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COMMENTARY

Signs of stress on soft surfaces

A commentary on: Cui, Y., F.M. Hameed, B. Yang, K. Lee, C.Q. Pan, S. Park, and M. Sheetz. 2015. Cyclic stretching of soft substrates induces spreading and growth. Nat Commun. 6:6333

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Abstract Cells experience mechanical stimuli during growth and differentiation and transduce these stimuli into biochemical signals that in turn regulate cell responses to the imposed forces. Reduced spreading and impaired stress fiber formation are indicators of the mechano-response to growth on soft elastic culture substrates. However, Cui and coworkers demonstrate that cell spreading and stress fiber formation on soft substrates is possible if simultaneous cyclic stretching compensates for the lack of substrate stiffness-induced cell stress. The stress(ed) response is dependent on cyclic stretch amplitude and frequency and, at least in part, mediated by myocardin related transcription factor A (MRTF-A) and Yes-associated protein (YAP). The study thus provides novel insight into the mechanisms of cell mechanosensing and how materials can be designed to mimic mechanical conditions of body tissues.

Keywords Elastic modulus · Strain · Cyclic stretch · Micropillars · YAP · MRTF · Mechanosensing · Stress fibers · Cell spreading

Cells experience mechanical stimuli during growth and differentiation and transduce these stimuli into biochemical signals that in turn regulate cell responses to the imposed forces. In their physiological tissue environments, cells are often embedded in extracellular matrix (ECM) with an elastic modulus ranging from ~0.1 to 20 kPa which is million times softer than standard tissue culture plates with GigaPascal stiffness. It is proposed that adherent cells sense the mechanical properties of their substrate by a tugging process and respond to stiff ECM by forming stress fibers (hence the name) and cell spreading (Discher et al. 2009). Since the seminal work of Pelham and Wang (Pelham and Wang 1997), numerous studies have observed and used reduced spreading and impaired stress fiber formation as indicators of the mechano-response to soft elastic substrates such as polyacrylamide and polyethylene glycol (PEG) hydrogels or silicone elastomers (Solon et al. 2007; Tee et al. 2011). Conversely, restricting cell size on stiff substrates was shown to reduce intracellular stress and cell stiffness (Godbout et al. 2013; Tee et al. 2011).

Cui and coworkers now demonstrate that cell spreading and stress fiber formation on soft substrates is possible if simultaneous cyclic stretching compensates for the lack of substrate stiffness-induced cell stress (Cui et al. 2015). To enable cyclic strain of substrates that are perceived as soft by fibroblasts, they used previously established soft submicron-diameter pillars (Fu et al. 2010; Ghassemi et al. 2012) in a microfabricated stretching device (Mann et al. 2012). As expected, cells adhered to static soft substrates but spreading and stress fiber formation did not occur. However, applying different stretch stimulation regimes induced cell spreading and stress fibre formation on soft substrates with maximal effects at a frequency of 0.1 Hz, 3 % strain amplitude and 6 h duration. These results are consistent with the previous finding that cyclic strain can compensate for experimentally induced loss of intracellular tension (Kaunas et al. 2005). Cui and coworkers show that cortical cell stiffening occurs in response to cyclic stretching, as appreciated from outward pushing of pillars in the substrate relaxation period close to the cells' leading edge where focal adhesions form. The underlying structural changes in the actin cytoskeleton are possibly the

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first step in preserving stressed features on soft substrates but these changes last only minutes once cyclic stretch is suspended. The mechanosensitive myocardin related transcription factor A (MRTF-A) (Olson and Nordheim 2010) and Yes-associated protein (YAP) have been shown previously to localize predominantly in the nucleus on stiff and in the cytosol of cells grown on soft substrates (Dupont et al. 2011). Because both factors translocate from the cytosol to the nucleus upon cyclic stretch on soft substrates, the authors suggest that they may be involved in compensating lack of stiffness with stretch. Indeed, knock-down of each factor individually was sufficient to eliminate cyclic strain-induced cell spreading on soft substrates; effects on stress fiber formation however were not assessed.

YAP nuclear translocation on soft substrates is also observed by Chaudhuri and coworkers (Chaudhuri et al. 2015) with the important distinction that the substrates used in their study were chosen to be viscoelastic rather than elastic. In contrast to elastic substrates, viscoelastic soft substrates exhibit stress relaxation after cell pulling, i.e., tensile stress decreases over time when strain remains constant. This substrate stress relaxation allows cell spreading and stress fiber formation in fibroblasts cultured on such on soft substrates. On basis of these findings, one may be tempted to conclude that the phases of relaxation periods and not the stretching components in the cyclic protocol induce stress fibers and spreading on the soft pillar substrates. However, substrate relaxation and cell relaxation are not equivalent. Regional relaxation of viscoelastic substrates necessarily leads to increasing density (stiffness) at the pulling point (focal adhesions). Very local regions of such densified and stiffened matrix may be sufficient to generate stress responses as recently suggested (Dingal et al. 2015). Whether cyclic relaxation alone can promote cell stress features on soft substrates could be assessed using highly elastic and expandable substrates that allow to relaxing cells gradually without a stretch component (Majd et al. 2011; Shafieyan et al. 2014).

Intriguingly, the cyclic strain-induced "stressed cell phenotype" persisted on soft substrates even when Cui et al. terminated the strain protocol and the lasting effect was enhanced with increasing duration of the preceding cyclic stretch. Such momentum is indicative of a mechanically induced memory that stabilizes the stressed cell phenotype, at least temporarily, against relaxation on soft substrates. Long-term mechanical memory has been shown previously to protect the stressed fibroblast phenotype imprinted by continuous culture on pathologically stiff elastomers (100 kPa) for several passages against subsequent switch to 5 kPa soft substrates for weeks rather than hours (Balestrini et al. 2012). Another study, using elastic substrates with phototunable stiffness also demonstrated a dosing effect of mechanical preconditioning - or priming – on fate decision of mesenchymal stromal cells (Yang et al. 2014) in a process involving YAP.

The mechanisms responsible for mechano-protection by formation of memory are only beginning to be understood. In their study, Cui and coworkers observe that MRTF-A translocation from the cytosol to the nucleus is subject to momentum by continuing even after suspending the stretch protocol was and only slowly returning to the initial low nuclear levels in a matter of hours. A comparable behaviour, although with slower kinetics was observed for nuclear accumulation of YAP. Both, MRTF-A and YAP control different target genes as co-transcription factors and respond to mechanical challenge by sensing the state of the actin cytoskeleton (Dupont et al. 2011; Olson and Nordheim 2010). However, they have not been shown (yet) to directly control the state of actin polymerization and stress fiber formation and it remains to be solved how these stress features are preserved in the absence of acute stress. Stress fiber formation on very soft hyaluronic acid gels substrates in the absence of substantial cell traction has been described but was attributed to signalling pathways independent of YAP (Chopra et al. 2014).

In conclusion, the study featured in this commentary and the work of Chaudhuri and coworkers, published in the same journal 4 days earlier (Chaudhuri et al. 2015), suggest that mechanosensing is more complex than cells just probing the resistance of an elastic substrate by pulling on it. Both studies provide important insight into how cells feel substrate mechanics and will be of great value to produce culture substrates or biomaterials to more accurately mimic in vivo conditions for cells exposed to dynamic and soft tissue surroundings. Gene expression studies and further investigating the involved signalling pathways may provide direct evidence on the regulatory mechanisms affect cell response on a soft substrate to the stimulatory forces.

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