Traction force microscopy for understanding cellular mechanotransduction

Sung Sik Hur1,2,*, Ji Hoon Jeong1,2,4, Myung Jin Bar1,3, Jae Hong Park3, Jeong Kyo Yoon1,2,* & Yongsung Hwang1,2,*
1Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan 31151, 2Department of Integrated Biomedical Science, Soonchunhyang University, Cheonan 31151, 3Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Soonchunhyang University, Cheonan 31151, Korea

Under physiological and pathological conditions, mechanical forces generated from cells themselves or transmitted from extracellular matrix (ECM) through focal adhesions (FAs) and adherens junctions (AJs) are known to play a significant role in regulating various cell behaviors. Substantial progresses have been made in the field of mechanobiology towards novel methods to understand how cells are able to sense and adapt to these mechanical forces over the years. To address these issues, this review will discuss recent advancements of traction force microscopy (TFM), intracellular force microscopy (IFM), and monolayer stress microscopy (MSM) to measure multiple aspects of cellular forces exerted by cells at cell-ECM and cell-cell junctional intracellular interfaces. We will also highlight how these methods can elucidate the roles of mechanical forces at interfaces of cell-cell/cell-ECM in regulating various cellular functions. [BMB Reports 2020; 53(2): 74-81]

INTRODUCTION

It is well established that mechanical forces around cells and cellular functions are closely related to each other under both physiological and pathological conditions (1, 2). These cellular forces are either generated from cells and then transmitted through actin stress fibers referred to as endogenous forces or transmitted from outside cells referred to as external forces (3). Advancements in the field of mechanobiology have provided much evidence that cells are able to sense and adapt to these mechanical forces around their microenvironment (4). It has been shown that mechanotransduction through cell-extra-cellular matrix (ECM) adhesions (5), cell-cell junctions (6), plasma membrane (7), glyocalyx (8), and nucleus (9) can modulate various cell behaviors such as cell spreading (10), proliferation (11), differentiation (12-14), migration (15, 16), morphogenesis (17), cancer progression (18, 19), and ECM remodeling (19, 20). Therefore, developing novel tools to discover how cells could dynamically sense and respond to these mechanical forces would be of great importance to understand the physiology and pathology in life science and bioengineering fields.

Over the years, studies on mechanical forces have extensively exploited functions of integrin-mediated FAs that can act as mechanotransducers between actomyosin stress fibers and ECMs-polymer-based cell culture substrates with varying stiffness (13). Moreover, it has been well established that the interplay among focal adhesions, cell surface integrins, and the stiffness of ECMs could play a significant role in regulating cell adhesion and spreading (21-23). For examples, it has been reported that the density of ECM ligands could control the spreading behavior of cells through focal adhesion (FA) assembly and that subsequent degree of cell spreading could regulate cellular functions through changes in cell shape, cytoskeletal tension, and Ras homolog family member A (RhoA) mediation (10, 24). In addition, seminal studies pioneered by Discher and Engler have reported that the stiffness of PAA-based hydrogels as cell culture substrates with tunable mechanical properties could determine the fate of human mesenchymal stem cells (hMSCs) by remodeling focal adhesion and cytoskeleton (12, 25). These hMSCs adhered onto either “soft” or “stiff” matrix could sense biophysical and mechanical cues of the matrix having a native tissue-like stiffness, resulting in undergoing lineage-specific differentiation of hMSCs into various cell types depending on tissue-like elasticity. They also further reported that mechanotransduction for regulating stem cell fates could be primarily determined by matrix stiffness, not by ECM tethering or porosity of substrates (12, 26). Indeed, a comprehensive understanding of mechanobiology requires novel tools to measure the forces between cells and ECMs, which are termed as traction forces and the methods to quantify these forces using microscopy-based techniques are known as traction force microscopy (TFM) (27).
Therefore, in this review, we will highlight recent advancements in TFM-based methods for understanding multiple aspects of cellular forces exerted by cells at cell-ECM interfaces as well as at junctional intracellular domains within cellular microenvironment. Specifically, we will also discuss how the TFM-based methods can further elucidate the roles of mechanical forces at interfaces of cell-cell/cell-ECM in controlling various cellular functions. The different approaches and methods introduced in this review are summarized in Table 1 and 2.

### Table 1. Summary of each TFM-based cellular force measurement analysis

| TFM Methods          | Target Forces | Dimension & Image acquisition | Substrate Materials       | Advantages                                                                 | Disadvantages                                                                 | Refs                  |
|----------------------|---------------|--------------------------------|---------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------|
| Deformable material-based 2D TFM | Cell-ECM | • Cells on 2D substrates | PAA, PDMS, PEG           | • Simple experimental setups to prepare cell culture substrate              | • Essential to have reference image without force for force analysis        | (27, 31) |
|                      |               | • Force measurement in 2D    |                           | • Tunable substrate stiffness with wide ranges by concentration of monomers and cross-linking agents | • Required intensive image processing and stress computation steps          | (28, 32, 34) |
|                      |               | • Epifluorescence microscopy |                           | • Scalable and economic                                                    | • Unable to measure normal (out-of-plane) forces                           | (36, 37) |
|                      |               |                              |                           | • Showing flat physiological surface                                       |                                                                          |                       |
|                      |               |                              |                           | • Most popular and well verified method                                     |                                                                          |                       |
|                      |               |                              |                           | • Deformable material-based TFM                                             |                                                                          |                       |
|                      |               |                              |                           | • Simple experimental setups to prepare cell culture substrate              | • Essential to have reference image without force for force analysis        | (27, 31) |
|                      |               |                              |                           | • Tunable substrate stiffness with wide ranges by concentration of monomers and cross-linking agents | • Required intensive image processing and stress computation steps          | (28, 32, 34) |
|                      |               |                              |                           | • Scalable and economic                                                    | • Unable to measure normal (out-of-plane) forces                           | (36, 37) |
|                      |               |                              |                           | • Showing flat physiological surface                                       |                                                                          |                       |
|                      |               |                              |                           | • Most popular and well verified method                                     |                                                                          |                       |
|                      |               |                              |                           | • Deformable material-based TFM                                             |                                                                          |                       |
|                      |               |                              |                           | • Simple experimental setups to prepare cell culture substrate              | • Essential to have reference image without force for force analysis        | (27, 31) |
|                      |               |                              |                           | • Tunable substrate stiffness with wide ranges by concentration of monomers and cross-linking agents | • Required intensive image processing and stress computation steps          | (28, 32, 34) |
|                      |               |                              |                           | • Scalable and economic                                                    | • Unable to measure normal (out-of-plane) forces                           | (36, 37) |
|                      |               |                              |                           | • Showing flat physiological surface                                       |                                                                          |                       |
|                      |               |                              |                           | • Most popular and well verified method                                     |                                                                          |                       |
|                      |               |                              |                           | • Deformable material-based TFM                                             |                                                                          |                       |
|                      |               |                              |                           | • Simple experimental setups to prepare cell culture substrate              | • Essential to have reference image without force for force analysis        | (27, 31) |
|                      |               |                              |                           | • Tunable substrate stiffness with wide ranges by concentration of monomers and cross-linking agents | • Required intensive image processing and stress computation steps          | (28, 32, 34) |
|                      |               |                              |                           | • Scalable and economic                                                    | • Unable to measure normal (out-of-plane) forces                           | (36, 37) |
|                      |               |                              |                           | • Showing flat physiological surface                                       |                                                                          |                       |
|                      |               |                              |                           | • Most popular and well verified method                                     |                                                                          |                       |
|                      |               |                              |                           | • Deformable material-based TFM                                             |                                                                          |                       |
|                      |               |                              |                           | • Simple experimental setups to prepare cell culture substrate              | • Essential to have reference image without force for force analysis        | (27, 31) |
|                      |               |                              |                           | • Tunable substrate stiffness with wide ranges by concentration of monomers and cross-linking agents | • Required intensive image processing and stress computation steps          | (28, 32, 34) |
|                      |               |                              |                           | • Scalable and economic                                                    | • Unable to measure normal (out-of-plane) forces                           | (36, 37) |
|                      |               |                              |                           | • Showing flat physiological surface                                       |                                                                          |                       |
|                      |               |                              |                           | • Most popular and well verified method                                     |                                                                          |                       |
|                      |               |                              |                           | • Deformable material-based TFM                                             |                                                                          |                       |
|                      |               |                              |                           | • Simple experimental setups to prepare cell culture substrate              | • Essential to have reference image without force for force analysis        | (27, 31) |
|                      |               |                              |                           | • Tunable substrate stiffness with wide ranges by concentration of monomers and cross-linking agents | • Required intensive image processing and stress computation steps          | (28, 32, 34) |
|                      |               |                              |                           | • Scalable and economic                                                    | • Unable to measure normal (out-of-plane) forces                           | (36, 37) |
|                      |               |                              |                           | • Showing flat physiological surface                                       |                                                                          |                       |
|                      |               |                              |                           | • Most popular and well verified method                                     |                                                                          |                       |

### Engineering Tools to Measure Cell-ECM Forces

**Deformable material-based TFM**

The first approach to determine cellular traction force using TFM was reported by Harris et al. (28). Since then, TFM has become one of the most successful techniques to quantify cell-ECM forces. Recent fundamental and technological advancements in TFM have significantly enlightened our understanding of mechanobiological parameters in controlling biochemical response and cellular mechanotransduction at cell-matrix interfaces (29). To investigate these parameters, various engineering tools have been developed to measure traction forces exerted by adhered cells onto deformable substrates such as polyacrylamide (PAA), polyethylene glycol (PEG).
BMB equations by computational engineering analyses such as

General, in this approach, adhered cell-driven subtle deformation of a substrate less than submicron scale is measured by tracking the displacement of embedded fluorescent beads within PAA hydrogels while adhered cells undergo cell spreading or migration (Fig. 1A) (32). Subsequently, traction forces are calculated using constitutive equations by computational engineering analyses such as standard finite element method (FEM) (33) and Fast Fourier Transform (FFT) (34).

Very recently, Razafiarison et al. (32, 35) utilized the aforementioned PAA hydrogel-based TFM method to unveil the relationship of mechanosensitivity of hMSCs to matrix stiffness with supramolecular self-assembly and topology of ECM ligands on biomaterial surfaces with respect to surface energy. The contribution of such relationship to stem cell lineage commitment was evaluated by TFM (32, 35). To validate the hypothesis that surface energy-driven ligand topology could regulate stem cell fates, they introduced hydrophobic-polydimethylsiloxane (PDMS) and its counterpart-

### Table 2. Summary of each IFM- or MSN-based cellular force measurement analysis

| IFM & MSN Methods | Target Forces | Dimension & Image acquisition | Basic method for IFM & MSN | Advantages* | Disadvantages* | Refs |
|-------------------|---------------|-------------------------------|---------------------------|-------------|----------------|------|
| Deformable material-based 2D IFM | • Cell-cell | • Intra-cellular | Cells on 2D substrate • Force measurement in 2D • Epifluorescence microscopy | Deformable material-based 2D TFM | - Enable to quantify forces on cell-cell junction and intracellular organelles, such as adherens junctions and nucleus | - Intracellular tension is measured as an average value in 1D, and therefore, 2D mapping is not possible | (46, 52) |
| Micropost-based 2D IFM | • Cell-cell | • Intra-cellular | Cells on 2D substrate • Force measurement in 2D • Epifluorescence microscopy | Micropost-based 2D TFM | - Enable to quantify forces on cell-cell junction and intracellular organelles, such as adherens junctions and nucleus | - Intracellular tension is measured as an average value in 1D, and therefore, 2D mapping is not possible | (51) |
| Deformable material-based 3D IFM | • Cell-cell | • Intra-cellular | Cells on 2D substrate • Force measurement in 3D • Confocal microscopy | Deformable material-based 3D (2.5D) TFM | - Enable to measure normal (out-of-plane) forces, allowing to understand cell behaviors in 3D • Enable to quantify forces on cell-cell junction and intracellular organelles, such as adherens junctions and nucleus | - Intracellular tension is measured as an average value in 1D, and therefore, 2D mapping is not possible | (47) |
| Deformable material-based 2D MSM | • Cell-cell | • Intra-cellular | Cells on 2D substrate • Force measurement in 2D • Epifluorescence microscopy | TFM 2D Micropost-based | - Enable to quantify forces on cell-cell junction and intracellular organelles, such as adherens junctions and nucleus • Enable to measure intracellular stress map in 2D, resulting in higher degree of spatial resolution | - Need to have heavy assumption that mechanical properties of intracellular components, including nucleus, plasma membrane, etc., are the same • Ignoring normal (out-of-plane) forces (no bending component) | (53, 54) |
| Deformable material-based 3D MSM | • Cell-cell | • Intra-cellular | Cells on 2D substrate • Force measurement in 3D • Confocal microscopy | Deformable material-based 3D (2.5D) TFM | - Enable to measure bending stresses, allowing to understand inter/intracellular behaviors in 3D • Enable to quantify forces on cell-cell junction and intracellular organelles, such as adherens junctions and nucleus • Enable to measure intracellular stress map in 3D | - Need to have heavy assumption that mechanical properties of intracellular components, including nucleus, plasma membrane, etc., are the same | (55) |

*IFM and MSN analyses are proceeded using the basic information acquired from TFM, therefore IFM and MSN inherit advantages and disadvantages of TFM-based force measurement analysis.

(PEG), and polydimethylsiloxane (PDMS) known to have linearly elastic and isotropic properties in response to external force (27, 30, 31). Among these materials, the most extensively used substrates in TFM thus far is a PAA-based elastic substrate firstly introduced by Dembo and his colleagues (27). Generally, in this approach, adhered cell-driven subtle deformation of a substrate less than submicron scale is measured by tracking the displacement of embedded fluorescent beads within PAA hydrogels while adhered cells undergo cell spreading or migration (Fig. 1A) (32). Subsequently, traction forces are calculated using constitutive equations by computational engineering analyses such as
Fig. 1. Traction force microscopy (TFM)-based cell-ECM force quantification. (A) Schematic diagram for typical TFM platform using deformable substrates, where fluorescence beads (orange dots) are embedded. Cells can adhere to the substrate through surface-conjugated ECMs or protein ligands (purple line). Traction forces (indicated by red arrows) exerted by cells can cause subtle deformation of a substrate, where traction forces can be measured by tracking the displacement of fluorescent beads within the substrate. (B) Traction force stress map showing human bone marrow-derived mesenchymal stem cells adhered onto hydrophobic-polydimethylsiloxane (PDMS) and hydrophilic-PDMS with polyethyleneoxide (PEO) (PEO-PDMS), with varying stiffness ranging from 0.2-0.3 kPa (soft, 70:1) to 5-6 kPa (intermediate, 60:1). Surfaces of these substrates were coated hydrophilic-PDMS with polyethyleneoxide (PEO) (PEO-PDMS). Their stiffness varied from 0.2-0.3 kPa (soft, 70:1) to 5-6 kPa (intermediate, 60:1). Surfaces of these substrates were coated with type I collagen (Fig. 1B). Their results indicated that both incorporation of collagen and the increase of matrix stiffness could escalate traction forces on both hydrophobic PDMS and hydrophilic PDMS. In addition, adhered cells on both matrices having intermediated stiffness (5-6 kPa) showed a spreading morphology, resulting in osteogenic lineage commitment. On the other hand, addition of hydrophilic moiety (PEO) to PDMS in a soft rigidity (0.2-0.3 kPa) decreased traction forces. More importantly, cell spreading was inhibited through surface energy-driven collagen assembly, thus promoting adipogenesis of hMSCs rather than osteogenesis. Taken together, these studies suggest that matrix stiffness alone could enable stem cells to differentiate into a certain lineage based on their native microenvironment having a tissue-elasticity. These studies also suggest that TFM can offer better understanding of how these stem cells sense matrix stiffness and their subsequent cell spreading and differentiation.

**Micropost-based TFM**

As an alternative to TFM using PAA hydrogel-based flat and continuous substrates, Chen and his colleagues have developed microfabricated post-array-detectors (mPADs) to manipulate spatial characteristics of substrates with tunable mechanical compliance (Fig. 1C and 1D). Subcellular traction force was calculated based on one-dimensional (1D) Hooke's law by measured deflection and spring constant of deformable posts (36). Very importantly, this study firstly suggested the possibility of tunable mechanical properties of micropost-based substrates by varying heights of deformable posts without changing their surface chemistry. In their follow-up studies using mPADs, Fu et al. (37) have investigated effects of micropost stiffness on cell morphology, cell traction force, and stem cell lineage commitment. As we discussed earlier regarding roles of matrix stiffness in stem cell differentiation, results also showed that cell surface areas, focal adhesions, and traction forces were all increased when the micropost became stiffer. Furthermore, they found a strong correlation between the traction force and stem cell lineage commitment into either osteogenic or adipogenic fate.

In another study, Kiran et al. (36) have elucidated roles of cytoskeletal tension in regulation of RhoA activity known to regulate actin fiber formation and actomyosin contractility using pulmonary artery endothelial cells. They utilized micropost-based TFM to confirm that cytoskeletal tension-mediated traction forces were critical to activate GTP-bound RhoA and its downstream effector, Rho-associated protein kinase (ROCK), which was validated by suppressing actin cytoskeletal tension of cells using blebbistatin and cytochalasin D.

Similar approaches have been applied to understand how traction forces could mediate cell shape changes such as cell spreading and flattening of human mesenchymal stem cells (hMSCs) and their differentiation into osteogenic lineage through RhoA/ROCK activation and cytoskeletal tension (38). In that study, Wang et al. (38) utilized mPADs with micro-
contact printing of fibronectin (FN) into substrates to restrict
the cell shape according to FN-patterned island size ranging
from 625 to 10000 μm². They demonstrated that the degree of
cell spreading was significantly higher in case of cells adhered
to FN-coated substrate with larger sizes and that these cells
could become highly stretched, resulting in increased actin
stress fiber formation and traction forces. These results suggest
that cell spreading could induce RhoA/ROCK signaling
pathway-dependent cytoskeletal traction force and eventually
promote osteogenic differentiation of hMSCs.

In combination, these diverse reports indicate that there is a
strong correlation between matrix stiffness and adhered cell-
induced traction forces. The degree of traction forces could become one of the determinants for switching stem cell fates
through cell spreading.

**Recent advancements on measuring three-dimensional (3D)
TFM (3D TFM)**

Cellular forces are known to predominantly occur in tangential
(in-plane) directions (X, Y) with an assumption that there are
no normal (out-of-plane) forces to the substrates beneath cells
(Fig. 1E) (4). Therefore, TFM has been extensively used to
calculate two-dimensional (2D) traction forces generated by
adhered cells onto 2D substrates. More recently, however,
several studies have reported 3D TFM techniques to quantify
both tangential and normal forces against 3D ECM by utilizing
z-stacked 3D images obtained from confocal microscopy (33,
39, 40). For example, Hur et al. (28) reported 3D TFM
methods to measure three-dimensional (3D) traction forces
and adherent cells on 2D substrates, thus often called as 2.5D, in both tangential and
normal directions. They were able to visualize 3D traction
forces particularly at the cell-cell junctional and intracellular
tensions in monolayers of vascular endothelial cells (Fig. 1F)
(4, 33). This method enabled embedded cells to penetrate,
stretch, and become physiologically similar cell shapes within
3D hydrogels. Furthermore, they clearly explained that FAs
were experiencing various out-of-plane rotational moments at
different regions of either migrating or spreading cells (41).

Although mapping multi-dimensional traction forces with
spatiotemporal manners is highly demanded, much less is
known about how to quantify 3D traction forces exerted by
cells within 3D microenvironments. To solve these issues,
novel approaches have been reported to quantify the
spatiotemporal nature of 3D traction forces exerted by cells
within 3D hydrogels, exhibiting linear elastic properties (41,
42). Legant et al. (43) firstly reported the most sophisticated
3D TFM methods by encapsulating GFP-expressing fibroblasts
into enzymatically degradable but linear elastic polyethylene
glycol (PEG) hydrogels, rather than highly non-linear 3D
biopolymers such as collagen, fibrin, and mixture of ECMs.

As an alternative approach to measure 3D traction forces in
native nonlinear and viscoelastic connective tissue-like micro-
environments, Steinwachs et al. (43) have utilized collagen-
based 3D matrices as physiologically equivalent platforms.
Their results showed that MDA-MB-231 breast carcinoma cells
embedded in 3D collagen gels produced almost constant
forces irrespective of the concentration or stiffness of collagen
(43). Similarly, Hall et al. (44) have investigated mechanical
interactions between encapsulated MDA-MB-231 breast carci-
noma cells and fibrous 3D collagen networks using 3D
single cell TFM methods. In that study, they established breast
tumor-like microenvironments with varying microstructures
and densities of 3D fibrous collagen networks exhibiting
nonlinear elasticity. Their results revealed a positive mechanical
feedback loop. They indicated that cells could locally
induce collagen fiber alignment, reinforce the collagen
network, and then mechanically reinforce 3D collagen
networks, which in return could create greater cellular traction
forces evident by significantly longer range of displacement
propagation (Fig. 1G and 1H). Altogether, these studies
highlight the great potential of 3D TFM methods to probe
intracellular multi-dimensional traction forces associated with
FAs, actin cytoskeleton, and ECM remodeling.

**NOVEL METHODS TO UNDERSTAND JUNCTIONAL OR INTRACELLULAR FORCES**

**Intracellular force microscopy (IFM)**

Soon after substantial progresses have been made in analyzing
intracellular traction forces via TFM, it has been suggested that the
same principle as TFM could be extended to interpret average
intracellular junctional and intracellular forces by applying the same
force balance principle. These methods are known as
intracellular force microscopy (IFM) and monolayer stress microscopy (MSM) (45-47). Emerging evidences have suggest-
ed that adherent cells could exert normal forces to beneath
substrates and that these forces are no longer ignorable. Thus,
there have been numerous attempts to decipher spatiotem-
poral regulations of 3D forces around cells (33, 48).

Furthermore, recent advances in IFM have unraveled import-
ant attributes of force transmission through cell-ECM and
cell-cell adhesions or intercellular junctions-mediated force
transmission to the ECM (49, 50). In addition, these IFM
methods offer new opportunities to assess intracellular and
intracellular forces in a group of cells such as cell-cell doublets
(46, 51) and monolayers of cells.

For example, to better understand endogenous intracellular
forces, cell-cell tugging junctional forces between pairs of ECs,
Liu et al. (51) enabled 2D IFM by utilizing microfabricated
arrays of microneedles to evaluate intracellular tugging forces
at cell-cell adherens junctions. Their study uncovered that
intracellular forces could alter the size of AJs of ECs. They also
found close correlations between adherens junction sizes and
subsequent forces (Fig. 2A and 2B). Similarly, Chien and their
colleagues (47) have expanded this idea into developing novel
3D IFM methods to quantify 3D cell-cell junctional and
intracellular forces of monolayers of ECs under static and
dynamic shear flow conditions (Fig. 2C and 2D) (47). They
investigated how fluid shear stresses could interplay with 3D cell-ECM, cell-cell, and intracellular forces of partially confluent or confluent monolayers of ECs, exhibiting both normal and tangential stresses exerted by monolayers of ECs. These results suggested that intracellular tension could be highly associated with chemo-mechanical feedbacks of ECs under the flow shear, allowing localization and growth of adherens junctions at cell-cell adherens junctions.

In a similar approach using IFM-based methods, Ng et al. (52) have demonstrated the dynamics of E-cadherin-associated basal force fluctuations at intercellular adherens junctions of epithelial cells and quantified the force transmission at the cell-cell adherens junctions during spontaneous epithelial cluster formation. In that study, they revealed that at the multi-cellular level intercellular forces, the following transfer through cells required orchestrated changes in cell-matrix adhesions and actomyosin contraction within cells and their neighbors. Furthermore, they revealed that intercellular forces and force exchanges among neighboring cells were increased by recruitment of E-cadherin at cell-cell adherens junctions evident by IFM. Altogether, these results indicate that formation of cell-cell adhesion junctions plays an important role in the exchange of forces among cells within clusters or monolayers of cells.

Monolayer stress microscopy (MSM)

It has been shown that IFM-based methods have great advantages with little assumptions required for mechanical properties of cellular materials such as nuclei, plasma membranes, actin cytoskeletons, and cell-cell junctions to calculate intercellular or intracellular forces at adherens junctions. However, intercellular or intracellular tensions measured by IFM-based methods are averaged in-plane (in 2D) and mapped linearly (in 1D). Therefore, stresses could not be mapped on a 2D plane (53, 54). To resolve these challenges, Tambe et al. (53, 54) have developed a novel method, monolayer stress microscopy (MSM), to quantify forces within and between cell sheets. It can analyze forces based on an assumption that cells are made of one large sheet with one stiffness or Young’s modulus. By employing MSM-based method, collective migration behaviors of endothelial and epithelial monolayers could be visible. Their results confirmed that collective migration of neighboring cells had to join forces together to transfer detectable stresses through cell-cell adherens junctions.

Very recently, Serrano et al. (55) have developed a new 3D MSM method to quantify the collective generation and transmission of intracellular stresses within monolayers of ECs in micropatterned islands with varying sizes and shapes, where cell monolayers undergo bending stresses and lateral deformations. Their results revealed that these lateral deformations to cell monolayers could develop over long distances, whereas bending-associated stresses at cell-cell adhesions were predominantly localized within a few cell lengths. Taken together, these studies suggest that novel approaches using MSM-based methods offer the possibility to understand collective migration behaviors of cell sheets and cell-cell adherens junctional forces.

CONCLUDING REMARKS

The past two decades have seen the development of a variety of approaches for understanding cellular mechanotransduction.
of methods to measure cell-generated forces on FAs, AJs, and intracellular organelles via actin stress fibers. These methods have elucidated many aspects of mechanisms through which cells migrate, proliferate, differentiate, remodel, and mechanosense their microenvironment. In this review, we provided an overview of recent advancements of TFM quantifying cell-ECM forces (or traction forces) exerted on integrin-based focal adhesions and IFM and MSN quantifying cell-cell and intracellular forces applied through E-cadherin-based adherens junctions. As mechanobiology becomes more important in life science and engineering, TFM and IFM will play a fundamental role in elucidating cell functions related to mechanical force responses in biological research field. There is no doubt that these TFM-/IFM-based novel methods for understanding roles of biomechanical forces at interfaces of cell-cell/cell-ECM will open doors to breakthrough technologies for revolutionizing regenerative medicine, disease modelling, and drug discovery.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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