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Biophysical Regulation of Cell Behavior—Cross Talk between Substrate Stiffness and Nanotopography

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ABSTRACT

The stiffness and nanotopographical characteristics of the extracellular matrix (ECM) influence numerous developmental, physiological, and pathological processes *in vivo*. These biophysical cues have therefore been applied to modulate almost all aspects of cell behavior, from cell adhesion and spreading to proliferation and differentiation. Delineation of the biophysical modulation of cell behavior is critical to the rational design of new biomaterials, implants, and medical devices. The effects of stiffness and topographical cues on cell behavior have previously been reviewed, respectively; however, the interwoven effects of stiffness and nanotopographical cues on cell behavior have not been well described, despite similarities in phenotypic manifestations. Herein, we first review the effects of substrate stiffness and nanotopography on cell behavior, and then focus on intracellular transmission of the biophysical signals from integrins to nucleus. Attempts are made to connect extracellular regulation of cell behavior with the biophysical cues. We then discuss the challenges in dissecting the biophysical regulation of cell behavior and in translating the mechanistic understanding of these cues to tissue engineering and regenerative medicine.

1. Introduction

A growing body of literature shows that cell fate can be dictated by the stiffness and topographical characteristics of the extracellular matrix (ECM). The ECM, which is constructed from diverse, nanometer-sized biomacromolecules including collagen, elastin, and fibronectin [1], often displays topography at nanoscales, as shown in Fig. 1(a) [2–8]. For example, collagen fibers, being several microns in diameter, are hierarchically structured from collagen fibrils of 10–300 nm in diameter [9,10]. The lung interstitial matrix displays an interrelated framework of nanoscale fibrous collagen and elastin proteins [8,11]. Depending on the composition of the ECM as well as on interstitial fluids [12], the ECM exhibits various degrees of stiffness, as shown in Fig. 1(b) [13–15]. The biophysical (stiffness and nanotopographical) cues, in concert with the spatiotemporally arranged biochemical and biomechanical cues, regulate cell phenotype and function.

The stiffness and nanotopographical characteristics of the ECM influence numerous developmental, physiological, and pathological processes *in vivo* [16–20]. For example, tissue stiffness can be altered by the disease state. The stiffness of mammary tissue increases from ~1 kPa in its normal condition to ~4 kPa during breast cancer [21]. Lung stiffness is lower in emphysema [22], but higher in fibrotic tissues than in the normal condition [23,24]. Moreover, fibroblasts respond to increases in matrix stiffness with promoted proliferation and collagen synthesis; the induced ECM stiffening can further promote, amplify, and perpetuate fibrosis via a positive feedback loop [24,25].

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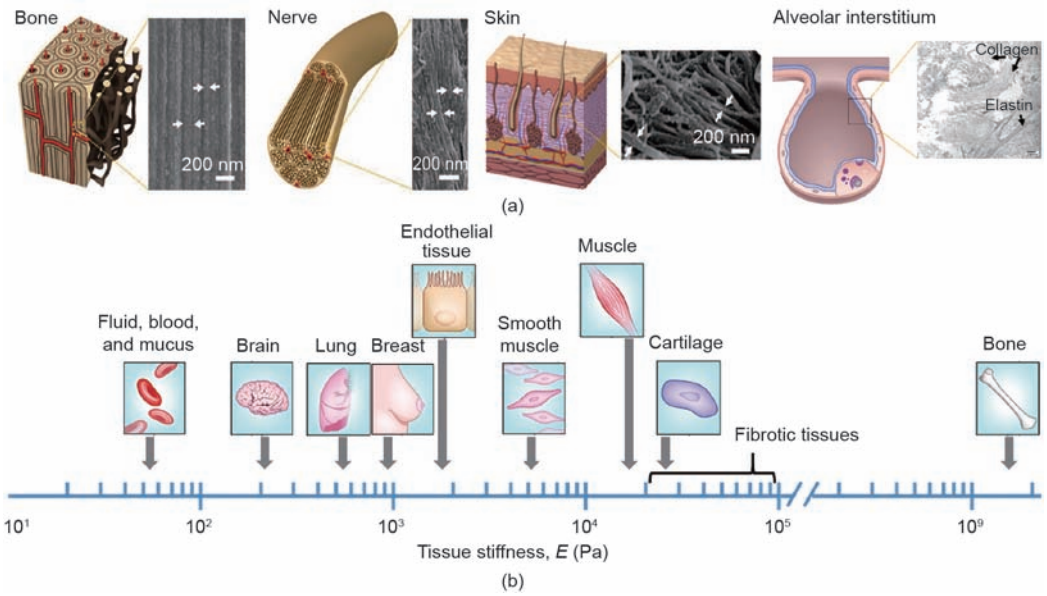

Fig. 1. Biophysical characteristics of human tissues. (a) Nanoscale structures displayed in various tissues. The arrows indicate various nanostructures. (Reproduced with permission from Ref. [6] for the graphical illustrations and scanning electron microscope (SEM) micrographs of bone, nerve, and skin. The graphical illustration and SEM micrographs of the alveolar interstitium are reproduced from Refs. [7] and [8], respectively) (b) Stiffness of human tissues. The fibrotic tissues become stiffer than those in normal conditions. (Reproduced with permission from Ref. [15])

Biophysical cues have therefore been applied to modulate almost all aspects of cell behavior [26]. Since the first report in 1997 [27], emerging compelling evidence has shown that substrate stiffness plays important roles in cell modulation and many biological processes [27–32]. For example, C2C12 mouse myoblasts exhibit definitive actomyosin striations only on polyacrylamide (PAAm) gels with a stiffness that is typical of normal muscle, but not on softer gel or stiffer glass substrate [33]. Furthermore, the neurogenic, myogenic, and osteogenic differentiation of human mesenchymal stem cells (hMSCs) can be facilitated by PAAm gels with stiffnesses matching those of brain, muscle, and collagenous bone, respectively [28]. Meanwhile, a large body of literature underscores the phenomenon that cellular responses are highly sensitive to nanotopography [34–39]. In addition to having a pronounced influence on cell morphology, nanotopographical cues could regulate cell proliferation and facilitate stem cell differentiation into certain lineages such as neuron [35,40,41], muscle [42], and bone [36,37].

Many excellent review articles discuss cellular responses to substrate stiffness [14,43,44] or topography [45–50]. However, despite similarities in phenotypic manifestations, the interwoven effects of stiffness and nanotopographical cues on cell behavior have not been well described [51]. Herein, we first review the effects of substrate stiffness and nanotopography on cell behavior, and then focus on intracellular transmission of the biophysical signals from integrins to nucleus. Attempts are made to connect extracellular regulation of cell behavior with the biophysical cues. We then discuss the challenges in dissecting the biophysical regulation of cell behavior and in translating the mechanistic understanding of these cues to tissue engineering and regenerative medicine.

2. Biophysical regulation of cell phenotype and function

2.1. Stiffness cues

A broad spectrum of materials has been adopted as substrates/matrices for cellular studies. These materials range from very hard metals such as titanium oxide (TiO_2; Young's modulus $E \approx 150$ GPa) [52], to hard glass (65 GPa) [53], to thermoplastic polymers such as polystyrene (PS; 2.3 GPa) [54] and poly(lactic-*co*-glycolic acid) (PLGA; 1.31 GPa for PLGA 50/50) [55], to elastomeric polymers such as polydimethylsiloxane (PDMS; 3.4 MPa) [56], and to soft hydrogels (from several pascals to several kilopascals), as shown in Fig. 2(a). In the literature, different terms such as elasticity, stiffness, rigidity, and shear modulus have been used to characterize the mechanical property of substrates. Elasticity is an intensive property of the material, while stiffness is an extensive property, depending on the material and the shape and boundary conditions. Throughout this review, the value in the brackets gives the Young's modulus of the substrate, unless otherwise specified.

2.1.1. Stiffness effects

With an increase in substrate stiffness, cells usually exhibit enhanced cell adhesion [57–60], enlarged cell spreading with defined actin organization [60–67], increased cellular contractility [60–68], decreased migration speed [69,70], and promoted proliferation [57,61,67,71,72]. For example, when hMSCs adhere onto collagen I-modified PAAm gels, paxillin-labeled adhesions change from undetectable diffuse focal complexes on soft gels (1 kPa), to punctate adhesions on gels with intermediate stiffness (11 kPa), to long, thin, and more stable focal adhesions on the stiffest gels (34 kPa) [28]. The expression of the focal adhesion protein vinculin in MC3T3-E1 osteoblasts on alginate gels increases 1.5-fold as the gel stiffness increases from 20 kPa to 110 kPa [57]. It has also been shown that NIH 3T3 fibroblasts on the stiffer collagen I-coated PAAm gels (7.69 kPa) are more dispersed and have better attachment, with > 80% of cells remaining after a centrifugation assay, as compared with the softer gels (2.68 kPa), which only have about 30% of cells remaining [58].

Although many studies show monotonic dependence of cell behavior on substrate stiffness, biphasic relations between cell adhesion [73], migration [59,74–76], and proliferation [77–79] and substrate stiffness have also been observed. On the one hand, when primary adult human dermal fibroblasts are grown on poly(ethylene glycol) (PEG) hydrogels, the average cell migration speed decreases significantly from 0.81 $\mu m \cdot min^{-1}$ on soft gels (95 Pa) to 0.38 $\mu m \cdot min^{-1}$ on stiff gels (4.3 kPa) [70]. In addition, when the Young's modulus of PAAm gels increases from 4.7 kPa to 14 kPa, NIH 3T3 fibroblasts