

Quantitative structural mechanobiology of platelet-driven blood clot contraction

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Abstract

© 2017 The Author(s). Blood clot contraction plays an important role in prevention of bleeding and in thrombotic disorders. Here, we unveil and quantify the structural mechanisms of clot contraction at the level of single platelets. A key elementary step of contraction is sequential extension-retraction of platelet filopodia attached to fibrin fibers. In contrast to other cell-matrix systems in which cells migrate along fibers, the "hand-over-hand" longitudinal pulling causes shortening and bending of platelet-attached fibers, resulting in formation of fiber kinks. When attached to multiple fibers, platelets densify the fibrin network by pulling on fibers transversely to their longitudinal axes. Single platelets and aggregates use actomyosin contractile machinery and integrin-mediated adhesion to remodel the extracellular matrix, inducing compaction of fibrin into bundled agglomerates tightly associated with activated platelets. The revealed platelet-driven mechanisms of blood clot contraction demonstrate an important new biological application of cell motility principles.

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References

- [1] Tutwiler, V., Wang, H., Litvinov, R. I., Weisel, J. W. & Shenoy, V. Interplay of platelet contractility and elasticity of fibrin/erythrocytes in blood clot retraction. *Biophys. J.* 4, 714-723 (2017).
- [2] Montesano, R. & Orci, L. Transforming growth factor beta stimulates collagenmatrix contraction by fibroblasts: Implications for wound healing. *Proc. Natl Acad. Sci. USA* 85, 4894-4897 (1988).
- [3] Kawada, N., Tran-Thi, T., Klein, H. & Decker, K. The contraction of hepatic stellate (Ito) cells stimulated with vasoactive substances. *Eur. J. Biochem.* 213, 815-823 (1993).
- [4] Webb, R. C. Smooth muscle contraction and relaxation. *Adv. Physiol. Educ.* 27, 201-206 (2003).
- [5] Garcia, J. G., Verin, A. D. & Schaphorst, K. L. Regulation of thrombin-mediated endothelial cell contraction and permeability. *Sem. Thromb. Hemost.* 22, 309-315 (1996).
- [6] Qiu, Y. et al. Platelet mechanosensing of substrate stiffness during clot formation mediates adhesion, spreading, activation. *Proc. Natl Acad. Sci. USA* 111, 14430-14435 (2014).
- [7] Litvinov, R. I., Farrell, D. H., Weisel, J. W. & Bennett, J. S. The platelet integrin α IIb β 3 differentially interacts with fibrin versus fibrinogen. *J. Biol. Chem.* 291, 7858-7867 (2016).
- [8] Cohen, I., Gerrard, J. M. & White, J. G. Ultrastructure of clots during isometric contraction. *J. Cell Biol.* 93, 775-787 (1982).
- [9] Petrich, B. G. et al. Talin is required for integrin-mediated platelet function in hemostasis and thrombosis. *J. Exp. Med.* 204, 3103-3111 (2007).
- [10] Schoenwaelder, S. M., Yuan, Y., Cooray, P., Salem, H. H. & Jackson, S. P. Calpain cleavage of focal adhesion proteins regulates the cytoskeletal attachment of integrin IIb3 (platelet glycoprotein IIb/IIIa) and the cellular retraction of fibrin clots. *J. Biol. Chem.* 272, 1694-1702 (1997).

- [11] Carr, M. E. Jr & Zekert, S. L. Abnormal clot retraction, altered fibrin structure, normal platelet function in multiple myeloma. *Am. J. Physiol.* 266, H1195-H1201 (1994).
- [12] Carr, M. E. & Zekert, S. L. Measurement of platelet-mediated force development during plasma clot formation. *Am. J. Med. Sci.* 302, 13-18 (1991).
- [13] Jen, C. J. & McIntire, L. V. The structural properties and contractile force of a clot. *Cell Motil.* 2, 445-455 (1982).
- [14] Liang, X. M., Han, S. J., Reems, J., Gao, D. & Sniadecki, N. J. Platelet retraction force measurements using flexible post force sensors. *Lab. Chip.* 10, 991-998 (2010).
- [15] Lam, W. A. et al. Mechanics and contraction dynamics of single platelets and implications for clot stiffening. *Nat. Mater.* 10, 61-66 (2011).
- [16] Qiu, Y., Ciciliano, J., Myers, D. R., Tran, R. & Lam, W. A. Platelets and physics: How platelets "feel" and respond to their mechanical microenvironment. *Blood Rev.* 29, 377-386 (2015).
- [17] Meshel, A. S., Wei, Q., Adelstein, R. S. & Sheetz, M. P. Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat. Cell Biol.* 7, 157-164 (2005).
- [18] Mohammadi, H., Janmey, P. A. & McCulloch, C. A. Lateral boundary mechanosensing by adherent cells in a collagen gel system. *Biomaterials* 35, 1138-1149 (2014).
- [19] Schwarz Henriques, S., Sandmann, R., Strate, A. & Koster, S. Force field evolution during human blood platelet activation. *J. Cell Sci.* 125, 3914-3920 (2012).
- [20] Tutwiler, V. et al. Kinetics and mechanics of clot contraction are governed by the molecular and cellular composition of the blood. *Blood* 127, 149-159 (2016).
- [21] Carr, M. E. Jr, Krischnaswami, A. & Martin, E. Method of using platelet contractile force and whole blood clot elastic modulus as clinical markers. U.S. Patent 7 192, 726 (2007).
- [22] Kim, J. H. et al. Mechanical tension drives cell membrane fusion. *Dev. Cell* 32, 561-573 (2015).
- [23] Discher, D. E., Janmey, P. & Wang, Y. L. Tissue cells feel and respond to the stiffness of their substrate. *Science* 310, 1139-1143 (2005).
- [24] White, J. G., Krivit, W. & Vernier, R. L. The platelet-fibrin relationship in human blood clots: An ultrastructural study utilizing ferritin-conjugated antihuman fibrinogen antibody. *Blood* 25, 241-257 (1965).
- [25] Cohen, I. & de Vries, A. Platelet contractile regulation in an isometric system. *Nature* 246, 36-37 (1973).
- [26] Cohen, I., Gerrard, J., Bergman, R. & White, J. The role of contractile filaments in platelet activation. *Protides Biol. Fluids*, 26, 555-566 (1979).
- [27] Wood, W. et al. Wound healing recapitulates morphogenesis in *Drosophila* embryos. *Nat. Cell Biol.* 4, 907-912 (2002).
- [28] Woolner, S., Jacinto, A. & Martin, P. The small GTPase Rac plays multiple roles in epithelial sheet fusion-dynamic studies of *Drosophila* dorsal closure. *Dev. Biol.* 282, 163-173 (2005).
- [29] Solon, J., Kaya-Copur, A., Colombelli, J. & Brunner, D. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell* 137, 1331-1342 (2009).
- [30] Zanet, J. et al. Fascin is required for blood cell migration during *Drosophila* embryogenesis. *Development* 136, 2557-2565 (2009).
- [31] Zanet, J. et al. Fascin promotes filopodia formation independent of its role in actin bundling. *J. Cell Biol.* 197, 477-486 (2012).
- [32] Kress, H. et al. Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity. *Proc. Natl Acad. Sci. USA* 104, 11633-11638 (2007).
- [33] Shibue, T., Brooks, M. W., Inan, M. F., Reinhardt, F. & Weinberg, R. A. The outgrowth of micrometastases is enabled by the formation of filopodium-like protrusions. *Cancer Discov.* 2, 706-721 (2012).
- [34] Jacquemet, G., Hamidi, H. & Ivaska, J. Filopodia in cell adhesion, 3D migration and cancer cell invasion. *Curr. Opin. Cell Biol.* 36, 23-31 (2015).
- [35] Schindelin, J. et al. Fiji: An open-source platform for biological-image analysis. *Nat. Methods* 9, 676-682 (2012).