Mechanics in Cell Adhesion and Motility on the Elastic Substrates

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1. Introduction

Cells determine their biological behaviors and fate, based on the interchange of various kinds of bioactive molecules and signals with extracellular surroundings. The signaling molecules and the regulatory factors for cell growth and differentiation such as hormones and cytokines are transferred among cells with different manners depending on their intrinsic transport property, interaction property for cells and intratissue localization of target cells, such as endocrine, paracrine, autocrine, juxtacrine, and matricrine1, 2). Within these categories of signal transferring manner, the last mentioned matricrine is the mode of regulation of cellular functions by the non-diffusable factors which are physically adsorbed or biochemically fixed on the solid phase of extracellular matrix (ECM). The systematic control of matricrine is of great importance for biomaterials engineering, because it is deeply related with tissue physiology governed by the interaction between cells and ECM. The cellular functional regulation by this kind of “solid phase factors” has been considered...
to include not only the effects of non-diffusible cytokine but also the contribution of cell adhesion complex which are formed from the initial interaction of integrin on the cell membrane with the cell adhesive proteins adsorbed on ECM such as fibronectin and vitronectin.

Adhesive cells generally autonomously regulate their spatio-temporal behaviors of adhesion, extension, transfer, growth and differentiation. These each behavior convolutes not only the results from intra/inter-cellular biochemical processes but also the contribution from dynamic interactions among the molecular assembled systems of cell membrane, cytoskeletal structures, and extracellular mechanical milieu. Therefore, the matricrine controls of cellular functions are deeply based on the concerted interactions between biochemical/physiological aspects and static/dynamic mechanical aspects of ECM. This kind of cellular functional control by mechanical field or stimuli, and investigation for its mechanism, which is called mechano-biology, is increasingly becoming to be the essential basis for the strategy of more biomimetic or bio-inspired designing for high-functional biomaterials. In this mini-review, we will describe recent movement on the designing of interface of elastic substrate to manipulate cellular mechano-biology together with our approaches on cell motility control.

2. Cell adhesion mechanics

Adhesion of cells onto two-dimensional substrate surface or with three-dimensional matrix is the first event that affects the subsequent cellular behaviors such as extension, transfer, growth and differentiation, as the uppermost stream of matricrine signaling. Investigation for intracellular biochemical processes triggered by the interaction between cell adhesive protein and integrin has been clarifying a series of assembling reactions of adhesion complex proteins and architectural reactions of cytoskeletal systems, and has accumulated knowledges on behaviors of proteins relating to these reactions. On the other hand, the process of cell adhesion has been known to be controlled not only by the biochemical and physicochemical factors from adhesion interface but also by the mechanical factors derived from structural features in subsurface and bulk region of extracellular matrix. Mechanistic understanding for such the interrelationship between the biochemical process and mechanical event has been extensively studied so far. Bellow, the cell adhesion mechanics on the two-dimensional substrate surface are described as the simple model system to focus such the mechanistic understanding.
Attachment of cells to substrate surface occurs at the tachetic-like protein assembled site of focal adhesion formed in the cell-substrate interface. Cell adhesive proteins adsorbed on the substrate surface bind to integrins on the cell membrane, which leads to the subsequent binding of talin and focal adhesion kinase to the intracellular domains of integrin 6, 7, 8. Moreover, assembling of these primary binding proteins induce integration of the secondary binding proteins such as vinculin, tensin, paxlin, α-actinin, etc, and formation of linking machinery for actin cytoskeleton 9. In other aspect, formation of the focal adhesion activates Rho family signaling proteins, and affects actin polymerization, their filaments bundling and contraction of actinomyosin systems of stress fibers connecting with the focal adhesion machineries 10, 11. The focal adhesion plays an essential role as the mechanosensor that sense extracellular mechanical milieu or stimuli and transmit the mechanical signal to cytoskeletal systems 12, 13.

What kind of mechanical interactions is generated at the focal adhesion site in the cell-substrate interface? Figure 1 schematically shows elemental forces acting at the focal adhesion. Focal adhesion complex undergoes the force generated in the same direction to the contractile force derived from actin cytoskeleton connected with the complex \( f_a \). This force loads the force in the same direction onto the substrate surface, which is called traction force \( f_b \). In the focal adhesion complex, reaction force to the traction force, i.e., counteractive traction force, appears in the inverse direction to the traction force, and make balance with the force from actin cytoskeleton \( f_c \). Here in the case that the substrate is elastic material, the focal adhesion complex dynamically acts to keep the force balance between the traction force and the restoring force generated by horizontal deformation of elastic substrate surface \( f_d \), which involves dynamic processes of the changes in the amount of assembled focal adhesion proteins and structural remodeling of the protein complex itself. Although the description of elemental forces of \( f_a \sim f_d \) is quite simple

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Fig. 1  Schematic representation of traction forces generated at focal adhesion site. a) FA: focal adhesion, TF: traction force, SF: stress fiber. b) \( f_a \): FA traction force induced by SF, \( f_c \): substrate traction force loaded by FA, \( f_d \): reaction force against the substrate traction force, \( f_c \): horizontal restorative force of elastic substrate. Cell adhesion occurs to quasi-statically keep the force balance of \( f_a = f_c \) and \( f_b = f_d \).
treatment, the actual dynamic aspects for keeping the balance between such the simple forces, for controlling the biochemical behaviors of focal adhesion complex, and for the interrelationship between cytoskeletal systems and extracellular mechanical milieu have not been understood very well to date.

Such the dynamic aspect of focal adhesion behavior is one of the essential issues in the cell/elastic substrate interaction. Because restoring force \( f_d \) does not appear on the commonly used hard substrate of plastics or glasses for cell culture, which exhibit no deformation in the adhesion interface, there is no need to consider mechanical dynamics of the substrate-side. On the other hand, behaviors of focal adhesion are essentially regulated by the dynamics of mechanical field on the elastic substrate. Cell culture elastic materials are important model system to investigate mechanical dynamics of cell adhesion behaviors on soft tissues.

To determine the absolute value of traction force, it is required to measure local micro-deformation of the elastic substrate with known elastic modulus (see Fig. 3). Mainly two types of methodology have been developed to measure the traction deformation in the cell-substrate interface. One is the method employing the fluorescent microbeads-embedded cell adhesive hydrogels developed by Dembo and Wang \(^{14,15}\), the other method applies the soft lithography-based PDMS substrate with nano-pillar structures reported by Geiger and collaborators \(^{16}\). In these methods, local micro-distortion of substrate surface induced by the traction behavior of cells is determined from the measurement of micro-displacement of distortion maker, i.e. microbeads in the former method and nanopillars in the latter. The traction force values generated at each focal adhesion site have been determined by these methods as several-tens nN per single focal adhesion, and it typically exhibits larger values in the peripheral region or pseudopodium of adhered cells \(^{14}\), especially in the leading edge \(^{15}\). In addition, traction force has been found to linearly increase with the increase of area of focal adhesion (ca. 4~5 nN/\( \mu m^2 \)) \(^{16,17}\). Concerning the correlation between substrate elasticity and cellular traction behaviors, it has been reported that on the surface with higher elastic modulus, maturation of the focal adhesion is enhanced \(^{18}\) and traction force is increased \(^{19}\).

![Measurement of local strain exerted by adhered cell (A - B) can determine the local distribution of the traction forces](image.png)

Fig. 3. Schematic representation of principle for traction force microscopy
How do cells detect the difference of the elastic modulus of the substrate surface and reflect the information to the control of traction force? Although the molecular mechanism for the elasticity-detection cannot ascribe to the behavior of specific single protein molecule and precise whole story for the issue has not been clarified yet, mainly three kinds of manner of transduction from detected mechanical input into biochemical signal are proposed and investigated.

The first mechanism proposed is the effect of mechanical conformational regulation of protein molecules involved in the mechanotransduction. For example, the integrin binding proteins of talin, α-actinin, fibronectin, etc., possess the repeated tandem-like amino-acid sequencing, and are gradually unfolded by a mechanical extension. The unfolded module of the protein works as a recognition site for other proteins, which induces the subsequent binding of downstream proteins and finally control the biomechanical activity of the proteins. Depending on the magnitude of loaded force, the modules with different mechanical response are unfolded in order, which enables semi-quantitative detection of force strength and signal transduction. The second mechanosensing mechanism is the effect of mechano-gating channel protein on cell membrane that can be mechanically opened and closed. Membrane tension, cytoskeletal contractile forces, and matrix-loading forces affect the open-close state of the mechano-gating channel, which regulates the inflow of the signal molecule. The third mechanism for mechanotransduction is known as “catch bonds”, which is the bond inversely strengthened by the aparting force for the pre-binding complex. FimH in the adhesion of bacteria E. coli, selectin in the adhesion of leucocytes are the examples of formation of catch bonds. All these mechanosensing mechanisms can be considered as the force-induced allosteric effect for regulation of protein activity induced by the external forces in the sense that the conformation of the protein molecule is altered by the mechanical factors and affect the biomechanical activity.

3. Cell mechanotaxis: control of cell motility mechanics on the elastic substrate

Dynamics of focal adhesions as described above that are governed by the traction force balance at each site generally affect the entire dynamical behavior of adhered cells, i.e., its motility. Cell motility is a critical basis of various dynamical behaviors of the cell ensemble in vivo: inflammation, wound healing, morphogenesis, and tumor metastasis, etc. Appropriate control of such biological processes has remained a longstanding goal of study in the fields of development of functional biomaterials and tissue
engineering. Establishment of surface engineering of biomaterials to regulate cell motility is strongly required in those fields as well as understanding of its mechanisms.

If no stimuli to induce directional movement are given in the systems, the adherent cells exhibit amoeboid movements as a random-walk crawling process. Directional cell movements are induced by various kinds of environmental gradients of: dissolved chemicals (chemotaxis) 32); surface-fixed biomolecules (haptotaxis) 33); light intensity (phototaxis) 34); electrostatic potential (galvanotaxis) 35); gravitational potential (geotaxis) 36); and surface elasticity (durotaxis or mechanotaxis) 37-39). From the perspective of development of functional biomaterial surfaces, haptotaxis and mechanotaxis are expected to be applicable for preparing an artificial extracellular matrix to manipulate cell motility because the nano/micro-distribution of surface-fixed biomolecules and surface elasticity can be designed using a bioengineering approach. While many studies have examined such the approaches using haptotaxis 40), numbers of reports on mechanotaxis have been quite limited to date and application of mechanotaxis has not been performed sufficiently. For example, a few previous reports showed that mechanotaxis was induced for an elasticity jump from the softer region to the harder region, such as from 1.8 kPa to 34 kPa 38), from 12 kPa to 2.5 MPa 38), and from 14 kPa to 30 kPa 37). While only these limited conditions have been reported, systematic condition of surface microelasticity distribution to induce mechanotaxis has not been established.

To systematically investigate the cellular mechanotaxis, at least the following three design requirements should be satisfied: a) Cell adhesive elastic substrate with similar degree of elasticity to living cell, preferably hydrogels. b) Systematic tunability of surface elasticity. c) well-designed elasticity boundary as sharp with the resolution of single cell’s adhered size. In the past studies on the mechanotaxis, there were no reports that satisfy all these requirements, especially the last item c, therefore the conditions to induce mechanotaxis could not be established.

For this study, we developed a photolithographic surface-microelasticity patterning method using photocurable styrenated gelatin for fabricating a cell-adhesive hydrogel with a microelasticity gradient surface 41), satisfying all the above-mentioned three design requirements. We prepared a microelastic gradient (MEG) gel with different absolute surface elasticities and elasticity jumps to investigate conditions of surface microelasticity to induce mechanotaxis. Surface elasticity and its two-dimensional distribution were characterized by microindentation tests using atomic force microscopy (AFM). An analysis of cell trajectories facilitated by time-lapse observations extracted three important surface
elasticity criteria on the elasticity jump and the absolute elasticity of MEG gels to induce mechanotaxis: 1) the high elasticity ratio between hard and soft regions; 2) the elasticity of the soft region to provide medium motility; 3) sharpness of the elasticity boundary. The effective induction of mechanotaxis was considered to be affected by the balance among these three factors. Within these criteria, the last condition plays the most essential role for driving mechanotaxis. To analyze the effect of sharpness of elastic boundary on the induction efficiency of mechanotaxis, MEG gels with the different sharpness of elastic boundary and the same magnitude of elasticity jump between softer and stiffer regions were fabricated. While on the diffuse elastic boundary cells did not exhibit any directional movements, marked enhancement of mechanotaxis was observed only on the discrete elastic boundary of 50 kPa/50 µm (Fig. 2).

A high elasticity jump in the sharp boundary can cause a considerable imbalance of traction forces generated at the focal adhesion sites between harder and softer regions because the high elasticity surface withstands a large traction force and induces lateral strain well; the low one engenders elastic breakdown of focal adhesion complexes under the same traction force. Because the degree of that imbalance is greater, the driving force for mechanotaxis is stronger. On the other hand, elasticity condition providing medium motility is effective for cells to find the boundary to induce mechanotaxis more rapidly in the intrinsic process of random-walk cellular movement. In general, reduced cell motility is observed on a very hard substrate. An overly hard surface adheres firmly to the cells and inhibits their motility, which lowers the probability of cells reaching the boundary.
Is mechanotaxis really induced by the surface mechanical factors? The biased cell movement towards stiffer region of elastic substrate surface might be attributable not to the surface mechanical conditions but to the surface chemical conditions. This is one of the main criticisms for the observation of mechanotaxis. Actually, it is difficult to prepare the gels that have more than two adjacent regions with different elasticities so as to keep similar surface-chemical conditions, because the gels with different elasticity may exhibit different cell adhesivity and different swelling property. Concerning the cell adhesivity, adsorbed amount of cell adhesive proteins such as fibronectin has been characterized by fluorescent-labeling and confocal microscopic observation for top surface of each region of elasticity gradient sample. Literature reported that in the case of PDMS systems the adsorbed amount of fibronectin is almost the same level\(^3\). Styrenated gelatin system also exhibit the similar character\(^4\).

4. Concluding remarks

In this mini-review, mechanobiological aspects of cell adhesion and motility on elastic substrate have been described. Mechanical regulation of cell functions based on the substrate-based matricrine signaling is recently becoming to be one of the essential factors for designing biomaterial surface and tissue engineering matrix, because it is significantly related to cell proliferation and differentiation as well as soluble factors such as cytokines do. For example, recently many reports have clarified that the stem cells sense the matrix elasticity and alter its lineage specification into different phenotype depending on the matrix elasticity\(^4\). If one can systematically design the nano/micro-mechanical condition of elastic substrate surface or matrix including precise arrangement of heterogeneous distribution of the mechanical properties, the mechanobiological behaviors of cells would be appropriately controlled. Such a systematic regulation of cell mechanobiology based on designing extracellular mechanical milieu in the artificial materials can be considered to serve for the development of novel functional cell manipulation materials, which can be called “mechanobio-materials”. Development of the mechanobio-materials will be expected as one of the frontier fields of biomaterials engineering in near future.

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