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The Effects of Stiffness, Fluid Viscosity, and Geometry of Microenvironment in Homeostasis, Aging, and Diseases: A Brief Review

Cells sense biophysical cues in the micro-environment and respond to the cues biochemically and biophysically. Proper responses from cells are critical to maintain the homeostasis in the body. Abnormal biophysical cues will cause pathological development in the cells; pathological or aging cells, on the other hand, can alter their micro-environment to become abnormal. In this minireview, we discuss four important biophysical cues of the micro-environment—stiffness, curvature, extracellular matrix (ECM) architecture and viscosity—in terms of their roles in health, aging, and diseases.

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1 Introduction

It had been widely known for a long time that specialized cells, such as sensory neurons of touch and hair cells in the ear can sense and respond to mechanical inputs from the environment;

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and that another set of specialized cells, such as skeletal muscle cells, cardiomyocytes, and smooth muscle cells, are capable of generating mechanical forces. However, a vast body of studies in the past two decades laid the foundation of modern mechanobiology, the essence of which is the notion that not restricted to the aforementioned specialized cells, every cell type is equipped with the capacity to sense the mechanical cues and/or stimuli, and to interact with the micro-environment by generating mechanical forces. In other words, mechanosignaling is an integral part of homeostasis, to maintain normal function of cells, and to govern cell fate.

Cells sense mechanical cues and stimuli from their surrounding micro-environment. The cues include, but not limited to, stiffness, viscosity, and geometry. Stiffness determines how deformable the substrate is where cells reside. Viscosity defines the resistance experienced by an object when moving in an aqueous environment. The most biologically relevant parameters of geometry at the molecular, cellular, and tissue scales are curvature and organization of constituent extracellular matrix (ECM) proteins. The ECM architecture can be characterized by parameters including density, pore size, and orientation of the ECM proteins. During progression of many diseases, micro-environment of affected tissues is altered biochemically and biophysically, leading to changes of values in stiffness, viscosity, curvature, and ECM architecture. For example, during cancer development, increased stiffness [1,2], elevated extracellular fluid (ECF) viscosity [3,4], altered architecture of fibrillar ECM proteins in stroma [5–7] and changed curvature [8] are often observed in the micro-environment of solid tumors. Fibrosis results in elevated stiffness [9–12] and altered ECM architecture [13,14].

To study the relation between alterations in stiffness, viscosity, ECM architecture, and curvature, many techniques have been developed to measure those physical parameters of the micro-environment. For stiffness measurement, methods implementing atomic force microscopy [15,16], magnetic tweezers [17,18], microfluidics [19,20], indentation [21,22], and magnetic resonance electrography [23], are commonly used depending on the length scale of the measurement [24]. For viscosity measurement, rheometers/viscometers [25–27], optical traps [28–30] and magnetic tweezers [31,32] are commonly used. ECM architecture can be surveyed using several imaging modalities, including histological visualization [33,34], internal reflectance microscopy [35–37], and second harmonic generation [38–41]. For local curvature measurement, image modalities such as computational tomography [8,42] and optical microscopy [13,43,44] have been used to acquire images of a region of interests, followed by the image analysis to determine the local curvature value at the desirable length scale. The advances in these techniques have allowed dynamic measurement with high spatial resolution *in vitro* and *in vivo*.

It should be noted that cells and tissues are a mixture of solid substances, consisting of insoluble biomolecules, and liquid, consisting of water and soluble biomolecules. As a result, cells and tissues usually exhibit dual characteristics belonging to both solids and liquids, and cannot be considered purely elastic or purely viscous most of the time. However, there are a handful of examples where elastic behaviors are dominant. Based on experimental observations, theoretical models depicting elastic, hyperelastic, viscoelastic, and poroelastic behaviors have been established [24]. Both viscoelastic and poroelastic models adopt dual characteristics of solids and fluids. The viscoelastic model examines the time-dependent response of the biomaterials. But the measurement approaches described above can be used to extract stiffness value in different models wherever an elastic element is included. Recent evidences showed that viscous dissipation, or known as stress relaxation in the tissues may be involved in modulating proliferation, differentiation, and gene expression [45,46]. The viscous element of the tissue also can also contribute to cell behaviors. For example, there is an optimal stress relaxation time intrinsic to the soft substrate to maximize cell spreading, whereas

stress relaxation time does not affect cell spreading on stiff substrates [47].

In this brief review, we will discuss the essential discoveries made using some of the measurement techniques mentioned above, which enable us to gain insights into the roles of stiffness, viscosity, and geometry of micro-environment in homeostasis, aging, and diseases.

2 Stiffness

Stiffness plays a key role in many cellular behaviors and functions. Stiffness of a certain object, defined as the resistance of the object to change in length, is determined by the structure of the object and intrinsic mechanical properties of the constituent materials [24]. The unit of stiffness is force per length (e.g., Newton/meter). It should be noted that most cells and tissues exhibit nonlinear elastic behaviors, such as strain stiffening, where stiffness is not a constant. Rather, their resistance to change in length is load-dependent. Moreover, many tissue types can be more accurately modeled as viscoelastic, where the response to load evolves in times because of the stress-relaxation process. Nonetheless, representative stiffness values corresponding to physiological loading can be extracted if an appropriate measurement method is selected, despite the nonlinear nature of the cells/tissues.

In biology and medicine, stiffness is broadly used to represent mechanical properties of the cells and tissues, because stiffness is implicated in many diseases, such as tumor progression and fibrosis [48]. The value of stiffness is found different in many types of diseased/aging cells or tissues, compared to their normal/young counterparts [9,22,49]. While the tumor mass of many cancer types tend to be stiffer than normal tissues, decreased stiffness has been observed in single disseminated breast cancer cells during metastatic progression [50]. It has been reported that excessive deposition of ECM proteins by cancer cells and fibroblasts in tumor stroma [51–53], as well as force-dependent ECM fibril alignment by cancer and stromal cells [5,54] contribute to increased stiffness in the tumor tissue. Note it has been observed that some cancer cells can generate higher forces than their normal counterparts, and such higher forces are exploited to advance tumor progression [54,55]. Importantly, stiffness of the substrate, where cells reside, regulates gene expression, cell differentiation, migration, and proliferation [56], and determines the stiffness of the cells growing in on the substrate [57]. Here, the effect of substrate stiffness is discussed in the context of homeostasis, aging, and diseases.

2.1 Cell Morphology, Differentiation, Migration Regulated by Stiffness. The cellular response to the varied substrate stiffness is manifested in their morphology, gene expression, and motility [58]. The process of cell spreading can be understood from the perspective of free energy minimization [59,60]. The total free energy, which is the sum of energy required to sustain cytoskeletal structure and order, and to maintain the tension between the cell and the substrate, is minimized during spreading. As the cell spreads, the energy required to maintain the cytoskeletal structure, which becomes flatter and flatter with the gradual formation of stress fibers, decreases; but the energy required to maintain the tension between the cell and the substrate increases, given more focal adhesions are formed at the interface. In general, cells seeded on stiffer substrates are flatter, with larger spread area and more stress fibers traversing across the cell body [61,62].

The effect of substrate stiffness on differentiation was first demonstrated in the studies by Discher and coworkers, where mesenchymal stem cells differentiated into neurons when plated on soft (0.1–1 kPa) substrate, and into myocytes when plated on substrates of intermediate stiffness (8–17 kPa), and into osteocytes when plated on stiff substrate (25–40 kPa) [16,63]. The underlying mechanism of stiffness-driven differentiation is associated with mechanosensitive gene expression [64]. More details about how stiffness affects gene expression are discussed in Sec. 2.2. Cell

Fig. 1 Cells change their movement modes on substrates with different stiffness. (a) On stiff substrates, strong actomyosin-substrate coupling stretches mechanosensitive focal adhesion proteins, leading to the turnover of focal adhesions, a force-dependent process. (b) On soft substrates, weaker actomyosin-substrate coupling causes focal adhesion to be tugged backward, making more space between the membrane and the actin networks, allowing actin polymerization at the leading edge to drive the plasma membrane to expand forward.

migration correlates with the stiffness of the substrate, on which cells undergo locomotion. In general, there is an optimal stiffness of the substrate to enable cells to move at the maximal speed [65–69]. Such relation is especially apparent in the mode of focal adhesion-based cell migration [70,71]. The dynamics of focal adhesion determines how fast a cell can anchor itself onto the substrate while shifting its center of mass, expanding the leading edge by actin polymerization, then displace itself from the current location on the substrate by myosin contraction to move forward once the center of mass is shifted.

When moving on 2D surface, myosin contractility, and actin polymerization pushing against the plasma membrane at the leading edge result in a constant actin flow, in the direction from the cell edge to the center, known as “retrograde flow” [72]. In this process, cells also exert forces that are transmitted to the ECM-coated surface through focal adhesions and transmembrane proteins, namely, integrin. When integrin is mechanically engaged to the ECM, resistance to the myosin contractility arises, thereby slowing actin retrograde flow but promoting actin polymerization. Sometimes strong contractile forces can disrupt the integrin-ECM engagement. As a result, competition between actin polymerization, retrograde flow, and adhesion strength determines the extent of leading edge expansion, and subsequent cell motility. The linkage between the actin cytoskeleton and ECM through integrin, known as “molecular clutch,” is a two-state machinery—engaged or unengaged.

On stiff substrate, focal adhesion is anchored onto the substrate via the catch bond [73,74], which is an engaged state between ECM molecules (e.g., fibronectin, collagen, vitronectin) [75] and integrin. At this engaged state, focal adhesion disassembly is force-dependent [76]. On soft substrate, focal adhesion is anchored onto the substrate via the slip bond [73,77] between ECM molecules and integrin, resulting in focal adhesion being tugged backward from the leading edge. When a cell moves using focal adhesions on a stiff surface, the actomyosin network is strongly coupled with the substrate via integrin binding to the ECM molecules; the traction forces generated by the actomyosin network are transmitted more effectively to focal adhesions to facilitate the turnover of the focal adhesion to propel cells forward (Fig. 1(a)). It should be noted that there exists an optimal range of substrate stiffness that enables maximal clutch engagement. Above the optimal stiffness, strong force loading on individual clutches result in engagement immediately followed by disengagement, before

additional integrin and FA molecules are allowed to recruit to the adhesion site [65,72]. As a result, many molecular clutches stay disengaged, a phenomenon known as “frictional slippage.” On the other hand, when a cell moves using focal adhesions on soft surface, the actomyosin network is weakly coupled with the substrate via integrin binding to the ECM molecules; the traction forces generated by the actomyosin network pull focal adhesions away from the leading edge [78], making more room for leading-edge expansion driven by actin polymerization (Fig. 1(b)). In other words, the substrate stiffness determines how fast actin polymerizes at the leading edge and how fast focal adhesions turn over, both contributing to cell motility. A cell type-dependent optimal substrate stiffness coordinating the two processes will result in maximal motility.

It has been shown that microtubules coordinate with actin filaments in many cellular processes, including mitosis [79,80], and cell motility [81]. However, the role of microtubules in stiffness sensing and stiffness-dependent cell migration is still yet to fully understood. Further investigation is required to elucidate whether and how microtubules are involved.

Furthermore, cells are known to move along the gradient of substrate stiffness, generally from low to high. This phenomenon, known as durotaxis, can be observed in single cells [82,83] or collective cell migration [84]. From the perspective of free energy, durotaxis is driven by the much lower free energy state associated with remodeled cytoskeletal structure on stiffer substrates, even the formation of more focal adhesions increases the strain energy [85]. Durotaxis plays an important role in development [86], innate immunity [87], cancer invasion [88], and epithelial-to-mesenchymal transition [89]. It has been suggested that cells use focal adhesion to survey the local stiffness of the substrate in proximity to guide the durotactic cell migration [90]. Exceptionally, it was observed that neurons tend to migrate from high to low stiffness [91].

2.2 Gene Expression is Regulated by Substrate Stiffness.

Substrate stiffness regulates the expression of certain mechanosensitive genes. Such mechanosensitive gene expression has been observed in cardiomyocytes [92], hepatocytes [93], vascular smooth muscle cells [94], and various types of cancer cells [95]. For example, relatively soft (~0.91 MPa) substrates promote gene expression of laminins (cell adhesion), MMP and TIMPS (ECM remodeling) in cardiomyocytes, whereas stiff substrates promote genes or proteins such as Nkx-2.5 and GATA-4 (133 MPa) [92]. Mechanosensitive transcription factors contribute to the stiffness-dependent gene expression. The most extensively studied mechanosensitive transcription factors are YAP and TAZ [58]. YAP/TAZ distributes in the nucleus when the cell is in contact with the stiffer substrate (15–40 kPa) and in the cytoplasm when the cell is in contact with the softer substrate (0.7–1 kPa) [96,97]. When in the nucleus, YAP/TAZ activates the Hippo signaling pathway, which governs the proliferation, apoptosis, and size control of the cell [98,99]. Recently, it has been shown that the mutant form of another transcription factor, p53 (mt p53), is also responsive to substrate stiffness. p53 is a tumor suppressor. However, some p53 mutants can facilitate aggressive tumor progression [100–105], thus the term gain-of-function p53 mutants. The gain-of-function p53 mutants are more stabilized against protein degradation when cells are in contact with the relatively stiffer substrate (50 kPa), but wild-type p53 is not affected by substrate stiffness [106,107].

2.3 The Implication of Tissue Stiffness in Aging Biology and Geriatric Medicine.

Tissue stiffness measured in the eye [108,109], blood vessel [94], lung [110], and skin [111] increases with age. The stiffness increase in these tissues corresponds to pathology including glaucoma [112], chronic respiratory diseases such as asthma, fibrosis, and pulmonary hypertension [110], atherosclerosis [113,114], fibrosis [115], stroke [116], aortic aneurysms [117], and cancer progression [118]. Aging-dependent stiffening

in tissues results from decreased water content, as well as alterations in ECM architectures. The alterations in ECM architectures are caused by elevated ECM deposition, crosslinking, and fiber orientation [112]. For example, crosslinking of collagen fibers and loss of water content stiffen the skin [119–121]. Given that tissues, in general, are stiffer in older individuals, and stiffness can drive tumor progression by promoting invasiveness [2,88,122,123] and gain-of-function p53 activity [100–105], tissue stiffness might be a contributing factor to more aggressive cancer development in elderly patients [61,63,124,125]. In addition, it was recently reported that the efficacy of anticancer drugs could be affected by the stiffness of ECM in which cancer cells grow [126], though the underlying molecular mechanism involved in elevated tumor progression in stiffer micro-environments has not been comprehensively investigated. More studies are in need to understand the complex relationship between aging, changing mechanical properties of the tissues, and the diseases prevalent in elderly patients.

3 Substrate Curvature

The effect of substrate curvature on cells and tissues is under active investigation. It has been observed that cytoskeletal organization can be modulated by substrate curvature at the submicron scale [127–129], leading to changes in cell migration and cell orientation. Specifically, cells may sense and preferentially migrate along nanopatterned gratings that are only hundreds of nanometers wide [128]. To date, several membrane- or cytoskeleton-associated proteins have been identified to be responsive to curvatures at the submicron scales [130–132]. For example, SrGAP2 and ArhGAP44 are proteins that sense membrane curvature through BAR domains and modulate cell protrusions through different downstream pathways [133,134]. However, at the micron-scale and above, it is yet to be elucidated how cells sense the curvature and respond correspondingly, given the curvature sensitive proteins are too small to detect curvature at the micron-scale. Yet, it has been shown cells indeed respond to curvature differences at the micron-scale or larger. For example, curvature ($1/50\ \mu\text{m}$) in intestinal villi enhance the barrier function of the intestinal epithelial cells by upregulating mucin and occluding expression [135]. It has also been shown that high curvatures ($1/25\ \mu\text{m}$) promote tumorigenicity and invasion of breast cancer cells in vitro [136,137].

Substrate curvature plays a profound role in physiological conditions. For example, tubular/ductal structures are commonly found throughout the body, like the trachea of the airway, blood and lymphatic vessels, renal tubules in the kidney, urethra that drains the urine, bile ducts that connect the liver and small intestine, and mammary ducts in the breast tissue. The local curvature along these ducts or tubules usually varies. To evaluate how the variation of the local curvature results in different cellular behaviors and functions, cells are cultured onto the surface of prescribed curvatures. The surface of specific curvatures can be fabricated using photolithography [128,129,136], molding [13,135], and bioprinting [13,138].

3.1 Cell Migration and Adhesion Regulated by Substrate Curvature. Substrate curvatures at the scale of microns or larger impose prominent effects on collective cell behaviors. With cells seeded on concaved surfaces, it is observed that epithelial cells move collectively with periodic relaxation when the curvature is high ($1/25\ \mu\text{m}$), whereas cells move continuously and 5 times faster when the curvature is low ($1/100\ \mu\text{m}$) [44]. In contrast, when cells are seeded on the convex surfaces, it is observed the collective movement of epithelial cells is faster when the curvature is high ($1/25\ \mu\text{m}$), compared to speed measured on curvatures smaller than $1/25\ \mu\text{m}$ [139]. When cells are plated on the outer surface of a cylinder (convex surface), cells are more elongated in alignment with the axis of the cylinder, if the curvature is larger than $1/45\ \mu\text{m}$ [140]. Substrate curvature affects not only migrating

Fig. 2 Curvature sensing at a multicellular scale. (a) On the flat surface, cell-cell tension generated by inward actomyosin contraction is balanced between two neighboring cells. Epithelium is securely attached to the substrate provided there is adequate cell-ECM adhesion. (b) On the concaved surface, the tension between two neighboring cells results in forces pointing to the direction of the apical side, stretching the bond between integrin and ECM. The actomyosin contractility at the apical side increases to achieve balance by counteracting the stretching force. If the stretching force cannot be balanced, epithelium with decreased cell-ECM adhesion strength will detach from the substrate.

epithelial sheets but also stationary epithelium. For example, increased cortical actin formation, E-cadherin expression, and myosin contractility can be detected in mammary gland epithelial cells growing on the concaved surface ($1/60\ \mu\text{m}$), compared to cells on the flat surface. Interestingly, the prominent cortical actin formation is abolished if there is no cell-cell contact, or after treating the cells with drug to inhibit signaling involving E-cadherin complex or nonmuscle myosin II, suggesting both adherens junction and cortical tension are involved in the curvature sensing [13].

Substrate curvature also affects behaviors and phenotypes at the single-cell level. For example, a reduced density of focal adhesions is observed when human mesenchymal stem cells are cultured on concaved surfaces, compared to the ones cultured on flat surfaces [141]. When single cells are seeded onto substrate with concaved and convex surfaces, it has been observed that single cells migrate toward the location of concaved surface (negative local curvatures), a behavior termed as curvotaxis [142]. Curvotaxis has been shown as the manifestation of interplay between nucleus sliding and actomyosin dynamics in the cell plated on the curved surface, where contractile actomyosin compresses on the nucleus, resulting in the nucleus sliding toward the closest curvature minimum [142].

3.2 Curvature Sensing at the Micron-Scale or Larger. The effects of curvature on cell migration and adhesion suggest curvature sensing at the micron-scale or larger possibly involves the force balance mediated by the actin cytoskeleton and adhesion molecules present at the cell surface. The structural integrity of the epithelium requires the balance between tension from cell-cell adhesion and force from cell-substrate adhesion [138,143–145] (Fig. 2(a)). Specifically, tension between cells in a concaved arrangement might result in forces to detach cells from the initial site of cell adhesion. Such detachment can be understood from the imbalance between tension from cell-cell adhesion and force from cell-substrate adhesion. Prominent junctional localization of E-cadherin is observed when tension between cells is high [146]. Higher junctional localization has been reported in the cell on the curved surface, which is likely caused by high tension between cells when grown on the curved surface [13]. Higher tension between cells requires increased cell-ECM adhesion to reach the force balance [143–145]. If the cell-ECM adhesion is not reinforced in proportion to the increased tension between cells on the curved surface, the epithelium might adjust its height and spread area to achieve balance [143], or detach from the substrate.

Interestingly, reduced focal adhesions were observed when cells were cultured on the curved surface compared to cells on the flat control [141]. With the weaker adhesion between the cells and the concaved substrate, the integrity of the epithelium is more likely to be breached, leading to the detachment of cells from the substrate. Indeed, lower cell–ECM adhesion and higher cell–cell tension in cells on the curved surface, detachment of Madin–Darby Canine Kidney cells has been observed [147]. To compensate the weaker adhesion caused by decreased cell–substrate adhesion, and to counteract the detaching forces, the cortical actomyosin network grow thicker at the apical part of the cell–cell junction, where myosin contractility is also upregulated [13], to exert contractile force toward the substrate and maintain sustainable tension between cells (Fig. 2(b)).

4 Extracellular Matrix Architecture

Cells generate forces to interact with their environment [148,149]. Macroscopically, forces deform ECM [150]; microscopically, forces may rearrange the organization of the ECM proteins, causing architectural alterations [5,151]. Cancer cells interact with the tumor micro-environment (TME) [152] using forces without exception. TME is a heterogeneous milieu. In addition to the tumor, TME comprises immune cells, fibroblasts, adipocytes, and endothelial cells [153–158], residing in the proximal ECM [159], known as stroma. Invasion, a critical step during metastasis, first depends on cancer cells migrating through the ECM network surrounding the primary tumor [40].

Interaction between ECM and cancer cells is important for tumor progression. An increase in ECM density is related to cancer development. For example, an increase in stromal collagen density can be detected in the mammography of early stage breast cancer patients [160,161]. It was demonstrated that ECM porosity determines the invasive behaviors of disseminated cancer cells. Dense ECM induce collective migration from the primary tumor, whereas more porous ECM allows single-cell invasion [162]. Moreover, during tumor progression, cancer cells stiffen ECM by secreting ECM modification agents including glycoproteins, prolyl-4-hydroxylase, lysyl-hydroxylase, and lysyl oxidase [163–167]. Stiffer ECM is associated with the activation of mechanosensitive pathways involving Rho signaling [168,169], which in turn promote proliferation, invasiveness [1,170], and drug resistance of the cancer cells [171].

In the past decade, the critical role of carcinoma-associated fibroblasts (CAFs) in tumor progression has been extensively documented [6,7,51,172–178]. CAFs are pathological stromal-dwelling cells promoting tumor progression by enhancing cancer cell proliferation, facilitating efficient metastasis [7,178–180], and protecting cancer cells against apoptosis [181,182]. Other than secreting chemical factors to promote tumor progression [155], CAFs are observed to establish contact with cancer cells and mechanically pull cancer cells toward stroma during the dissemination process, initiating the first step of invasion [183].

The CAFs are an integral component of the tumor micro-environment in a variety of cancer types, including lung cancer, breast cancer, and prostate cancer [184–186], which are three most commonly diagnosed types of cancer worldwide [187]. At the inception of cancer [188], cancer cells secrete CAF-promoting factors, such as cytokines (e.g., TGF- β), micro-RNAs, and exosomes containing cytokine and/or micro-RNA to transform normal stroma-dwelling cells into CAFs [189–193]. The detection of CAFs at the early stage, when cancer cells are not yet invasive and secrete low level of MMP [194,195], suggests that CAF-promoting factors are delivered to reach the stromal cells by diffusion through the stromal ECM. It was observed that early stage cancer cells can generate contractile forces to realign the ECM fibers in a radially parallel fashion [37]. The alignment of ECM fibers in the TME enhances the originally slow diffusion of macromolecules [196,197] and exosomes [37] (Fig. 3). Such enhancement in transport can reduce the time for exosomes or other

Fig. 3 ECM alignment enhances stroma-ward diffusion of CAF-promoting factors, induces CAFs, and provides a track for CAFs to migrate toward the tumor. Left: initially, isotropic ECM limits diffusion of CAF-promoting factors. Right: aligned ECM by cancer cells allows CAF-promoting factors to diffuse more efficiently along the aligned ECM fibrils to reach stroma and induce stromal-residing cells to exhibit CAF phenotypes. The CAFs subsequently move toward the tumor along the aligned ECM fiber to further promote tumor progression.

molecules reaching the stromal cells by four orders of magnitude reduction [197], effectively inducing CAFs, which in turn promote cancer invasion [40]. In addition, the aligned ECM fibers might also serve as a medium to transduce mechanical forces in long ranges (further than 300 μ m) [198,199], effectively activating mechanosignaling pathways associated with CAF phenotypes [200,201]. Synergistically, ECM alignment, along with increased ECM deposition and subsequent extensive ECM crosslinking by cancer cells, elevate the ECM stiffness.

5 Extracellular Fluid Viscosity

Another critical component of the micro-environment is the ECF, also known as interstitial fluid. Throughout the body, cells are surrounded by and move through biological fluids that span orders of magnitudes of viscosity (Fig. 4), including mucus [202], saliva, blood, and synovial fluid [203], to name a few. Fluctuations in lipid and protein secretion and hydration levels can lead to changes in fluid viscosity [204], and abnormal viscosity contributes to various disease states. Mucins are large, heavily glycosylated proteins responsible for the viscosity of mucus, and changes in mucin production and mucus viscosity are associated with cystic fibrosis, asthma, and health risks related to smoking [202,204,205]. Aging can lead to elevated fibrinogen and albumin concentrations in blood, thereby increasing the osmolarity and viscosity of plasma, which is thought to contribute to coronary heart disease [206,207]. The increase in plasma osmolarity could, in turn, increase ECF viscosity due to the osmotic pressure balance between plasma and ECF. During the wound healing process, the viscosity of pus has been used by physicians as an indicator of its quality [208]. Abnormal ECF viscosity is also implicated in cancer, where leaky vasculature and matrix degradation within the tumor could lead to elevated viscosity [4]; additionally, both secreted and transmembrane mucins are overexpressed in many malignancies [209–211].

Compared to other physical parameters of the micro-environment, such as stiffness, ECM architecture, and substrate curvature, the effect of viscosity on cell behavior is relatively unexplored. This is likely due, at least in part, to the difficulty of measuring viscosity *in vivo*. Bulk fluids like mucus and blood can be reliably measured *ex vivo* with conventional macrorheological techniques, but techniques for localized measurements of viscosity *in vivo* have only recently emerged [212]. Molecular rotors

Fig. 4 Physiological relevance of fluid viscosity in the human body

[213] and helical nanobots [214] have been used to probe viscosity both within the cell and in the extracellular environment, as well as in the tumor micro-environment. These studies have shown that ECF viscosity is greater, typically by several orders of magnitude, than that of water and of normal cell culture medium [212–214].

Still, challenges remain in the application of these techniques. In viscous fluids consisting of large polymers or proteins, the effective viscosity is relative, and depends on the size of the objects moving through the fluid [215]. For example, in methyl-cellulose solutions, nanoparticles diffuse freely and do not experience the bulk viscosity [215]; in a biological context, this can be seen with mucus, through which proteins are often able to pass freely [202]. It follows that the technique used for probing viscosity must be appropriate for the length scale of interest. For example, lateral diffusion of membrane proteins might not be affected by bulk ECF viscosity, but a cell migrating through the same ECF could be.

There have been several pioneering studies on the effects of ECF viscosity on cell behavior, both theoretical and experimental. In early studies, where cells were suspended in and moving through or across viscous fluids or dehydrated mucus, it was observed that the migration of macrophages [216] and neutrophils [217] was hindered. More recent work has shown that phenotypically mesenchymal cells migrating on 2D surfaces and biomimetic substrates while *immersed* in viscous ECF generate stronger cell–substrate adhesion and migrate more quickly [218–220]. Although the more recent findings may seem to contradict the earlier studies, that is not necessarily the case. Viscous ECF likely differentially affects adherent cells migrating on solid substrates and nonadherent cells migrating through fluids, as these involve distinct mechanisms of cell motility.

A hypothetical model of adherent, crawling cells predicts that increased ECF viscosity either slows cell motility or has no effect, depending on the strength of cell adherence [221]. However, the opposite has been shown experimentally [218–220]. This suggests that migrating cells in viscous ECF may not act as inert moving objects; rather, there may be an adaptive mechanism that allows

cells to modify their behavior in response to elevated ECF viscosity.

In a recent study, it was observed that the direction of galvano-taxis of cells in viscous fluids is dependent on the size of the polymer used as a thickening agent [222]. Another study found that enhanced cell–substrate adhesion in viscous ECF was dependent not on the molecular weight or size of the polymer but on bulk viscosity only [219]. Untangling and interpreting these results is challenging. It is possible that a number of factors, including mode of cell motility and ECF composition, contribute to the cellular response to ECF viscosity, and there remain many important questions to be answered.

6 Conclusion

Cells interact with their micro-environment by sensing external mechanical cues and responding to them. When the interactions are properly regulated to attain normal physiological functions, homeostasis in the body is maintained. But pathologically altered stiffness, viscosity, ECM architecture, and curvature can start a vicious cycle that deviates farther and farther from the norm over time. For example, the stiffening of the tumor micro-environment activates the mechanosignaling pathways in cancer cells to deposit more collagen or secrete ECM crosslinking agents [223], resulting in even stiffer micro-environment. To restore the altered mechanical properties of the micro-environment might lead to slowing or stopping the disease progression, though it is an underexplored topic. In the future, if technologies can be developed to reduce ECM stiffness, to decrease body fluid viscosity, to prevent ECM remodeling, or to modify local curvatures in the tissue, they might prove useful in aid of disease treatment. It is little known whether curvature plays any role during the process of aging. Given significant changes in tissue stiffness and osmotic pressure of the interstitial fluid as one ages, the curvature effect on geriatric disease is a topic of potential importance for future study.

Due to the complex and heterogeneous nature of tissues, a comprehensive landscape survey, in terms of mechanical properties, on any tissue of any organ is still lacking. With advances in measurement technology, such survey becomes a possibility in the near future. Mapping mechanical properties, such as stiffness, relaxation time, fluid viscosity, and substrate curvature, in both normal and abnormal tissues, at the micron or smaller length scales, can provide insights to the physical changes during disease progression. By correlating the mechanical properties mapped in the diseased tissues with the local gene expression profiles in cells, powerful insights will be gained regarding the effect of the altered mechanical cues in the pathological micro-environment. With this newly gained knowledge, better models that more realistically represent the mechanochemical behaviors *in vivo* will emerge.

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