

# Editorial: Understanding molecular interactions that underpin vascular mechanobiology

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## ABSTRACT

Cells are exposed to a variety of mechanical forces in their daily lives, especially endothelial cells that are stretched from vessel distention and are exposed to hemodynamic shear stress from a blood flow. Exposure to excessive forces can induce a disease, but the molecular details on how these cells perceive forces, transduce them into biochemical signals and genetic events, i.e., mechanotransduction, and integrate them into physiological or pathological changes remain unclear. However, seminal studies in endothelial cells over the past several decades have begun to elucidate some of these signals. These studies have been highlighted in *APL Bioengineering* and elsewhere, describing a complex temporal pattern where forces are sensed immediately by ion channels and force-dependent conformational changes in surface proteins, followed by biochemical cascades, cytoskeletal contraction, and nuclear remodeling that can affect long-term changes in endothelial morphology and fate. Key examples from the endothelial literature that have established these pathways include showing that integrins and Flk-1 or VE-cadherin act as shear stress transducers, activating downstream proteins such as Cbl and Nck $\beta$  or Src, respectively. In this Editorial, we summarize a recent literature highlighting these accomplishments, noting the engineering tools and analysis methods used in these discoveries while also highlighting unanswered questions.

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Mechanical cues, including shear stress, activate a variety of signaling cascades and induce downstream gene programs that regulate endothelial cell (EC) functions and pathophysiological processes, including atherosclerosis when forces become aberrantly high.<sup>1–3</sup> Activation largely occurs in discrete steps from initial force sensing, conversion to biochemical signals, cell contraction, and concluding with nuclear remodeling. This general outline has been established over the last three decades and integrates discrete observations from several key luminaries in the field. For example, ECs rearrange themselves under a flow, and this is caused by cytoskeletal remodeling to minimize strain during shear stress.<sup>4–10</sup> Separately, the EC nuclear transduction induces microRNA expression<sup>11</sup> and differential histone modifications<sup>12</sup> in athero-susceptible regions of branches, a high curvature, and a disturbed flow. In between these portions of the pathway, plasma membrane,<sup>13,14</sup> ion channels,<sup>1</sup> and membrane receptors (e.g., integrins,<sup>15,16</sup> GPCR,<sup>17</sup> Cadherin,<sup>18,19</sup> PECAM-1,<sup>19–21</sup> VCAM-1,<sup>22,23</sup> and ICAM-1<sup>22</sup>) perceive mechanical cues and transduce them into molecular signaling cascades, i.e., mechanotransduction. While many classic reviews have highlighted specific contributions from noted

luminaries as well as how each type of force impacts an endothelial cell function,<sup>24–29</sup> here we focus our commentary on the contributions of Dr. Shu Chien on the occasion of his retirement.

In a series of papers published in *APL Bioengineering*, colleagues noted contributions from Dr. Chien across the complex temporal sequence of endothelial mechanotransduction events. Starting with events at the membrane, Zhu and co-workers<sup>30</sup> note early observations that solid, uniaxial strain can induce a perpendicular alignment of intracellular actin stress fibers via the Rho signaling. By stretching elastic membranes to mimic vessel hoop strain, Chien *et al.* found that actin stress fibers aligned perpendicular to the direction of applied strain but that realignment could be blocked by interrupting molecular signaling cascades, e.g., RhoA, ROCK, and mDia.<sup>31</sup> These pathways are critical to modulating cytoskeletal assembly kinetics, and a subsequent work has shown that catch-slip bonds within the cytoskeleton<sup>32</sup> regulate many of the morphological observations in the original work from Chien and co-workers. Fluid shear stress may also use contractility-mediated pathways to achieve the perpendicular alignment; shear stress, not receptor ligation, causes Flk-1 phosphorylation

and association with Cbl in an effort to realign cells,<sup>33</sup> although additional downstream pathways could also be influenced by receptor ligation,<sup>34</sup> though again pathways diverge when Flk-1 recruits the adapter protein Nck $\beta$  chemically vs ERK and JNK mechanically.<sup>35</sup> Also downstream is cell–cell transducers, including the non-receptor tyrosine kinase Src kinase. A seminal work from the Chien lab used a fluorescence resonance energy transfer (FRET)-based biosensor<sup>36,37</sup> to visualize mechanotransduction upon mechanical stimulation. The laser-tweezer induced traction pulled integrins on the cell surface, resulting in the directional wave propagation of Src activation at 18 nm/s along the plasma membrane, propagating in the direction opposite to the pulling force and possibly due to cytoskeletal polymerization.<sup>38</sup>

While these previous examples are emblematic of physiologically forces, it is important to understand how these early molecular mechanisms change to effectuate a disease, e.g., atherosclerosis. He and co-workers<sup>39</sup> note the use of oscillatory shear stress (OS) in Dr. Chien's work as a tool to mimic the disturbed flow patterns. While they are typically found at regions of a high vessel curvature or bifurcation, OS can reproducibly induce hallmarks of atherosclerosis found in more complex mouse models.<sup>40</sup> He *et al.* further note Dr. Chien's finding that OS can exacerbate inflammation by causing endothelial cells to secrete monotype chemokines,<sup>41</sup> by accumulating a lipid, and by activation of TNF- $\alpha$  and other related pathways.<sup>42</sup> Yes-associated protein (YAP) has also been shown to be mechano-sensitive,<sup>43</sup> and it has recently been associated with YAP-dependent activation of inflammatory genes.<sup>44</sup> In addition to the presence of OS noted by He and co-workers,<sup>39</sup> Bulter<sup>45</sup>—in a final manuscript in this collection from *APL Bioengineering*—notes that OS effects are particularly sensitive to a shear stress magnitude and rate-of-change, and that the net effect is very different endothelial cell-mediated vasodilations. For example, step- and ramp-shear stress applications cause vastly different vasodilation: a step application causes a transient peaked whereas a slow ramp over the same observation window causes much less dilation;<sup>46</sup> membranes<sup>17</sup> and receptor tyrosine kinases<sup>33</sup> appear to be rate limiting mechanotransducers identified in part by Dr. Chien, suggesting the possible disease connections to endothelial mechanobiology. Finally, Kaunas<sup>47</sup> further describes the use of models, particularly negative feedback models, to understand endothelial remodeling to OS. Using the mechanobiological measurements from the work of Dr. Hur *et al.*,<sup>48</sup> Tondon and Kaunas,<sup>49</sup> Trepap *et al.*,<sup>50</sup> and Yeung *et al.*,<sup>51</sup> Kaunas proposes a model resulting in two predictions for endothelial cells exposed to OS: (1) oscillating strain rates become exceedingly high such that actomyosin crossbridges cannot dissipate tension changes fast enough, causing stress fiber disassembly parallel and accumulation perpendicular to the strain field and (2) low strain rates can be dissipated by crossbridge cycling, causing no change in stress fibers.<sup>47</sup> The model present therein expands upon these conclusions, but Kaunas is quick to suggest that further understanding of all of the mechanotransductive pathways discussed (and not) by Chien and others will require increasingly complex, mathematical-based models in the future.

From these perspectives, we believe one can get not just an impression of the state of endothelial cell mechanotransduction and the impact that Dr. Shu Chien and other luminaries have had on it, but also appreciate the influence that endothelial mechanobiology has had on medicine. Most notably, the field has identified novel, mechanically sensitive microRNAs,<sup>11,39</sup> histone modifications,<sup>12</sup> and signaling

pathways<sup>15–23,36,37,43,44</sup> involved in the development of atherosclerotic plaques<sup>1–3</sup> in regions of a high shear stress and vessel dilation. Despite these observations, there are still many unanswered questions about endothelial cell mechanobiology regarding how certain forces are sensed, converted into specific biochemical signals, and integrated into a cellular response. Moreover, few drugs, if any, target the mechano-sensitivity of these proteins and RNAs, and this will be a key challenge as the field matures. However, observations in the literature and in this collection suggest that endothelial mechanobiology would not be where it is today without Dr. Chien's contributions. Perhaps a key to the field's future success and impact on human health will be Dr. Chien's 7 “C” principles: Compassion, Commitment, Comprehension, Creation, Communication, Cooperation, and Consummation.

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## AUTHOR DECLARATIONS

### Conflict of Interest

The authors have no conflicts to disclose.

## DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## REFERENCES

- Y. S. Li, J. H. Haga, and S. Chien, “Molecular basis of the effects of shear stress on vascular endothelial cells,” *J. Biomech.* **38**, 1949–1971 (2005).
- P. F. Davies, J. A. Spaan, and R. Krams, “Shear stress biology of the endothelium,” *Ann. Biomed. Eng.* **33**, 1714–1718 (2005).
- Y. C. Boo and H. Jo, “Flow-dependent regulation of endothelial nitric oxide synthase: Role of protein kinases,” *Am. J. Physiol. Cell Physiol.* **285**, C499–508 (2003).
- B. P. Helmke, A. B. Rosen, and P. F. Davies, “Mapping mechanical strain of an endogenous cytoskeletal network in living endothelial cells,” *Biophys. J.* **84**, 2691–2699 (2003).
- S. Li *et al.*, “Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases,” *J. Biol. Chem.* **272**, 30455–30462 (1997).
- A. M. Malek and S. Izumo, “Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress,” *J. Cell Sci.* **109**(4), 713–726 (1996).
- T. Morita, H. Kurihara, K. Maemura, M. Yoshizumi, and Y. Yazaki, “Disruption of cytoskeletal structures mediates shear stress-induced endothelin-1 gene expression in cultured porcine aortic endothelial cells,” *J. Clin. Invest.* **92**, 1706–1712 (1993).
- B. P. Helmke and P. F. Davies, “The cytoskeleton under external fluid mechanical forces: Hemodynamic forces acting on the endothelium,” *Ann. Biomed. Eng.* **30**, 284–296 (2002).
- B. P. Helmke, D. B. Thakker, R. D. Goldman, and P. F. Davies, “Spatiotemporal analysis of flow-induced intermediate filament displacement in living endothelial cells,” *Biophys. J.* **80**, 184–194 (2001).
- B. P. Helmke, R. D. Goldman, and P. F. Davies, “Rapid displacement of vimentin intermediate filaments in living endothelial cells exposed to flow,” *Circ. Res.* **86**, 745–752 (2000).
- Y. Z. Jiang, E. Manduchi, J. M. Jimenez, and P. F. Davies, “Endothelial epigenetics in biomechanical stress: Disturbed flow-mediated epigenomic plasticity in vivo and in vitro,” *Arterioscler. Thromb. Vasc. Biol.* **35**, 1317–1326 (2015).
- J. Dunn *et al.*, “Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis,” *J. Clin. Invest.* **124**, 3187–3199 (2014).

- <sup>13</sup>P. J. Butler, G. Norwich, S. Weinbaum, and S. Chien, "Shear stress induces a time- and position-dependent increase in endothelial cell membrane fluidity," *Am. J. Physiol. Cell Physiol.* **280**, C962–969 (2001).
- <sup>14</sup>J. B. McCabe and L. G. Berthiaume, "Functional roles for fatty acylated amino-terminal domains in subcellular localization," *Mol. Biol. Cell* **10**, 3771–3786 (1999).
- <sup>15</sup>S. Jalali *et al.*, "Shear stress activates p60src-Ras-MAPK signaling pathways in vascular endothelial cells," *Arterioscler. Thromb. Vasc. Biol.* **18**, 227–234 (1998).
- <sup>16</sup>E. Tzima, M. A. del Pozo, S. J. Shattil, S. Chien, and M. A. Schwartz, "Activation of integrins in endothelial cells by fluid shear stress mediates Rho-dependent cytoskeletal alignment," *Embo J.* **20**, 4639–4647 (2001).
- <sup>17</sup>M. Chachisvilis, Y. L. Zhang, and J. A. Frangos, "G protein-coupled receptors sense fluid shear stress in endothelial cells," *Proc. Natl. Acad. Sci. U. S. A.* **103**, 15463–15468 (2006).
- <sup>18</sup>H. Miao *et al.*, "Effects of flow patterns on the localization and expression of VE-cadherin at vascular endothelial cell junctions: In vivo and in vitro investigations," *J. Vasc. Res.* **42**, 77–89 (2005).
- <sup>19</sup>E. Tzima *et al.*, "A mechanosensory complex that mediates the endothelial cell response to fluid shear stress," *Nature* **437**, 426–431 (2005).
- <sup>20</sup>Z. Bagi *et al.*, "PECAM-1 mediates NO-dependent dilation of arterioles to high temporal gradients of shear stress," *Arterioscler. Thromb. Vasc. Biol.* **25**, 1590–1595 (2005).
- <sup>21</sup>I. Fleming, B. Fisslthaler, M. Dixit, and R. Busse, "Role of PECAM-1 in the shear-stress-induced activation of Akt and the endothelial nitric oxide synthase (eNOS) in endothelial cells," *J. Cell. Sci.* **118**, 4103–4111 (2005).
- <sup>22</sup>J. J. Chiu *et al.*, "Shear stress increases ICAM-1 and decreases VCAM-1 and E-selectin expressions induced by tumor necrosis factor- $\alpha$  in endothelial cells," *Arterioscler. Thromb. Vasc. Biol.* **24**, 73–79 (2004).
- <sup>23</sup>T. Nagel, N. Resnick, W. J. Atkinson, C. F. Dewey, Jr., and M. A. Gimbrone, Jr., "Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells," *J. Clin. Invest.* **94**, 885–891 (1994).
- <sup>24</sup>P. F. Davies, "Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology," *Nat. Clin. Pract. Cardiovasc. Med.* **6**, 16–26 (2009).
- <sup>25</sup>K. M. Gray and K. M. Stroka, "Vascular endothelial cell mechanosensing: New insights gained from biomimetic microfluidic models," *Semin. Cell Dev. Biol.* **71**, 106–117 (2017).
- <sup>26</sup>J. Ando and K. Yamamoto, "Vascular mechanobiology: Endothelial cell responses to fluid shear stress," *Circ. J.* **73**, 1983–1992 (2009).
- <sup>27</sup>S. Chien, "Mechanotransduction and endothelial cell homeostasis: The wisdom of the cell," *Am. J. Physiol. Heart Circ. Physiol.* **292**, H1209–1224 (2007).
- <sup>28</sup>S. Li, N. F. Huang, and S. Hsu, "Mechanotransduction in endothelial cell migration," *J. Cell Biochem.* **96**, 1110–1126 (2005).
- <sup>29</sup>S. Weinbaum, X. Zhang, Y. Han, H. Vink, and S. C. Cowin, "Mechanotransduction and flow across the endothelial glycocalyx," *Proc. Natl. Acad. Sci. U. S. A.* **100**, 7988–7995 (2003).
- <sup>30</sup>C. Zhu, C. Y. Lee, and L. V. McIntire, "From cellular to molecular mechanobiology," *APL Bioeng.* **4**, 010902 (2020).
- <sup>31</sup>R. Kaunas, P. Nguyen, S. Usami, and S. Chien, "Cooperative effects of Rho and mechanical stretch on stress fiber organization," *Proc. Natl. Acad. Sci. U. S. A.* **102**, 15895–15900 (2005).
- <sup>32</sup>C. Y. Lee *et al.*, "Actin depolymerization under force is governed by lysine 113: Glutamic acid 195-mediated catch-slip bonds," *Proc. Natl. Acad. Sci. U. S. A.* **110**, 5022–5027 (2013).
- <sup>33</sup>Y. Wang *et al.*, "Interplay between integrins and FLK-1 in shear stress-induced signaling," *Am. J. Physiol. Cell Physiol.* **283**, C1540–1547 (2002).
- <sup>34</sup>Y. Wang *et al.*, "Shear stress and VEGF activate IKK via the Flk-1/Cbl/Akt signaling pathway," *Am. J. Physiol. Heart Circ. Physiol.* **286**, H685–692 (2004).
- <sup>35</sup>Y. Wang *et al.*, "Selective adapter recruitment and differential signaling networks by VEGF vs. shear stress," *Proc. Natl. Acad. Sci. U. S. A.* **104**, 8875–8879 (2007).
- <sup>36</sup>A. Y. Ting, K. H. Kain, R. L. Klemke, and R. Y. Tsien, "Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells," *Proc. Natl. Acad. Sci. U. S. A.* **98**, 15003–15008 (2001).
- <sup>37</sup>J. Zhang, Y. Ma, S. S. Taylor, and R. Y. Tsien, "Genetically encoded reporters of protein kinase A activity reveal impact of substrate tethering," *Proc. Natl. Acad. Sci. U. S. A.* **98**, 14997–15002 (2001).
- <sup>38</sup>Y. Wang *et al.*, "Visualizing the mechanical activation of Src," *Nature* **434**, 1040–1045 (2005).
- <sup>39</sup>M. He, M. Martin, T. Marin, Z. Chen, and B. Gongol, "Endothelial mechanobiology," *APL Bioeng.* **4**, 010904 (2020).
- <sup>40</sup>S. Chien, J. J. Chiu, and Y. S. Li, "Focal adhesion kinase phosphorylation in flow-activation of endothelial NF-kappaB. Focus on "Focal adhesion kinase modulates activation of NF-kappaB by flow in endothelial cells," *Am. J. Physiol. Cell Physiol.* **297**, C800–C801 (2009).
- <sup>41</sup>Y. J. Shyy, H. J. Hsieh, S. Usami, and S. Chien, "Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium," *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4678–4682 (1994).
- <sup>42</sup>Y. Liu *et al.*, "Shear stress activation of SREBP1 in endothelial cells is mediated by integrins," *Arterioscler. Thromb. Vasc. Biol.* **22**, 76–81 (2002).
- <sup>43</sup>S. Dupont *et al.*, "Role of YAP/TAZ in mechanotransduction," *Nature* **474**, 179–183 (2011).
- <sup>44</sup>K. C. Wang *et al.*, "Flow-dependent YAP/TAZ activities regulate endothelial phenotypes and atherosclerosis," *Proc. Natl. Acad. Sci. U. S. A.* **113**, 11525–11530 (2016).
- <sup>45</sup>P. J. Butler, "Mechanobiology of dynamic enzyme systems," *APL Bioeng.* **4**, 010907 (2020).
- <sup>46</sup>P. J. Butler, S. Weinbaum, S. Chien, and D. E. Lemons, "Endothelium-dependent, shear-induced vasodilation is rate-sensitive," *Microcirculation* **7**, 53–65 (2000).
- <sup>47</sup>R. Kaunas, "Good advice for endothelial cells: Get in line, relax tension, and go with the flow," *APL Bioeng.* **4**, 010905 (2020).
- <sup>48</sup>S. S. Hur *et al.*, "Roles of cell confluency and fluid shear in 3-dimensional intracellular forces in endothelial cells," *Proc. Natl. Acad. Sci. U. S. A.* **109**, 11110–11115 (2012).
- <sup>49</sup>A. Tondon and R. Kaunas, "The direction of stretch-induced cell and stress fiber orientation depends on collagen matrix stress," *PLoS One* **9**, e89592 (2014).
- <sup>50</sup>X. Trepal *et al.*, "Universal physical responses to stretch in the living cell," *Nature* **447**, 592–595 (2007).
- <sup>51</sup>T. Yeung *et al.*, "Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion," *Cell Motil. Cytoskeleton* **60**, 24–34 (2005).