
Article:
Boldock, L., Wittkowske, C. and Perrault, C.M. orcid.org/0000-0003-2230-6994 (2017) Microfluidic traction force microscopy to study mechanotransduction in angiogenesis. Microcirculation, 24 (5). e12361. ISSN 1073-9688,

https://doi.org/10.1111/micc.12361

MR. LUKE BOLDOCK (Orcid ID : 0000-0002-6296-9736)

Received Date : 30-Nov-2016

Revised Date : 12-Jan-2017

Accepted Date : 31-Jan-2017

Article type : Invited Reviews

--- INVITED REVIEW ---

Microcirculation

Special Issue on "New and Emerging Tools for Studying the Microvasculature – Microfluidics in Vascular Research"

MICROFLUIDIC TRACTION FORCE MICROSCOPY TO STUDY MECHANOTRANSDUCTION IN ANGIOGENESIS

Boldock, Luke[*]; Wittkowske, Claudia[*]; Perrault, Cecile M.[#]

Department of Mechanical Engineering and INSIGNEO Institute for in silico Medicine,

University of Sheffield, UK

* Both authors contributed equally to this work.

[#] **Corresponding author:**

Cecile M Perrault, PhD

Insigneo Institute for in silico Medicine

Room C+05

The Pam Liversidge Building

Sir Frederick Mappin Building

Mappin Street

Sheffield S1 3JD

Phone: +44 (0)114 2220154

c.perrault@sheffield.ac.uk

ABSTRACT

The formation of new blood vessels from existing vasculature, angiogenesis, is driven by coordinated endothelial cell migration and matrix remodelling in response to local signals. Recently, a growing body of evidence has shown that mechanotransduction, along with chemotransduction, is a major regulator of angiogenesis. Mechanical signals, such as fluid shear stress and substrate mechanics, influence sprouting and network formation, but the mechanisms behind this relationship are still unclear. Here, we present cellular traction forces as possible effectors activated by mechanosensing to mediate matrix remodelling,

and encourage the use of traction force microscopy to study mechanotransduction in angiogenesis. We also suggest that deciphering the response of endothelial cells to mechanical signals could reveal an optimal angiogenic mechanical environment, and provide insight into development, wound healing, the initiation and growth of tumours, and new strategies for tissue engineering.

Keywords: Traction force microscopy, microfluidics, extracellular matrix, mechanotransduction, angiogenesis, endothelial cells

LIST OF ABBREVIATIONS

EC	Endothelial cells
ECM	Extracellular matrix
PA	Polyacrylamide
PDMS	Polydimethylsiloxane
TFM	Traction force microscopy

1. INTRODUCTION

Angiogenesis, the complex process by which new blood vessels form from pre-existing ones, is essential during embryogenesis, development and tissue repair. It is similarly crucial for the development of a number of pathologies, including the formation and growth of cancerous tumours [1]. In regenerative medicine, angiogenesis has been a long-standing challenge for the creation of large tissues and whole organs. Therefore, a better understanding of angiogenesis' mechanism is expected to impact medicine widely, from improved tissue engineering to novel cancer treatments. Angiogenesis can be described as the rearrangement, migration and proliferation of endothelial cells (EC) from existing blood vessels into the surrounding extracellular matrix (ECM), forming new capillary networks [2]. In addition to biochemical factors which have been studied most extensively in the past [3], the mechanical microenvironment of EC has recently been shown to play a fundamental role in angiogenesis (Figure 1). EC are located in a mechanically dynamic setting, not only subjected to fluid flow-induced shear stress but also surrounded by ECM of varying stiffness. These external forces influence EC behaviour, modulating their ability to generate traction forces, for instance. These internal forces in turn enable cell migration and cell-induced matrix remodelling. Although fluid shear stress, substrate stiffness and traction forces have been studied individually on EC in vitro, few studies have aimed at combining them to establish their simultaneous influence on cells. Here, we consider examples where techniques for traction force measurement are combined with established microfluidic platforms, routinely used to apply shear stress to adherent cells. This relatively new and ongoing research endeavours to present a more complete biomechanical picture of angiogenesis and the link between flow and matrix remodelling, and could guide the development of microfluidic platforms specifically designed for this purpose.

2. THE MECHANICAL ENVIRONMENT OF ANGIOGENESIS

Angiogenesis is driven by EC which are located in a dynamic environment and respond to a wide range of external forces. These include shear stress generated by the friction of flowing blood, strain caused by wall deformation due to the pulsatile nature of the flow, and hydrostatic pressure. Here, we focus on shear stress, to which EC are particularly sensitive and responsive; its magnitude and consistency has a significant effect on cell behaviour and gene regulation [4]. In addition, we consider the effect of the mechanical properties of the ECM, and internal traction forces. The link between these mechanical stimuli and angiogenesis are summarised below.

2.1 SHEAR STRESS AND ANGIOGENESIS

Physiological shear stress in most vasculature lies within 0.1 to 5 Pa, but in microcirculation it can be as low as 0.01 Pa [5] and lower still at the tips of sprouting, developing vessels. Previous research found that sprouting was triggered above a shear stress threshold of approximately 1 Pa, via either luminal or transmural flow [6]. However, when EC were exposed to extremely low shear stress levels (10^{-3} to 10^{-5} Pa) in a separate study, proliferation was high but no cytoskeletal reconstruction was observed [7]. Since cell alignment and network formation require cytoskeletal reconstruction [8], it has been concluded that the flow rates may have been too low to trigger shear stress sensing mechanisms and activate flow-mediated angiogenesis. Continuous flow stimulation was also found to be required to not only maintain vessel sprouting, but to prevent rapid retraction [6][9].

There are limitations to be considered in the in vitro models used for shear stress studies. For example, while angiogenesis can occur through sprouting (the migration of EC into tissue towards an angiogenic stimulus) and splitting (the separation of existing vessels by reorganisation of EC), most in vitro models focus on the former. Furthermore, in vitro models do not always consider the different haemodynamic conditions within arteries and veins, or the different responses of arterial and venous cells given identical conditions. In an embryonic animal model, shear stress only inhibited sprouting in the venous network, not within the arterial network [10]. This differing behaviour may explain instances of shear stress inhibiting sprouting angiogenesis in vitro [11].

The precise mechanisms linking shear stress and angiogenesis currently remain unclear. Yet, it is known that one of the initial steps in shear-induced angiogenesis involves the combined action of several mechanotransducer molecules, including cell-cell [12] and cell–ECM adhesion complexes [13]. It is thus relevant to also establish the role of the cells' substrate on angiogenesis.

2.2 ECM MECHANICAL PROPERTIES AND ANGIOGENESIS

During angiogenesis, EC are known to interact with their surrounding environment and ECM stiffness has been shown to have a profound effect on endothelial cell behaviour, particularly migration, proliferation [14] and network assembly [15]. Similarly, the influence of ECM stiffness on angiogenesis can be investigated by altering the substrates' mechanical properties in vitro (for example, synthesising polyacrylamide (PA) gels of varied stiffness [15], or incorporating polyethylene glycol hydrogels [16][17]).

Angiogenesis studies with substrates of a wide range of stiffness have shown that softer, more compliant substrates (Young's modulus < ~1 kPa) encourage the assembly of stable networks, capillary-like structures and lumen formation [16][18], while stiffer substrates (> ~1 kPa) do not see any network assembly, but rather a uniform spreading of cells and growth to confluence [15] [17]. An intermediate state has also been identified around the transition point of 1 kPa, with some cells connecting to one another amongst others growing in patches [15].

It has been noted that glass, polystyrene and laboratory plastics, with moduli of several megapascals (10^9), fall well outside of the angiogenic range, and that – perhaps unsurprisingly – the elastic modulus of the angiogenic compliant substrates is similar to many soft tissues [19]. Yet, it is also clear that the mechanical properties of the substrate are not the only influences of angiogenesis, as other physical properties of the ECM, including its density [20] and topography [21] also control network formation in vitro. Nor is it likely that mechanical properties are the predominant influences. Indeed, Califano and Reinhart-King showed that by reducing substrate-bound collagen I concentration from 100 μg/ml to 1 μg/ml, it is possible to induce network formation on relatively stiff PA substrates (2.5 kPa – 10 kPa) [18]. Conversely, on very compliant substrates (140 Pa), network stability can be decreased by treating cells with growth factors [15]. Chemical stimulation thus appears to dominate stiffness-induced mechanical stimulation of angiogenesis.

At this point, we can establish that shear stress and mechanical stiffness of the substrate influence angiogenesis, and can be used to modulate sprouting. However, it is unclear how those external mechanical forces encourage cells to physically alter and shape their environment to create new vessels. One theory is that mechanical forces modulate the

ability of cells to generate traction forces, thus allowing them to restructure their surroundings, and in turn enabling vessel growth.

2.3 THE ROLE OF TRACTION FORCES ON ECM REMODELLING AND ANGIOGENESIS

Unlike external forces such as shear stress and substrate stiffness, described above, traction forces are generated by EC themselves. They involve the coordinated action of various cell organelles (e.g. stress fibres, actomyosin, and actin filament bundles) and are transmitted to the substrate through protein structures, such as focal adhesions, used by the cells to anchor themselves to their surrounding ECM [22]. Traction forces could play a fundamental role in angiogenesis as they drive a wide range of related EC processes, including migration and ECM remodelling [23], which have been shown to be required for blood vessel formation and maintenance [24]. Traction forces can also facilitate cell-cell communication by transmitting mechanical stresses in compliant substrates [25]. It would not be surprising that traction forces could influence angiogenesis, as cells need to migrate and extensively remodel their environment during the formation of new vessels. Currently, few studies (described in the following section) have investigated the link between shear stress, the mechanical properties of substrates, and the generation of endogenous traction forces in EC and, to our knowledge, none have specifically assessed the role of external forces on traction forces in angiogenesis.

3. TRACTION FORCE MICROSCOPY

Traction force studies were first developed following the realisation that cells placed on thin films would deform them. The first qualitative reports estimated fibroblast traction forces by observing silicone membrane wrinkling [26]. The approach was further developed into a more sophisticated quantitative method referred to as 'traction force microscopy' (TFM) [27].

3.1 MEASURING CELL TRACTION FORCES

TFM uses the displacement of fiducial fluorescent markers embedded within an elastic substrate to map the location, magnitude and direction of forces exerted by cells against the substrate (Figure 2). PA gels are most commonly used as substrates as their stiffness can be readily controlled [28]. Other substrates used for TFM include ultra-soft polydimethylsiloxane (PDMS) [29] and gelatine [30]. PA gels and PDMS require treatment with ECM proteins such as fibronectin and collagen to support cell attachment. Upon attachment, traction forces exerted by the cells generate displacements within the substrate. These displacements can be quantified by taking images of the same location with and without cells (i.e. 'force loaded' and 'force unloaded'). By comparing the two images, a displacement field can be calculated based on the movement of the fluorescent markers on the surface of the elastic substrate [31]. Depending on the mechanical properties of the substrate, the measured deformations are converted into strain fields and traction forces. A wide range of different computational methods have been developed which are reviewed elsewhere in more detail [32][33]. In addition to planar substrates, traction forces have also been calculated by analysing the displacement of deformable PDMS micro-posts [34][35][36]. Despite TFM on both flat hydrogel substrates and micro-posts sharing the same aim, their different methodology can lead to differences in force calculations [37]. When performing TFM, the effect of surface treatments should also be considered as this may affect traction forces, cell spread area and focal adhesion area [36]. For example, Yeung et

al observed that matrix stiffness is not the only cue for cell morphology and motility as EC spread less on collagen-coated gels compared to fibronectin-coated gels [28].

3.2 TRACTION FORCES UNDER STATIC CONDITIONS

In contrast to early qualitative silicone membrane assays, TFM enables a spatial and temporal resolution of traction forces. Traction forces in single EC are not uniformly distributed throughout the adhered area, but correlated to the location of focal adhesions, primarily located around the edge of the cells and most strongly expressed at the end of pseudopodia [38]. This polarisation enables EC to generate substrate strains which can be sensed by their neighbours. These strains are thought to guide endothelial migration, drawing cells closer together and thereby guiding network self-assembly [39]. In line with single cell observation, traction forces are heterogeneously distributed within endothelial monolayers, with cell-cell junctions showing the greatest magnitude [40]. Traction forces also appear to differ between EC types. Tip and stalk cells, arising from asymmetric cell division, exert very different forces from each other. This disparity drives angiogenic sprouting via coordinated collective cell migration [41][42]. Tumour-derived capillary EC grown on fibronectin-coated PA gels displayed greater traction forces (~100 nN) than healthy EC (~50 nN). The increased traction forces might enable tumour-derived cells to form tubular networks more readily [43]. EC traction forces also depend positively on matrix stiffness [23]. Therefore, higher traction forces of tumour-derived cells might be associated with matrix stiffening of tumour tissue [44].

3.3 TRACTION FORCES UNDER DYNAMIC CONDITIONS

The effect of shear stress on EC has been the focus of much research, and as such a range of microfluidic in vitro systems for the application of flow have been developed, some specific to the study of angiogenesis and others repurposed to that aim. Comprehensive reviews already exist, in which these techniques are described in detail [45]. In brief, systems range from common planar flow chambers [7], in which shear stress is applied via the laminar flow of fluid between two parallel plates, to simple circular channels [46], microvascular networks [47] and patient-specific geometries.

In vitro fluidic systems allow the simultaneous use of separate techniques (for example, the application of both shear stress and cyclic substrate stretch), and recently have been coupled with TFM to quantify cellular stress changes under the influence of flow. TFM requires flow to be applied within planar parallel plate models, with the lower plate comprising the deformable substrate. Both elastic PA [48][49][50][51] and micro-post arrays [34][35][52] have been used to perform TFM under flow, applied by passive, gravity-driven flow [50], syringe- [48], peristaltic- and piston-pumps [51] at rates required to generate physiologically relevant levels of shear stress.

Currently, analysis of traction forces under flow has produced conflicting, often contradictory results. Both Conway et al (using a micro-post assay, under 1.5 Pa shear stress) [52], and Steward et al (1.2 kPa PA gel, 1 Pa shear stress) [49] report a decrease of traction forces of 25% and 6% respectively, compared to static conditions. Ting et al [34] also observed a decrease in force, but for disturbed flow only – traction forces were increased when cells were exposed to laminar flow.

Additional studies support the contrary hypothesis that shear stress is linked to increased traction force. Lam et al (micro-post assay, 2 Pa shear stress) [35] saw an increase in cytoskeletal contractility, which was maintained for approximately 4 hours before returning to baseline – the same time period during which the cells were aligning and elongating parallel to flow. EC migration and increased traction forces were observed by Shiu et al (28 kPa PA gel, 1.2 Pa shear stress) [50]. The largest increases were found at the front and rear of cells, perpendicular to flow direction – the leading advancing edge and the area of detachment.

Perrault et al applied shear stress of between 0.014 and 0.133 Pa, an order of magnitude lower than previous studies and more representative of venous and interstitial flow [48]. Once again, increased traction forces were seen under shear stress. However, altering the level of shear – achieved through the use of a channel of varying height and changing the flow rate – had no significant effect. In agreement with the previously described works, a tendency for traction forces to orientate perpendicular to flow direction, and to return to baseline levels once the flow stimuli was removed, was identified.

Finally Hur et al [51], studying EC on a PA substrate of Young's modulus 3.63 kPa, found that the increase in intracellular tensions under flow was beyond levels necessary to simply balance the applied shear. Tension was shown to be 3.5 times higher than required under oscillatory flow (0.05 ±0.4 Pa) and almost 8 times higher than required under laminar flow (1.2 Pa). It would thus appear that the modulation of traction force with flow is not an attempt by the cells to re-establish a mechanical equilibrium state, but rather to actively respond to this mechanical stimulation by physical alteration of their substrate.

3.4 FUTURE DEVELOPMENTS IN TRACTION FORCE MICROSCOPY

TFM is currently primarily performed on flat substrates exposed to uniform hydrodynamic conditions within parallel plate flow chambers. Yet, planar models are poor approximations of natural vessel geometry and hinder the application of three-dimensional hydrogel substrates which better mimic the ECM. In vitro models can be enhanced by better representing in vivo anatomy through the use of realistic vascular structure. However, while traction forces of cells on two-dimensional substrates can be measured in three dimensions ('2.5D TFM') [51], applying TFM techniques to cells within non-planar models, or a three-dimensional ECM, is more complex [53]. Challenges, such as the tracking of markers within three dimensions, and the consideration of anisotropic substrate properties, could be overcome with the implementation of improved computational algorithms. These can provide even greater resolution, and aid the development of reference-free approaches by removing the need to detach cells to obtain the reference location of trackers [54]. Increased accuracy is vital to look deeper at the sub-cellular level, to study the coordination of cytoskeletal structure and sites of mechanoreceptor activation and traction force generation [35][50].

At the macro-scale, most TFM algorithms are optimised for single cell studies, even though the application of TFM to confluent monolayers provides more physiologically relevant data than single-cell analysis [51]. Future improvements in this area would allow the exploration of cell-cell stresses during migration and network formation, key aspects of angiogenesis, which is, by its nature, a multi-cellular process.

4. CONCLUSION

Angiogenesis is known to be influenced by flow-induced shear stress and matrix mechanics. The development of microfluidic systems and the use of substrates of various rigidities have helped to establish these relationships. It is, however, unclear how those external mechanical forces can lead to matrix remodelling, which is required to alter and shape the ECM into nascent vessels. Cellular traction forces are a plausible and viable mechanism that would enable cells to link mechanical stimulation and matrix remodelling.

Traction force microscopy is an effective tool to examine cellular forces and ECM interaction. When considering EC in particular, the ability to integrate TFM with fluid systems makes it even more valuable, as the addition of flow-generated shear stress more closely mimics the natural cell environment. This combination will continue to yield increasingly reliable data as further improvements allow TFM to be applied with more advanced microfluidic platforms, which model accurate vessel geometries and mechanical properties, cell co-cultures and realistic fluid dynamics.

The mediation of EC behaviour, morphology and biochemical response by shear forces, ECM mechanical properties and intercellular stresses, and the impact on vessel formation, is not entirely understood; the interplay between these stimuli even less so. The combined results of shear stress, substrate mechanics and traction force studies, could point to the existence of optimal mechanical angiogenic environments (Figure 3), and the techniques described here may provide valuable insight into this intricate relationship.

REFERENCES

[1] C. Giverso and P. Ciarletta, “Tumour angiogenesis as a chemo-mechanical surface instability,” *Sci. Rep.*, vol. 6, p. 22610, 2016.

[2] B. Vailhé, X. Ronot, P. Tracqui, Y. Usson, and L. Tranqui, “In vitro angiogenesis is modulated by the mechanical properties of fibrin gels and is related to αvβ3 integrin localization,” *Vitr. Cell. Dev. Biol. - Anim.*, vol. 33, no. 10, pp. 763–773, 1996.

[3] P. Carmeliet and R. K. Jain, “Molecular mechanisms and clinical applications of angiogenesis,” *Nature*, vol. 473, no. 7347, pp. 298–307, 2011.

[4] J. W. Wragg, S. Durant, H. M. Mcgettrick, K. M. Sample, S. Egginton, and R. Bicknell, “Shear stress regulated gene expression and angiogenesis in vascular endothelium,” *Microcirculation*, vol. 21, no. 4, pp. 290–300, 2014.

[5] D. Kaiser, M. A. Freyberg, and P. Friedl, “Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells,” *Biochem. Biophys. Res. Commun.*, vol. 231, no. 3, pp. 586–590, 1997.

[6] P. A. Galie, D.-H. T. Nguyen, C. K. Choi, D. M. Cohen, P. A. Janmey, and C. S. Chen, “Fluid shear stress threshold regulates angiogenic sprouting,” *Proc. Natl. Acad. Sci.*, vol. 111, no. 22, pp. 7968–7973, 2014.

[7] J. Y. Park, J. B. White, N. Walker, C. H. Kuo, W. Cha, M. E. Meyerhoff, and S. Takayama, “Responses of endothelial cells to extremely slow flows,” *Biomicrofluidics*, vol. 5, no. 2, p. 22211, 2011.

[8] G. K. Kolluru, S. Sinha, S. Majumder, A. Muley, J. H. Siamwala, R. Gupta, and S. Chatterjee, “Shear stress promotes nitric oxide production in endothelial cells by sub-cellular delocalization of eNOS: a basis for shear stress mediated angiogenesis,” *Nitric Oxide*, vol. 22, no. 4, pp. 304–315, 2010.

[9] S. Egginton, A. Hussain, J. Hall-Jones, B. Chaudhry, F. Syeda, and K. E. Glen, “Shear stress-

induced angiogenesis in mouse muscle is independent of the vasodilator mechanism and quickly reversible," *Acta Physiol.*, vol. 218, no. 3, pp. 153–166, 2016.

[10] G. Chouinard-Pelletier, E. D. Jahnsen, and E. A. V Jones, "Increased shear stress inhibits angiogenesis in veins and not arteries during vascular development," *Angiogenesis*, vol. 16, no. 1, pp. 71–83, 2013.

[11] J. W. Song and L. L. Munn, "Fluid forces control endothelial sprouting," *Proc. Natl. Acad. Sci.*, vol. 108, no. 37, pp. 15342–15347, 2011.

[12] F. Orsenigo, C. Giampietro, A. Ferrari, M. Corada, A. Galaup, S. Sigismund, G. Ristagno, L. Maddaluno, G. Y. Koh, D. Franco, V. Kurtcuoglu, D. Poulikakos, P. Baluk, D. McDonald, M. G. Lampugnani, and E. Dejana, "Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo," *Nat. Commun.*, vol. 3, p. 1208, 2012.

[13] S. Egginton, "In vivo shear stress response," *Biochem. Soc. Trans.*, vol. 39, no. 6, pp. 1633–1638, 2011.

[14] L. Krishnan, C. C. Chang, S. S. Nunes, S. K. Williams, J. A. Weiss, and J. B. Hoying, "Manipulating the microvasculature and its microenvironment," *Crit. Rev. Biomed. Eng.*, vol. 41, no. 2, pp. 91–123, 2013.

[15] R. L. Saunders and D. A. Hammer, "Assembly of human umbilical vein endothelial cells on compliant hydrogels," *Cell. Mol. Bioeng.*, vol. 3, no. 1, pp. 60–67, 2010.

[16] D. Hanjaya-Putra, J. Yee, D. Ceci, R. Truitt, D. Yee, and S. Gerecht, "Vascular endothelial growth factor and substrate mechanics regulate in vitro tubulogenesis of endothelial progenitor cells," *J. Cell. Mol. Med.*, vol. 14, no. 10, pp. 2436–2447, 2010.

[17] J. Sun, N. Jamilpour, F.-Y. Wang, and P. K. Wong, "Geometric control of capillary architecture via cell-matrix mechanical interactions," *Biomaterials*, vol. 35, no. 10, pp. 3273–3280, 2014.

[18] J. P. Califano and C. A. Reinhart-King, "A balance of substrate mechanics and matrix chemistry regulates endothelial cell network assembly," *Cell. Mol. Bioeng.*, vol. 1, no. 2–3, pp.

122–132, 2008.

[19] C. A. Reinhart-King, “How matrix properties control the self-assembly and maintenance of tissues,” *Ann. Biomed. Eng.*, vol. 39, no. 7, pp. 1849–1856, 2011.

[20] L. T. Edgar, C. J. Underwood, J. E. Guilkey, J. B. Hoying, and J. A. Weiss, “Extracellular matrix density regulates the rate of neovessel growth and branching in sprouting angiogenesis,” *PLoS One*, vol. 9, no. 1, p. e85178, 2014.

[21] L. E. Dike, C. S. Chen, M. Mrksich, J. Tien, M. W. George, and D. E. Ingber, “Geometric control of switching between growth , apoptosis , and differentiation during angiogenesis using micropatterned substrates,” *Vitr. Cell. Dev. Biol. - Anim.*, vol. 35, no. 8, pp. 441–448, 1999.

[22] K. Katoh, Y. Kano, and S. Ookawara, “Role of stress fibers and focal adhesions as a mediator for mechano-signal transduction in endothelial cells in situ,” *Vasc. Health Risk Manag.*, vol. 4, no. 6, pp. 1273–1282, 2008.

[23] J. P. Califano and C. A. Reinhart-King, “Substrate stiffness and cell area predict cellular traction stresses in single cells and cells in contact,” *Cell. Mol. Bioeng.*, vol. 3, no. 1, pp. 68–75, 2010.

[24] E. Kniazeva and A. J. Putnam, “Endothelial cell traction and ECM density influence both capillary morphogenesis and maintenance in 3-D,” *Am. J. Physiol. Cell Physiol.*, vol. 297, no. 1, pp. C179–C187, 2009.

[25] C. A. Reinhart-King, M. Dembo, and D. A. Hammer, “Cell-Cell Mechanical Communication through Compliant Substrates,” *Biophys. J.*, vol. 95, no. 12, pp. 6044–6051, 2008.

[26] A. K. Harris, P. Wild, and D. Stopak, “Silicone rubber substrata: a new wrinkle in the study of cell locomotion,” *Science (80-.).*, vol. 208, no. 4440, pp. 177–179, 1980.

[27] M. Dembo and Y.-L. Wang, “Stresses at the cell-to-substrate interface during locomotion of fibroblasts,” *Biophys. J.*, vol. 76, no. 4, pp. 2307–2316, 1999.

[28] T. Yeung, P. C. Georges, L. A. Flanagan, B. Marg, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver, and P. A. Janmey, “Effects of substrate stiffness on cell morphology, cytoskeletal

structure, and adhesion," *Cell Motil. Cytoskeleton*, vol. 60, no. 1, pp. 24–34, 2005.

[29] T. Das, T. K. Maiti, and S. Chakraborty, "Traction force microscopy on-chip: shear deformation of fibroblast cells," *Lab Chip*, vol. 8, no. 8, pp. 1308–1318, 2008.

[30] A. D. Doyle and J. Lee, "Simultaneous, real-time imaging of intracellular calcium and cellular traction force production," *Biotechniques*, vol. 33, no. 2, pp. 358–364, 2002.

[31] J. H.-C. Wang and J.-S. Lin, "Cell traction force and measurement methods," *Biomech. Model. Mechanobiol.*, vol. 6, no. 6, pp. 361–371, 2007.

[32] B. Sabass, M. L. Gardel, C. M. Waterman, and U. S. Schwarz, "High resolution traction force microscopy based on experimental and computational advances," *Biophys. J.*, vol. 94, no. 1, pp. 207–220, 2008.

[33] S. V Plotnikov, B. Sabass, U. S. Schwarz, and C. M. Waterman, "High-resolution traction force microscopy," *Methods Cell Biol.*, vol. 123, pp. 367–394, 2014.

[34] L. H. Ting, J. R. Jahn, J. I. Jung, B. R. Shuman, S. Feghhi, S. J. Han, M. L. Rodriguez, and N. J. Sniadecki, "Flow mechanotransduction regulates traction forces, intercellular forces, and adherens junctions," *Am. J. Physiol. Hear. Circ. Physiol.*, vol. 302, pp. H2220–H2229, 2012.

[35] R. H. W. Lam, Y. Sun, W. Chen, and J. Fu, "Elastomeric microposts integrated into microfluidics for flow-mediated endothelial mechanotransduction analysis," *Lab Chip*, vol. 12, no. 10, pp. 1865–1873, 2012.

[36] J.-J. Han, H.-F. Tan, C. Feng, W.-K. Wee, S.-Y. Tee, and S.-M. Tan, "Data on cell spread area and directional contraction in human umbilical vein endothelial cells on fibronectin and on collagen type I-coated micro-posts," *Data Br.*, vol. 6, pp. 803–810, 2016.

[37] A. J. S. Ribeiro, A. K. Denisin, R. E. Wilson, and B. L. Pruitt, "For whom the cells pull: hydrogel and micropost devices for measuring traction forces," *Methods*, vol. 94, pp. 51–64, 2016.

[38] C. A. Reinhart-King, M. Dembo, and D. A. Hammer, "Endothelial cell traction forces on RGD-derivatized polyacrylamide substrata," *Langmuir*, vol. 19, no. 5, pp. 1573–1579, 2003.

[39] J. P. Califano and C. A. Reinhart-King, "The effects of substrate elasticity on endothelial cell

network formation and traction force generation," *Conf. Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.*, vol. 2009, pp. 3343–3345, 2009.

[40] E. T. Valent, G. P. van Nieuw Amerongen, V. W. M. van Hinsbergh, and P. L. Hordijk, "Traction force dynamics predict gap formation in activated endothelium," *Exp. Cell Res.*, vol. 347, no. 1, pp. 161–170, 2016.

[41] G. Costa, K. I. Harrington, H. E. Lovegrove, D. J. Page, S. Chakravartula, K. Bentley, and S. P. Herbert, "Asymmetric division coordinates collective cell migration in angiogenesis," *Nat. Cell Biol.*, vol. 18, no. 12, pp. 1292–1301, 2016.

[42] Y. Du, S. C. B. Herath, Q.-G. Wang, D.-A. Wang, H. H. Asada, and P. C. Y. Chen, "Three-dimensional characterization of mechanical interactions between endothelial cells and extracellular matrix during angiogenic sprouting," *Sci. Rep.*, vol. 6, p. 21362, 2016.

[43] K. Ghosh, C. K. Thodeti, A. C. Dudley, A. Mammoto, M. Klagsbrun, and D. E. Ingber, "Tumor-derived endothelial cells exhibit aberrant Rho-mediated mechanosensing and abnormal angiogenesis in vitro," *Proc. Natl. Acad. Sci.*, vol. 105, no. 32, pp. 11305–11310, 2008.

[44] D. T. Butcher, T. Alliston, and V. M. Weaver, "A tense situation: forcing tumour progression," *Nat. Rev. Cancer*, vol. 9, no. 2, pp. 108–122, 2009.

[45] C. A. Davis, S. Zambrano, P. Anumolu, A. C. B. Allen, L. Sonoqui, and M. R. Moreno, "Device-based in vitro techniques for mechanical stimulation of vascular cells: a review," *J. Biomech. Eng.*, vol. 137, no. 4, p. 40801, 2015.

[46] S. T. Hsiao, T. Spencer, L. Boldock, S. D. Prosseda, I. Xanthis, F. J. Tovar-Lopez, H. van Buesekamp, R. Y. Khamis, N. Foin, N. Bowden, A. Hussain, A. Rothman, V. Ridger, I. Halliday, C. Perrault, J. Gunn, and P. C. Evans, "Endothelial repair in stented arteries is accelerated by inhibition of Rho-associated protein kinase," *Cardiovasc. Res.*, 2016.

[47] Y. Zheng, J. Chen, M. Craven, N. W. Choi, S. Totorica, A. Diaz-Santana, P. Kermani, B. Hempstead, C. Fischbach-Teschl, J. A. López, and A. D. Stroock, "In vitro microvessels for the study of angiogenesis and thrombosis," *Proc. Natl. Acad. Sci.*, vol. 109, no. 24, pp. 9342–9347, 2012.

[48] C. M. Perrault, A. Brugues, E. Bazellieres, P. Ricco, D. Lacroix, and X. Trepat, “Traction forces of endothelial cells under slow shear flow,” *Biophys. J.*, vol. 109, no. 8, pp. 1533–1536, 2015.

[49] R. Steward, D. Tambe, C. C. Hardin, R. Krishnan, and J. J. Fredberg, “Fluid shear, intercellular stress, and endothelial cell alignment,” *Am. J. Physiol. Cell Physiol.*, vol. 308, no. 8, pp. C657–C664, 2015.

[50] Y.-T. Shiu, S. Li, W. A. Marganski, S. Usami, M. A. Schwartz, Y.-L. Wang, M. Dembo, and S. Chien, “Rho mediates the shear-enhancement of endothelial cell migration and traction force generation,” *Biophys. J.*, vol. 86, no. 4, pp. 2558–2565, 2004.

[51] S. S. Hur, J. C. del Alamo, J. S. Park, Y.-S. Li, H. A. Nguyen, D. Teng, K.-C. Wang, L. Flores, B. Alonso-Latorre, J. C. Lasheras, and S. Chien, “Roles of cell confluency and fluid shear in 3-dimensional intracellular forces in endothelial cells,” *Proc. Natl. Acad. Sci.*, vol. 109, no. 28, pp. 11110–11115, 2012.

[52] D. E. Conway, M. T. Breckenridge, E. Hinde, E. Gratton, C. S. Chen, and M. A. Schwartz, “Fluid shear stress on endothelial cells modulates mechanical tension across VE-cadherin and PECAM-1,” *Curr. Biol.*, vol. 23, no. 11, pp. 1024–1030, 2013.

[53] W. J. Polacheck and C. S. Chen, “Measuring cell-generated forces: a guide to the available tools,” *Nat. Methods*, vol. 13, no. 5, pp. 415–423, 2016.

[54] M. Bergert, T. Lendenmann, M. Zündel, A. E. Ehret, D. Panozzo, P. Richner, D. K. Kim, S. J. P. Kress, D. J. Norris, O. Sorkine-Hornung, E. Mazza, D. Poulikakos, and A. Ferrari, “Confocal reference free traction force microscopy,” *Nat. Commun.*, vol. 7, p. 12814, 2016.

Figure 1: Schematic representation of the local mechanical environment of angiogenesis. Endothelial cells are subjected to external stimuli, including shear stress and extracellular matrix (ECM) stiffness. In response to these cues, endothelial cells generate traction forces themselves, enabling matrix remodelling and enhancing cell-cell communication, both integral parts of angiogenesis.

Figure 2: Traction force microscopy (TFM). A: Schematics of typical techniques. TFM is commonly performed on elastic substrates with embedded fluorescent markers (left) or on flexible micro-posts (right). B: TFM protocol. *For dynamic flow environments elastic substrates or micro-posts could be integrated in a microfluidic chamber.

Figure 3: Angiogenic effects in relation to mechanical conditions (shear stress and substrate stiffness) including results from traction force studies. Green icons indicate an increase in network formation, sprouting, or traction force; red a decrease; yellow intermediate or insignificant change. While angiogenic behaviour can be seen on compliant substrates under static conditions, a stiffness threshold may exist above which shear stress must be applied to induce angiogenesis-related function (green background). *Denotes oscillatory or disturbed flow, numbers within icons refer to citations.