CELLULAR MECHANOBIOLOGY

Highly parallel single-cell force cytometry

Single-cell traction-force measurements performed on fluorescently labelled elastomeric surfaces can probe the physiological and pathological behaviour of thousands of cells in a single experiment.

Agnes M. Resto-Irizarry and Jianping Fu

Adherent mammalian cells attach to the surrounding extracellular matrix (ECM) through integrin-mediated focal adhesions. After initial attachment, the cells use their cytoskeletal contractile machinery to exert mechanical force (cell-traction force) onto the ECM through the focal adhesions. Recent studies have revealed that cells also use traction forces to probe the mechanics of the ECM, which in turn elicits downstream intracellular signalling pathways that regulate the cells' behaviour, such as migration and differentiation[1,2]. Importantly, deficiencies in traction-force generation are associated with the pathogenesis of several disorders[3,4]. However, despite an increasing appreciation of the role of cell-traction forces in the regulation of cell functions, tools available to measure traction forces are limited.

At the single-cell level, cell-traction forces are small (in the piconewton to nanonewton range), and occur across length scales on the order of nanometres and micrometres, making it technically challenging to measure the forces directly. Over the past 20 years, several imaging-based methods have been developed for single-cell traction-force measurements. The most widely used techniques are traction force microscopy (TMF)[5] and elastomeric micropost arrays (EMAs)[6]. Both can achieve subcellular resolution and were established mostly for traction-force measurements on two-dimensional (2D) substrates. However, given the inherently multidisciplinary expertise required to conduct and interpret measurements using TMF or EMAs, these techniques have been implemented in only a few research laboratories. Also, there is limited success in automation of TMF and EMAs for the high-throughput screening of contractility agonists or antagonists[7,8]. Specifically, EMAs require high-resolution microfabrication[6], and TMF requires an additional imaging step to reveal positions of fiduciary markers in unstressed substrates[5]. Consequently, both methods are limited by their low throughput (less than 100 individual cells under typical conditions), which is associated with expensive imaging and computational costs. Recently, developed molecular force sensors promise high-resolution, direct force measurements on intracellular proteins bearing forces across the ECM–integrin–cytoskeleton linkage[9], but the interpretation and validation of the forces measured with molecular force sensors remain challenging. The details, advantages and disadvantages of various existing single-cell traction force methods have been recently summarized[10,11].

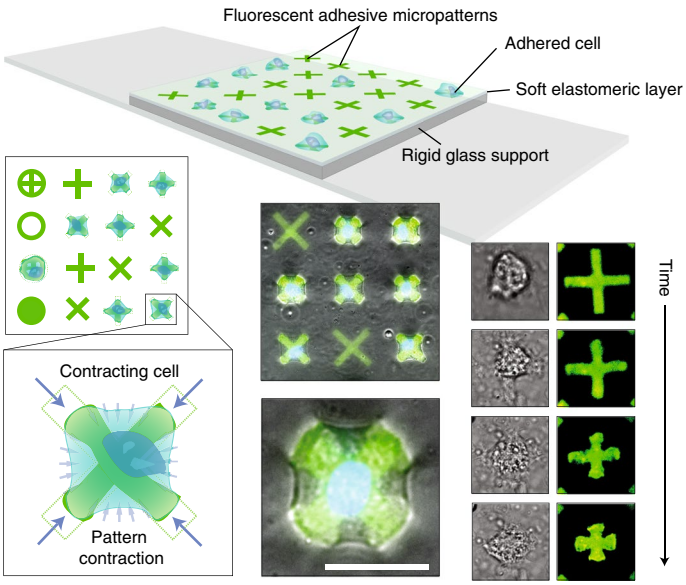

Fig. 1 | Fluorescently labelled elastomeric contractible surface for single-cell force cytometry. Top: single cells adhere to functionalized adhesive micropatterns embedded in an elastomeric film supported on thin glass, and are imaged with a fluorescence microscope. Bottom left: top view of multiple patterns and zoom-in of a cell contracting an 'X' pattern with inwardly displaced edges. Bottom middle: overlay of fluorescent and phase-contrast images of adhesive micropatterns. Bottom right: time-lapse microscopy (left, phase-contrast images; right, fluorescent images) of a contracting cell and its underlying micropatterns. Scale bar, 25 μm. Figure reproduced from ref. [12], Macmillan Publishers Ltd.

Writing in *Nature Biomedical Engineering*, Dino Di Carlo and colleagues now report a scalable whole-cell traction-force measurement tool that achieves 100-fold greater throughput in measuring single-cell forces than existing single-cell tools[12]. The device, termed fluorescently labelled elastomeric contractible surface (FLECS), consists of a large array of fluorescently labelled, X-shaped adhesive patterns coated on a glass-supported, thin elastomeric film. The adhesive patterns, with specifically designed sizes and shapes, allow the attachment of single cells to achieve a uniform cell-spreading area. Traction forces exerted by each cell cause the underlying adhesive pattern to deform and bend towards the pattern's centre. Using fluorescence-microscopy and image-analysis algorithms, inward displacements of adhesive patterns caused by single cells can be quantified as a coarse measurement of whole-cell traction force (Fig. 1). Importantly, Di Carlo and colleagues show that the FLECS system can be seamlessly integrated with the multi-well plate format to achieve highly parallelized assays compatible with existing automation workflows and screening infrastructure.

To demonstrate the resolution and utility of FLECS for cell 'mechanophenotyping',

Di Carlo and co-authors first analysed and compared whole-cell traction forces of human mesenchymal stem cells and their differentiated progeny, as well as human airway smooth muscle (HASM) cells treated with different concentrations of blebbistatin, a small molecule that inhibits myosin II activity and thus reduces cell-traction force. The cell-traction data obtained were consistent with previous studies[13], thus indicating that FLECS can resolve population-wide contractile phenotype differences. The authors also studied single-cell traction forces of primary HASM cells isolated from patients with fatal asthma, and compared the forces to those exerted by HASM cells from healthy patients of the same age, race and gender. They found that, as expected, HASM cells from asthmatic patients exhibited a higher baseline contractile tone and showed a greater contractile-force increase in response to treatment with bradykinin, a contractility stimulant. Interestingly, the authors identified a subgroup of HASM cells from healthy individuals that exhibited greater contractile force reversal when further treated with formoterol, the asthma standard-of-care β2-adrenoceptor agonist, revealing single-cell heterogeneity in the contractile phenotype of HASM cells. A noteworthy finding by the authors is the absence of a correlation between agonist-induced maximum contraction and peak calcium response. At present, the study of asthma and its possible remediation is carried out with emphasis on contraction of HASM cells in bronchoconstriction and airway hyperresponsiveness. The results of Di Carlo and co-authors's study strongly call into question some high-throughput screening assays using calcium-sensitive dyes as an indirect measure of HASM contractility. In addition, these results highlight that high-throughput single-cell traction-force measurements combined with live-cell imaging could be used to monitor intracellular signalling and uncover new molecular insights on traction-force-generation mechanisms.

Moreover, Di Carlo and colleagues used FLECS to study phagocytic force generation in single primary human monocyte-derived macrophages. They specifically examined the effects of stimulation with subclasses of immunoglobulin G antibodies and the impact of pharmacological inhibition of actin polymerization and phosphoinositide 3-kinase (PI3K) on the contractile forces involved in the closure of the phagocytic cup. The study showed that PI3K is directly involved in phagocytic force generation. This finding is especially important to inform the development of drugs that rely on PI3K inhibition with possible negative effects on phagocytosis and, therefore, on a patient's immune system. One example highlighted by Di Carlo and colleagues is CAL-101, a drug being developed for chronic lymphocytic leukaemia that works by inhibiting PI3K. The variety of assays conducted in this study is a testament to the versatility of FLECS and its potential for applications in fundamental biological research investigating biomechanical forces at the single-cell level.

The trade-off between resolution and the cost of implementation and analysis (and thus overall throughput) for single-cell traction-force measurement needs to be properly balanced when addressing different biological questions and applications. This is especially true when considering single-cell heterogeneity, particularly when single-cell traction force needs to be evaluated in different disease states or under different chemical treatments, or for the identification of rare populations of disease-relevant cells from clinical samples[14]. In this regard, the high-throughput capabilities and user-friendly fabrication and data analysis of FLECS are significant advantages over TMF and EMAs. The fabrication process in FLECS could be readily adjusted for mass production and incorporated into existing automation workflows to achieve high-throughput, high-content screens with cellular force as the target. Previous studies have shown that single-cell traction force can be used as a non-invasive marker for monitoring cell phenotype or even as an early predictor of cell-fate decisions[13]. Hence, high-throughput methods for single-cell traction-force measurements such as FLECS should find promising applications in drug screening, diagnostics and regenerative medicine[14].

As pointed out by Di Carlo and colleagues, TMF and EMAs remain superior for single-cell traction-force measurements in terms of subcellular resolution and their ability to attribute measured subcellular traction forces directly to individual focal adhesions[15]. Identifying the most useful method, taking into account the trade-off between resolution and throughput, will depend on the specific biological questions and applications at hand. Nevertheless, continuous development of cell-traction-force measurement tools as a critical component of mechanomedicine will require interdisciplinary collaboration and innovation. As mechanomedicine continues to grow and mature, the focus will shift from tool development to understanding cellular forces as effectors and regulators of cells and tissues. There is also significant interest in the development of methods for the measurement of traction forces of cells embedded in 3D gels (a more physiologically relevant environment), as the phenotypes and shape of cells in 2D and 3D environments can differ substantially. ❐

Agnes M. Resto-Irizarry[1] and Jianping Fu[1,2,3]*
[1]*Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA.* [2]*Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA.* [3]*Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA.*
**e-mail: jpfu@umich.edu*

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Competing interests

The authors declare no competing financial interests.