms of mechanotransduction. We will describe the key parameters that have been placed under synthetic control and the biology that has been revealed by these capabilities (Fig. 1b). In particular, we will focus on materials that enabled investigations into the impact of stiffness, degradability and viscoelasticity on cell behavior in setting where cells are culture on the surface of such materials (2D) and embedded within them (3D). We will also comment on ongoing developments that may lead to new concepts and paradigms in the field of mechanobiology in the coming years.

Substrate stiffness and surface topography regulate cell morphology, function and fate

Historically, the role of physical forces in regulating bone tissue remodeling and embryonic development was already appreciated by Wolff, Roux and Thompson¹², yet it was not until the 1980's before the importance of mechanical forces in controlling cell behavior was welcomed by the broader scientific community¹⁹. Instead of using rigid tissue culture dishes, Harris and Stopak seeded cells on soft silicone films, a material platform that enabled one to witness cells generating forces via wrinkling of the underlying substrates during cell spreading and migration^{20,21}. Although often credited as the first study showing that non-muscle cells exerted traction forces, their work also demonstrated that (1) mechanobiology, a nascent field that until then predominantly relied on animal models, could be studied *in vitro*; and (2) materials could be employed as a tool to study mechanotransduction, a groundbreaking approach prelude the era of modern mechanobiology.

Since then, multiple covalently crosslinked polymer hydrogels were developed as cell-compatible substrates to study the mechanics of cell adhesion and migration, including polyacrylamide (PAAm) gels and polydimethylsiloxane (PDMS), arguably the two most extensively used materials in the field. The advent of a variety of surface chemistries enabled the synthetic coupling of ECM proteins to the surface of these substrates, allowing one to tune the density of conjugated biological ligands. In addition, the stiffness of the substrates could be tuned by directly changing the ratio of polymer and cross-linker solution, curing

temperature or duration of curing. Unlike native fibrous matrix, these synthetic hydrogels exhibit linear elastic behavior, such that the bulk modulus (stiffness) is not affected by matrix deformation or strain rate²². Such synthetic characteristics offered the opportunity to decouple biochemical signals from substrate stiffness, a feature that was unattainable in native ECM-derived hydrogels.

Using this material toolbox, a remarkable degree of insight in how cells sense and respond to mechanical forces has been revealed. Perhaps the most important discovery was that matrix rigidity alone can regulate cell morphology, function and fate²³. Cells adherent to stiff substrates display larger spreading area, enlarged focal adhesions, actin stress fiber assembly and proliferate more as compared to cells adherent on softer substrates^{24,25}. Seminal work from Discher and co-workers revealed that bone marrow stromal cells (BMSCs) can differentiate into different lineages as a function of substrate stiffness. BMSCs on very soft hydrogels preferentially adopted a neurogenic phenotype, while cells on intermediate stiff substrates switched on transcription of genes associated with myogenic differentiation, and cells on stiff substrates committed to the osteogenic lineage²⁶. Thus, matching the substrate stiffness to the mechanics of specific tissues of interest was sufficient to prime BMSCs into tissue-relevant lineages^{23,27}. In addition to the physiologic role for stiffness, aberrantly high matrix stiffness was found to promote epithelial-mesenchymal transition (EMT) of epithelial cells and migration of cancer cells to metastasize^{17,18,28,29}. Application of approaches to spatially control either crosslinker concentration or the amount of UV-exposure to the underlying substrates resulted in substrates with multiple stiffness's at different locations or stiffness gradients. When seeding cells on such materials, it was discovered that cells preferentially migrate to the stiffer substrates, a phenomenon coined as durotaxis³⁰⁻³². Taken together, these studies demonstrate that matrix stiffness is a critical physical parameter that mediates normal and pathological states of cells.

The strain-independent, elastic properties of PAAm and PDMS not only supported studies of stiffness, but also allowed for the measurement of traction forces generated by cells. In a method referred to as traction force microscopy (TFM)³³, deformations in the material and corresponding forces were detected by tracking fiduciary beads embedded in PAAm substrates^{31,34}, micropatterned features on PDMS^{35,36}, or bending of micropillars^{37,38}. Using TFM, it was discovered that adherent cells continuously probe their physical environment and change the degree of force generation depending on the stiffness of the substrate. That is, cells pull harder on stiff versus soft matrices, allowing cells to match their stiffness to the stiffness of the substrate^{24,39}.

Degradable crosslinks: the missing link to study cell mechanics in three dimensions.

Although the simplicity of 2D platforms provides a reductionist approach to study mechanotransduction, cells in many physiological settings are embedded within a 3D matrix. This limitation has spurred substantial efforts towards developing cytocompatible materials to support encapsulated (3D) culture^{4,40}. In contrast to 2D substrates that are polymerized before cells are seeded, encapsulating cells in 3D requires the material

components and crosslinking procedures to be biocompatible. Given the toxicity of the crosslinkers and acrylamide monomer, traditional PAAm gels are therefore unsuitable for encapsulating cells in 3D. Analogously, PDMS is a non-aqueous material and therefore cannot be used for this purpose. Advances in macromolecular chemistry and material science has enabled the design of new biocompatible materials to impart the hierarchical structure, biological complexity and dynamic mechanical properties of natural ECM^{41–43}.

Pioneering work from Hubbell and co-workers adapted a Michael-type addition reaction as crosslinking chemistry to form poly(ethylene glycol) (PEG)-based 3D hydrogels. The mild and cytocompatible yet efficient nature of the chemical reaction permitted *in vitro* encapsulation of various cell types, demonstrating successful transition from 2D substrate cell culture platform to 3D cell-encapsulation hydrogel system⁴⁴. The synthetic nature of these polymers preserves the well-defined relationship between modulus and crosslinking density and permits tunability of stiffness independent of the concentration of conjugated ligands. Tuning the crosslinking density yields a wide range of hydrogel mechanical properties. In addition, bioactive ligands derived from natural ECM can be directly conjugated during cell encapsulation and hydrogel formation processes to support cell viability, spreading and matrix degradation and remodeling in 3D, which explains why this design strategy quickly populated the biomaterials field. Building on similar strategies, polysaccharide (e.g., hyaluronan, dextran, gelatin and alginate) based materials have also been widely introduced for 3D cell culture, and advances such as oxime and click chemistry have been introduced as additional cross-linking chemistries^{43,45}. Compared to PEG molecules, these polysaccharides provide many more sites on the backbone available for chemical modifications, offering greater flexibility for tuning ligands and stiffness⁴⁶.

Intriguingly, early attempts to encapsulate cells in 3D hydrogels revealed a completely opposite trend compared to traditional 2D culturing substrates. In contrast to planar surfaces, embedded cells in soft matrices spread well and display a polarized morphology, but remained round in stiff hydrogels, suggesting that in addition to stiffness, matrix degradation – the breakage of crosslinks *via* passive hydrolysis or cell-mediated enzymatic cleavage – is a key component in regulating cell morphology in 3D microenvironment^{47,48}. It was further shown that the differentiation of hMSCs was regulated by scaffold degradability⁴⁹. However, when encapsulated in non-degradable ionically crosslinked alginate hydrogel, lineage commitment of these MSC was determined by matrix stiffness⁵⁰. The physical crosslinking mechanism in this case allows significant cellular reorganization of the material and adhesion ligand presentation. The apparently divergent responses of cells in non-degradable versus degradable hydrogels underscores the importance of materials with distinct crosslinking mechanisms, molecular structure and dimensionality in order to elucidated how cells are transducing their environment.

Despite the promising success in recapitulating the tunability of material parameters in 3D, it remains a major challenge to precisely tune mechanical properties in fully synthetic matrices without interfering with other physiochemical factors. Increasing bulk hydrogel stiffness is generally associated with increasing crosslinking density which concomitantly alters the matrix porosity, degradation, medium diffusion, mass transport, and thus the swelling properties of the gel. Swelling of hydrogels, typically more dramatic for soft

matrices, has hindered the ability to generate structures with precise geometries, and important to mechanobiology, might introduce unwanted mechanical stresses in the system. To address this problem, we recently developed a synthetic dextran hydrogel with tunable hydrophobicity as a means to control non-swelling/swelling features. Using this system to tune hydrogel porosity independent of matrix degradability and stiffness, we showed that matrix degradability governs the mode of 3D endothelial cell invasion from single-cell to multicellular, strand-like invasion required for angiogenesis⁵¹. This example illustrates again how gaining control of confounding material properties provides a path to better understanding how specific material parameters are being transduced by cells.

Transient mechanics and non-linear elasticity regulate cellular function

Most mechanobiology studies have relied on hydrogels that exhibit simple linear elastic mechanics. However, more complex mechanical properties exist in many native ECMs, including non-linear mechanics due to their fibrous nature, as well as time-dependent effects such as plasticity and viscoelasticity⁵².

One approach to introduce the fibrous character of native ECMs has been explored using electrospinning, a method used to fabricate fibrous matrices with tunable mechanics and user-defined fiber architecture with physiologically relevant dimensions and scales^{53,54}. Nonlinear elastic characteristic of fibrillar network promoted increased focal adhesion signaling by recruiting local fiber assembly on soft fibrous substrates, revealing an inverted mechanosensing mechanism compared to traditional 2D hydrogel surfaces^{53,54}.

To impart viscoelastic features, David Mooney and colleagues used ionic-mediated non-covalent crosslinking strategy to form alginate hydrogels. In contrast to covalent bonds, ionic bonds can break under physiological stresses rendering plastic deformation of the material, which enabled the discovery of cells spreading and proliferation even on soft substrates to comparable extents of cells on stiff substrates. It appears that the ability of cells to rearrange the material allows cells to recruit adhesive ligands, locally enhancing integrin clustering^{55,56}. Such unconventional findings implicate that time-dependent mechanical characteristics can serve as an additional physical regulator in directing cell behavior, ECM remodeling and stem cell fate.

Temporal control of material properties to study mechanotransduction

The recent development of light-based conjugation strategies has allowed for material properties to be changed non-invasively during an experiment. Pioneering work from Anseth and coworkers demonstrated that they could decorate 3D PEG hydrogels with photo-labile nitrobenzyl ether-derived acrylate moieties, allowing for on-demand scaffold degradation *via* external photo-irradiation stimuli. This chemistry approach allowed them further to demonstrate the capabilities to trigger encapsulated stem cells to spread into the gel and differentiate at will⁵⁷. In a follow up study, the team incorporated a thiol-ene photoreactive chemistry (reactive to visible light) to conjugate biological ligands and the previously established photo-cleavable reaction (reactive to UV light) to degrade the polymer backbone, in PEG hydrogels that were crosslinked *via* a strain-promoted azide-alkyne cycloaddition

(SPAAC) method⁵⁸. Using these orthogonal crosslinking strategies together demonstrated the capability of spatiotemporal regulation of multiple materials properties (e.g., integrin-binding ligand presentation and network erosion) in real time. The photo-cleavable chemistry also permitted *in vitro* modulation of hydrogel mechanical properties such as reducing the modulus of an initially stiff substrate to a softer one while cells remained adhered to the substrate. This combination revealed the role of microRNA-21 and Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) in transducing mechanical properties, and imparting some mechanical memory, in influencing stem cell plasticity. This work illustrates how new synthetic capabilities have uncovered previously unappreciated insights into how cells respond to their local environment. In this case, the use of switchable mechanics was essential to demonstrate that cells can remember, for a time, their past mechanical conditions^{59,60}. Matrix stiffening using this strategy has recently revealed that it can trigger activation of hepatic stellate cells into a fibrotic phenotype, suggesting the importance of such stiffening in the development of liver fibrosis^{61,62}.

Outlook

The evolution of mechanobiology studies highlights the ongoing development and advancement in material approaches to further understand how cells transduce material cues in ever more complex environments⁶³. Initial studies were on linear elastic 2D substrates, and the field has begun to move into 3D encapsulating materials, fibrous materials, and even those with time-dependent mechanical properties. Such transitions emphasize the importance of recapitulating the complexities of native ECMs over multiple length scales as an essential step towards understanding how mechanical cues regulate cellular function and stem cell fate. Despite the exciting progress in biomaterial design, there are still very few successes that truly demonstrate the ability to simultaneously and independently regulate biochemical, structural and mechanical cues within one single synthetic platform. Such challenges can be leveraged by engineering novel biocomposites with integrated material properties to capture the heterogeneous and anisotropic characteristics of the ECM. Although, emerging trends using photo-sensitive molecular switches to manipulate mechanical properties *in vitro* have successfully demonstrated dynamic modulation of mechanical stiffness of synthetic ECM in both 2D and 3D, such exogenous stimuli are not yet readily applicable to *in vivo* studies due to absorption of light in deep tissues/organs. Therefore, novel chemistries such as non-covalent reversible reactions that can be additionally manipulated to respond to physiological stimuli (e.g., temperature, electrical potential, pH and enzymatic activity) have begun to gain increasing attention as alternative hydrogel cross-linking strategies to offer dynamic mechanical tunability that can be applied in *in vivo* settings^{42,43,45}. Furthermore, other promising classes of materials, such as self-assembling peptide-/polypeptide-based materials, are also being developed to build complex fibrous scaffolds with hierarchical structures and non-linear viscoelastic mechanics, offering additional platforms to probe complex mechanical events (e.g., molecular association, energy dissipation etc.) in more native ECM-like settings^{64–66}. Finally, the limitation of long-term cell encapsulation due to degradation of these materials requires the design of novel strategies to maintain the mechanical integrity of the material by matching the kinetics

of matrix degradation with the rate of ECM production. Extending the experimental lifetime of 3D culture systems from days to weeks will be critical for connecting short-time mechanosensing and biochemical signaling to long-term effects of these signals on cell function and tissue development. The future of this field is bright, as innovations in materials continue to drive our understanding of mechanobiology ever forward.

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Figure 1.

Designer approaches to engineer biomaterials for mechanobiology. (a) Schematic of a cell embedded in a fibrous-rich, mechanically anisotropic 3D microenvironment; (b) multiple designer parameters to orthogonally control specific properties of synthetic analogs of extracellular matrices, such as crosslinking density, matrix degradability, fiber architecture, and viscoelastic mechanics.