Review

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Stem cell mechanobiology: diverse lessons from bone marrow

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A stem cell niche is defined by various chemical and physical features that influence whether a stem cell remains quiescent, divides, or differentiates. We review mechanical determinants that affect cell fate through actomyosin forces, nucleoskeleton remodeling, and mechanosensitive translocation of transcription factors. Current methods for physical characterization of tissue microenvironments are summarized together with efforts to recapitulate niche mechanics in culture. We focus on mesenchymal stem cells, particularly in osteogenesis and adipogenesis, and on blood stem cells – both of which reside in mechanically diverse marrow microenvironments. Given the explosion of efforts with pluripotent stem cells, the evident mechanosensitivity of clinically relevant, multipotent marrow cells underscores an increasing need to examine and understand *in vivo* and *in vitro* physical properties on length scales that cells sense.

In vitro approaches to mimic and characterize the extracellular matrix (ECM) in the stem cell niche

Stem cells are plastic in that they have the potential to differentiate into multiple lineages. The control of stem cell fate has been classically attributed to growth factors that regulate transcription factors. However, because stem cells do not exist in isolation *in vivo*, additional environmental factors are now being recognized as likely regulators of stem cell fate. Indeed, multipotent stem cells or progenitors are present in many adult tissues [1] and reside in an environment known as a niche that helps to maintain the cells in a naïve state until prompted to differentiate. The niche combines tissue-specific matrix and nearby differentiated cells with key soluble molecules, all of which the stem cell probes and interprets when deciding to undergo differentiation [2]. Mechanotransduction is also now being studied as a differentiation mechanism in which cells physically probe their surroundings with mechanical forces that alter protein organization and ultimately gene expression.

A role for ECM mechanics in determining cellular function – including stem cell differentiation – has been extensively studied, in part by using reductionist *in vitro* approaches that simplify the complex mechanical properties of tissue. For example, collagens are the most abundant proteins in metazoans, but they display complex mechanics; collagen fibrils are semi-flexible biopolymers with non-linear elastic behavior and, when crosslinked, form strain-stiffening networks [3]. The development of biomimetic culture systems depends on methods to measure the mechanical properties of both biological and synthetic systems with high spatial resolution, such as rheology, micropipette aspiration, and atomic force microscopy (AFM), as described in Box 1. We discuss here how these techniques provide insight into the roles of ECM, actomyosin contractility, nuclear mechanics, and mechanosensitive pathways in determining stem cell commitment to specific lineages. We describe some of the mechanical properties of tissues that increasingly motivate the characterization and control of biomimetic platforms at nanoscales to understand the role of the ECM and mechanotransduction in stem cell biology, with a particular focus on bone marrow stem and progenitor cells.

Influence of matrix mechanics on differentiation of bone marrow cells

Mesenchymal stem cells (MSCs) contribute to an osteoprogenitor population of cells, which differentiate into osteoblasts that produce the osteoid matrix at the interface between bone marrow and calcified collagen (Figure 1A)

Glossary

Elasticity: a property of a material that causes it to be restored to its original state after deformation. Stiffness describes how resistant an object is to deformation.
Monotonic function: a function that, within a given interval, either only increases or decreases with the argument.
Strain: the deformation of an object in response to stress. When an object is deformed, distances between points within the object change. Strain describes the change in distance between points relative to the separation of the points before deformation. It is a tensor because it is a function of the orientation on the faces of a cube of material within the object.
Stress: a physical quantity describing the forces acting through a given cross-sectional area in an object subjected to strain. Stress is also a tensor.
Storage and loss moduli: in viscoelastic materials, they are measures of the stored energy (elastic component) and the energy dissipated as heat (viscous component).
Traction forces: the forces that cells exert on their surroundings.
Viscoelasticity: a property of a material that displays both viscous and elastic characteristics when deformed.
Viscosity: a measure of the resistance of a fluid to gradual deformation by 'shearing' stresses (i.e., when layers within a fluid are moved laterally with respect to each other).
Young's modulus: the ratio of stress to strain for simple extensional deformation; a measure of elasticity.

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Box 1. Common techniques for measuring mechanical properties of ECM, cells, and the nucleus

Rheological methods

The material properties of natural or synthetic gels can be characterized using rheological methods. Measurements can be made of the complex modulus G* = G′ + *i*G″ under shear stress, where the storage modulus G′ describes the elastic component and the loss modulus G″ describes the viscous contribution. Extension of any substance, such as a nucleus aspirated into a micropipette, is characterized in terms of E*, and a convenient metric of stiffness is approximated as the root-mean-square of E* on a given time scale for deformation. Gels formed from different cytoskeletal and extracellular proteins exhibit strain-stiffening for small to intermediate strains, measured with a cone and plate rheometer [3] (Figure IA). The deformations of tissues, cells, or nuclei can be measured on micron scales as they are drawn into a micropipette under negative pressure. Optical microscopy is used to image the deformations over time and often the proteins of interest are fluorescently labeled. The nucleus in Figure IB is from a cell expressing GFP-tagged lamin A protein (GFP-LamA). This method was used to show that nuclei stiffen during differentiation [83], embryonic heart tissues stiffen during development [84], and lamina composition determines the viscoelastic response of nuclei [52–54].

Atomic force microscopy (AFM)

AFM is a widely used instrument to measure a variety of forces between a sample and a nano-sized probe [85]. The working principal behind the method is to raster-scan a surface with a small probe at the end of a flexible cantilever. Interactions with the sample cause the cantilever to bend and its deflection is detected by measuring the position of a laser beam reflected from the back of the lever (Figure IC). AFM can be used for force spectroscopy or 'force mode.' With this application, the tip approaches the sample surface vertically, and is then retracted. When the tip indents the sample, a force indentation curve is recorded that can be used to obtain the properties of the material under compression. When the probe is retracted, material properties that are under stretching can be measured, or proteins that are unfolding under tension can be examined. Using the Hertz model for contact mechanics of elastic solids and its modifications for different geometries, one can extract Young's modulus *E* from force–indentation curves (Figure ID). Another application of AFM is imaging structures at high resolution such as the organization and assembly of matrix proteins. The AFM image in Figure IE shows the topography of nano-fibrils in a thin molecular crosslinked collagen film. Moreover, AFM can be used for 'force mapping' (Figure IF). With this method, force curves are recorded at an array of points across the sample. Elasticity maps of ECM, cells, or tissues on stiff or flexible substrates can be generated. The image in Figure IF shows *E* of an MSC on a flexible, molecularly thin collagen film.

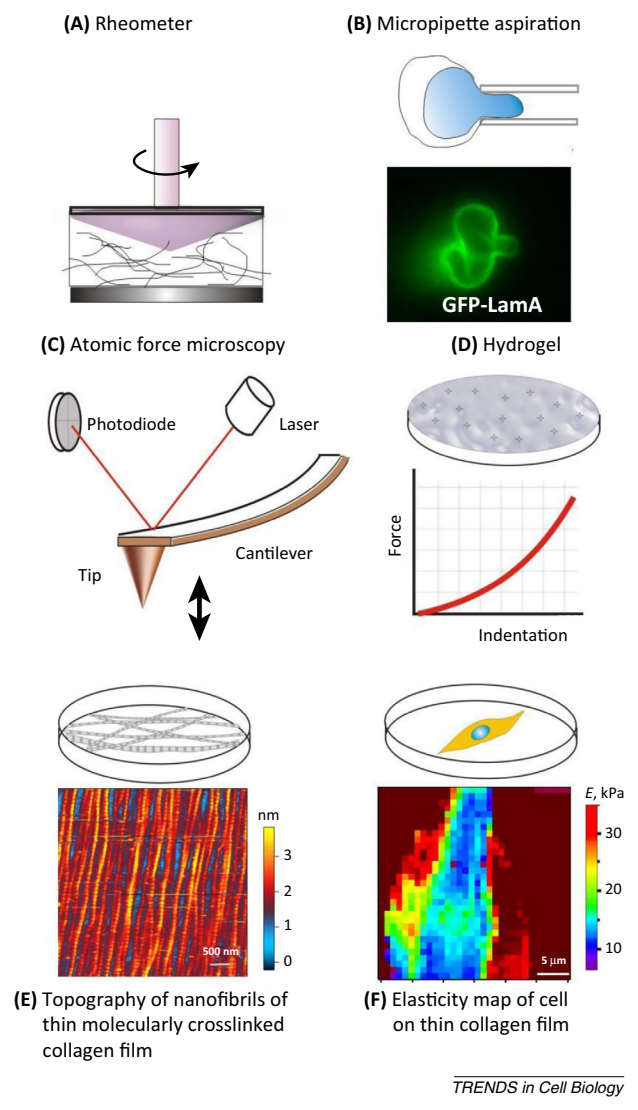

Figure I. Common techniques for measuring the mechanical properties of ECM, cells, and nuclei.

[4]. Osteoid contains fibrillar collagen, non-collagenous proteins, and proteoglycans, all of which are crosslinked by enzymes secreted by osteoblasts. With time, the matrix thickens and mineralization is initiated through the deposition of apatite (calcium phosphate mineral) crystals [5]. The nanoscale composition and topology of the bone matrix (Figure 1B) defines how cells experience stresses at the subcellular level. Matrix fibrillar and non-fibrillar proteins, and apatite crystals, have nanoscale dimensions and their structure is likely to affect matrix nanomechanics. Calcium phosphate crystals grow between fibrils but can also be found embedded within the fibrils themselves [6]. Interestingly, the orientation of the apatite crystals, rather than the density of the mineral, correlates most strongly with the stiffness of this nascent bone [7].

Isolated from marrow, MSCs are an adherent cell type that spreads on tissue-culture plastic or glass. In culture with the proper soluble factors, MSCs are multipotent; in addition to their osteogenic and chondrogenic potential, they certainly possess an adipogenic potential [8,9]. Adipocytes are common in human bone marrow [10] and, based on findings reviewed below, it is tempting to speculate that the soft marrow cavity is conducive to adipogenesis whereas the stiffer surface of bone influences osteogenesis. The latter is likely an important function of MSCs, and there is some evidence that marrow adipocytes – perhaps also differentiated from marrow MSCs – contribute to the regulation of hematopoiesis [11,12].

Hematopoietic stem and progenitor cells (HSPCs) isolated from bone marrow are non-adherent compared to MSCs, and HSPCs are also capable of growing in suspension *in vitro*. Nonetheless, interactions *in vivo* with the surrounding marrow cells and the ECM seem unavoidable. Indeed, HSPCs express surface adhesion receptors, including integrins and cytoskeletal components such as actomyosin that, in principle, enable the sensing of physical forces and ECM stiffness from the microenvironment. Historically,