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Citation for published version (APA):

Kurniawan, N. A., & Bouten, C. V. C. (2018). Mechanobiology of the cell–matrix interplay: catching a glimpse of complexity via minimalistic models. *Extreme Mechanics Letters*, 20, 59-64.
<https://doi.org/10.1016/j.eml.2018.01.004>

DOI:

[10.1016/j.eml.2018.01.004](https://doi.org/10.1016/j.eml.2018.01.004)

Document status and date:

Published: 01/04/2018

Document Version:

Accepted manuscript including changes made at the peer-review stage

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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- The final published version features the final layout of the paper including the volume, issue and page numbers.

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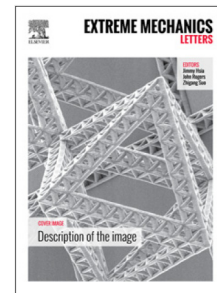
PII: S2352-4316(17)30186-4
DOI: <https://doi.org/10.1016/j.eml.2018.01.004>
Reference: EML 343

To appear in: *Extreme Mechanics Letters*

Received date: 1 November 2017
Revised date: 14 January 2018
Accepted date: 17 January 2018

Please cite this article as: N.A. Kurniawan, C.V.C. Bouten, Mechanobiology of the cell–matrix interplay: Catching a glimpse of complexity via minimalistic models, *Extreme Mechanics Letters* (2018), <https://doi.org/10.1016/j.eml.2018.01.004>

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Mechanobiology of the cell–matrix interplay: catching a glimpse of complexity via minimalistic models

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Abstract

Biological tissues present a grand challenge for mechanicians. Not only are their mechanical properties complex; they show a strong spatiotemporal heterogeneity due to ongoing and active remodeling of the living matter they are composed of: cells and extracellular matrix (ECM). The main sensors and effectors in this process are the cells, which direct tissue structure and mechanics by changing their own behavior and by producing and reorganizing the ECM. Vice versa, the ECM exhibits unique mechanical signatures associated with its fibrous polymer network that can modulate cell behavior. Grasping the full complexity of this reciprocal mechanical interaction between cells and the ECM is key for understanding physiological tissue function and maladaptation. An emerging approach is to explain the role of tissue biomechanics one component at a time and gradually (re)build tissue complexity. Here we highlight how this approach has been valuable in providing new insights in the relative and combined roles of cells and matrix, and in raising new questions into the origins of cellular and tissue responses. The answers may offer new approaches for mechanically driven tissue regeneration and biomaterial design.

Keywords:

Reconstituted ECM networks
Tissue equivalent
Remodeling
Collagen
Cell–matrix interaction
Mechanosensing

Introduction

A biological tissue is a composite material, mainly consisting of cells and extracellular matrix (ECM), mutually organized to perform a specialized function. Mechanical functioning is governed by the properties of the ECM as well as the interactions between the ECM and the resident tissue cells. The ECM is scaffolded by a network of fibrous proteins, mainly collagen and elastin, embedded in a gel of proteoglycans, glycoproteins, and water. At the macro-scale, the relative amount of these molecular constituents endows the tissue with tensile strength, resilience, and resistance against compressive forces, while further fine-tuning of load-bearing properties takes place via modification of fiber architecture, including collagen fiber length, diameter, anisotropy, and cross-linking [1]. At the micro-scale, the intricate architecture of the fiber-reinforced porous hydrogel offers a rich microenvironment for the cells, providing and stimulating them with a variety of mechanical and chemical cues.

Biological tissues show a fascinating adaptive response to changes in growth and mechanical demands. The principle sensors and effectors in this process are the cells. During early tissue development, they respond to mechanical stimuli by proliferating, migrating, and by laying down ECM. Later, they respond by changing their own morphology and function (e.g., contraction, adhesion) and by orchestrating the composition and structure of the ECM—a process referred to as remodeling. These continual, bidirectional interactions between the cells and the ECM are known to be facilitated by focal adhesion complexes. These complexes not only form a physical linkage between the ECM molecules and the intracellular cytoskeleton [2], but also act as a force transmitter and a mechanosensor [3], triggering a cascade of mechanical and chemical signaling in the cell that determine cellular decision making [4]. Thus, the cells are key modulators of mechanically-driven tissue formation and remodeling and actively control tissue architecture and mechanics via mechanosensing and mechanotransduction strategies. How cells sense and affect their mechanical environment is only partly known and is a topic of intense study in the emerging field of mechanobiology [5].

The cell's mechanosensing ability has also added another level of complexity in bridging the gap between functions at the cell and tissue levels, because it means that to fully understand tissue mechanical response we have to take into account not only the properties of the cells and the matrix, but also cell–matrix interactions. In vitro studies of three-dimensional (3D) minimal models designed to mimic the individual components of biological tissues has been a powerful approach to understand the material behavior of (the components of) the ECM and tissues. In this perspective article, we reflect on recent findings obtained using this minimalistic approach that have shed new light on tissue mechanobiology and the complex interplay between ECM and cells (Fig. 1). We discuss how these findings shape the future directions of cell–matrix mechanobiology and open a gateway to a multiscale understanding of cell and tissue mechanics, which is of relevance for mechanically-driven tissue engineering and materials design.

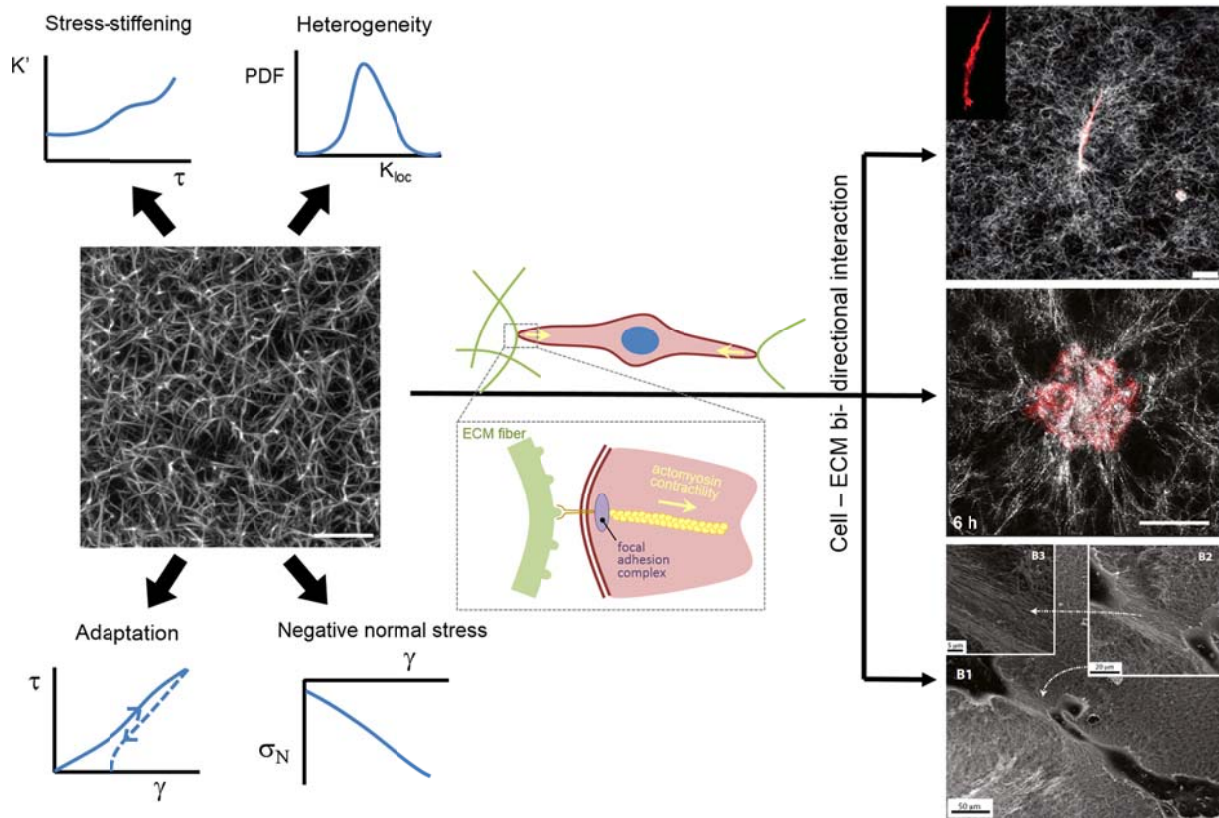


Figure 1. Deconstructing tissue mechanobiology. Tissues (*right panel*) are formed and supported through the dynamic bidirectional interactions between the ECM (*left panel*) and the resident cells that exert physical forces to the surrounding ECM via adhesion-mediated cell traction (*center panel*). The *left panel* illustrates that the (cell-free) ECM itself exhibits characteristic mechanical signatures, for example nonlinear stress-stiffening behavior (top left; K' : differential elastic modulus, τ : applied shear stress), heterogeneous local stiffness (top right; PDF: probability distribution function, K_{loc} : local stiffness), network adaptation (bottom left; γ : shear strain), and negative normal stress or reverse Poynting effect (bottom right; σ_N : normal stress). See text for more details about these phenomena. The image at the center depicts an (cell-free) *in vitro* reconstituted fibrin network as an example of ECM network; scale bar = 20 μm . The *right panel* shows examples of cell-ECM mechanical interactions: single 3T3 fibroblasts can remodel the pericellular ECM fibers (top; scale bar = 10 μm ; figure reproduced from ref. [6] with permission from Elsevier), multicellular 3T3 fibroblast tissues align their surrounding ECM (middle; scale bar = 50 μm ; figure reproduced from ref. [7] with permission from Elsevier), two mammary acini interacting over long distance by aligning ECM fibers in between (bottom; figure reproduced from ref. [8]).

Mechanics lessons from *in vitro* minimal models

ECM equivalents

Constructing minimal models of the ECM to study its role in cell and tissue functions has broadly employed three types of approach. The first focuses on isolating *single properties* of the ECM, such as stiffness, pore size, and gross tissue curvature, typically using simple, synthetic materials for which these properties can be easily tunable, such as polyacrylamide [9]. This approach is especially advantageous in unravelling the precise molecular mechanisms underlying specific cellular response to individual ECM physical properties, as has been comprehensively addressed in recent reviews [10-12]. A potential pitfall is the translatability of the findings to *in vivo* situations, as isolating a single ECM physical property at a time implies that the potentially important interplay with other properties is overlooked, and in practice often enforces the other properties to be entirely unphysiological. The

second approach focuses on recapitulating the *in vivo composition* of the ECM, using decellularized tissues. This approach is promising for promoting site-specific tissue regeneration, since the decellularized ECM retains the biochemical complexity, nanostructure, and inductive properties of the native matrix [13]. Various decellularization protocols are currently being developed, and the current lack of control over the amount and organization of the ECM components makes it challenging to identify the mechanisms by which the ECM mediates cell behavior [14]. The third approach focuses on reconstituting *single components* of the ECM, such as collagen, fibronectin, and fibrin, thereby preserving the overall ECM architecture and cell compatibility without the complexity of the complete ECM composition. Here the intrinsic presence and interplay of different ECM physical properties is both a strength and a weakness, since it limits the independent tunability of the individual properties [15]. For example, in reconstituting networks of collagen or fibrin fibers, the protein concentration, ligand density, network pore size, fiber size, and network stiffness are often interlinked, and specific gel polymerization protocols are often required to independently tune one of these factors without significantly affecting the others [16-19]. Importantly, characterization of these reconstituted ECM itself has shown unexpectedly rich mechanical behaviors, whose mechanobiological contributions to cell response and tissue functions are still not fully understood.

Since 2D substrate stiffness has been shown to directly affect various vital cell behaviors, such as adhesion, differentiation, proliferation, and migration [20-24], mechanical characterization of 2D cell culture substrates and 3D reconstituted ECM networks has become an integral part in these studies. It is worth noting that, compared to culture dishes and glass slides (stiffness in the GPa range) but also hard (bone ~GPa) and soft tissues in the body (0.1–100 kPa), *in vitro* reconstituted ECM networks (collagen, Matrigel, fibrin) typically fall in the lower stiffness range (1 Pa–1 kPa) [23]. This is likely related to the fact that these networks are reconstituted typically by self-assembly of purified ECM proteins, resulting in low protein density and sparse, disorganized fiber networks, in contrast to the dense, highly-organized structure of physiological ECM. Theories of biopolymer physics predict that the stiffness of random semiflexible fiber networks is strongly determined by the protein content c , following a scaling dependence of c^α , where $2 < \alpha < 3$, as has also been verified by experiments [25-29]. Such direct relation can be useful for designing ECM-based biomaterials with desired mechanical properties.

A key mechanical feature of fibrous biopolymer networks is that they often exhibit nonlinear behavior, meaning that the stiffness significantly changes as the networks are increasingly stretched [30]. The origin and extent of this stiffening vary, depending on the precise micro- and nano-scale structure of the network. For example, collagen networks initially softens when subjected to shear stress, due to the dynamic nature of the fiber cross-linking [31], and then stiffens in a stress-dependent but concentration-independent manner [32], due to the bending resistance of the constituent fibers. This nonlinear stiffening response may be physiologically important, including in the presence of cells. The low initial stiffness can prevent high-stiffness-induced mesenchymal phenotype and differentiation of cells [33], which have been associated with pathological processes like fibrosis [34] and tumor invasion [35]. At the same time, the stiffening at large deformations can directly modulate cell–matrix interactions [36] and enhance tissue mechanical integrity by preventing excessive deformations/damage. In contrast to the mechanics of collagen, fibrin networks—the ECM scaffold in blood clots—also strongly stiffen (up to 100-fold) but in an intriguing multi-stage process [27,37,38]. At small deformations, the networks stiffen due to reduction of entropic fluctuation as the thermal slacks in the fibers are pulled out, whereas at large deformations the fibers themselves stiffen following the well-established worm-like-chain polymer response. This hierarchical stiffening behavior endows fibrin with an extensive gel extensibility (up to 200–300% strains), which can contribute to its physiological necessity to withstand blood shear flow [39].

The structure-dependent mechanics of ECM fiber networks highlights the importance of closely examining their microstructure. The pore size of these networks typically falls in the micrometer scale, within the range of cell sizes. Interestingly, the stochastic nature of the self-assembly of these networks results in significant spatial heterogeneity in their structure, which directly translates to

striking heterogeneity in the local mechanical properties (spanning 2 orders of magnitude) [40,41] and stresses [42]. This raises the question whether cells sense the local or global ECM properties; a recent study suggests, for instance, that mesenchymal stem cell spreading and differentiation are directed by local fiber stiffness rather than global ECM stiffness [43]. Alternatively, cells may sample and integrate the diverse local mechanical information to “estimate” the global tissue stiffness [44]. Moreover, the spatial heterogeneity also leads to non-affine (i.e., non-uniform) deformations [45-47], which are particularly relevant for explaining the biomechanics of whole tissues [48,49].

Recently, the structure of ECM fiber networks has been shown to underlie not only their intricate elastic properties, but also a few more exotic characteristics. Collagen and fibrin networks have been demonstrated to remember and mechanically adapt to strain history [50], by virtue of their multiscale remodeling at the network, fiber, and molecular levels [51]. Remarkably, this remodeling occurs consistently under multiple deformation types, including shear, compression, and extension [50-54], and is especially relevant to consider under recurring or cyclic mechanical loading, which frequently takes place *in vivo* in many load-bearing tissues. This can explain their viscoelastic and plastic behaviors [51,55,56] and, at the cell level, it has also been hypothesized to be a mechanism by which cells mechanically remodel the ECM [6,57,58]. Another interesting feature of ECM fiber networks is their tendency to contract when deformed—an unusual material phenomenon referred to as negative normal stress [59,60] or reverse Poynting effect [61]. While this behavior has previously been attributed to the semiflexible nature of the fibers [62], recent evidence suggests a more universal structural parameter, network porosity, to be the determining factor for the switch between positive and negative normal stresses [63]. If true, this can be a key link between the mechanics of *in vitro* reconstituted ECM networks and the dense ECM in tissues.

Tissue equivalents

To understand the mechanical interactions between cells and the ECM in tissues, a convenient minimal model often employed is by seeding cells in gels made from reconstituted ECM proteins [64], forming *in vitro* tissue equivalents [65]. The gel provides a 3D environment that can better promote *in-vivo*-like cellular activity than 2D cultures [66]. Aside from the (long-term) chemical modification by the cells by depositing, cross-linking, and degrading the ECM, the cells also physically remodel the ECM immediately after they are added to the gel. Cells exert traction forces to the ECM that result in local ECM densification and anisotropy, as well as global gel compaction [67,68].

When cellular traction forces are sufficiently high to overcome the mechanical resistance from the ECM, the pericellular ECM fibers are translocated and reoriented, increasing the local fiber density and initiating large-scale fibrillary rearrangement [69]. Aided by the ECM’s viscoplasticity, these physical rearrangements result in irreversible, location-dependent ECM reorganization and increase in the local ECM mechanical properties [70]. The extent of local ECM remodeling is dependent on the pericellular condition [71], and increasing cell seeding density leads to an overall increase in the global gel stiffness in a contractility-dependent manner [72]. A prime example of the *in vivo* relevance of such ECM remodeling is in cancer, where one physical hallmark of tumor invasion is dense remodeled networks of taut collagen fibers around the tumor [73], which not only promote malignant transformation [74] but also facilitate metastatic migration of the cancer cells towards the blood vessels and the surrounding tissue [75].

The ability of cells to contract ECM gels has been exploited to construct microtissues [76] that can serve as a standalone tissue model for studying cell forces. Using this *in vitro* platform, our group has shown that the resulting ECM microstructure, (an)isotropy, and cellular orientation are strongly affected by the configuration of the spatial constraints and the application of external mechanical stretch in a ECM-density-dependent manner [77,78], suggesting an interplay between active force generation, mechanosensing, and structure-guided cell reorientation in the microtissues. Moreover, the morphology and orientation of the seeded cells, which are dependent on the degree of ECM alignment, also influence the homogeneity of contraction [79], indicating the importance of both cell and ECM

organization in translating local cell forces to large-scale coordinated contraction of the whole microtissue.

The cell-induced changes in the local ECM mechanical properties feed back to regulate cell behavior via mechanosensing. Changes in the ECM stiffness regulate the expression and intracellular organization of proteins associated with cell contractility and adhesion [80,81], which in turn alter the magnitude and direction of traction forces and therefore local ECM remodeling [82,83]. As a result, cell-induced ECM remodeling can result in directional cell migration [84-86] as well as spatiotemporally heterogeneous behavior of individual cells within a population [87]. Furthermore, this remodeling can be sensed by remote cells and has been proposed to mediate mechanical cell-cell communication over long distances (several cell diameters) [88,89]. Interestingly, a recent study showed that mechanical signaling alone (i.e., in the absence of electrical signaling) between heart muscle cells—via ECM—can coordinate the beating of whole embryonic heart tissue [90], highlighting the potential importance of ECM as a mechanical signal transducer in tissues. These findings suggest that cells in tissues actively seek a preferred or optimal mechanical state together with their surrounding ECM (i.e., tensional or mechanical homeostasis) that can best support their functions, as has been hypothesized in the fields of cancer [91] and vascular biology [92].

Where we stand and outstanding questions

The use of in vitro minimal models has clearly revealed many fundamental insights about the mechanobiological functions of ECM and simplified tissues that would have been hidden otherwise. To conclude this Perspective article, we briefly reflect on 4 focus points that pave the way to both the new possibilities and the challenges lying ahead for bringing the field closer to in vivo situations and technological advancement in biomedicine.

Mechanoactivity of seemingly passive components

Minimalistic ECM models have revealed that the biomechanics of seemingly passive structural components is not only surprisingly rich, but can also have direct mechanobiological impacts on cell and tissue behavior. Aside from commonly-studied ECM properties (such as pore size, bulk stiffness, and density), other—perhaps subtler—ECM properties (such as nonlinear behavior, inelasticity, and negative normal stress) have only started to be appreciated. How these properties mediate cell-matrix interactions are largely still unexplored, though we anticipate this to be a subject of intensive research in the coming years. What seems to be clear is that the structure of these components governs their mechanical characteristics; at the same time their mechanical response is responsible for the dynamics of the structure. Function and form go hand-in-hand. On the one hand, this emphasizes the need to better characterize the dynamic structure of ECM in vivo across length scales, for example by optimizing the spatiotemporal resolution of non-invasive imaging modalities. On the other hand, this opens new avenues for directing cell and tissue response, for example in the context of functional tissue regeneration [93], using designed (synthetic) biomimetic ECM structure to actively modulate cell and tissue function. The first step for this is to better understand the mechanisms by which cells respond to physical and mechanical cues from the environment [12].

Cell-matrix cooperativity: the whole is greater than the sum of its parts

Although the mechanobiology of each component in the system (cell and ECM) already poses fascinating new questions, the interplay between these players can evidently further lead to emergent behaviors that are only beginning to be appreciated. In moving towards the tissue level, the cooperativity between the cell and the ECM, as well as between cells through the ECM, needs to be explored. This is particularly important as cells also synthesize and deposit their own matrix as well as break down the endogenous ECM, creating a self-healing and highly adaptive system. We anticipate that computational modeling, in conjunction with observational and validatory experiments, will play

a major role in establishing the link between the mechanobiology at the nanoscale (protein), microscale (cell), and macroscale (tissue).

Spatiotemporal evolution in mechanobiology

The bidirectional interactions between cells and ECM in tissues necessarily results in spatiotemporal evolution of the cells and the ECM. This aspect has been largely overlooked so far, partly due to the focus on studying cell response to (static) individual properties of the ECM. Two approaches can potentially mitigate this. First, cells and tissues can be cultured in dynamic condition, with external stimuli that can be controlled over time [94,95]. Second, cells can be cultured in specifically-designed dynamic, stimuli-responsive substrates or scaffolds, in which the cell response and the accompanying ECM remodeling can be simultaneously monitored [96]. Insights obtained using these time-resolved approaches can be particularly of relevance for understanding the progression of disease pathology (e.g., cancer plasticity [97]) and for improving the regeneration of functional tissues.

Potential of exploiting mechanobiology for biomaterial design and tissue regeneration

Alongside its contribution to understanding the fundamental principles of cell and tissue (patho)physiology, mechanobiology offers a unique approach for regenerative medicine, by exploiting the physical and mechanical properties of the cell and ECM for directing their biological function. Such an approach is already starting to pay dividends for in vitro functional tissue regeneration [98], and we expect it to also help us move towards controlling in situ regeneration [99]. Although many currently available synthetic materials lack various characteristics of natural ECM networks, as noted earlier, there is a growing effort to recapitulate ECM mechanical properties, such as nonlinear behavior [100], for potential biological use. The potential of using mechanobiological principles as a driving force for creating ordered 3D tissues is a truly exciting prospect.

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